NUTRIENT COMPOSITION, ANTIOXIDANT AND HYPOGLYCEMIC EFFECT OF BITTER GOURD (*Momordica charantia* L.)

by

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(2012-24-101)

THESIS

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DECLARATION

I hereby declare that this thesis entitled "**NUTRIENT COMPOSITION, ANTIOXIDANT AND HYPOGLYCEMIC EFFECT OF BITTER GOURD (*Momordica charantia* L.)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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LIST OF ABBREVIATIONS

%	Per cent
°C	degree celsious
µg	Micro gram
AACC	Approved Methods of Analysis
AAS	Atomic absorption spectroscopy
AMPK	Adenosine -5- monophosphate kinase
AOAC	Association of Official Agricultural Chemistry
APA	Abrus precatorius
DGB	Dark green big
DGS	Dark green small
DM	Diabetes Mellitus
DNA	Deoxyribo nucleic acid
DPPH	2,2- Diphenyl-1-picryl hydrazine
DV	Daily value
EDTA	Ethylene Diamine Tetra Acetic acid
et al	and others
Fig	Figure
g	Gram
HCl	Hydrochloric acid
HIV	Human Immuno Deficiency Virus
HMP	Hexose Mono Phosphate
HPLC	High Performance Liquid Chromatography
Kcal	kilo calories
LDL	Low density lipoprotein
LGB	Light green big

LGS	Light green small
MAA	Maackia nigra
MAP 30	Momordica anti HIV protein
mcg	Micro gram
mg	Milligram
MGC	Microgial cells
min	Minutes
ml	Milliter
mm	Mllimeter
NaOH	Sodium hydroxide
NIN	National Institute of Nutrition
NP	Nei paval
PPAR γ gene	Peroxisomeproliferator activated receptor gamma
PTP	Protein Tyrosine Phosphate
RDA	Recommended Dietary Allowance
RIP	Ribosome inactivating protein
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
RTS	Ready To Serve
SCRIP	Single chain ribosome inactivating protein
SGOT	Serum Glutamic Oxaloacetate Pyruvate Transaminase
SGPT	Serum Glutamic Pyruvate Transaminase
SOD	Super oxide dismutase
STZ	Streptozotozin
US	United States
USDA	United State Department of Agriculture
VFPCK	Vegetable and Fruit Promotion Council Kerala
WHO	World Health Organization

NBT	Nitro Blue Tetrazolium
NADH	Nicotinamide Adenine Dinucleotide
HPTLC	High Performance Thin Layer Chromatography
H_2SO_4	Sulphuric acid
UV	Ultra violet
hrs	Hours
$FeCl_2$	Ferric chloride
NA	Nutrient agar
EMB	Eosin methylene blue
Cfu	Colonies forming unit
BMI	Body Mass Index
WHR	Waist hip ratio
FBS	Fasting blood sugar
PPBS	Post prandial blood sugar
HbA_1C	Glycosylated haemoglobin
GI	Glycemic index
GL	Glycemic load
GTT	Glucose tolerance test
AUC	Area under curve
GAE	Gallic acid equivalent
TAC	Total antioxidant capacity
WBM	White Bitter Melon

Introduction

1. INTRODUCTION

The increasing food demands of the world population have overwhelmed the available land resources. Vegetables constitute a significant part of the food basket. They are cheap source of energy and rich sources of phytochemicals and nutrients such as carbohydrates, protein, carotene, ascorbic acid, calcium, iron, and trace elements. Bitter gourd (*Momordica charantia)* is one of the most popular vegetable in South Asia, which belongs to the family *cucurbitaceae*. The Latin name *Momordica* means *"*to bite" referring to the jagged edges of the leaves, which appear as if they have been bitten. It is regarded as one of the world's major vegetable crops and has great economic importance. Bitter gourd grows in tropical and subtropical areas, including parts of East Africa, Asia, Caribbean, and South America, where it is used not only as a food but also as a medicine. Furthermore, Indians have traditionally used the leaves and fruits as a medicine to treat diabetes, colic and to heal skin sores and wounds (Paul et al., 2009). This vegetable plant, *Momordica charantia*, a group comprises of about 130 genera and 800 species and contains an array of biologically active phytochemicals. These include triterpenes, proteins, steroids and antioxidants (Taylor, 2002). Recently antioxidants and secondary metabolites are attracting a great deal of attention for their effects in preventing diseases due to oxidative stress, that leads to degeneration of cell membranes and leads to many pathological diseases. Bitter gourd also known as balsam pear, karela, or bitter melon is a fast growing tropical vegetable crop. In India, it is cultivated in an area of 26,004 ha with a production of 1,62,196 tons and the productivity level is 6.23 t/ha (Kokate et al., 2002). All parts of plant, especially roots, leaves, fruits and seeds are widely used in traditional medicine throughout Asia, East Africa and South America (Gbeassor et al., 2006).

Bitter gourd is reported to be a good source of phenolic compounds, which possess potent antioxidant activity (Aminah and Anna, 2011). Fruits are relatively high in proteins, minerals and vitamins and many other nutrients

required in the human diet (Ali et al., 2008) which are necessary for maintaining proper health. The fruits contain, 83.2 per cent water, 2.9 per cent protein, 1.0 per cent fat, 1.4 per cent mineral matter, 1.7 per cent fiber, 9.8 per cent carbohydrates, 0.05 per cent calcium, 0.14 per cent phosphorus, 9.4 per cent iron and traces of magnesium. The presence of secondary metabolites such as alkaloids, saponins, tannins, glycosides and polyphenols in the *Momordica charantia* may contribute to its medicinal value. The unripe fruit is eaten in various ways, but the ripe fruit is too bitter. It has many proven medical uses. The leaves, flowers, and seeds are more extensively used in folk medicines. *Momordica charantia* has immense medicinal properties due to the presence of beneficial phytochemicals which are known to have antibiotic, antimutagenic, antioxidant, antiviral, antidiabetic and immune enhancing properties (Grover and Yadav, 2004). A compound known as charantin, present in the bitter gourd is used in the treatment of diabetes in reducing blood sugar level and is used for making nutraceuticals (Akuwuoh et al., 2005).

Antioxidants and secondary metabolites play a major role in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological diseases (Ahmed and Beigh, 2009). Moreover, recent investigations have shown that the antioxidants with free radical scavenging properties of plant origin could have great importance as therapeutic agents in ageing process and free radical mediated diseases (Zhang et al., 2009). The herbal plants are considered as useful means to prevent or ameliorate certain disorders such as diabetes, atherosclerosis and other complication (Scartezzini and Speroni, 2000). According to WHO (2010) about 82 per cent population depends upon herbal drugs and these medicines are gaining popularity because of having less side effects and being low priced (Kumar, 2001). Among the herbal resources, usefulness of bitter gourd or bitter melon as food and medicine has long been known to people. The juice of bitter gourd has been shown to possess hypoglycemic activity (Sitasawad et al., 2000) and many diabetic patients drink this juice in the morning as a natural remedy.

Diabetes mellitus (DM) is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels or hyperglycemia. (Lew and Zonszein, 2010). It is considered as one of the five leading causes of death in world (Joseph and Jini, 2011). Diabetes mellitus is a major global health problem. The rise in its prevalence from 171 million in 2000 to 366 million in 2030 causing world wide concern (Shaw et al., 2010). Being a major degenerative disease, diabetes is found in all parts of the world and is becoming the third most lethal disease affecting mankind which increasing rapidly. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million individuals worldwide (WHO, 2010). DM is spread widely, not only in developed countries, but also in developing countries (Hossain et al., 2007). Diabetes is emerging as terrible burden of Kerala due to rapid changes in life style. Thankappan et al. (2010) observed that Kerala is emerging as the capital of life style diseases of India with the prevalence of hypertension, diabetes and obesity, reaching levels comparable to those in Western Countries.

Hence, the present study "Nutrient composition, antioxidant and hypoglycemic effect of bitter gourd (*Momordica charantia* L.)" was attempted with the objective to estimate the chemical, nutrient composition, phytochemicals and antioxidant activities of the selected bitter gourd types and to assess its hypoglycemic effect on type 2 diabetes mellitus patients.

2. REVIEW OF LITERATURE

Medicinal plants and its products continue to be an important aid for alleviating the ailments of human kind (Joseph and Raj, 2010; Singh et al., 2012). Since ancient times, plants and plant extracts were used to combat various ailments. Many traditional medicines in use are derived from medicinal plants and which are used for medicinal purposes around the world. Literature available on different aspects related to the present study entitled "Nutrient composition, antioxidant and hypoglycaemic effect of bitter gourd (*Momordica charantia* L.) is reviewed under following headings.

2.1 Nutrient composition of bitter gourd

2.2 Bioactive compounds of bitter gourd

2.3 Nutraceutical properties of bitter gourd

2.4 Antidiabetic effect of bitter gourd

2.5 Culinary uses and value added products of bitter gourd

2.6 Bitter gourd and its toxic effects

2.1 NUTRIENT COMPOSITION OF BITTER GOURD

Bitter gourd is very low in calories but dense with precious nutrients. It is an excellent source of vitamins B_1, B_2, and B_3, Vitamin C, magnesium, folic acid, zinc, phosphorus, manganese, and has high dietary fiber (Keding and Krawinkel 2006). It is rich in iron, contains twice the beta-carotene of broccoli, twice the calcium of spinach, and twice the potassium of a banana (Aboa et al., 2008; Wu and Ng, 2008).

The vegetable is very low in calories, providing just 17 calories per 100g. Nevertheless, its pods are rich in phytonutrients like dietary fiber, minerals, vitamins and anti-oxidants (Klomann et al., 2010).

Islam and Jlaluddin (2005) reported that freeze dried gourd flesh was high in lysine compared to soy protein isolate. Flesh was relatively low in glutamic acid and arginine. Essential amino acids, including threonine, valine, methionine, isoleucine, leucine, and phenylalanine are comparable in amount to soy proteins and other legume proteins.

The immature fruit are eaten as vegetables and are a good source of Vitamin C, Vitamin A, phosphorus and iron (Sultana and Bari, 2003; Paul et al., 2009). The bitter flavour is due to the alkaloid momordicin produced in fruit and leaves.

Balasubramanian et al. (2007) reported that fresh bitter gourd is an excellent source of vitamin-C (100 g of raw pod provides 84 mg or about 140 per cent of RDA). Vitamin-C, one of the powerful natural antioxidants, helps the body in scavenging deleterious free radicals. The biggest nutritional benefit from bitter gourd is its vitamin C content. According to Nutrition Data one cup of raw bitter gourd contains 78.1 mg of vitamin C, which is 130 per cent of the DV (Daily Value). The National Institute of Health explains that vitamin C is an antioxidant vitamin that plays a role in building the immune system, and maintaining healthy tissues, skin, gums and blood vessels. Bitter gourd contains another powerful antioxidant vitamin i.e., Vitamin A. Although it is in a significantly smaller amount 9 percent of the daily value, Vitamin A works closely with vitamin C to boost antioxidant benefits. The nutrient composition of bitter gourd is given in Table 1.

Table 1. Nutrient composition of bitter gourd

Nutrients	Amount/100g
Energy (Kcal)	25.0
Carbohydrate (g)	4.20
Protein (g)	1.60
Dietary fiber (g)	0.80
Total fat (g)	0.20
Vitamins	
Vitamin A equiv. (µg)	126.0
Thiamine (mg)	0.07
Riboflavin (mg)	0.09
Niacin (mg)	0.50
Vitamin C (mg)	88.0
Minerals	
Calcium (mg)	20.0
Iron (mg)	0.61
Magnesium (mg)	36.10
Phosphorus (mg)	70.0
Potassium (mg)	152.0
Sodium (mg)	17.80
Zinc (mg)	464.0

(NIN, 1995)

Bitter gourd is an excellent source of health benefiting flavonoids such as ß-carotene, α-carotene, lutein, and zea-xanthin (Sathishsekar, 2005). It also contains a good amount of vitamin A, which acts as protective scavengers against oxygen-derived free radicals and reactive oxygen species (ROS) that play a role in aging, cancers and various disease processes. Fernandes et al. (2007) suggested that fresh pods are an excellent source of folates, contain about 72 µg/100g (Provides 18 per cent of RDA). Folate helps to reduce the incidence of neural tube defects in the newborns when taken by mothers during early pregnancy.

The University of California at San Francisco reported that most adults in the United States get less than half of the recommended daily value for fiber, which is between 25 and 30 mg. One cup of bitter gourd contains 2.6 g of fiber, which is about 10 per cent of the daily value. As with the vitamin and mineral

content, even though it may seem like an insignificant amount of fibre (Jiratchariyakul et al., 2001).

One cup of bitter melon offers some nutritional benefit in the form of minerals as well, although not nearly as much as it does with vitamins. The most abundant mineral present is potassium, with 275 mg or 8 percent of the daily value. According to the University of Maryland Medical Center, potassium is necessary for the proper functioning of the heart muscle, electrical impulses, muscle contractions, cells, tissues and digestive system. (Sathishsekar, 2005).

Chaudhari et al. (2009) stated that the extract of *Momordica charantia* significantly reduces serum glutamic pyruvate transaminase (SGPT), and serum glutamic oxaloacetate transaminase (SGOT) in rats. The hepatoprotective activity of *M. charantia* leaves may be attributed to the presence of flavonoids and ascorbic acid.

Khan and Anderson (2003) pointed out that essential oil of the seed of *M. charantia* showed antibacterial and antifungal activities due to the presence of *trans*-nerolidol (61.6 per cent of the total oil). Clinically and experimentally, leaf extracts (methanol and ethanol) of *M. charantia* have demonstrated a broad spectrum of antimicrobial activity (Braca et al., 2008).

2.2 BIOACTIVE COMPOUNDS OF BITTER GOURD

The plant contains several biologically active compounds, chiefly momordicin I and momordicin II, and cucurbitacin B (Semiz and Sen, 2007). The plant also contains several bioactive glycosides including alkaloids, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, guanylate cyclase inhibitors, gypsogenin, hydroxytryptamines, karounidiols, lanosterol, lauric acid, linoleic acid, linolenic

acid, momorcharasides, momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordicosides, momordin, momordolo, multiflorenol, myristic acid, nerolidol, oleanolic acid, oleic acid, oxalic acid, pentadecans, peptides, petroselinic acid, polypeptides, proteins, ribosome-inactivating proteins, rosmarinic acid, rubixanthin, spinasterol, steroidal glycosides, stigmasta-diols, stigmasterol, taraxerol, trehalose, trypsin inhibitors, uracil, vacine, v-insulin, verbascoside, vicine, zeatin, zeatin riboside, zeaxanthin, zeinoxanthin. Amino acids-aspartic acid, serine, glutamic acid, thscinne, alanine, g-amino butyric acid and pipecolic acid (Kimura et al., 2005).

Table 2. Phytochemicals present in bitter gourd

Source	Phytochemicals	Reference
Plant body	Momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins, multiflorenol,	(Umesh et al., (2005) and Budrat and Shotipruk,(2008)
Leaf	Glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids	(Chaudhari et al., 2009).
	Momordicine, charantin, polypeptide- p insulin, ascorbigen, Amino acids – aspartic acid, serine, glutamic acid,	Au et al.(2000) (Wako ,2005) and kobori et al. (2008)

Fruit	threonine, glutamic acid, threonine, alanine, g-amino butyric acid and pipecolic acid, luteolin, Fatty acids – lauric, myristic, palmitic, palmitoleic, stearic,oleic,linoleic, linolenic acid	
Seeds	Urease, Amino acids – valine, threonine methionine, isoleucine, leucine, phenylalanine, glutamic acid	Horax et al. (2005) and Semiz and Sen (2007)

Bitter gourd notably contains phyto-nutrient, polypeptide-P; a plant insulin known to lower blood sugar levels. In addition, it composes hypoglycemic agent called charantin acid (Harinantenaina et al., 2006). Charantin increases glucose uptake and glycogen synthesis in the cells of liver, muscle and adipose tissue (Bano et al., 2011). The pharmacological properties of p-insulin are similar to those of insulin (Singh et al., 2011). Together, these compounds are thought to be responsible for reduction of blood sugar levels in the treatment of type-2 diabetes.

Kobori et al. (2008) pointed out that bitter gourd contains a unique phyto-constituent that has been confirmed to have a hypoglycemic effect called charantin. There is also another insulin-like compound known as polypeptide P which has been suggested as insulin replacement in some diabetic patients.

Aminah and Anna (2011) reported that bitter gourd contains a lectin that has insulin-like activity due to its non protein-specific linking together to insulin receptors. This lectin lowers blood glucose concentrations by acting on peripheral tissues and, similar to insulin's effects in the brain, suppressing appetite. This

lectin is likely a major contributor to the hypoglycemic effect that develops after eating bitter gourd.

Sathishsekar (2005) opined that bitter gourd is an excellent source of phenolic compounds, antioxidants, and antimutagen. This can find application in food products and dietary supplements. The phenolic extracts showed high inhibition effect to prevent lipid oxidation. These natural plant phenolics can be a good antioxidant which may be applied in many food systems to enhance and maintain food quality (Cai et al., 2003).

Horax et al. (2005) reported that *M. charantia* extracts possess potent antioxidant and free radical scavenging activities and this may be due to the presence of phenolic and flavonoid compounds like, galic acid, tannic acid, catechin, caffeic acid, p-coumaric, gentisic acid, chlorogenic acid and epicatechin.

Au et al. (2000) clinically and experimentally demonstrated that the leaf extract of *M. charantia* have the ability to increase resistance against viral infections and to provide immunostimulant effects. Several isolated phytochemicals, e.g. α and β-momorcharin, lectin, MRK 29 and MAP 30, have been documented to have *in vitro* antiviral activity against epstein-barr, herpes, HIV, coxsackievirus B_3 and polio-viruses and among them MAP 30 have promising anti HIV activity.

Momordica charantia extract and its several isolated compounds like momordin I, Id and Ie, α and β momorcharin and cucurbitacin B as well as MAP 30- have shown anticancer activity against lymphoid leukemia, lymphoma, choriocarcinomamelanoma, breast cancer, skin tumor, prostatic cancer, squamous carcinoma of tongue and larynx, human bladder carcinomas and Hodgkin's disease (Braca et al., 2008). Kobori et al. (2008) have depicted α-eleostearic acid present in the seed extract of this plant and its dihydroxy derivative strongly

inhibited the growth of cancer and fibroblast cell lines like HL60 leukemia and HT29 colon carcinoma.

Literature review revealed the experimental documentation of abortifacient properties of *Momordica* proteins and momorcharins in early and midterm pregnancy (Wako , 2005).

Wang and Ng (2001) observed that momordin and its aglycone, oleanolic acid are active principles with antirheumatoid activity and mild hypotensive response with momordin. In another study, *M. charantia* prolonged. prothrombin time by inhibiting activation of factor X by factor VIIa-tissue factor complex or factor IXa (Chaudhari et al.,2009).

Umesh et al. (2005) reported that *M. charantia* and its isolated compounds increase the activity of adenosine-5-monophosphate kinase (AMPK), an enzyme that facilitates cellular glucose uptake and fatty acid oxidation. Hypoglycemic agents in *M. charantia* promote efficient oxidation of glucose into fuel, and conversion into starch. During glucose shortages, fats/fatty acids are used as fuel. Continued demand for energy in the absence or shortage of glucose causes fat cells to release their fat contents to maintain energy balance. This increased fatty acid oxidation eventually leads to weight loss. The most important bio active compounds present in bitter gourd are following.

2.2.1 Charantin

Charantin is a typical cucurbitane type triterpenoid in bitter gourd and is a potential substance with antidiabetic properties (Keding and Krawinkel, 2006; Patel et al., 2011). Pitiphanpong et al. (2007) demonstrated that charantin could be used to treat diabetes and can potentially replace treatment. It is a mixture of two compounds, namely sitosteryl glucoside and stigma steryl glucoside (Pitiphanpong et al., 2007). Chen et al. (2009) isolated 14 cucurbitane triterpenoids, kuguacins, including two pentanorcucurbitacins,

octanorcucurbitacin and trinorcucurbitacins along with six known analogues from the vines and leaves of bitter gourd.

Studies have reported that the compound is more effective than the oral hypoglycemic agent (Cousens, 2008). In a study, two aglycones of charantin were isolated and identified as sitosterol and sigmastadienol glycosides, however, when tested separately for their hypolglycemic effects *in vivo*, these two constituents did not produce any notable change in blood glucose levels (Harinantenaina et al., 2006). This is an indicator that charantin may contain other specific components, yet to be identified, that are responsible for the hypoglycemic activity observed in diabetes.

2.2.2 Polypeptide - p

Bitter gourd is one of the most commonly used vegetable that contains polypeptide – p and is used to control diabetes naturally (Hellolife, 2008). Polypeptide – p or p- insulin is insulin like hypoglycemic protein, shown to lower blood glucose level in gerbils, langurs and humans when injected subcutaneously (Tayyab et al., 2012).

The p –insulin works by mimicking the action of human insulin in the body and thus may be used as plant insulin replacement in patients with type 1 diabetes (Paul and Raychaudhuri, 2010). Recently (Wang, 2007) have cloned and expressed the 498 bp gene sequence coding for the bitter gourd polypeptide – p gene and have also proved the hypoglycemic effect of the recombinant polypeptide in alloxan induced diabetic mice (Wang, 2007). The oral intake of the extract from bitter gourd seed does produce hypoglycemic effect in streptozotocin (STZ) induced type – 1 diabetic rat (Wehash et al., 2012). This indicates that compounds in bitter gourd seeds other than p – insulin may also be effective in the treatment of type – 1 diabetes.

2.3 NUTRACEUTICAL PROPERTIES OF BITTER GOURD

Bitter gourd has been used in various Asian and African herbal medicine systems for a long time (Basch et al., 2003). During the last few decades tremendous research work has been carried out with *Momordica charantia* extract to see the antihelmintic, antibacterial, antibiotic, antidiabetic, anti- inflammatory, anti microbial, antileukemic, antimutagenic, antimycobacterial, antioxidant, antitumor, antiulcer, antiviral, aperitive, aphrodisiac, astringent, carminative, cytostatic, depurative, hormonal, hypocholesterolemic, hypotensive, hypotriglyceridemic, hypoglycemic, immunostimulant, insecticidal, lactogogue, laxative, purgative, refrigerant, stomachic, tonic and vermifuge properties.

2.3.1 Alzheimer's disease

Horax et al. (2005) reported that four lectins (Abrus precatorius (APA), Maackia amurensis (MAA), Bitter gourd and Sambucus nigra (SNA) have been used to identify glycohistochemically the microglial cells (MGC) activation in autoptic brain samples from Alzheimer's disease subjects. Three of these lectins including the one from bitter melon have utilized as microglial cell markers for the first time.

2.3.2 Anticancer

Compounds extracted from bitter gourd, α-eleostearic acid (from seeds) and 15,16-dihydroxy-α-eleostearic acid (from the fruit) have been found to induce apoptosis of leukemia cells *in vitro* (Kohno et al., 2004). Diets containing 0.01 per cent bitter gourd oil (0.006 per cent as α-eleostearic acid) were found to prevent azoxymethane-induced colon carcinogenesis in rats (Kobori et al., 2008). Researchers at Saint Louis University claim an extract from bitter gourd, commonly eaten and known as karela in India, cause a chain of events which helps to kill breast cancer cells and prevent them from multiplying (Ratna, 2010).

Kohno et al. (2004) reported that a novel phytochemical in bitter melon has clinically demonstrated the ability to inhibit an enzyme named guanylate cyclase.

This enzyme is thought to be linked to the pathogenesis and replication of not only psoriasis, but leukemia and cancer as well. Other phytochemicals that documented with cytotoxic activity are a group of ribosome-inactivating proteins named alpha- and beta-momorcharin, momordin, and cucurbitacin B. A chemical analog of bitter gourd protein named MAP-30 can inhibit prostate tumor growth (Semiz and Sen, 2007). Numerous *in vitro* studies have also demonstrated the anti-cancerous and anti-leukemic activity of bitter melon against numerous cell lines including liver cancer, human leukemia, melanoma and solid sarcomas (Leung et al., 2009).

Semiz and Sen (2007) elucidated the aqueous extract killed human leukemia lymphocytes in dose dependent manner. Experimental studies reported that, water extract blocked the growth of rat prostate carcinoma and hot water extract of the entire plant inhibited the development of mammary tumors in mice (Semiz and Sen, 2007).

2.3.3 Antihelmintic

Momordica charantia was more effective than piperazine in the treatment of Ascaridia galli (Sathish et al., 2010).

Bitter gourd is used as a folk medicine in Togo to treat gastrointestinal diseases and extracts have shown activity *in vitro* against the nematode worm *Caenorhabditis elegans* (Beloin et al., 2005).

2.3.4 Antimalarial

Bitter gourd is traditionally regarded in Asia as useful for preventing and treating malaria. Tea from its leaves is used for this purpose also in Panama and Colombia. In Guyana, bitter gourd are boiled and stir-fried with garlic and onions. This popular side dish known as corilla is served to prevent malaria. Laboratory studies have confirmed that species related to bitter gourd have antimalarial activity, though human studies have not yet been published (Wako, 2005).

Singh et al. (2006) studied that the *Momordica charantia* was shown good larvicidal activity. The mosquito larvicidal property of *Momordica charantia* against three mosquito species – Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti.

2.3.5 Antiviral

In Togo, the plant is traditionally used against viral diseases such as chickenpox and measles. Tests with leaf extracts have shown *in vitro* activity against the herpes simplex type 1 virus, apparently due to unidentified compounds other than the momordicins (Dey et al., 2006).

Bitter gourd (and several of its isolated phytochemicals) also has been documented with *in vitro* antiviral activity against numerous viruses including Epstein-Barr, herpes, and HIV viruses. In an *in vivo* study, a leaf extract demonstrated the ability to increase resistance to viral infections as well as to provide an immunostimulant effect in humans and animals (increasing interferon production and natural killer cell activity) (Islam et al., 2005).

Laboratory tests suggest compounds in bitter melon might be effective for treating HIV infection. Jiratchariyakul et al. (2001) reported that compounds isolated from bitter gourd have either been proteins or lectins, having impact on HIV infected people. Oral ingestion of bitter melon possibly could offset negative effects of anti-HIV drug. (Nerurkar et al., 2006).

Islam et al. (2005) pointed out that two proteins known as alpha- and beta-momorcharin (which are present in the seeds, fruit, and leaves) have been reported to inhibit the HIV virus *in vitro*. In one study, HIV-infected cells treated with alpha- and beta-momorcharin showed a nearly complete loss of viral antigen while healthy cells were largely unaffected. In 1996 the inventors of the chemical protein analog MAP-30 filed a U.S. patent, stating it was "useful for treating tumors and HIV infections.

More recently, Sylvia Lee-Huang, a biochemist at the NYU School of medicine, isolated another protein, MAP30 (Momordica anti-viral protein of 30 Daltons) that also has anti-HIV activity. This discoveries have considerable potential for the treatment of HIV, possibly providing new drugs to complement those already used to treat HIV or cheaper alternatives for those who can't access the drugs already available (Lako et al., 2007).

The antiviral activity observed *in vitro* has been attributed to MAP30. This protein has been reported to inhibit HIV viral integrate and to cause irreversible relaxation of supercoiled viral nucleic acids, these changes render viruses unable to integrate themselves into host cell genomes. Reduced rates of T-lymphocyte infection with HIV type-1 and reduced rates of viral replication in infected cells have also been reported *in vitro* (Paul et al., 2009). The MAP30 gene has been cloned and expressed, and the recombinant protein re-MAP30 has properties *in vitro* similar to those of native MAP30. However, the antiviral activity of MAP-30 has not been studied in humans.

Anti viral activities of ribosome inactivating proteins from *Momordica charantia* an interesting paradigm emerges which may safely be used in treating viral diseases. It has been reported that ribosome inactivating proteins are member of the single chain ribosome inactivating protein (SCRIP) family which act irreversibly on ribosome by removing adenine residue from eukaryotic ribosomal RNA. Various activities of ribosome inactivating proteins include anti – tumor, broad anti viral, ribonuclease and deoxyribonuclease (Puri et al., 2009).

2.3.6 Cardio protective

Studies in mice indicated that bitter gourd seed may have a cardioprotective effect by down-regulating the NF-κB inflammatory pathway (Gadang et al., 2011).

Pratibha et al. (2006) reported that the treatment of diabetic rats with *M. charantia* extract resulted in significant reduction of blood lipid levels (total cholesterol and triglycerides) in diabetic rats. *M. charantia* also ameliorate PI-

associated apoB and lipid abnormalities in HepG2 cells. Compounds in *M. charantia* improve lipid profiles. They reduce liver secretion of apolipoprotein B (Apo B) – the primary lipoprotein of low-density "bad" cholesterol; reduce apolipoprotein C- III expression, the protein found in very-low density cholesterol which turns into LDL/bad cholesterol; and increases the expression of apolipoprotein A-1 (ApoA1) - the major protein component of high density "good" cholesterol. It also lowers cellular triglyceride content.

Kumar et al. (2010) stated that the *Momordica charantia* increase the activity of adenosine 5 monophosphate kinase (AMPK), an enzyme that facilitate cellular glucose uptake and fatty acid oxidation and reduces the triglyceride and total cholesterol content.

2.3.7 Diabetes

Manjamalai et al. (2010) pointed out that bitter gourd contains a hypoglycemic compound (a plant insulin) that is highly beneficial in lowering sugar levels in blood and urine. Bitter gourd juice has been shown to significantly improve glucose tolerance without increasing blood insulin levels.

In 1966, Lotlikar and Rao extracted from the plant a substance, which they called charantin, which had hypoglycaemic effect on normal and diabetic rabbits. Bitter gourd has been found to increase insulin sensitivity (Sridhar et al., 2008). In 2007, a study by the Philippine Department of Health determined a daily dose of 100 mg per kilogram of body weight is comparable to 2.5 mg/kg of the antidiabetes drug glibenclamide taken twice per day (GMA News, 2007). Tablets of bitter gourd extract are sold in Philippines as a food supplement and exported to many countries (GMA News, 2007).

Other compounds in bitter melon have been found to activate the AMPK, the protein that regulates glucose uptake (a process which is impaired in diabetics) (Shetty et al., 2005). Bitter gourd also contains a lectin that has insulin-like

activity due to its nonprotein-specific linking together to insulin receptors. This lectin lowers blood glucose concentrations by acting on peripheral tissues and, similar to insulin's effects in the brain, suppressing appetite. This lectin is likely a major contributor to the hypoglycemic effect that develops after eating bitter gourd (Sridhar et al., 2008). Feeding trials with insulin resistant or Type II diabetic rats and mice have shown that bitter gourd helps to prevent or reverse insulin resistance (Nerurkar et al. 2008, Klomann et al. 2010).

Sathishsekar (2005) Klomann et al. (2010) reported that high sugar concentrations from Type I and Type II diabetes increase the risk of inflammation and oxidation in the whole body, leading to blindness, diabetic feet, kidney disease, stroke, or heart attack. Consuming bitter gourd can help to prevent these complications, as it not only decreases blood sugar levels, but also has some antioxidative properties.

Investigations were carried out to evaluate the effect of Momordica charantia on the glucose tolerance of maturity onset diabetic patients. Kalia (2005) found that the fruit juice of M. charantia was found to significantly improve the glucose tolerance of 73 per cent of the patients investigated while the other 27 per cent failed to respond.

Singh et al. (2014) studied the effects of long term feeding of acetone extract of *M. charantia* (whole fruit powder) on alloxan diabetic albino rats. Acetone extract given orally daily lowered the blood sugar and serum cholesterol levels to normal range after 15 to 30 days in alloxan diabetic rats. The blood sugar once lowered after 30 days treatment did not increase even after 15 days of discontinuation of the treatment.

A study tested the effect of eating powdered whole bitter gourd for one week in people with type II diabetes. Fasting blood sugar levels and blood sugar levels measured after consuming 50 g of pure glucose were significantly lower

after consumption of bitter gourd. The average fasting blood sugar level decreased from 248 mg/dl to 155 mg/dl (Belinda 2003 ; and Ajayi et al., 2011).

2.3.8 Antiobesity

According to Microbiome and Me (2012) bitter gourd in combination with Chinese yam has been shown to contribute to weight loss. Over a period of 23 weeks, those eating the diet containing bitter gourd lost 7 kilos. In mice and rats, bitter gourd has been shown to reduce hypertension (Singh et al., 2004), plasma cholesterol and plasma lipids (Nerurkar et al., 2008).

Umesh et al. (2005) reported that *in vivo* studies, bitter gourd fruit or seed have been shown to reduce total cholesterol and triglycerisin both the presence and absence of dietary cholesterol. In one study, elevated cholesterol and triglyceride levels in diabetic rats were returned to normal after 10 weeks of treatment (Shetty et al., 2005). The fruit and seed of bitter gourd have demonstrated (in animal studies) to lower blood cholesterol levels.

2.3.9 High fat diet – induced brain oxidative stress

Bitter gourd ameliorated high fed diet-associated changes in blood brain barrier permeability. High fat diet-induced brain oxidative stress was also significantly reduced by bitter gourd supplementation (Sridhar et al., 2008).

2.3.10 Indigestion and constipation

Klomann et al. (2010) reported that bitter gourd stimulates easy digestion and peristalsis of food through the bowel until it is excreted from the body, thus helps in relieving indigestion and constipation problems.

Sathish et al. (2010) pointed out that *Momordica charantia,* is also found in China, where it is known as Chinese Bitter Melon. It has been used in traditional Chinese medicine as an appetite stimulant and a treatment for gastrointestinal infection.

2.3.11 Anti- ulcer activity

The traditional use of *Momordica charantia* in the treatment of ulcers is supported by research, suggesting the dried powdered fruits in filtered honey have significant and dose dependent anti ulcerogenic activity against ethanol induced ulcerogenesis in rats (Matsuda et al., 1999). Gurbuz et al. (2000) demonstrated that momordin I_c (10 mg/kg) potentially inhibited ethanol induced gastric mucosal lesions.

2.3.12 Blood disorders

Shetty et al. (2005) suggested that bitter gourd juice is highly beneficial for treating blood disorders like blood boils and itching due to toxemia.

2.3.13 Eye problems

A study conducted by Paul et al. (2009) opined that high beta-carotene and other properties in bitter gourd makes it one of the finest vegetable-fruit that helps to alleviate eye problems and improving eyesight.

2.3.14 Hangover

Bitter melon juice may be beneficial in the treatment of a hangover for its alcohol intoxication properties. It also helps to cleanse, repair and nourish liver problems due to alcohol consumption (Taylor, 2002).

2.3.15 Immune booster

Grover and Yadav (2004) reported that *Momordica charantia* extracts and its isolated constituents have a variable effect on the immune system .However, its immunostimulant activity has been attributed to increase the interferon production and natural killer activity. The bitter gourd juice helps to build immune system and increase body's resistance against infection (Keding and Krawinkel, 2006).

2.3.16 Piles

Bitter gourd juice is good for treating piles (Haque et al., 2011).

2.3.17 Psoriasis

Au et al. (2000) suggested that regular consumption of bitter gourd juice has also been known to improve psoriasis condition and other fungal infections like ring-worm and athletes feet.

Sathish et al. (2010) opined that a novel phytochemical in bitter gourd has clinically demonstrated the ability to inhibit an enzyme named guanylate cyclase. This enzyme is thought to be linked to the pathogenesis and replication of psoriasis.

2.3.18 Respiratory disorders

Drinking of two ounces of fresh bitter gourd juice mixed with a cup of honey diluted in water daily will improve asthma, bronchitis and pharyngitis (Bose et al., 2002).

2.3.19 Toxemia

Bitter gourd contains beneficial properties that cleanse the blood from toxins. It is also helpful in ridding jaundice for the same reasons (Shetty et al., 2005).

Paul et al. (2010) studied that the *Momordica charantia* decrease the genotoxic activity of methylnitrosamine, methanesulfonate and tetracycline, as shown by the decrease in chromosome breakage.

2.3.20 Liver and Biliary system

Momordica charantia fruit is useful in sub acute cases of liver and spleen. Another method for carcinogen- induced lipid peroxidation in liver and DNA damage in lymphocytes were reduced by following treatment of *M. charantia*

(Virdi et al., 2003). The fruit extract was found to significantly active liver enzymes glutathionine peroxidase and catalase, which showed a depression following exposure to the carcinogen. The result suggest the preventive role of water soluble constituent of *M. charantia* fruit during carcinogenesis, which is mediated effect on enzymes of biotransformation and detoxification system of host (Sathish et al., 2010).

2.3.21 Hypotensive and anti prothrombin activity

Wang and Ng, (2001) observed mild hypotensive response with Momordin. In another study, *M. charantia* prolonged prothrombin time by inhibiting activation of factor X by factor VIIa tissue factor complex or factor IXa (Kumar et al., 2010).

2.3.22 Anxiolytic activity

Ganesan et al. (2008) studied that the oral administration of 5 ml kg- 1 propylene glycol methanol extract of dried leaves of *Momordica charantia* Linn was investigated for anxiolytic activities in animal models.

Anxiolytic activity of methanol extract of dried leaves of *Momordica charantia* Linn was tested by elevated plus maze test. The results showed a significant anxiolytic effect comparable, with diazepam in all the tested doses (Ganesan et al., 2008).

2.3.23 Wound healing activity

Sharma et al. (2009) reported that *Momordica charantia* fruit powder, in the form of an ointment (10 per cent w/w dried powder in simple ointment base) showed a statistically significant response ($P < 0.01$) in terms of wound contracting ability, wound closure time, period of epithelisation, tensile strength of the wound and regeneration of tissue at wound site when compared with the control group, and these results were comparable to those of reference drug

povidone iodine ointment in an excision, incision and dead space wound model in rats.

2.4 ANTI DIABETIC EFFECT OF BITTER GOURD

Bitter gourd and its various extracts and components are believed to exert their hypoglycaemic effects via different physiological, pharmacological, and biochemical modes (Taylor, 2002). The possible modes of hypoglycemic actions of bitter gourd and its various extracts and compounds are its hypoglycaemic effect (Cumming et al., 2004), stimulation of peripheral and skeletal muscle glucose utilization (Jeong et al., 2008), inhibition of intestinal glucose uptake (Nerurkar et al., 2006), inhibition of adipocyte differentiation (Ragasa, 2011), suppression of key gluconeogenic enzymes (Singh et al., 2011), stimulation of key enzyme of HMP path way and preservation of islets β cell and their functions (Gadang et al., 2011; Fuangchana et al., 2011 and Wehash et al., 2012).

According to Kim and Kim (2011) bitter gourd extract suppressed the activation of mitogen – activated protein kinase (MPAKs) including stress activated protein kinase (SPAK), p^{38} and $p^{44/42}$, and the activity of NF- KB in MIN_6N_8 cells. A similar study suggested that bitter gourd improves the serum and liver lipid profile and serum glucose levels by modulating PPAR – γ gene expression (Gadang et al., 2011). According to Ragasa et al. (2011) cholesterol 5 α – stigma – 7-en 3β-ol were isolated as sterols from bitter gourd having significant hypoglycaemic effects. Bitter gourd was identified to possess a potent neuroprotective activity against global cerebral ischemia reperfusion induced neuronal injury and consequent neurological deficits in diabetic mice (Malik et al., 2011). Protein tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signalling, has served as a potential drug target for the treatment of type 2 diabetes (Hoang et al., 2010).

Bitter gourd, its extracts and isolated components are believed to exert their hypoglycaemic effects via different physiological and biochemical

processes. These include insulin secretagogue like effect, stimulation of skeletal muscle and peripheral cell glucose utilization, inhibition of intestinal glucose uptake, inhibition of adipocyte differentiation, suppression of key gluconeogenic enzymes, stimulation of key enzymes, HMP pathway and preservation of pancreatic islet cells and their functions.

2.4.1 Preservation of pancreatic β cells and insulin secretion

It was previously demonstrated by Jeevathyaparan et al. (1995) that oral administration of bitter gourd could lead to the secretion of insulin from endocrine pancreatic β cells. This observation was further confirmed by Ahmed et al. (2004) who investigated the effect of daily oral administration of bitter gourd juice and the distribution of γ, β and δ cells in the pancreas of STZ- induced diabetic rats using immune histochemical methods. The feeding of alcoholic extract from bitter gourd showed definite improvement in the islets of Lanngerhans (Singh et al., 2008).

Physiological experiments have also shown that bitter gourd can stimulate insulin secretion from the endocrine pancreas and elicit glucose uptake in the liver (Jeong et al., 2008). Current evidence therefore indicates that the recovery and subsequent increase in the number of insulin producing cells followed by release of insulin may be part of the several pathways by which bitter gourd exerts its hypoglycaemic effects. In addition to the properties mentioned above, bitter gourd and its extracts may possess cell like proliferation and growth like properties similar to that of insulin (Parmar et al., 2011).

2.5 CULINARY USES AND VALUE ADDED PRODUCTS OF BITTER GOURD

In Northern India, it is often prepared with potatoes and served with yogurt on the side to offset the bitterness, or used in sabzi. In North Indian cuisine, it is stuffed with spices and then cooked in oil. In Southern India, it is used in the dishes *thoran* (mixed with grated coconut), *theeyal* (cooked with

roasted coconut) and *pachadi* (which is considered as medicinal food for diabetics) (Kubola and Siriamorpaun, 2008). In Pakistan and Bangladesh, bitter melon is often cooked with onions, red chili powder, turmeric powder, salt, coriander powder and a pinch of cumin seeds (Kubola and Siriamorpaun, 2008).

Bitter gourd is generally consumed cooked in the green or early yellowing stage. The young shoots and leaves of the bitter melon may also be eaten as greens. Bitter gourd is often used in Chinese cooking for its bitter flavor, typically in stir-fries (often with pork) and soups, and also in tisanes. It has also been used in place of hops as the bittering ingredient in some Chinese and Okinawan beers (Bagchi and Indrani, 2006).

Bitter melon is a significant ingredient in Okinawan cuisine, and is increasingly used in mainland Japan. It is popularly credited with Okinawan life expectancies being higher than the already long Japanese ones. In Indonesia, bitter melon is prepared in various dishes, such as gado-gado, stir fried, cooked in coconut milk or steamed (Belinda, 2000).

In Vietnam, raw bitter gourd slices are consumed with dried meat floss and bitter melon soup with shrimp. Bitter gourds stuffed with ground pork are served as a popular summer soup in the south (Belinda, 2000). In the Philippines, bitter melon may be stir-fried with ground beef and oyster sauce, or with eggs and diced tomato. The dish *pinakbet*, popular in the Ilocos region of Luzon, consists mainly of bitter melons, eggplant, okra, string beans, tomatoes, lima beans, and other various regional vegetables (Belinda, 2000).

Value addition of bitter gourd can be done by dehydration. Thin slices can be dehydrated and this technology is adopted in a small scale for domestic purposes. A better quality product can be prepared if driers are used for dehydration. In addition slices of this fruit can be preserved in brine solution (Mudgal, 2009).

2.5.1 Bitter gourd extract

Momortin is a product extracted from the fruits of *Momordica* charantia, containing high purity of natural charantin. Metabolic and hypoglycemic effects of bitter gourd extracts have been demonstrated in cell culture, animal and human studies. Most studies have shown a blood-glucose lowering effect of the fruit of bitter gourd when fed orally. Momordica charantia inhibits glucose uptake by the gut and stimulate glucose uptake by skeletal muscle cells. Moreover, it also helps to preserve islet ß cells and ß cell functions, normalizes the systolic blood pressure and reduces oxidative stress (Hossain et al., 2007).

Bitter gourd extract is cultivated in Asia, Africa and South America. Extract of this vegetable is being popularized as a dietary supplement in Western Countries, since it is known to contain additional glycosides such as mormordin, vitamin C, carotenoids, flavonoids and polyphenols (Semiz and Sen 2007).

2.5.2 Dehydrated bitter gourd powder

Bitter gourd powder is widely used in natural food supplement such as food for people with diabetes and the vast number of consumer's health food, functional food. Also can be used for advanced cosmetic special additives and pharmaceutical raw materials (Hossain et al., 2007). Bitter gourd powder is used for soups, sauces, gravy, stock cubes, flavorings, seasonings, health drinks, flavors, dietary supplement etc.

2.5.3 Ready-to-eat bitter gourd chips

Bitter gourd chips were prepared and popularized among rural and urban areas as snack foods. The process for manufacturing of bitter gourd chips involves cutting of bitter gourd to 0.25–0.30 cm thick slices, followed by cooking in 0.1% sodium bicarbonate solution at 100°C for 30–40 min (Mudgal, 2009).

2.5.4 Herbal blended lemon bitter gourd RTS beverage

Three varieties of RTS beverage was prepared by blending bitter gourd juice with lemon juice; sample A have bitter gourd juice and lemon juice in same ratio, sample B have lemon juice about one fourth part of bitter gourd juice, sample C was control which was prepared by without preservative to compare the product shelf life. In all three sample 90% of water was added. These samples were evaluated initially and after that at the interval of 15, 30 and 60 days for sensory and chemical analysis viz. taste, color, flavor, appearance, pH, TSS, ascorbic acid, titrative acidity, microbial analysis, and overall acceptability. After the analysis it was found that both blend had high nutritive value and a shelf life of approximately 60 days which can be increased by providing better technique and condition. Out of three samples, A was found to be best (Rani, 2008). Other value added products are bitter gourd pickles, *Momordica Charantia* capsule, frozen bitter gourd, bitter gourd juice and soft drink and skin care cream.

Blanching of sliced bitter gourd resulted in considerable losses of radical scavenging activity, total phenolic content and vitamin C (Myojin and Enami, 2008). In frozen storage at 18^0 C showed that blanching of bitter gourd improves the retention of radical scavenging activity and total phenolic during subsequent frozen storage but Vitamin C losses and quick freezing at 40^0 C not causes any decrease in radical scavenging activity, total phenolic and vitamin C content without proceeding blanching (Mudgal, 2009). Mechanical dehydration decreases moisture content and vitamin C but it improves the shelf life quality (Wang, 2007; Myojin and Enami, 2008).

2.6 BITTER GOURD AND TOXIC EFFECTS

2.6.1 Endocrine

Bitter gourd has been found to lower blood glucose levels in animal studies and in several methodologically weak human trials. Proposed mechanisms include

insulin-like effects, stimulation of pancreatic insulin secretion, decrease hepatic gluconeogenesis, increased hepatic glycogen synthesis, increased peripheral glucose oxidation (Lancet, 2000). Two cases were reported in children ie, hypoglycemic coma and convulsions after administration of a bitter gourd tea (Jayasoorya et al., 2000).

2.6.2 Gastrointestinal.

The seeds and outer rind of bitter gourd contain a toxic lectin that inhibits protein synthesis in the intestinal wall. However, this has not been correlated with clinical signs or symptoms in humans (Sultana and Bari, 2003).

2.6.3 Hematologic.

Individuals with glucose-6-phosphate dehydrogenase deficiency are at risk of developing favism after ingesting bitter melon seeds (Semiz and Zen, 2007).

2.6.4 Hepatic

Significant increases in y-glutamyl transferase and alkaline phosphatase have been observed in animals after oral administration of bitter gourd fruit juice and seed extract (Semiz and Zen, 2007).

Excessive consumption of bitter gourd may cause mild abdominal pain or diarrhoea. Diabetics taking hypoglycemic drugs will need to alter the dosage of their drugs if they consume bitter gourd on a regular basis (Leu and Zonszein, 2010).

Pregnant women should avoid taking too much bitter gourd or its juice as it may stimulate the uterus that may lead to constrictions in uterus preterm labour (Leu and Zonszein,2010).

Two factors naming abortifacient and emmenagogue properties are abundantly available in bitter gourd. Bitter gourd tea should be avoided by people with heartburns and ulcer problems as it can worsen the conditions (Sultana and Bari, 2003).

2.6.5 Thyroid function

Panda and Kar (2000) reported that alcoholic extract of *Momordica charantia* fruits was found to enhance T3, T4 was reduced. Since two higher doses inhibited thyroid hormone concentrations and increased hepatic lipid peroxidation, *M. Charantia* fruit extract, when used in excess may prove to be harmful with respect to thyroid function and lipid peroxidation.

3. MATERIALS AND METHODS

The present study entitled "Nutrient composition, antioxidant and hypoglycemic effect of bitter gourd (*Momordica charantia* L.)*"* was conducted in three experiments. First experiment encompasses an assessment of various parameters like chemical, nutrient composition, antioxidant activity and phytochemical analysis of the selected bitter gourd types.

3.1. CHEMICAL, NUTRIENT COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE SELECTED BITTER GOURD TYPES

3.1.1. Materials selected

Four types of commercially cultivated bitter gourd viz., light green small, light green big, dark green small, dark green big along with *Nei paval* were selected for the study (Plates 1 to 5). First two types were collected from VFPCK, Kalliyoor and the second two types were collected from local market in Trivandrum and *Nei paval* was collected from Madurai, Tamil Nadu.

The fresh bitter gourd fruits were cut in to thin slices and dried in an cabinet drier below 40^{o} C and was processed into powder form with the help of mixer. The powdered bitter gourd of the five types was stored at ambient condition in zip lock pouches. The chemical, nutritional composition and antioxidant activities of the selected bitter gourd types were carried out both in fresh and processed form.

3.1.2 Assessment of chemical and nutritional composition

In the present study macro and micro nutrients present in the fresh and powdered bitter gourd fruits were estimated. Nutrients such as total carbohydrate, protein, acid profile including essential amino acids, non-essential amino acids, sulphur containing amino acids, vitamins *viz.* vitamin C, β carotene and

Plate 1. Light green big

Plate2. Light green small

Plate3. Dark green big

Plate4. Dark green small

Plate5. Nei paval

folic acid were estimated. Minerals and trace elements such as calcium, phosphorus, sodium, potassium, iron, manganese, copper and zinc were also determined. In the present study, phytochemicals such as alkaloids, tannins, flavonoids, saponins, polyphenols, charantin and lectin were estimated. Antioxidant activity such as total antioxidant activity, DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide radical scavenging activity were also ascertained. Apart from the above nutrients moisture, fibre and total ash were estimated in the fresh and powdered bitter gourd.

3.1.2.1 *Protein*

The nitrogen content of bitter gourd samples was estimated by micro Kjeldahl's wet digestion method. The nitrogen values were multiplied by the factor 6.25 to get the crude protein content (AOAC, 2000).

3.1.2.2 *Evaluation of protein quality*

The quality of proteinaceous food depends on its amino acid composition in relation to the protein content and digestibility (Ghosh and Chakravarthy, 1990). Amino acid composition may also serve as a good relative measure to compare with other food stuffs of established nutritive value.

The amino acid content of bitter gourd was estimated by HPLC method. Total of 15 amino acids (including 8 essential and sulphur containing amino acid) have been evaluated from five dry bitter gourd powder samples.

3.1.2.3 *Crude fibre*

Crude fibre content was determined according to AACC method (2000).

3.1.2.4 *Total carbohydrates*

Total carbohydrates were estimated following the anthrone method as described by Sadasivam and Manickam (2008).

3.1.2.5 *Moisture*

Moisture content was estimated by the method of AOAC (1990).

3.1.2.5 *β carotene*

β carotene was estimated by the method of Sadasivam and Manickam (2008).

3.1.2.6 *Vitamin C*

Vitamin C was estimated by the method of Ranganna (2001).

3.1.2.7 *Folic acid*

Folic acid was estimated by the method of HPLC. 10g of each bitter gourd fruit sample weighed and made into homogenized in mortar with pestle and transferred into conical flask and 25 ml of extraction solution was added, kept on shaking water bath at 70 ^{0} C for 40 min. thereafter, the sample was cooled down, filtered and finally the volume was made up to 50 ml with extraction solution. Then sample filtered through 0.45 µm filter tips and aliquots of 20 µl from this solution was injected into the HPLC by using auto sampler.

Analytical reversed phase C 18 column was used for the separation. Mobile phase consisting of a mixture of buffer and methanol in the ratio of 96:4 was delivered at a flow rate of 1 ml/ min with UV detection at 210 nm. The mobile phase was filtered through 0.22 µm membrane filter, sonicated and degassed before use. Analysis was performed at room temperature. All the prepared bitter gourd fruit sample solutions were first chromatographed to ensure that interfering peaks were not present. 20 µl aliquots of the standard solution of folic acid and sample solutions were injected and estimated the amount of folic acid.

3.1.2.8 *Calcium (Ca)*

Calcium was estimated by the method of Sadasivam and Manickam (2008). Fill the burette with 0.02N EDTA solution up to zero mark. Take 10 ml of ash solution of sample in conical flask. Add 2 ml of 0. 01N NaOH as buffer solution and 4 to 5 drops of Eriochrome black – T indicator. Titrate it against 0.02N EDTA solution, the colour change will be wine red to blue. Note the burette reading and repeat it twice or thrice till concordant values were obtained.

3.1.2.9 *Total minerals*

The samples were powdered and digested with concentrated nitric acid in a microwave digester. Total minerals such as phosphorus (in spectrophotometer), sodium and potassium (in flame photometer), iron, manganese, copper and zinc content of the digest were estimated in AAS using hydride vapor generator method suggested by Jackson (1973).

Qualitative estimation of Phytochemicals

3.1.2.10 *Preparation of the extract*

Collected fresh fruits were washed with tap water two to three times to remove dust particles and cut into small pieces. From this took 25g fruit and ground it well with the help of pestle and mortar and the extraction of bitter gourd was carried out by using 50 ml of each ethanol, methanol, acetone and distilled water as solvents. Extracts were centrifuged at 5000 rpm for 20 minutes. The supernated extracts were kept overnight for incubation at room temperature.

Phytochemical screening

The fruit extract was subjected to preliminary phytochemical screening using the methods described by Evans (1996).

3.1.2.11 *Test for Tannins*

In 1.0 ml of plant extract, equal volume of Ferric chloride ($FeCl_3$) or bromine water was added. Formation of reddish brown or greenish black precipitate indicates the presence of tannins.

3.1.2.12 *Test for Flavonoids*

To 3 ml of sample extract in a test tube add 10 ml of ethyl acetate and heated over a steam bath for 3 minute. The mixture was filtered and 4ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Appearance of yellow colouration was observed indicating the presence of flavonoids.

3.1.2.13 ***Test for Phenolic compounds***

Dilute ammonia solution was added to the sample extract and the production of reddish to yellow colour indicates the presence of phenolic compounds.

3.1.2.14 ***Test for Alkaloids***

For the screening of alkaloids, 1.0 ml of extract was mixed in 5 ml of dil. hydrochloric acid on a steam bath. The mixture was filtered and 1.0 ml of Mayer's reagent was added to 1.0 ml of filtrate in a separate tube. A cloudy slightly yellow colour indicates the presence of alkaloids.

3.1.2.15 ***Test for Saponins***

Frothing test was used to determine the presence of saponin in fruit extract of *Momordica charantia.* A 0.2 ml of extract was mixed with 5.0 ml of distilled water and shaken for 20 minute. Persistence of foams indicates the presence of saponins.

3.1.2.16 ***Test for Steroids***

The screening for the presence of steroid in the fruit extract was performed by Liebermann-Burchard's test. 0.5 ml of extract was dissolved in 2 ml of acetic anhydride and cooled in ice. 1 ml of Conc. H_2SO_4 was added and formation of blue green ring was considered positive for presence of steroids.

3.1.2.17 ***Test for Cardiac glycoside***

The screening of cardiac glycoside was done by using Legal's method in which 1 ml extract was dissolved in 5 ml of pyridine an add 2 drops of 2 per cent Sodium Nitroprusside and 2 drops of 20 per cent NaOH. A deep red colour faded to brown indicates presence of cardenolide.

3.1.2.18 *Test for Phlobatinnins*

For the screening of phlobatinnins, 1.0 ml of extract was boiled in 2ml of 1 per cent aqueous HCl. A red precipitate indicates the presence of phlobatinnins.

3.1.2.19 *Test for Anthraquinones*

Borntrager's test was used for the screening of anthraquinone. 2 g extract in 10 ml of ethanol was steamed for 5 min and filtered, 2 ml filtrate was added to 2ml chloroform, shaken thoroughly, chloroform layer was taken off and 5 ml of distilled water was added and shaken with 5ml of dilute ammonia solution. Absence of red colour in ammonia upper phase indicates the absence of anthraquinones.

Quantitative Phytochemical analysis

3.1.2.20 *Total polyphenols*

Method suggested by Sadasivam and Manickam (2008) was used for estimating total phenols. The absorbance of the blue colour developed after experiment was measured at 660nm on double beam UV-visible spectrophotometer. Total polyphenols were calculated with the help of standard curve of 0.1mg/mL tannic acid and expressed as gram 100 g^{-1} dry weight.

3.1.2.21 *Tannins*

Tannins were estimated by the method suggested by Sadasivam and Manickam (2008). After completion of reaction absorbance were measured at 700 nm on double beam UV-visible spectrophotometer (Shimadzu-190). A blank was prepared with water instead of the sample. Water soluble tannins were estimated and calculated with the help of standard curve of tannic acid (0.1mg/mL) and expressed as gram $100g^{-1}$ of dry weight.

3.1.2.22 *Alkaloids*

Method suggested by Harborne (1973) was used for estimating alkaloids. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

3.1.2.23 *Flavonoids*

Flavonoid was determined by the method of Bhom and Kocipai (1994). 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3.1.2.24 *Saponins*

Method suggested by Obdoni and Ochuko (2001) was used for estimating saponins. 20 g of sample powder was taken in a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hrs with continuous stirring at about 55°C. The mixture was filtered and the residue re extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5%

aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

3.1.2.25 *Charantin*

Charantin was estimated by the HPLC method. About 15g of dry powder of samples were subjected to successive solvent extraction in a soxhlet apparatus for 8hrs using ethanol to get concentrated extract. The filtrate was subjected to evaporation and dried extract was collected and used for Analytical HPLC analysis. In this method aluminium backed silica gel 60 F_{254} plates were used as stationary phase and toluene ethyl, acetate, methanol and formic acid (68:20:10:02) as a optimized mobile phase. Developed chromatogen was scanned at 525 nm, the wavelength of maximum absorption for charantin after derivatization with anisaldehyde sulphuric acid reagent. Regression analysis of the calibration data showed an excellent linear relationship between peak area vs drug concentration. Linearity was found to be in the range of 100-500 ng/ band. The suitability of developed HPLC method for estimation of charantin was established by validating it as per the ICH guide line. Standard Charantin (Sigma) was used as reference marker.

3.1.2.26 *Lectin*

The method adopted for the determination of lectins is the colorimetric method as reported in the Manual of Food quality (AOAC, 1990). Here, the samples were ground into slurry and 1g of the slurry sample was weighed into a crucible. Ten milliliter of distilled water was added followed by the addition of 1 ml concentrated H_2SO_4 and the mixture allowed standing for one hour. The solution was made up to 50 ml using distilled water. Five milliliter of the extract was pipetted into a test tube and 1ml of Schiffs reagent added to the test tube. A portion of the solution in the test tube was put into cuvette and the absorbance measured at 510 nm. The value of lectin in each sample is estimated from the standard curve of the lectin.

3.1.2.27 *Antioxidant assays*

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. They act as a defense system against oxidative damage in our bodies and are helpful in avoiding chronic diseases and the effects of aging (Oliveri, 2000).

Bitter gourd is regarded as an antioxidant rich vegetable with beneficial properties for the circulatory, respiratory, digestive and nervous systems according to the Indian indigenous system of medicine (Horax et al., 2005). Antioxidant property was determined by the following methods.

a. Antioxidant activity

The antioxidant activity was determined according to the thiocyanate method with slight modifications (Oliveri, 2000). For the stock solution, 10 mg of ascorbic acid was dissolved in 10 ml water. Then the solution of standards samples (100mg/l) in 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6) was added to 2.5 ml of linoleic acid emulsion. Fifty ml linoleic acid emulsion contained Tween-20, linoleic acid and potassium phosphate buffer (0.04 M, pH 7.6). On the other hand, 5.0 ml of control contained 2.5 ml of linoleic acid emulsion and 2.5 ml of potassium phosphate buffer (0.04 M, pH 7.6). Each solution was then incubated at 37°C in a glass flask in the dark. At 24 h intervals during incubation, 0.1 ml of this incubation solution was added to 4.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely 3 min after 0.1 ml of 0.02 M FeCl2 in 3.5% (w/v) HCl was added to the reaction mixture, the absorbance of the red colour was measured at 500 nm in a spectrophotometer. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Inhibition \%} = [(A0 - A1)/A0 \times 100],$$

Where A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of standards.

b. Diphenyl Picryl Hydrazyl (DPPH) radical scavenging activity

Free radical scavenging activity of sample to characterize antioxidant activity was estimated as suggested by Blois (1958). Different amount of the methanolic, ethanolic, acetate, petroleum ether extracts of the sample was taken and DPPH (0.1 mM dissolved in methanol) was mixed together and the reaction mixture was left in dark room for 20 minutes. The absorbance was measured at 517 nm against the blank prepared by mixing DPPH and methanol. The antioxidant activity was expressed in terms of per cent inhibition of DPPH free radicals using the following equation:

$$DPPH\,radical\,scavenging\,activity\,(\%) = \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100$$

Where, $\mathrm{Abs}_{\mathrm{control}}$ = absorbance of DPPH solution (blank) and $\mathrm{Abs}_{\mathrm{sample}}$ = absorbance of sample. The IC_{50} of each sample (concentration in µg/ml required to inhibit DPPH radical formation by 50 per cent) has also been calculated.

c. Hydroxyl radical scavenging activity

In order to assess the hydroxyl free radical scavenging activity of the methanolic, ethanolic, acetate and petroleum ether extracts of the bitter gourd samples, the deoxyribose method was used, as described by Halliwell et al. (1996), with some slight modifications. The reaction mixture contained phosphate buffer (20 mM, pH 7.4), (60 mM) deoxyribose, (10mM) hydrogen peroxide, (1 mM) ferric chloride, (1.04 mM) EDTA, different amount of powered samples and (2mM) ascorbic acid. The reaction mixtures were incubated for 1hr at 37°C, after which 17 mM trichloro acetic acid (TCA) was added. The mixture was then boiled for 15 minutes, ice cooled and measured for absorbance at 532 nm.

Plate6.Best type selected- Light green big

Plate7. Processing steps

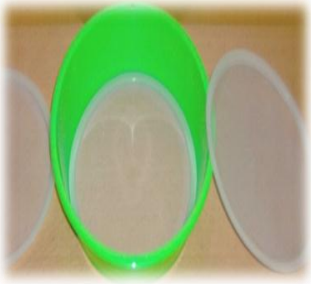
Soaking and washing

Cutting

Drying by electrical drier

Poedering

Sieving



Bitter gourd powder

Bitter gourd powder in Zip lock pouches

Distilled water in lieu of extract was utilized as blank and the sample solution without added deoxyribose was used as a sample blank.

d. Superoxide anion radical scavenging activity

Superoxide anion scavenging activity was measured based on the method described by Robok and Gryglewski (1988). Superoxide radicals were generated in a PMS-NADH system via the oxidation of NADH and then assayed by the reduction of nitro blue tetrazolium (NBT). The superoxide radicals were generated in reaction mixture containing sodium phosphate buffer (100 mM, pH 7.4) containing 150µM NBT, 468 µM NADH solution in sodium phosphate buffer and different concentrations of methanolic, ethanolic, acetate and petroleum ether extracts of the samples. To this 60 µM phenozine metho sulphite (PMS) solution was added. The reaction mixture was incubated for 5 minutes at 25°C and the absorbance was measured at 560 nm.

3.3 Quality evaluation and processing of bitter gourd

3.3.1 Processing of bitter gourd

Among the five types studied in the first experiment, commercially available bitter gourd light green big was selected for processing (Plate 6). The variety was collected from VFPCK outlet, Trivandrum. In the present study, cleaned bitter gourd fruits were soak in turmeric water for one hour and again cleaned in fresh water and cut in to thin slices and dried below 50 ^{0} C in the electric drier 6 – 7 hrs. Care was taken to avoid the cross contamination from other foreign particles. Properly dried bitter gourd fruits were powdered by the help of mixer and sieved properly and stored under ambient condition for the further clinical analysis (Plate 7). The quality evaluation of the processed bitter gourd powder such as chemical, nutrient, phytochemicals, antioxidants, yield ratio and storage studies was conducted.

3.4 Storage studies

To assess the shelf-life quality of the bitter gourd powder, it was kept in zip lock pouches at ambient conditions for a period of six months. Storage study was conducted initially and at the end of each month. The following parameters were assessed.

3.4.1 Moisture

Moisture content was estimated by the method of AOAC (1990).

3.4.2 Peroxide value

Method suggested by Sadasivam and Manickam (2008) was used for estimating peroxide value.

3.4.3 Microbial contamination

The stored bitter gourd powder was assessed for the presence of various micro-organisms that included bacteria, fungus, Escherichia coli and actinomycetes over a period of six months. Serial dilutions of the samples followed by spread plating were employed to estimate the population of viable micro-organisms in the developed product (Johnson and Curl, 1972).

Ten gram of the bitter gourd powder was transferred aseptically to 90 ml sterile water blank and suspended thoroughly by mixing. Further progressive serial dilution up to 1000 fold of the suspension was done in sterile water by serially transferring 1ml samples each into 9 ml water blanks.

Nutrient agar (NA), Kenknights agar, Martin rose Bengal agar and EMB agar medium were used for culturing of bacteria, actinomycetes, fungi and E.coli respectively. Plates were poured and allowed for solidification. 0.1 ml of the suspension from each dilution was then transferred on to the solidified agar medium using a sterile pipette and spread evenly with a sterile glass spreader. The whole procedure was done aseptically in a laminar air flow chamber. Plates were

then kept for incubation at room temperature. Colonies appearing in the plates were recorded after the next day in the case of bacteria and after 4 days for fungi and seven days for actinomycetes. The microbial load of the samples was then expressed as cfu/g.

3.5 Yield ratio of bitter gourd

The yield ratio of the bitter gourd powder was computed using the formula

$$\textit{Yield ratio} = \frac{\text{Weight of dried bitter gourd powder (kg)}}{\text{Weight of fresh bitter gourd (kg)}}$$

Processing loss was calculated using the formula

$$\frac{\text{Weight of fresh bitter gourd (kg)} - \text{Weight of dried bitter gourd (kg)}}{\text{Weight of fresh bitter gourd (Kg)}}$$

3.6 Cost of production

Cost of the developed bitter gourd powder was analyzed based on input cost i.e. cost of bitter gourd used for processing, cost of packaging material and output cost (10 per cent of the cost of product was added as overhead charges for fuel and labour to the total input cost).

3.7 Experiment III: Evaluation of clinical efficacy of the bitter gourd powder

In order to assess the therapeutic value of bitter gourd powder, supplementation study was carried in which bitter gourd in its powered form

prepared in the laboratory was given to selected ten human subjects having diabetes mellitus and hyperlipidemia.

3.7.1 Selection of subjects for case study

In the present experiment subjects were selected for investigating the effect of bitter gourd powder on disease condition *viz.* hyperglycemia and hyperlipidemia. For each disease condition, subjects with similar clinical parameters were selected. Subjects were identified through personal interview based on the following criteria.

i) Willingness to co -operate in the study
ii) Age between 45-55 years
iii) Subjects with similar sex for each disease condition
iv) Blood profile of the subjects

Disease condition	Blood profile
Hyperglycemia	Fasting blood sugar 140 mg/dl & above
Hyperlipidemia	Total blood cholesterol 200 mg/dl & above

v) Persons who are not under medication for hyperglycemia and hyperlipidemia.

More than 50 respondents were interviewed for the preliminary screening among the faculty members of the College of Agriculture, Vellayani and also from outside hospitals. A list of subjects having hyperglycemic and hyperlipidemic were prepared. From the above list, person under medication and subjects with co-morbidities were deleted. Subjects having both disease conditions were identified for the case studies from both sex. After selection, preliminary informations regarding their medical history, socio economic background, dietary and life style pattern were collected through a suitably structured pre tested questionnaire.

3.7.2 Socio economic profile

The socio economic profile of the subjects such as socioeconomic status, religion and family background in general has a very distinct part to play in determining attitude and food consumption, health and behavioral pattern of the individual (Arrora, 1991).

The socio economic profile collected from the subjects were family size, type of family, educational status, occupation of family members, total monthly income, income spent on food and health care etc. Questionnaire used is appended (Appendix I).

3.7.2.1 *Age*

It refers to the number of calendar years completed by the respondents at the time of interview. This variable measured directly by asking the respondent the number of years he/she completed at the time of investigation (Sindhudevi, 1994).

3.7.2.2 *Family Income*

Monthly family income from all sources was taken into account for measuring this variable.

3.7.2.3 *Family size*

In the present study, family size was measured by taking into consideration the specific number of members in the family of the respondents.

3.7.2.4 *Type of family*

Family type means nuclear family, joint type family or extended family. Nuclear family consists of husband, wife and their unmarried children whereas joint family is composed of grand parents and their married children and married sons and daughters with their spouse.

3.7.2.5 ***Educational status***

It is defined as the formal education attained by the respondent.

3.7.2.6 ***Nutritional and medical status***

According to Kamath (1986) nutritional status is defined as the state of health enjoyed as a result of nutrition. It is one of the critical indicators of health, therefore regular nutritional assessment is important to maintain the health of respondents (Mourya and Jaya, 1997).

Nutritional status of the selected respondents was assessed through anthropometry. Anthropometry provides the single most universally applicable, inexpensive technique for assessing the size, proportions and composition of the human body. Anthropometry has been accepted as an important tool for the assessment of nutritional status (Vijayaraghavan, 1987). Anthropometric measurements such as height, weight, waist circumference, hip circumference were taken as per the following procedures.

3.7.2.8 ***Height***

Height or the total length apart from nutritional and environmental factors is influenced by hereditary factors. The extend of height deficit in relation to age as compared to regional standards is regarded as a measure of the duration of malnutrition (Gopaldas and Sheshadri, 1987).

To determine height, an anthropometric rod was used. The rod was fixed vertically on a smooth wall, perpendicular to the ground taking care to see that the floor was smooth.

The subjects were asked to remove their slippers and to stand with centre of the back touching the wall with feet paralleled and heels, buttocks, shoulder and back of head touching the wall. The head was held comfortably erect, the arms hanging close by the side. A smooth, thin ruler was held on the top of the

head in the centre crushing the hair at angles to the wall and the height read off from the lower edge of the ruler to the nearest 0.1 cms. An average of the three measurements was taken as final measurement of height of the respondents.

3.7.2.9 ***Weight***

Weight is the measurement of body mass (Rao and Vijayaraghavan, 1996). According to Kaul and Nyamongo (1990) a change in body weight may be the result of changes in the health of an individual, changes in food consumed or even changes in one's physical activity.

For weighing, platform weighing balance was used as it is portable and is convenient to use in the field. The weighing scale was checked periodically for accuracy. The scale was adjusted to zero before each measurement. The subjects having minimum clothing were asked to stand on the platform of the scale, without touching anything and looking straight ahead. The weight was recorded to the nearest of 0.5 kg. Each reading was taken thrice to ensure correctness of the measurement.

3.7.2.10 ***Body Mass Index (BMI)***

BMI is expressed as the ratio of weight to height square i.e. Weight (kg) / Height ^{2} (m) was used as a good parameter to grade chronic energy deficiency (James et al., 1988).

$$BMI = \frac{Weight(Kg)}{Height(m^2)}$$

3.7.2.11 ***Waist-Hip ratio***

The Waist Hip Ratio (WHR) reflects the proportion of body fat located intradominally as opposed to that in the subcutaneous region (Lean et al., 1995). Waist was measured above the umbilicus meaning the narrowest circumference and hip was measured in the broadest area of hip. After documenting the waist

and hip measurements of the respondents their WHR was calculated by dividing the circumference of the waist by the circumference of the hip (Chadha et al., 1995).

3.7.2.12 *Dietary Pattern*

Dietary pattern have been used to identify typical combinations of food which is associated with disease risk. According to Swaminathan (1993) through diet surveys, information on nutrient intake level, source of nutrients, food consumption pattern and preferences of the subjects could be collected. Food habits of the respondents were collected in order to understand whether diet has any influence on their disease condition.

3.7.2.13 *Life Style Pattern*

Life styles are group specific forms of how individuals live and interpret their lives in a social context. Life style pattern include the personnel habits, stress and strain in the daily life, type of food they consume. Life style pattern has its own effect on the health of an individual. Data regarding the habit of doing exercise as well as the stress and strain faced by the subjects were also recorded.

3.8 Health profile

Details on the medical history of the subjects, use of medicines were collected. The FBS, PPBS and lipid profile of the selected respondents were determined.

3.9 Conduct of feeding trial

Bitter gourd powder supplement was distributed to the subjects for consumption based on their individual calorie requirement and disease condition. Subjects were given ten gram zip lock pouches of bitter gourd supplement distributed on a weekly basis. Investigator made a good rapport among the respondents and ensured the incorporation of supplement daily in their diet. Investigator also helped to tackle any problem if arise during the course of incorporation of bitter gourd powder supplement. Investigator has made interaction with the respondents personally and through telephone to know whether the subjects were consuming the supplement regularly. The supplementation study was conducted for a period two months.

3.9.1 Assessing the efficacy of the supplement on the Blood profile of the selected subjects

Feeding trial over a given period of time is considered as the most reliable method to determine the impact of the food. The feeding experiment was conducted for a period of two months to assess the efficacy of bitter gourd powder on hyperglycemia and hyperlipidemia. Blood profile of the subjects such as FBS, PPBS, HbA_1C and total cholesterol were recorded before the introduction of the supplement and after 30^{th} and 60^{th} day of supplementation. Along with these, glycemic index and glycemic load were also ascertained.

3.9.2 Glycemic index

3.9.2.1 *Conduct of Glucose Tolerance Test (GTT) among the subjects*

For the conduct of GTT, the subjects were asked to fast overnight. Blood samples were collected from the finger tips for determining their fasting blood glucose level. In order to get the glucose tolerance, the subsequent blood glucose was recorded at 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes and 120 minutes. Procedure followed is appended (Appendix 2).

3.9.2.2 ***Computation of glycemic index of the subjects***

To find out the glycemic index of the subjects, each subject were given portions of test food (bitter gourd powder) and glucose containing 25g available carbohydrate on separate occasions after an overnight fasting.

Blood samples were taken from the finger tips before the administration of test food and glucose to access the fasting blood sugar level of the subjects. After the administration of test meal, blood glucose level of the subjects were analyzed independently at half hour interval up to two hours (1/2 hr, 1 hr, 11/2 hr and 2 hr).

Blood glucose concentration of the subjects was determined by using a glucometer.

The response of the reference food mainly glucose and also that of the test food administered on the subjects were plotted, against time‘t’.

The area under curve (AUC) thus obtained was found out from test food as well as for reference food (glucose). The Glycemic Index (GI) of test food is computed as the ration of AUC of test food and AUC of reference food.

$$G.I = \frac{\text{AUC (Test food)}}{\text{AUC (Reference food)}} \times 100$$

Using the glycemic index, glycemic load (GL) was also calculated. The formula used for finding out the glycemic load was

$$G.L = \frac{G.I \times \text{Available carbohydrate in the portion size}}{\text{AUC (Reference food)}} \times 100$$

3.10 Statistical Analysis

In order to obtain meaningful interpretation, the generated data was subjected to suitable statistical analysis such as ANOVA and Paired ‘t’ test.

4. RESULTS

Results of the present study entitled "Nutrient composition, antioxidant and hypoglycemic effect of bitter gourd (*Momordica charantia* L.) were presented under the following headings.

4. 1. Nutrient composition

4. 2. Phytochemical analysis

4. 3. Antioxidant properties

4. 4. Shelf life quality

4. 5. Yield ratio and processing loss

4. 6. Cost of production

4. 7 Clinical efficacy of the bitter gourd powder

4. 1 NUTRIENT COMPOSITION

To assess the chemical/ nutritional composition of the bitter gourd types in the fresh and dried form, the following parameters were determined i.e. total carbohydrate, protein, dietary fibre, moisture, amino acid profile, β carotene, vitamin C, folic acid, calcium, phosphorus, sodium, potassium, iron, manganese, copper and zinc. The protein content of fresh and dry bitter gourd types are presented in Table 3.

Table 3. Protein content of bitter gourd types

Types	Protein (g/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	2.06^{a}	30.11^{a}
Light green small (LGS)	1.81^{b}	28.74^{b}
Dark green big (DGB)	1.55^{cd}	24.52^{d}
Dark green small (DGS)	1.61^{c}	24.55^{c}
Nei paval (NP)	1.51^{d}	24.55^{c}
CD (0.05)	**0.069**	**0.028**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Proteins are nitrogen-containing substances that are formed by amino acids. They serve as the major structural component of muscle and other tissues in the body. In addition, they are used to produce hormones, enzymes and haemoglobin (Alibhai et al., 2006). Proteins can also be used as energy. However they are not the primary choice as an energy source. For proteins to be used by the body they need to be metabolized into their simplest form, amino acids. There have been 20 amino acids identified that are needed for human growth and metabolism. Vegetable proteins, when combined to provide for all of the essential amino acids, provide an excellent

source for protein considering that they will likely result in a reduction in the intake of saturated fat and cholesterol (Aiking, 2011).

Table 3 showed that highest protein content was found in Light green big(2.06 g) and was significantly different from other types. The lowest protein content was found in NP fresh (1.51 g) and was on par with dark green big (1.55 g). The protein content of dark green big fresh sample (1.55 g) was on par with dark green small fresh type (1.61 g). The protein content of LGB fresh type (2.06 g) was significantly different from LGS, DGB,DGS and NP fresh types. In the case of bitter gourd dried, highest protein content was found in light green big dried type (30.11 g) and was significantly different from LGB, DGB, DGS and NP. The lowest protein content was found in DGB dried type (24.52 g) and was significantly different from LGB, LGS, DGS, and NP dried types. The protein content of DGS (24.55 g) was on par with NP dried type (24.55 g) and no significant difference was noticed between DGS and NP dried samples. The dry matter protein content of LGB (30.11 g) was significantly different from other four dried types i.e. LGS, DGB, DGS and NP.

Evaluation of protein quality

The nutritive value of protein depends to an important degree on the relation of amino acids in its molecules to those required for building new tissues. If the amino acid composition of a food substance meets the amino acid composition of a tissue, the food protein is of a high quality.

In the present study, to evaluate the protein quality of bitter gourd powder, 15 amino acids were estimated and the results are depicted in Table 4 and 5.

Table 4. Essential and sulphur containing amino acid content of bitter gourd powder

Essential amino acid	**Bitter gourd dried (n moles/ 100 ml)**				
	Light green big	**Light green small**	**Dark green big**	**Dark green small**	***Nei paval***
Histidine	28	25	17	17	27
Threonine	23	21	19	11	8
Methionine	28	14	26	14	8
Valine	18	11	16	11	14
Phenylalanine	11	4	8	7	10
Isoleucine	12	7	10	6	6
Leucine	11	6	10	7	7
Lysine	26	13	25	19	6
Total Essential Amino Acid (TEAA)	**157**	**101**	**131**	**92**	**86**

The present study revealed that all the total essential amino acids were highest in light green big (157 n moles/ 100 ml) and lowest in *Nei paval* (86 n moles/ 100 ml).

Table 5. Non essential amino acid content of bitter gourd powder

Non essential amino acid	**Bitter gourd dried (n moles/ 100 ml)**				
	Light green big	**Light green small**	**Dark green big**	**Dark green small**	***Nei paval***
Aspartic acid	12	7	11	5	6
Glutamic acid	19	16	9	9	15
Serine	29	15	22	21	28
Glycine	21	18	17	17	19
Arginine	75	36	68	66	72
Alanine	80	41	78	70	78
Tyrosine	30	19	22	18	29
Total Non Essential Amino Acid (TNEAA)	**266**	**152**	**227**	**206**	**247**

The present study revealed that all the total non essential amino acids were highest in light green big (266 n moles/ 100 ml) and lowest in light green small (152 n moles/ 100 ml).

Table 6. Carbohydrate content of bitter gourd types

Types	Carbohydrate (g/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	5.71[c]	43.15[c]
Light green small (LGS)	8.22[a]	60.13[a]
Dark green big (DGB)	5.21[e]	42.68[e]
Dark green small (DGS)	5.35[d]	42.73[d]
Nei paval (NP)	6.11[b]	50.08[b]
CD (0.05)	**0.019**	**0.046**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Carbohydrates are an important part of our diet since they are the body's primary source of energy. Carbohydrates mainly come from plant foods such as grains, fruits and vegetables. Experts recommend 45 per cent to 65 per cent of our total calories come from carbohydrates (Riboli and Horel, 2003). Most of our carbohydrates should be nutrient dense specially whole grains and fiber containing fruits and vegetables. For example, a balanced diet that is about 2,000 calories should include at least six servings of grains, five servings of vegetables and four servings of fruit daily (Slavin, 2008). The Scientific update also considered the relationship between dietary carbohydrate and cardiovascular disease, disorders of carbohydrate metabolism and cancer (Cummings and Stephen, 2007). A wide range of intakes of carbohydrate-containing foods is acceptable in the context of dietary patterns, which are protective against cardiovascular disease, diabetes and prediabetic states (Elia and Cummings, 2007).

Highest carbohydrate content was found in light green small fresh (8.22 g) and significant differences in carbohydrate content were noticed among fresh bitter gourd types. The lowest carbohydrate content was found in DGB fresh type (5.21 g) and was significantly different from LGB, LGS, DGS, NP fresh samples (Table 6). The results of bitter gourd dried revealed that, highest carbohydrate content was found in light green small dried type (60.13 g) and was significantly different from LGB, DGB, DGS and NP dried types. The lowest carbohydrate content was found in DGB dried (42.68 g) and was significantly different from LGB, LGS, DGS, NP dried types (Table 6). In dried form, the carbohydrate content of LGB, DGS and NP were 43.15 g/ 100g, 42.73 g/ 100g and 50. 08 g/ 100g respectively. The carbohydrate content of fresh and dried bitter gourd types were significantly different from each other.

Table 7. Fibre content of bitter gourd types

Types	Fibre (g/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	1.12^{b}	18.80^{b}
Light green small (LGS)	1.21^{a}	20.31^{a}
Dark green big (DGB)	0.91^{e}	15.53^{e}
Dark green small (DGS)	1.02^{d}	17.14^{d}
Nei paval (NP)	1.08^{c}	17.95^{c}
CD(0.05)	**0.016**	**0.026**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

The term “dietary fibre” is now well accepted by health professionals because of a significant amount of scientific evidence showing that consumption of dietary fibre reduces the risk of developing specific chronic diseases or conditions. Most

prominent of these are coronary heart disease, type 2 diabetes, certain types of cancers, as well as obesity (Jones and Varady, 2008). Dietary fibre is an extremely important component of a balanced diet (Jones and Varady, 2008). It has numerous functions in the human body and is also linked to the prevention of many diseases. Vegetables and fruits (any plant product for that matter) contain both soluble and insoluble fibre, but depending on the type and degree of ripeness of vegetable or fruit, the soluble to insoluble fibre ratio may vary.

Table 7 reveals the fibre content of fresh and dry bitter gourd types. The fibre content of fresh bitter gourd types differ significantly each other. The fibre content of bitter gourd both in fresh and dry was found highest in light green small (1.21 g and 20. 31g respectively) and lowest in dark green small (0.91 g and 15.53 g in fresh and dry types respectively).The fibre content of LGB, DGS and NP were 1.12 g/ 100g, 1.02 g/ 100g and 1.08 g/ 100g respectively in fresh whereas in dried, the carbohydrate content of LGB, DGS and NP were 18.80 g/ 100g, 17.14 g/ 100g and 17.95 g/ 100g respectively.

Table 8. Moisture content of bitter gourd types

Types	Moisture (%/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	90.40^{a}	6.61^{a}
Light green small (LGS)	90.00^{b}	6.30^{b}
Dark green big (DGB)	86.29^{c}	4.93^{c}
Dark green small (DGS)	85.40^{e}	4.07^{e}
Nei paval (NP)	85.59^{d}	4.28^{d}
CD(0.05)	**0.021**	**0.045**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Foods can be spoiled by food microorganisms or through enzymatic reactions. Within the food, bacteria, yeast, and molds must have a sufficient amount of moisture around them to grow and cause spoilage. Reducing the moisture content of food prevents the growth of these spoilage-causing microorganisms and slows down enzymatic reactions that take place within food (Andress and Harrison, 2006). The combination of these events helps to prevent spoilage in dried food.

Table 8 showed that highest moisture content was found in Light green big (90.40 per cent) and was significantly different from LGS, DGB, DGS and NP fresh types. The lowest moisture content was found in DGS fresh (85.40 per cent) and was significantly different from LGB, LGS, DGB, NP fresh types (Table 8). The moisture content of LGS, DGB and NP fresh samples were 90 per cent/100g, 86.29 per cent/ 100g and 85. 59 per cent/ 100g respectively. In the case of bitter gourd dried, highest moisture content was found in large green big (6.61 per cent/ 100g) and was significantly different from LGS, DGB, DGS and NP dried types. The lowest moisture content was found in DGS (4.07 per cent/ 100g) and was significantly different from LGB, LGS, DGB, NP dried types. The moisture content of LGS, DGB and NP dried types were 6. 30 per cent/ 100g, 4.93 per cent/ 100g and 4.28 per cent/ 100g respectively.

Table 9 Beta carotene content of bitter gourd types

Types	Beta carotene (mcg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	136.17[d]	90.14[d]
Light green small (LGS)	136.10[e]	89.94[e]
Dark green big (DGB)	139.20[c]	98.22[c]
Dark green small (DGS)	139.81[b]	98.69[b]
Nei paval (NP)	140.03[a]	98.93[a]
CD(0.05)	**0.019**	**0.032**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Carotene protects plant cells against the destructive effects of ultra violet light. β-Carotene is an antioxidant. Numerous observational studies have found that people who ingest more carotenoids or more fruits and vegetables have a reduced risk of several chronic diseases including, cancer, cardiovascular disease, age-related macular degeneration and cataract (Olson,1999).

The statistical analysis of the data revealed that there exist a significant difference in beta carotene of all the fresh and dry bitter gourd types. The beta carotene content was found highest in neipaval both in fresh and dry samples (140.03 mcg/ 100g and 98.93 mcg/ 100g respectively) (Table 9). The data also revealed that lowest beta carotene content was found in light green small both in fresh and dry samples (136.10 mcg/100g and 89.94 mcg/100g respectively). The process of drying and powdering significantly reduced the amount of β carotene in the dried samples as compared to the fresh types.

Table 10. Vitamin C content of bitter gourd types

Types	Vitamin C (mg/ 100 g)	
	Fresh	Dried
Light green big (LGB)	98.20[a]	30.42[a]
Light green small (LGS)	98.10[b]	30.15[b]
Dark green big (DGB)	97.90[d]	28.24[d]
Dark green small (DGS)	97.92[c]	28.89[c]
Nei paval (NP)	97.91[c]	27.98[e]
CD(0.05)	**0.013**	**0.015**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Ascorbic acid is one of the important and essential vitamins for human health. It is needed for many physiological functions in human biology. Fresh fruits, vegetables and also synthetic tablets supplement the ascorbic acid requirement of the body (Frei and Traber, 2004). However, stress, smoking, infections and burns deplete the ascorbic acid reserves in the body and demands higher doses of ascorbic acid supplementation. Based on available biochemical, clinical and epidemiological studies, the current RDA for ascorbic acid is suggested to be 100–120 mg/day to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in healthy individuals (Frei and Traber, 2004). In view of its antioxidant property, ascorbic acid and its derivatives are widely used as preservatives in food industry. Many health benefits have been attributed to ascorbic acid namely antioxidant, anti-atherogenic and anti-carcinogenic activity.

In this study, the highest vitamin C content was noticed in LGB fresh type (98.20 mg) and was significantly different from the LGS, DGB, DGS and NP fresh types. The vitamin C content of DGS (97.92 mg) and NP (97.91 mg) fresh

types were on par with each other. The results of bitter gourd dried revealed that highest vitamin C content was found in LGB (30.42 mg) and lowest in NP (27.98 mg). The data when analyzed statistically revealed a significant difference among the vitamin C content of all the bitter gourd dried samples analyzed (Table 10). The heat treatment during drying process drastically reduced the amount of vitamin C in bitter gourd fruit samples.

Table 11. Folic acid content of bitter gourd types

Types	Folic acid (µg/ 100 ml)	
	Fresh	**Dried**
Light green big (LGB)	0.10[a]	0.87[a]
Light green small (LGS)	0.08[b]	0.79[b]
Dark green big (DGB)	0.04[c]	0.61[c]
Dark green small (DGS)	0.02[d]	0.42[d]
Nei paval (NP)	0.02[d]	0.40[d]
CD(0.05)	**0.088**	**0.025**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Folic acid is necessary for the growth and repair of every cell in the body. Folic acid is needed for the growth and repair of hair, skin and nails (Walls et al., 2007). Folic acid is an essential B vitamin; therefore, everyone needs it in order to stay in good health. Folic acid is water soluble, therefore it passes through the body very quickly. Other than being needed to create and regenerate cells in the body, it also has protective effects. Studies revealed that folic acid reduces the risk of certain cancers, cardiovascular diseases including coronary heart disease and stroke, and cognitive diseases or mental conditions such as Alzheimer's disease, age-related dementia or cognitive decline and depression (Huang et al., 2007).

The results revealed that there was a significant difference in folic acid content of bitter gourd types. The folic acid content of bitter gourd was found least in dark green small and neipaval sample both in fresh and dried (0.02 µg/ml,0.42 and 0.40 µg/ml respectively) where as it was found maximum in light green big both in fresh and dried bitter gourd samples (0.10 µg/ml and 0.87 µg/ml). The folic acid content of DGS fresh type (0.02 µg/ml) was on par with NP fresh (0.02 µg/ml) and in the case of dried samples DGS (0.42 µg/ml) was on par with NP (0.40 µg/ml). (Table 11).

Table 12.Calcium content of bitter gourd types

Types	Calcium (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	25.44[a]	69.16[a]
Light green small (LGS)	24.11[b]	60.47[b]
Dark green big (DGB)	22.78[d]	49.68[c]
Dark green small (DGS)	22.79[d]	46.96[d]
Nei paval (NP)	22.91[c]	49.61[c]
CD(0.05)	**0.029**	**0.504**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Different minerals have different benefits, so no mineral can be termed as more beneficial or less beneficial than another. All minerals, even trace ones, are critical for the proper functioning of the body. Most of the minerals aid in body metabolism, water balance, and bone health, but they can participate in hundreds of other small ways to effectively boost health as well (Wargovich, 2000).

This vital mineral also boosts bone health (prevents osteoporosis), relieves arthritis, improves dental heath, and relieves insomnia, menopause, premenstrual syndrome, and cramps. Furthermore, it is important in preventing or treating obesity, colon cancer, acidity, heart diseases, and high blood pressure (Wargovich, 2000).

Table 12 showed that highest calcium content was found in light green big (25.44 mg/ 100g and 69.16 mg/100 g respectively) both in the case of fresh and dried samples. The lowest calcium content was found in dark green big fresh (22.78 mg) and DGS fresh (22.79) and both of them were on par with each other and they were significantly different from LGB, LGS and NP (Table 12). In the case of bitter gourd dried, lowest calcium content was observed in DGS (46.96 mg) and was significantly different from all the other dried samples (Table 12). The amount of calcium present in DGB and NP were 49.68 mg and 49.61 mg respectively and were on par with each other.

Table 13. Phosphorus content of bitter gourd types

Types	Phosphorus (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	79.64^{a}	424.75^{a}
Light green small (LGS)	44.35^{c}	234.43^{c}
Dark green big (DGB)	62.43^{b}	335.99^{b}
Dark green small (DGS)	39.24^{e}	208.42^{cd}
Nei paval (NP)	39.75^{d}	181.08^{d}
CD(0.05)	**0.388**	**42.253**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Phosphorus is integral in reducing muscle weakness, improving bone health, boosting brain function, correcting sexual weakness, aiding in dental care, and optimizing body metabolism (Wargovich, 2000).

There was a significant difference in phosphorus content of bitter gourd fresh and dried samples. Highest phosphorus content was noticed in light green big both in fresh and dry samples (79.64 mg and 424.75 mg respectively) (Table 13).The phosphorus content of LGS, DGB, DGS and NP fresh types were 44.35 mg/ 100g, 62.43mg/ 100g, 39.24mg/ 100g and 35.75mg/ 100g respectively (Table 13). In dried ones, the lowest phosphorus content was found in NP (181.08 mg) and was on par with DGS (208.42 mg).

Table 14. Sodium content of bitter gourd types

Types	Sodium (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	20.12[a]	100.76[a]
Light green small (LGS)	19.86[b]	98.96[b]
Dark green big (DGB)	4.13[e]	21.66[d]
Dark green small (DGS)	4.37[d]	21.74[d]
Nei paval (NP)	5.94[c]	34.19[c]
CD(0.05)	**0.031**	**0.561**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Sodium is a widely used mineral a key to water balance, preventing sunstroke, improving brain function, relieving muscle cramps, and preventing premature aging.

Data on sodium content (Table 14) revealed a significant difference among the bitter gourds types at five per cent level. The sodium content was found

highest in light green big both in fresh and dry samples (20.12 mg/ 100g and 100.76mg/ 100g respectively) while it was found lowest in DGB fresh type (4.13 mg).

Table 15. Potassium content of bitter gourd types

Types	Potassium (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	154.67[d]	823.58[d]
Light green small (LGS)	149.96[e]	784.47[e]
Dark green big (DGB)	171.59[c]	851.93[c]
Dark green small (DGS)	172.88[b]	894.14[b]
Nei paval (NP)	174.46[a]	905.76[a]
CD(0.05)	**0.999**	**1.857**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Potassium can correct low blood sugar, regulate blood pressure, prevent heart diseases, increase water flow in the body, alleviate muscle disorders and cramps, boost brain function, manage diabetes, correct kidney disorders, and manage arthritis (Wargovich, 2000). As a vasodilator, it reduces the tension in the blood vessels, and ensures the proper distribution of oxygen to vital organ systems, thus protecting against cardiovascular diseases.

The statistical analysis of the data revealed that there exists a significant difference in potassium content of fresh and dry bitter gourd types. In fresh bitter gourd types, the potassium content ranged between 149.96 to 174.46 mg where as it ranged between 784.47 to 905.76 mg in the case of dried bitter gourd. The potassium content was found highest in *Nei paval* both in fresh and dry samples (174.46 mg/

100g and 905.76 mg/ 100g respectively) where as the lowest content of potassium was found in light green small both in the case of fresh and dry samples (149.96 mg/ 100g and 784 .47 mg/ 100g respectively) (Table 15).

Table 16. Iron content of bitter gourd types

Types	Iron (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	0.68^{d}	4.56^{d}
Light green small (LGS)	1.52^{b}	8.19^{b}
Dark green big (DGB)	0.45^{e}	2.44^{e}
Dark green small (DGS)	1.11^{c}	7.76^{c}
Nei paval (NP)	2.14^{a}	11.26^{a}
CD(0.05)	**0.037**	**0.055**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$)

Iron is a key element of haemoglobin formation, body metabolism, muscle activity, anaemia, brain function, immunity, insomnia, restless leg syndrome, and the regulation of body temperature (Oyarzun et al., 2001).

Table 16 revealed that highest iron content was found in fresh neipaval (2.14 mg) and was significantly different from other types. The lowest iron content was found in DGB fresh type (0.45 mg). All the five fresh bitter gourd samples were significantly differ in their iron content. In the case of dried bitter gourd samples, the highest iron content was noticed in NP (11.26 mg) and lowest value in DGB (2.44 mg).

Table 17.Manganese content of bitter gourd types

Types	Manganese (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	34.57[a]	184.56[a]
Light green small (LGS)	29.15[b]	177.78[b]
Dark green big (DGB)	22.74[c]	153.61[c]
Dark green small (DGS)	19.39[d]	150.31[d]
Nei paval (NP)	18.41[e]	140.31[e]
CD (0.05)	**0.056**	**0.012**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Manganese plays an important role in the management of body metabolism, osteoporosis, reducing fatigue, reproduction, sprains, inflammation, brain function, and epilepsy (Oyarzun et al., 2001).

In this study, the highest manganese content was noticed in LGB fresh type (34.57 mg) and was significantly different from LGS, DGB, DGS and NP fresh samples. The lowest manganese content was noticed in NP (18.41 mg). The results of bitter gourd dried revealed that highest manganese content was found in LGB (184.56 mg) and lowest in NP (140.31 mg). The data when analyzed statistically revealed a significant difference among the manganese content of all the bitter gourd dried samples (Table 17).

Table 18. Copper content of bitter gourd types

Types	Copper (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	40.17[a]	200.17[a]
Light green small (LGS)	36.13[b]	193.14[b]
Dark green big (DGB)	34.75[c]	186.21[c]
Dark green small (DGS)	30.10[e]	150.11[d]
Nei paval (NP)	30.81[d]	145.21[e]
CD(0.05)	**0.277**	**0.011**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

This mineral improves brain function, soothes arthritis, helps in skin care, eliminates throat infections, corrects haemoglobin deficiency, prevents heart diseases, and boosts immunity (Matkovic, 2007). Copper is necessary for nerve metabolism, nerve transmission, many enzyme reactions, blood vessels, fighting inflammation, cholesterol levels, absorption of other minerals such as iron, and cardiovascular health in general (Oyarzun et al., 2001).

Table 18 revealed that highest copper content was found in Light green big fresh type(40.17 mg) and was significantly different from LGS, DGB, DGS and NP fresh types. The lowest copper content was found in DGS (30.10 mg) and was significantly different from LGB, LGS, DGB and NP. The copper content in bitter gourd samples of fresh LG, DG and NP were 36.13 mg/100g, 34.75 mg/ 100g and 30.81 mg/ 100g respectively (Table 18). In the case of bitter gourd dried, highest copper content was found in light green big (200.17 mg/ 100g) and was significantly different from LGS, DGB, DGS and NP. The lowest copper content was found in NP (145.21 mg/ 100g) and was significantly different from LGB, LGS, DGB, DGS. The

copper content of LGS, DGB and DGS was 193.14 mg/ 100g, 186.21 mg/ 100g and 150.11 mg/ 100g respectively in the case of bitter gourd dried samples.

Table 19. Zinc content of bitter gourd types

Types	Zinc (mg/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	90.41[a]	361.13[a]
Light green small (LGS)	84.30[b]	321.13[b]
Dark green big (DGB)	72.45[c]	300.26[c]
Dark green small (DGS)	70.14[d]	294.17[d]
Nei paval (NP)	60.04[e]	241.18[e]
CD(0.05)	**0.018**	**0.011**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Zinc is an essential component of more than ten important enzymatic functions of the body, and without zinc, the body will quickly lose overall function and results in a number of health concerns, including an inability to heal wounds, store insulin, fight off disease, develop proper growth patterns, as well as defend against a variety of skin infections (Chanoine, 2003).

Data on zinc content (Table 19) revealed that there was a significant difference among the bitter gourds types at five per cent level. The zinc content was found highest in light green big both in fresh and dry samples (90.41 mg/ 100g and 361.13 mg/ 100g respectively) while it was found lowest in NP fresh type (4.13 mg) and NP dried type (241.18 mg).

4. 2. PHYTOCHEMICAL ANALYSIS

A number of dietary antioxidants exist beyond the traditional vitamins collectively known as phytonutrients or phytochemicals which are being increasingly appreciated for their antioxidant activity (Vermerris and Nicholson, 2006). Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine (Arts and Hollman, 2005). Recently reported that phytochemical extracts from fruit and vegetables have strong antioxidant and anti proliferative effects and proposed that the combination of phytochemicals in fruit and vegetables is critical to powerful antioxidant and anticancer activity (Sun et al., 2002).

The result of the phytochemical screening of the bitter gourd fruit extracts in different solvents of ethanolic, methanolic, acetone aqueous, petroleum ether, and chloroform is presented in Table 20. The results revealed the presence/ absence of tannins, flavonoids, phenols, alkaloids, saponins,steroids, cardiac glycosides, phlobatinnins and anthraquinones.

Table 20. Phytochemical screening of bitter gourd

Phyto chemical	Reagent	Nature of colour change	Types of solvent					
			Ethanol	Methanol	Acetone	Aqueous	Petroleum ether	Chloroform
Tannin	Sample extract+ $FeCl_3$	Reddish brown	++	++	++	-	-	-
Flavonoid	Sample extract+Ethylacetate+ Dil. ammonia	Yellow colouration	++	++	++	++	-	-
Phenols	Sample extract+Dil. ammonia solution	Reddish colouration	++	++	-	++	-	-
Alkaloids	Sample extract+ Dil. HCl+ Mayer's reagent	Cloudy slight yellow colouration	++	++	++	++	-	++
Saponins	Sample extract+ Distilled water	White foams	++	++	++	++	++	++
Steroids	Sample extract+acetic acid+Conc. H_2SO_4	Blue green ring	++	++	++	-	++	++
Cardiac glycoside	Sample extract+Pyridine+SodiumNitroprusside+NaOH	Red colouration	++	++	++	-	-	++
Phlobatinnins	Sample extract+HCl	Red precipitate	++	++	++	-	-	++
Anthraquinones	Sample extract+Ethanol+ Chloroform+ Distilled water+ Ammonia	Absence of red colour	-	-	++	-	-	-

++ Presence, - Absent

Results of phytochemical screening revealed that tannin was found in ethanol, methanol and acetone solvents, but it was absent in aqueous, petroleum ether and chloroform. Presence of flavonoid produces a yellow colouration and was present in ethanol, methanol, acetone and aqueous solvents and was absent in petroleum ether and chloroform.

Phenols were present in ethanol, methanol and aqueous solvents and were absent in acetone, chloroform and petroleum ether. Presence of phenol produces a reddish colouration in fresh bitter gourd extracts. Table 20 depicted that alkaloids were present in all the solvents except petroleum ether and produces a slight yellow colouration. Presence of saponin was identified in all the solvents and continuous shaking gives white foams. Steroids were present in all the solvents and produces blue green ring except aqueous. The above results pointed out that cardiac glycosides and phlobatinnins were absent in aqueous and petroleum ether and were present in all the other solvents. The results also revealed the absence of anthraquinones in acetone solvent and was present in all the other solvents.

After the confirmation of presence of tannin, flavonoids, phenolic compounds, alkaloids and saponins by preliminary phytochemical tests, the fresh bitter gourd samples were taken for quantitative estimation of polyphenols, flavonoids, alkaloids, tannins,saponins and lectins.

Polyphenol content of bitter gourd types (fresh and dry) is shown in Table 21.

Table 21. Polyphenol content of bitter gourd types

Types	Polyphenol (mg/ 100 g)	
	Fresh	Dried
Light green big (LGB)	18.76[a]	74.67[a]
Light green small (LGS)	16.50[b]	66.88[b]
Dark green big (DGB)	12.66[cd]	49.92[c]
Dark green small (DGS)	12.40[d]	49.53[d]
Nei paval (NP)	12.80[c]	49.89[c]
CD(0.05)	**0.312**	**0.031**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Epidemiological studies correlate flavonoid intake with a reduced incidence of chronic diseases, such as cardiovascular disease, diabetes and cancer. Polyphenols provide health benefits by several mechanisms, including the elimination of free radicals, the protection and regeneration of other dietary antioxidants (e.g. vitamin E) and the chelation of pro-oxidant metals. The nature and content of phenolics varies dramatically among plants, which are mainly esterified or glycosylated (Percival et al., 2006).

Polyphenols possess beneficial properties, such as antioxidant, immune modulatory actions and anti-cancer and antibacterial activity. In recent years there is an upsurge in the food industry related to newer developments about the role of phenolic component as antioxidant, anti-mutagenic, and scavenging activity on free radicals and prevention of pathologies such as cancer and cardiovascular heart disease (Liu, 2004).

Highest polyphenol content was noticed in light green big both in the fresh and dried forms (18.76 mg and 74.67 mg respectively) and was significantly differ from all the other fresh and dry bitter gourd samples.The lowest polyphenol content was observed in DGS fresh type (12.40 mg) and was on par with DGB (12.66 mg). The lowest polyphenol content was observed in DGS dried (49.53 mg) and was significantly differ from other bitter gourd dry samples. In the case of bitter gourd samples (dried), the polyphenol content of DGB (49.92 mg) and NP (49.89 mg) was on par with each other.

Flavonoid content of bitter gourd types (fresh and dry) is presented in Table 22.

Table 22. Flavonoid content of bitter gourd types

Types	Flavonoid (%/ 100 g)	
	Fresh	**Dried**
Light green big (LGB)	0.87[a]	3.51[a]
Light green small (LGS)	0.43[b]	2.01[b]
Dark green big (DGB)	0.12[c]	0.48[d]
Dark green small (DGS)	0.11[c]	0.45[d]
Nei paval (NP)	0.35[b]	1.60[c]
CD(0.05)	**0.100**	**0.035**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

There has been increasing interest in the research of flavonoids from dietary sources, due to growing evidence of the versatile health benefits of flavonoids through epidemiological studies. As occurrence of flavonoids is directly associated with human daily dietary intake of antioxidants, it is important to evaluate flavonoid sources in food (Clifford and Cuppett, 2000). Fruits and vegetables are the main dietary sources of flavonoids for humans, along with tea and wine (Nijveldt et al., 2001). Nevertheless, research on the health aspects of flavonoids for humans is expanding rapidly. Many flavonoids are shown to have antioxidative activity, free-radical scavenging capacity, coronary heart disease prevention and anticancer activity, while some flavonoids exhibit potential for anti–human immunodeficiency virus functions (Ren et al., 2003)

In the present study, the highest flavonoid content was found in light green type both in the fresh and dried forms (0.87 per cent and 3.51 per cent

respectively).The quantitative amount of flavonoids in LGS (0.43 per cent) was on par with NP fresh samples (0.35 per cent) and DGB (0.12 per cent) on par with DGS (0.11 per cent) (Table 25). In the case of dried bitter gourd samples, the lowest flavonoid content was observed in DGS (0.45 per cent) and was on par with DGB (0.45 per cent).

Alkaloid content of bitter gourd types (fresh and dry) is depicted in Table 23.

Table 23. Alkaloid content of bitter gourd types

Types	Alkaloid (% / 100 g)	
	Fresh	**Dried**
Light green big (LGB)	0.27[a]	1.13[a]
Light green small (LGS)	0.31[a]	1.29[a]
Dark green big (DGB)	0.10[d]	0.43[c]
Dark green small (DGS)	0.16[c]	0.70[b]
Nei paval (NP)	0.22[b]	0.88[b]
CD (0.05)	**0.048**	**0.0193**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$)

Alkaloids are natural, organic substances that are predominantly found in plants. They are active components of numerous medicinal plants or plant-derived drugs as well as poisons; their structural diversity and different physiological activities are unmatched by any other group of natural products (Das and Mishra, 2011). From the beginning of civilization, alkaloids have been of great interest to humans because of their pronounced physiological and medicinal properties. Alkaloid-containing plant extracts have been used in all cultures as potions, medicines, and poisons (Katare et al., 2000).

The alkaloid content of bitter gourd samples (fresh) ranged between 0.10 per cent to 0.27 per cent. Alkaloid content of sample LGB (0.27 per cent) was on par with LGS (0.31 per cent). In the case of bitter gourd (dried) samples, the alkaloid content ranged between 0.90 per cent to 1.01 per cent and significant differences exist between varieties LGB, LGS, DGB and NP.

Table 24 revealed the tannin content of selected bitter gourd types (fresh and dry).

Table 24. Tannin content of bitter gourd types

‘ Types	Tannin (mg / 100 g)	
	Fresh	**Dried**
Light green big (LGB)	0.51^{b}	0.92^{c}
Light green small (LGS)	0.60^{a}	0.98^{b}
Dark green big (DGB)	0.51^{b}	0.91^{d}
Dark green small (DGS)	0.51^{b}	0.90^{d}
Nei paval (NP)	0.61^{a}	1.01^{a}
CD (0.05)	**0.012**	**0.013**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Jackson, 2003). Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances (Gry et al., 2007). Tannins

have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, and modulate immune responses. The dosage and kind of tannins are critical to these effects (Donovan et al., 2006).

In the present study, the highest tannin content was observed in NP fresh type (0.61 mg/ 100g) and was on par with LGS (0.60 mg/ 100g) and lowest content was found in LGB, DGB and DGS and was on par with each other (0.51 mg, 0.51 mg and 0.51 mg respectively) (Table 24). In the case of dried bitter gourd samples the highest amount of tannin was found in NP (1.01 mg) and lowest amount was found in DGB (0.91 mg) and DGS (0.90 mg) and were on par with each other.

Saponin content of bitter gourd types (fresh and dry) is presented in Table 25.

Table 25.Saponin content of bitter gourd types

Types	Saponin (% / 100 g)	
	Fresh	**Dried**
Light green big (LGB)	0.68^{a}	2.83^{a}
Light green small (LGS)	0.43^{b}	2.35^{c}
Dark green big (DGB)	0.12^{c}	2.44^{b}
Dark green small (DGS)	0.11^{c}	2.41^{b}
Nei paval (NP)	0.35^{b}	1.24^{d}
CD(0.05)	**0.030**	**0.051**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

Table 25 showed that the highest amount of saponin was found in light green big both in the case of fresh and dry samples (0.68 per cent and 2.83 per cent

respectively). Saponin content of light green small fresh (0.43 per cent) was on par with neipaval (0.35 per cent) and dark green big (0.12 per cent) was on par with dark green small (0.11 per cent)) (Table 27). The lowest amount of saponin was present in NP in dried type (1.24 per cent) and was significantly differ from all the other four types. The amount of saponins present in DLG (2.44 per cent) was on par with DGS (2.41 per cent) in the case of dried samples.

Lectin content of bitter gourd types (fresh and dry) is depicted in Table 26.

Table 26.Lectin content of bitter gourd types

Types	Lectin (mg / 100 g)	
	Fresh	**Dried**
Light green big (LGB)	0.30^{c}	1.14^{c}
Light green small (LGS)	0.30^{c}	1.15^{bc}
Dark green big (DGB)	0.36^{b}	1.16^{b}
Dark green small (DGS)	0.37^{a}	1.17^{a}
Nei paval (NP)	0.35^{b}	1.16^{b}
CD(0.05)	**0.012**	**0.011**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

The data revealed that lectin content of bitter gourd types ranged between 0.30mg-0.37mg in the case of bitter gourd fresh types where as it ranged between 1.14mg- 1.17mg in the case of bitter gourd dried types. It was observed that light green big bitter gourd had minimum lectin content both in the case of fresh and dried types (0.30mg and1.14mg) respectively. Highest lectin content was observed in dark green small type both in the case of fresh and dried types (0.37mg and 1.17mg) respectively.

Table 27 revealed the charantin content of bitter gourd types (fresh and dry)

Table 27. Charantin content of bitter gourd types

Types	Charantin (µg / 100 ml)	
	Fresh	**Dried**
Light green big (LGB)	0.18[a]	1.39[a]
Light green small (LGS)	0.17[b]	1.33[b]
Dark green big (DGB)	0.13[d]	1.27[c]
Dark green small (DGS)	0.10[e]	1.20[e]
Nei paval (NP)	0.15[c]	1.22[d]
CD(0.05)	**0.009**	**0.012**

Mean values denoted by different letters in the same column are significantly different ($p<0.05$).

The results of above table revealed that there was a significant difference in charantin content of fresh and dried bitter gourd types. The charantin content was found to be highest in light green big both in the case of fresh and dried samples (0.18 µg/ml and1.39 µg/ml) followed by light green small fresh and dried bitter gourd samples (0.17µg/ml and 1.33 µg/ml respectively). The charantin content was found to be minimum in dark green small both in fresh and dried bitter gourd samples (0.10 µg/ml and 1.20 µg/ml respectively). The results of ANOVA table revealed that charantin content of bitter gourd samples were significantly different from each other ($P > 0.05$).

4. 3. ANTIOXIDANT PROPERTIES

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions but removing free radical intermediates, and inhibit other oxidation reactions (Johansen et al., 2007).

4.3.1. DPPH radical scavenging activity

Free radical scavenging is one of the known mechanism by which antioxidants inhibit lipid peroxidation (Blokhina et al., 2003). The DPPH radical scavenging activity has been extensively used for screening antioxidants from fruits and vegetable juices or extracts (Sanchez, 2002).

Free radical scavenging activities of the bitter gourd types were studied by the DPPH assay in different types of solvents . Table 28 and 29 illustrates the results of the DPPH activity among the bitter gourd fresh and dry samples. The IC_{50} value was calculated from the graph (it was noted as the concentration of sample needed to scavenge the free radicals at 50 per cent inhibition).

Table 28. DPPH radical scavenging activity of fresh bitter gourd types

Types	IC_{50} Values (µg/ml)			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	53.66	52.98	52.33	50.88
Light green small (LGS)	53.97	53.09	55.34	52.33
Dark green big (DGB)	55.98	53.76	58.90	59.09
Dark green small (DGS)	54.90	52.15	57.89	55.22
Nei paval (NP)	54.77	53.09	55.65	53.33

The findings revealed that Light green big sample had the highest DPPH activity with an IC_{50} value of 50.88 µg/ml in methanol solvent (i.e. 50.88 µg/ml of Light green big bitter gourd sample is needed to scavenge the free radical of DPPH), followed by light green small (52.15 µg/ml) in acetone media. The lowest DPPH radical scavenging activity was found in dark green big (59.09 µg/ml) in methanol solvent.

Table 29. DPPH radical scavenging activity of fresh bitter gourd types

Types	IC_{50} Values (µg/ml)			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	50.79	50.10	50.22	50.98
Light green small (LGS)	50.90	51.23	50.31	53.12
Dark green big (DGB)	53.21	53.21	55.76	57.88
Dark green small (DGS)	52.78	52.15	53.22	56.22
Nei paval (NP)	50.98	51.34	52.45	54.20

In the case of bitter gourd dried samples, highest DPPH activity with an IC_{50} value of 50.10 µg/ml was reported in light green big dried type and lowest DPPH activity with an IC_{50} value of 57.88 µg/ml was observed in dark green big types with the help of linear regression equation (Table 29).

4.3.2 Hydroxyl radical scavenging activity

The scavenging of H_2O_2 by extracts may attribute to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water (Nabavi et al., 2008). Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells. Addition of H_2O_2 to cells in culture can lead to transition metal ion dependent OH radical mediated oxidative DNA damage (Aljudi and Kamaruddin, 2004). Thus, removing hydrogen peroxide as well as superoxide anion is very important for protection of pharmaceuticals and food products (Gulcin et al., 2007).

Hydroxyl and superoxide radical are the two most representative free radicals. In cellular oxidation reactions, superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of cell damaging free radicals and oxidizing agents. However, the damaging action of the hydroxyl radical is the strongest among free radicals. Synthetic compound are found to be strong radical scavengers but usually they have side effects (Zhou and Zheng, 1991).

Neutralization of this radical activity by naturally occurring substances mainly by supplementation of food having antioxidant property is becoming one of the most acceptable modes of modern therapy (Pal et al., 2010). The assay is based on quantification of the degradation product of 2- deoxyribose by condensation with TBA. This method involves in vitro generation of hydroxyl radicals using Fe+/ascorbate/EDTA/H_2O_2 system using Fenton reaction. Scavenging of this hydroxyl radical in presence of antioxidants is measured (Elizabeth and Rao, 1990).

Table 30. Hydroxyl radical scavenging activity of fresh bitter gourd type

Types	IC_{50} Values (µg/ml)			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	56.02	52.32	50.95	52.98
Light green small (LGS)	56.78	52.98	53.22	58.90
Dark green big (DGB)	58.76	55.67	58.89	65.33
Dark green small (DGS)	57.82	54.87	56.34	64.12
Nei paval (NP)	55.32	53.97	54.22	62.55

The hydroxyl radical scavenging activities of fresh bitter gourd samples were shown in the Table 30. In this study, the hydroxyl radical scavenging activity of light green was found to be highest in fresh bitter gourd samples with an IC_{50} values of 50.95 µg/ml, whereas it was found to be minimum in fresh samples with an IC_{50} value of 65.33 µg/ml. Ascorbic acid was used as reference compound and its IC_{50} value was 3.90 µg/ml.

Table 31. Hydroxyl radical scavenging activity of dried bitter gourd type

Types	IC_{50} Values (µg/ml)			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	50.68	50.10	50.66	55.77
Light green small (LGS)	51.34	51.64	50.98	53.44
Dark green big (DGB)	52.84	53.45	55.34	59.56
Dark green small (DGS)	52.02	52.34	53.22	57.62
Nei paval (NP)	52.13	52.89	51.76	56.14

The findings revealed that Light green big sample had the highest hydroxyl radical scavenging activity with an IC_{50} value of 50.10 µg/ml in acetone solvent (i.e. 50.10 µg/ml of Light green big bitter gourd sample is needed to scavenge the free radical of hydroxyl), followed by light green small (50.98 µg/ml) in ethanol solvent media. The lowest DPPH radical scavenging activity was found in dark green big (59. 56 µg/ml) in methanol solvent.

4.3.3 Super oxide anion-radical scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non- enzymatic reaction such as autoxidation by catecholamines. In the present study, superoxide radical reduces NBT to a blue coloured formazan that is measured at 560 nm (Panda and Kar, 2000). Superoxide

anions indirectly initiated lipid peroxidation as a result of superoxide and hydrogen peroxide serving as precursors of single O_2 and hydroxyl radicals (Okuda et al., 2003).

The super oxide radical scavenging activity of fresh bitter gourd type is presented in Table 32.

Table 32. Super oxide radical scavenging activity of fresh bitter gourd type

Types	**IC_{50} Values (µg/ml)**			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	54.12	52.76	52.33	54.31
Light green small (LGS)	50.36	58.65	50.67	54.60
Dark green big (DGB)	57.82	61.22	58.90	59.11
Dark green small (DGS)	56.87	60.88	54.10	55.17
Nei paval (NP)	55.89	60.12	52.99	55.27

Light green small sample showed higher superoxide anion radical scavenging activity with an IC_{50} value of 50.36 µg/ml and 50.67 µg/ml in fresh samples, in solvents like petroleum ether and ethanol respectively. The results of above table revealed that dark green big possessed lowest superoxide anion radical scavenging activity in fresh and samples with an IC_{50} value of 61.22 µg/ml respectively.

Table 33. Super oxide radical scavenging activity of dried bitter gourd type

Types	IC_{50} Values (µg/ml)			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	52.34	50.12	49.65	51.61
Light green small (LGS)	50.89	49.76	45.23	50.71
Dark green big (DGB)	56.90	57.23	53.99	53.61
Dark green small (DGS)	54.76	52.33	50.87	50.94
Nei paval (NP)	52.90	51.21	49.99	51.98

Light green small dried sample showed higher superoxide anion radical scavenging activity with an IC_{50} value of 45.23 µg/ml and 49.76 µg/ ml in dried samples, in solvents ethanol and acetone respectively. The results of above table revealed that dark green big possessed lowest superoxide anion radical scavenging activity with an IC_{50} value of 57.23 µg/ml respectively.

4.3.4 Total antioxidant activity

Antioxidants can protect the human body from free radical and ROS effects. They retard the progress of many chronic diseases as well as lipid peroxidation (Gulcin, 2007). Being enzymatic or non enzymatic species, antioxidant molecules are classified in different categories. Antioxidants are major compounds that protect the quality of life by retarding the oxidation process through scavenging

free radical produced during many natural events. Although their ultimate aim is removal of ROS, they may use different mechanism depending on their structure and site of action. Antioxidants are also able to act by up regulating the expression of the genes encoding the antioxidant enzymes, repairing oxidative damage caused by radical and increasing elimination of damaged molecules (Wood et al., 2006).

The total antioxidant activity of fresh bitter gourd samples is depicted in Table 34.

Table 34. Total antioxidant activity of fresh bitter gourd type

Types	**IC_{50} Values (µg/ml)**			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	54.17	51.22	50.09	54.23
Light green small (LGS)	55.67	53.34	52.34	56.76
Dark green big (DGB)	61.90	60.98	56.88	59.76
Dark green small (DGS)	60.98	59.77	54.76	54.38
Nei paval (NP)	58.90	58.30	50.65	55.00

The results of above table revealed that antioxidant activity ranged with an IC_{50} values of 50.09 µg/ml to 61.90 µg/ml in fresh bitter gourd samples and maximum antioxidant capacity was observed in Light green big (50.09 µg/ml) and minimum antioxidant capacity was observed in dark green big fresh samples (61.90 µg/ml).

Table 35. Total antioxidant activity of dried bitter gourd types

Types	IC_{50} Values (µg/ml)			
	Petroleum ether	**Acetone**	**Ethanol**	**Methanol**
Light green big (LGB)	52.33	50.78	50.07	50.13
Light green small (LGS)	53.45	50.90	51.34	51.23
Dark green big (DGB)	58.90	54.12	55.67	52.37
Dark green small (DGS)	57.77	53.21	53.21	51.98
Nei paval (NP)	54.22	52.22	50.90	52.13

The above table revealed that in the case of bitter gourd dried samples, the highest antioxidant activity was observed in light green big type (50.07 µg/ml) in acetone solvent and lowest antioxidant capacity was noticed in dark green big (58.90 µg/ml) sample in petroleum ether as solvent obtained with the help of linear regression equations.

4. 4. Shelf life quality

Shelf life qualities are essential parameters to be assessed, since they determine the suitability of a particular ingredient for product development (Livingstone et al., 1993). Among the five types studied in the 1st experiment, based on nutrient analysis and commercial availability bitter gourd light green big was selected for processing. The variety was collected from VFPCK outlet, Kalliyoor. In

the present study, cleaned bitter gourd fruits were cut in to thin slices and dried below 40 ^{0} C in the electric drier till it is crisp. Care was taken to avoid the cross contamination from other foreign particles. Properly dried bitter gourd fruits were powdered by the help of mixer and sieved properly and stored under ambient condition for the further clinical analysis. The quality evaluation of the processed bitter gourd powder such as chemical, nutrient, phytochemicals, antioxidants, yield ratio and storage studies was conducted.

Bitter gourd powder undergo changes with respect to chemical constituents, which will give an indication of the deteriorative changes occurring in the powder. The stored bitter gourd powder was examined for chemical changes such as moisture, peroxide value and increase in microbial load. Shelf life quality of bitter gourd powder was ascertained by storing it in zip lock pouches for a period of six months at ambient condition. The parameters like moisture, peroxide value and microbial growth were analyzed initially and at the end of each month.

4.4.1 Moisture and peroxide during storage

Moisture content is one of the most commonly measured properties of food materials. Knowledge of the moisture content is often necessary to predict the behaviour of foods during processing.

Peroxide value gives an indication about the extent of peroxidation taking place in the stored food materials. The acceptability of a food product depends on the extent to which deterioration has occurred and oxidative rancidity is a major cause of food deterioration. This in turn represents a major cause of loss of nutritional quality as well as cause of concern for food safety, as the oxidative fats in a very high dosage have been shown to have toxic effects (Sen. and Sen., 2009). The extent of peroxidation in the stored items was estimated and the values obtained are presented in the Table 36.

The results of the moisture and peroxide during storage is presented in Table 36.

Table 36. Moisture and peroxide during storage of bitter gourd powder

Month	Moisture (%)	Peroxide (meq/ kg)
Initial	6.59	0
Ist	6.60	0
2nd	6.63	0
3rd	6.64	0
4th	6.65	0
5th	6.66	0.10
6th	6.68	0.12
Mean	**6.63**	**0.031**

From the above table, it was observed that there was a slight increase in moisture content during storage. The mean moisture level of bitter gourd powder after six month storage was 6.63 per cent. The initial moisture content was 6.59 per cent and after six months of storage it increased to 6.68 per cent.

The peroxide content was not observed in the first four months of the study. The above mean table elicited that the bitter gourd powder recorded peroxide content in the 5th month (0.10 meq/100g) and 6th month (0.12 meq/100g) of storage. However it could be noted that the peroxide content in the bitter gourd powder was much minimal than the permitted limits. This shows that, bitter gourd can be stored for a period of six months without any discriminate changes, thereby giving them a higher shelf life and microbial value.

4.4.2 Assessment of microbial growth in stored bitter gourd powder

Microbial population in developed food products is important as it determines the quality and safety of food products. The microbiological safety of food is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication (Beckers, 1988). Food products that have been subjected to an adequate heat treatment during processing are free of vegetative pathogens. So it is regarded as safe. Microbial analysis of stored products were done to ascertain the shelf life of the products. The products were stored at ambient condition for 6 month. The microbial evaluation was done initially and at 30 days interval up to 6 months. The growth of bacteria, fungi, actinomycetes and E-coli were determined using Nutrient Agar (NA), Rose Bengal (RB), Ken Knight's Agar (KEN) and Eosine Methylene Blue (EMB). This was done by the serial dilution of the samples followed by pour plating techniques suggested by Johnson and Curl (1972). The microbial load of stored bitter gourd powder was estimated and the values obtained are depicted in the Table 37.

Table 37. Microbial load of stored bitter gourd powder (cfu/g)

Microbes	Initial	1st Month	2nd Month	3rd Month	4th Month	5th Month	6th Month
Bacteria	Nil	Nil	Nil	Nil	Nil	1×10^7	2×10^7
Actinomycetes	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Fungi	Nil	Nil	Nil	Nil	Nil	Nil	Nil
E-Coli	Nil	Nil	Nil	Nil	Nil	Nil	Nil

It is evident from the above table that during the storage of six months period, no actinomycetes were found to be appeared in the bitter gourd powder. But bacterial colonies were observed from the 5^{th} month (1×10^7 cfu/g) and 6^{th} month (2×10^7 cfu/g)

and was present only in negligible level. No Fungi and E-coli were detected in bitter gourd powder during the period of six month of storage. It must also brought into the consideration that, among the replicate samples analyzed bacterial colonies was notable only in one sample randomly selected for analysis. So it could be concluded that, it might be due to cross contamination during the period of bulk production of the bitter gourd powder.

4. 5. Yield ratio and processing loss

When bitter gourd was converted into powdered form it was noticed that from 1Kg fresh bitter gourd with seeds around 50g powder (without seed) was obtained. The yield ratio was found to be 0.05.

Processing loss was also computed and the results revealed that the processing loss was found to be 95 per cent.

4. 6. Cost of production

Cost of production of bitter gourd was calculated (based on the price of bitter gourd+ 10% overhead charges) and it was found that for 1 Kg bitter gourd powder, the cost would be around Rs.100/ 100 g.

4. 7. Clinical efficacy of bitter gourd powder supplementation

The clinical efficacy of the bitter gourd powder was ascertained through case studies. The study was conducted in Trivandrum among randomly selected respondents from a list comprising of more than fifty members having hyperglycemia and hyperlipidemia which is highly prevalent in our state.

From the list of more than fifty members, people in the early stages of diseases like hyperglycemia and hyperlipidemia were screened. Subjects who were not on medication were again scrutinized. Willingness of participation of the subjects throughout the study was confirmed. The final list of the subjects for the study was in

the age group of 45-55 years with different gender and without any other complications.

Five female and five male subjects were selected for the case study. Bitter gourd powder was given to the subjects for a period of two months. After the selection process, preliminary information regarding their socio economic profile, health status, life style pattern and nutritional status were collected through a suitably structured questionnaire given in Appendix I.

Results of case study

Ten human subjects (five males and five females) in the age group of 45-55 years were selected for the case study (Subject A to J). Individual case study reports were presented in Appendix II.

4.7.1 Socio economic profile

Table 38. Socio economic profile of respondents

Age	45-55
Females	5
Males	5
Educational status	Plus two - PG
Family income (monthly)	Rs. 20, 000 – 90, 000
Occupational status	Govt. employee Business Private Housewives

Ten human subjects (five males and five females) having diabetes in the age group of 45-55 years and not on medication were selected for the case study (Subject A- J). All the respondents were Hindu and were from nuclear family.

The monthly income of the respondents ranged from 20,000 to 90,000. Their educational qualification ranged from plus two to PG, with occupation three subjects were Govt. employee, two business man, two housewives and three respondents worked in private sector .

4.7.2 Life style pattern

Table 39. Life style pattern of respondents

Duration of disease	6 month – 4 years
No. of members having diabetes	Eight
Other diabetes related diseases	Hyperlipidaemia Hypertension Hypotension
Activity level	One
Exercise Habit (daily)	Sedentary
Smoking	No
Alcohol	No

Duration of the diseases revealed that three respondents were having diabetes for the past 3 years, whereas four respondents were having diabetes for the last one year, one respondent was for the past 6 months, another one having diabetes for the

past 2 years and last one respondent having diabetes for the past four years. Except two respondents, all others were having diabetic history. From the obtained data revealed that five respondents were having hyperlipidemia at present and 5 respondents were having slightly hyperlipidemia. One respondent having hypertension and another one respondent having hypotension at present. All the respondents have no exercise habit except subject one and all the respondents were non vegetarian dietary habit except one respondent.

4.7.3 Dietary pattern

Table 40. Dietary pattern of respondents

Dietary habit	Vegetarian – 1 Non- vegetarian 9
Average calorie intake	1598 Kcal – 3100 Kcal
Daily consumption of various food groups	Cereals, pulses, milk and milk products, oil, fruits and vegetables
Frequency of meals	3 – 4 times
Oil used for cooking	Coconut oil, sunflower oil and rice bran oil

Frequency of consumption of various food groups in the diet revealed that all respondents were included cereals, pulses, vegetables, milk, milk products and tea daily. The average intake of energy as calculated from their diet was ranged between 1598 Kcal – 3100 Kcal respectively. All the respondents were non vegetarian dietary habit except one respondent. The meal frequency pattern of the respondents revealed that all of them have food 3 to 4 times in a day. All the respondents used different types of cooking oils like coconut oil, sunflower oil and rice bran oil due to health concern.

4.7.5 Anthropometric data

Table 41. Anthropometric data of respondents

Height (cm)	150 - 180
Weight (kg)	48 - 86
Body Mass Index (BMI)	20.50 – 32.04
Category	Over weight (3) Grade I obesity (3) Grade II obesity (2) Grade III obesity (1) Normal (1)
Waist hip ratio	0.86 – 0.99

The anthropometric data of the respondents revealed that three respondents were coming under over weight category, with a waist-hip ratio of).97, 0.99 and 0.94 and three respondents were coming under grade 1 obesity category, and two respondents were coming under grade II obesity category and one respondent was under grade III obesity category. Whereas one respondent was having normal BMI category, with a waist hip ratio of 0.92. The waist hip ratio of the respondents ranged between 0.86- 0.99.

4.7.6 Effect of bitter gourd supplementation

The subjects were willingly participated in the supplementation study and was not taking any oral hypoglycemic agents for controlling the disease. Bitter gourd supplement prepared in the laboratory were distributed to the subjects for a period of two months. Close observation was made by the investigator and ensured that the subjects were consuming the supplement promptly. The efficacy of the bitter gourd supplement was assessed by monitoring blood sugar (fasting blood sugar, post prandial blood sugar, HbA_1C) and blood cholesterol levels at different intervals, initially, 30^{th} day and 60^{th} day. The respondents having 5g bitter gourd powder in 50 ml water every day early morning and night before food. The respondents opined that the bitterness of the bitter gourd powder is less than that of fresh bitter gourd juice. The blood sample of the subjects were collected and blood profile was analyzed and the details are given in the Table 42 to 44 and details are given in Appendix II.

Table 42. Clinical assessment of respondents initially (Before treatment)

Subjects	Fasting blood sugar (FBS) mg/ dl	Post prandial blood sugar (PPBS) mg/ dl	Glycosylated Haemoglobin (HbA_1C) %	Total cholesterol (TC) mg/ dl
A	160	210	6.8	250
B	200	240	7.1	300
C	175	200	7.5	205
D	127	159	6.2	219
E	196	249	8.7	295
F	170	210	6.9	260
G	170	225	7.4	212
H	145	195	6	246
I	175	225	6.9	236
J	200	265	8	294
Mean	**171.8**	**217.8**	**7.15**	**251.7**

Before treatment ten respondents blood parameters were assessed (fasting blood sugar, post prandial blood sugar, glycosylated haemoglobin and total cholesterol). The above table 42 results revealed that the mean value of FBS of ten subjects were 171.8 mg/ dl, PPBS were 217.8 mg/ dl, glycosylated haemoglobin was 7.15 per cent

and total cholesterol was found to be 251.7 mg/ dl. The results of the blood parameters revealed that all the ten respondents were hyperglycemic.

Table 43.Intermitent clinical assessment of respondents (30th day)

Subjects	Fasting blood sugar (FBS) mg/ dl	Post prandial blood sugar (PPBS) mg/ dl	Total cholesterol (TC) mg/ dl
A	140	180	225
B	180	215	260
C	160	185	180
D	110	131	195
E	180	220	240
F	151	186	217
G	130	190	175
H	140	160	217
I	170	209	204
J	163	223	250
Mean	**152**	**189**	**216**

The 30th day of bitter gourd powder supplementation again the blood parameters of the ten respondents were assessed and found that there was a reduction

in fasting blood sugar 152 mg/ dl, post prandial blood sugar decreased to 189 mg/ dl than the initial value and total cholesterol were reduced to 216 mg/ dl respectively.

Table 44. Clinical assessment of respondents after supplementation (60th day)

Subjects	Fasting blood sugar (FBS) mg/ dl	Post prandial blood sugar (PPBS) mg/ dl	Glycosylated Haemoglobin (HbA_1C) %	Total cholesterol (TC) mg/ dl
A	108	128	5.3	184
B	147	164	6.5	221
C	138	169	6.5	143
D	101	119	5.3	140
E	170	200	7.6	219
F	120	114	6	183
G	105	136	6.1	135
H	130	155	5.8	180
I	160	178	6.2	183
J	141	196	6.8	212
Mean	**132**	**155.9**	**6.12**	**180**

The 60th day of bitter gourd powder supplementation again tha blood parameters of the ten respondents were assessed and found that there was a reduction in fasting blood sugar 132 mg/ dl, post prandial blood sugar decreased to 155.9 mg/ dl, glycosylated haemoglobin 6.12 per cent and total cholesterol 180 mg/ dl respectively. The results showed that reduction in all blood parameters

Table 45. Changes in blood glucose and cholesterol level of ten respondents

Group	Fasting blood sugar (FBS) mg/dl (Mean)	Post Prandial blood sugar (PPBS) mg/dl (Mean)	Glycosylated Haemoglobin (HbA_1C) (%) (Mean)	Total cholesterol (TC) mg/dl (Mean)
Pre test	171.8	217.8	7.15	251.7
Post test	132	155.9	6.21	180.0
Paired t – test	6.83*	8.42*	7.93*	24.10*

*Significant at 5 per cent level

Data on Table 45 revealed that the mean fasting blood sugar of pre test was found to be 171.8 mg/dl while for the post test it was decreased to 132 mg/dl. Result of the paired t- test showed significant changes in fasting blood sugar at 5 per cent level. From the value obtained for post test it, was clear that there was significant changes in fasting blood sugar levels. The mean value for post prandial blood sugar of pre test was found to be 217.8 mg/ dl while for the post test it was decreased to 155.9 mg/ dl. Results of the paired t- test showed that the changes in post prandial blood sugar was significant at 5 per cent level. There was a significant difference of 5 per cent level in the mean score for glycosylated haemoglobin. During pre test the value was found to be 7.15 per cent and it decreased to 6.21 per cent after supplementation. Statistical analysis of the data on total cholesterol revealed a

significant difference at 5 per cent level. The mean initial total cholesterol level of the subjects under study was 251.7 mg/dl. After the supplementation, the total cholesterol level was decreased to 180 mg/dl.

4.7.6 Glucose Tolerance Test (GTT)

Fig. shows the GTT of the ten subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

4.7.8 Glycemic index

The goal of the clinical management of the type 1 and type 2 diabetes is to control metabolic abnormalities in order to prevent both acute (hyperglycemia and hypoglycemia) and long term (retinopathy, neuropathy, nephropathy and cardio vascular diseases CVD) complications without negatively affecting quality of life (Anne, 2003). Achieving and maintaining blood glucose (BG) level as close to normal as possible is crucial for the prevention of long term complications in type 1 and type 2 diabetes and requires an intensive approaches to management. Nutrition is the utmost importance in intensive diabetes management and has been described as the corner stone of care.

Table 46 shows mean area under plasma glucose responses curve (AUC) for test food (bitter gourd powder) and reference food (glucose). As indicated in table 46 glucose had an AUC of 340 mm^2 whereas test food had an AUC of 220 mm^2.

Table 46. Mean area under the curve and glycemic index of test food and reference food

Food	Area under the curve (mm^2)
Glucose (Reference food)	**340**
Bitter gourd powder (Test food)	**220**
Food	**Glycemic index**
Glucose	**100**
Bitter gourd powder	**64**

Based on AUC, the glycemic index of the test food (bitter gourd powder) was determined which is depicted in Table 46. As shown in Table 46, bitter gourd powder was having a GI of 64 which was around 36 per cent less than that of glucose. The results of glycemic load revealed that bitter gourd powder had a glycemic load of 39.

5. DISCUSSION

Discussion of the present study entitled “Nutrient composition, antioxidant and hypoglycemic effect of bitter gourd (*Momordica charantia* L.) were presented under the following headings.

5.1 Nutrient composition

5.2 Phytochemical analysis

5.3 Antioxidant properties

5.4 Shelf life quality

5.5 Yield ratio and processing loss

5.6 Cost of production

5.7 Clinical efficacy of the bitter gourd supplementation

India is the largest producer of medicinal herbs and appropriately called the Botanical garden of the world (Mungole, 2010). The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health has been widely observed.

Bitter gourd has important role as a source of carbohydrate, proteins, vitamins, minerals and other nutrients in human diet (Ali et al., 2008) which are necessary for maintaining proper health. Knowledge of the chemical constituent of plants is desirable for the discovery of therapeutic agents and in discovering the actual value of folklore remedies. Traditionally, screening methods have been used to study the pharmacological effects of phytochemical compounds. Plants play a prominent role in maintenance of human health and used as medicine, since ancient times. According to World Health Organization, (WHO) plant extracts are used as folk medicine in traditional therapies of 80 per cent of the world's population (Singh et al., 2012).

5.1 NUTRIENT AND CHEMICAL COMPOSITION

To assess the chemical and nutritional composition of the selected bitter gourd types in the fresh and dried form, the following parameters were determined i.e. total carbohydrate, protein, dietary fibre, moisture, amino acid profile, β carotene, vitamin C, folic acid, calcium, phosphorus, sodium, potassium, iron, manganese, copper and zinc.

5.1.1 Protein

Proteins are essential component of diet needed for survival of animals and human beings. Its basic function is to supply adequate amounts of required amino acids for nutrition (Pughalanthal et al., 2004). Protein deficiency causes growth retardation, muscle wasting, oedema, abnormal swelling of the belly and collection of fluids in the body (Perkinz et al., 2005).

In the present study, highest protein content was found in light green big fresh (2.06 g) and was significantly different from other types. The lowest protein content was found in NP (1.51 g) and was on par with DGB fresh type (1.55 g). In the case of bitter gourd dried, highest protein content was found in LGB (30.11 g) and the lowest in DGB (24.52 g) (Fig 1 & 2).

It has been reported that protein-calorie malnutrition is a major factor responsible in nutritional pathology (Roger et al., 2005). According to Pearson (1976), plant food that provide more than 12 per cent of its calorific value from protein are considered as good source of protein. Furthermore, adults, pregnant and lactating mothers daily required 34-56 g, 50- 60 g and 71 g of protein respectively (Roger et al., 2005). The results of this work showed that good amount of protein are present in all the five types of bitter gourd samples.

According to Bakare et al. (2010) the amount of crude protein was 27.88 g/ 100 g in bitter gourd dried samples. A study conducted by Chunduri (2013) on antioxidant and nutritional analysis of edible cucurbitacae vegetables in India revealed that fresh bitter gourd contained 0.96 g protein per 100g.

Khalid (2010) developed a neutraceutical bitter gourd tea powder and was also good in protein content (21.83 per cent). A study conducted by Khalid (2010) on nutritional analysis and antioxidant activity of bitter gourd from Pakistan found that flakes and peel had highest concentration of protein (20.37 per cent and 20.66 per cent) as compared to seed (19.06 per cent).

Bitter gourd fruit proteins like momordin, alpha- and beta-momorcharin and cucurbitacin B were also tested for possible anticancerous effects. A chemical analog of these *M. charantia* proteins has been developed, patented, and named "MAP-30"; its developers reported that it was able to inhibit prostate tumor growth (Renuka, 2012). Some of the proteins like alpha- and beta-momorcharins have been reported to inhibit HIV infections (Renuka, 2012).

Fig 1.Protein and carbohydrate contents of bitter gourd types (fresh)

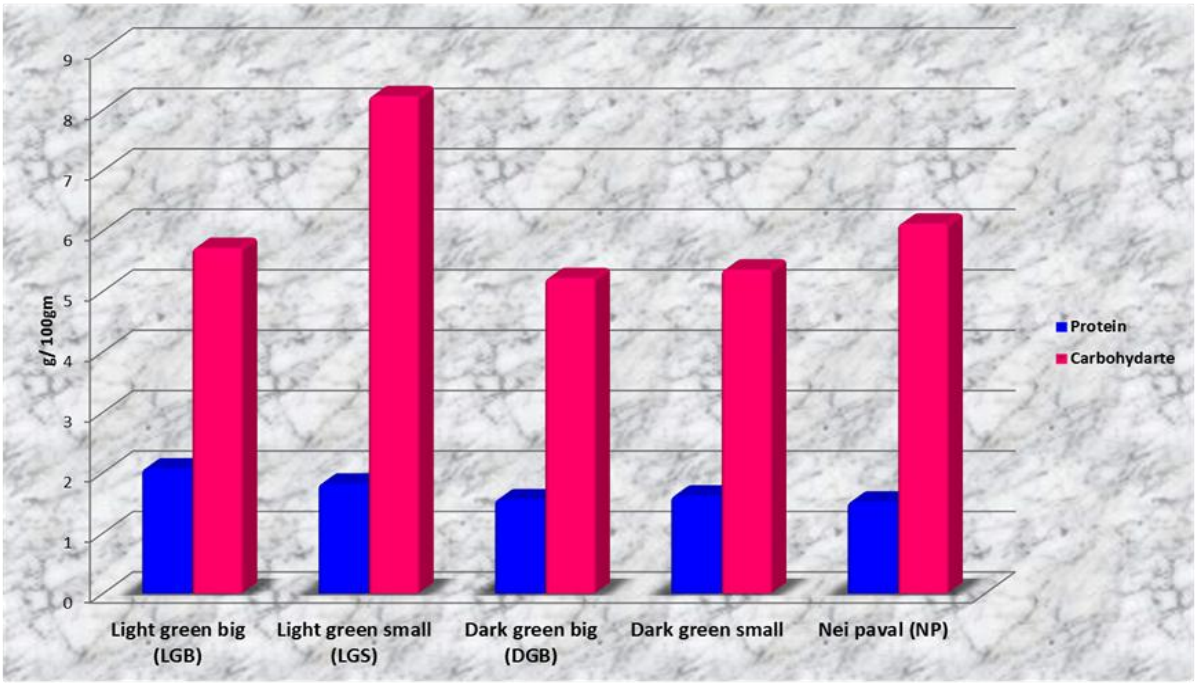
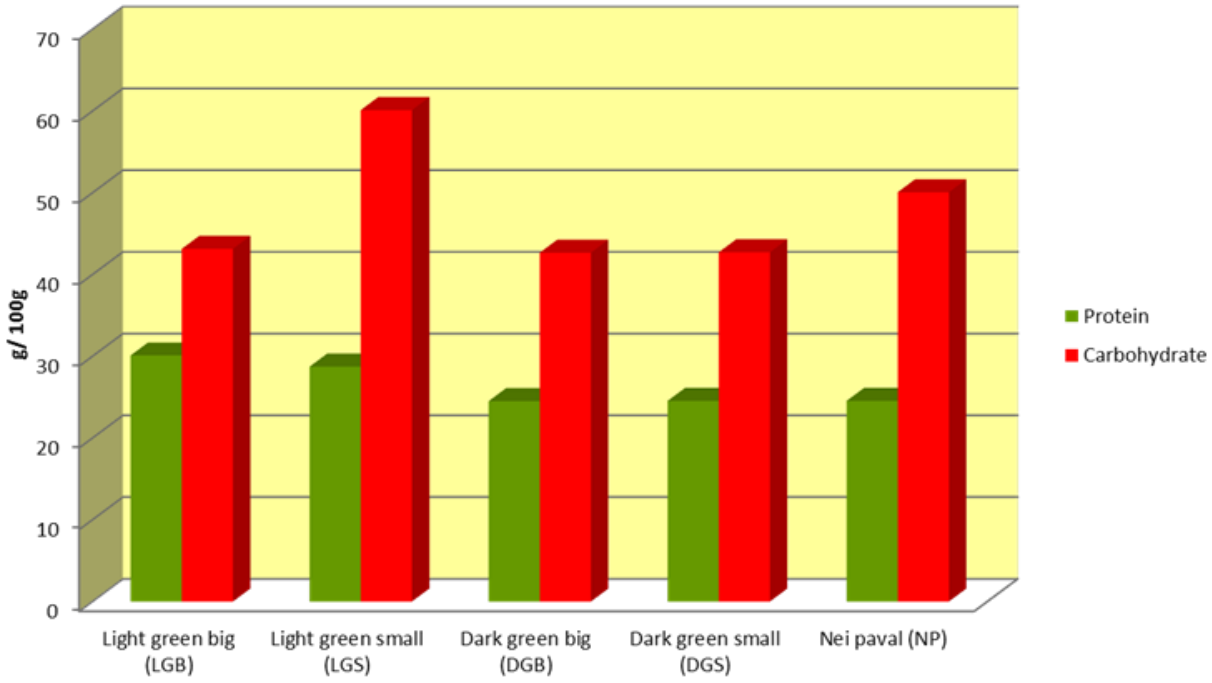


Fig 2.Protein and carbohydrate contents of bitter gourd types (Dried)



5.1.2 Evaluation of protein quality

Kendler (2006) observed that several amino acids are considered to be essential nutrients for maintaining normal cardiovascular function because inadequate levels of some amino acids under different pathophysiological conditions are associated with heart dysfunction.

The present study revealed that all the total essential amino acids were highest in light green big (157 n moles/ml) and lowest in *Nei paval* (86 n moles/ml) respectively. The total non essential amino acids were highest in large white dried (266 n moles/ml) and lowest in light green small dried type (206 n moles/ ml) (Fig 3 & 4).

The study is in agreement with the findings of Islam et al. (2005) who had reported that white varieties had higher protein contents than the green varieties. Compared with soy protein, most of the essential amino acid contents of bitter melon were similar as in soy proteins. Some amino acids such as alanine, glycine, and valine were relatively higher in bitter melon flesh than in soy protein.

Appleton (2002) reported that amino acid arginine, is a precursor for the synthesis of nitric oxide in virtually all cell types, and is also believed to exert cardiovascular benefits. The plasma levels of arginine are reduced in diabetic patients and have been linked to disturbances in both fasting and post challenge glucose levels (Meege et al., 2010). Nout and Ngoddy (2001) identified that certain amino acids may be synthesized and the availability of B group vitamins may be improved.

5.1.3 Carbohydrates

The carbohydrates are pivotal nutrients required for a balanced diet. Highest carbohydrate content was found in light green small fresh (8.22 g) and significant differences in carbohydrate content were noticed among fresh bitter gourd types. The lowest carbohydrate content was found in DGB fresh type (5.21 g). The

Fig 3. Essential amino acid contents of bitter gourd powder

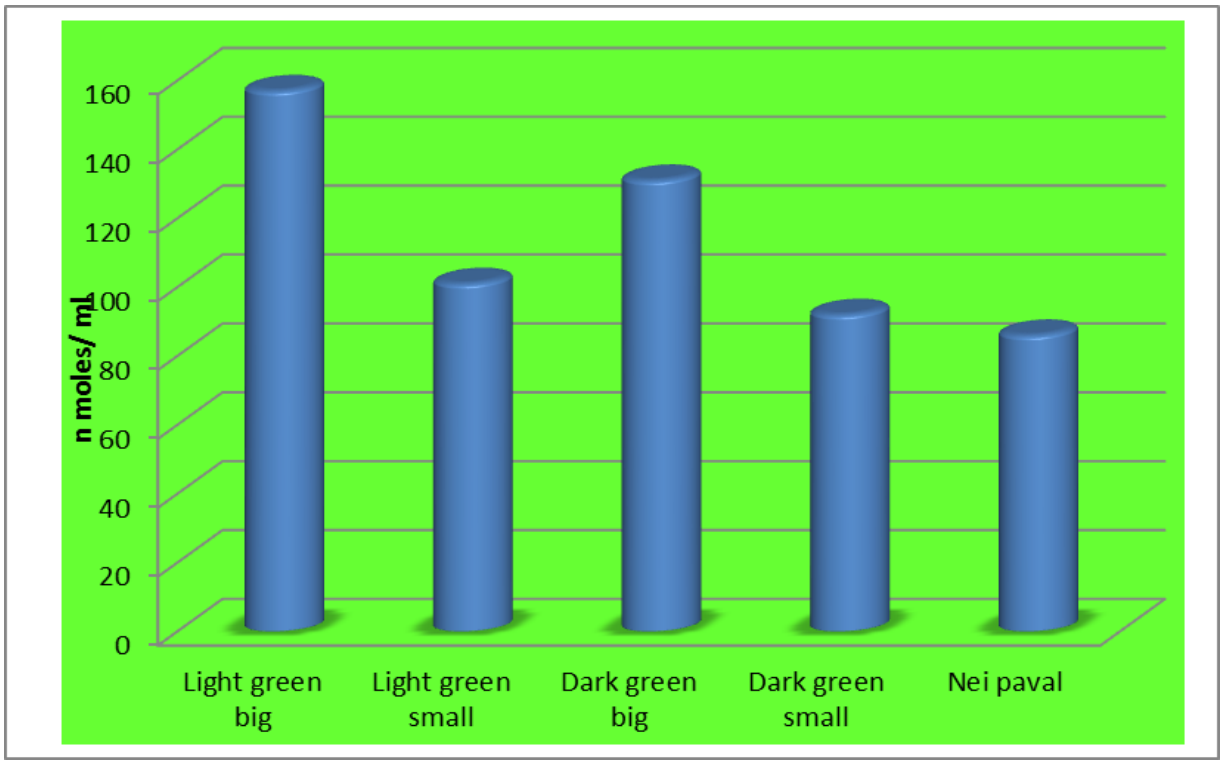
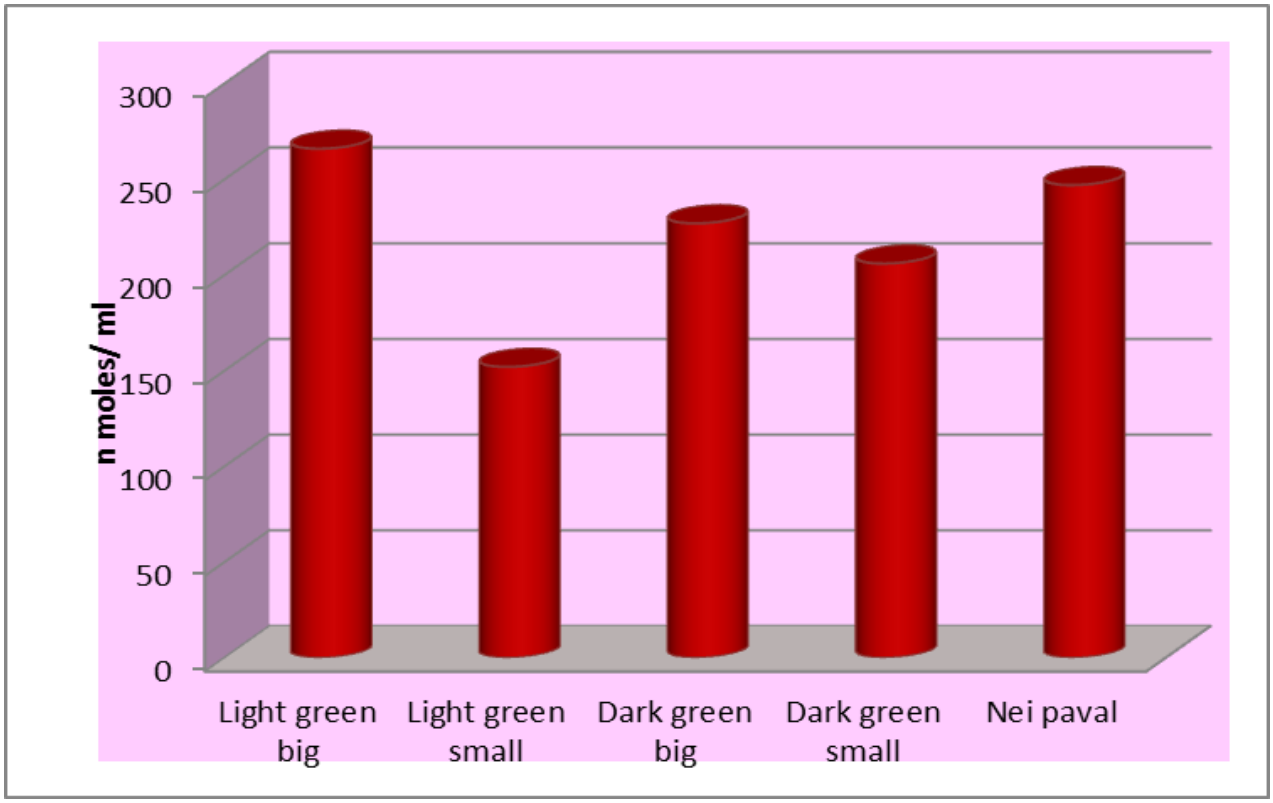


Fig 4. Non essential amino acid contents of bitter gourd powder


300
250
200
150
100
50
0
n moles/ ml
Light green big
Light green small
Dark green big
Dark green small
Nei paval

results of bitter gourd dried revealed that highest carbohydrate content was found in light green small dried (60.13 g) and was significantly different from other four types and the lowest carbohydrate content was found in DGB (42.68 g). Samples with low carbohydrate content might be ideal for diabetic and hypertensive patients requiring low sugar diets (Khalid, 2010). The findings coincide with the results of Bello et al. (2014) who had found that cucurbitacaeous fruits contain a normal amount of carbohydrates ranged from 47.62- 60.21 per cent on dry weight basis.

According to Khalid (2010) bitter gourd consists of 42.54 per cent of carbohydrate on dry weight basis. Bakare et al. (2010) opined that proximate analysis shows that the leaf and fruit of bitter gourd fruit are good source of carbohydrate (34.31 per cent), these may serve as source of energy and nutrients for the body metabolic activities in addition to its medicinal properties.

Rose et al. (2014) opined that fresh carbohydrate (0.61 g) were present in different cucurbitaceae family. As far as carbohydrate is concerned, cucurbitaceae family are not concerned as a good source of carbohydrate but after dehydration the carbohydrate content of the bitter gourd was comparable with many of the carbohydrate rich cereals and vegetables (Pallavi and Dipika, 2010).

5.1.4 Fibre

Fibres have been regarded as a bioactive compound with functional properties and named as nutraceuticals that enhances human physiological performance by preventing or treating diseases and disorders (Wildman, 2001). They undergo fermentation in the digestive tract and accelerate bowel movements, reduce cholesterol synthesis and absorption, increase mineral absorption, improve the anti-oxidative defense system and help to prevent some disorders such as constipation and colorectal cancer (Anderson, 2009).

Dietary fibers present in bitter gourd can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Ishida et al., 2000). *Momordica charantia* could be valuable sources of dietary fibre in human nutrition.

In the present study, bitter gourd fruits contain good amounts of fibre ranging from 0.91 g/100g to 1.12 g/100g in fresh and 15.53 g/100g to20.31 g/100g in dry samples. The results are in accordance with the studies carried out by Rose et al. (2014). The amount of fibre present in fresh bitter gourd samples was similar to the findings of Bakare et al. (2010).

A study done by Khalid et al. (2010) found out that dried samples of bitter gourd contain17.77 per cent of fibre. Another study carried out by Aziz et al. (2011) observed that fresh bitter gourd contain 1.8 g fibre in flesh and 1.9 g fibre in the skin.

5.1.5 Moisture

Understanding the water content of material is a common interest and concern to many diverse industries. Moisture content is important for the processing and handling of pharmaceuticals, food, personal care products and specialty chemicals. The amount of available water will also dictate the shelf life and stability of many systems. For example, the presence of water in food greatly impacts its susceptibility to chemical, enzymatic and microbial activity (Jangam and Mujumdar, 2010).

In the present study, highest moisture content was found in light green big both in the case of fresh and dried samples (90.40 per cent and 6.61 percent respectively) and lowest moisture content was found in dark green small both in the

case of fresh and dried samples (85.40 per cent and 4.07 per cent). The results are in accordance with the studies carried out by Ullah et al. (2011).

According to Islam et al. (2005), moisture level in bitter gourd ranges between 93.5 per cent to 53.3 per cent in different Indian and Chinese varieties. A study done by Gayathri (2014) on nutritional values and antioxidant properties of powdered *Momordica charantia* and *colocasia esculenta* found out that dried samples of bitter gourd samples contained 6.14 per cent of moisture. These findings are also in line with our results.

A study conducted by Khalid et al. (2010) reported that moisture content of each part of bitter gourd were different. Considering the overall percentage of moisture composition, it was highest in flakes followed by peel and seed (4.15, 4.09 and 4.72 per centages respectively).

5.1.6 Beta carotene

Among the various natural pigments, carotenoids comprise a large family of more than 700 structures (Britton et al., 2004) and are synthesized in plants and other photosynthetic organisms. Most carotenoids can be derived from a 40 carbon basal structure which includes a polymer chain contains 3 to 15 conjugated double bonds and these influences the antioxidants activity of carotenoids (Saura and Goni, 2006).

The results of the present study revealed that there exist significant differences in beta carotene content of fresh and dried bitter gourd samples. The beta carotene content was found highest in neipaval both in fresh and dried samples (140.03 mcg and 98.93 mcg) and lowest beta carotene content was found in light green small both in the case of fresh and dried samples (136.10 mcg and 89.94 mcg respectively). Studies have also shown that the carotene losses were directly dependent on the method of drying (Pallavi and Dipika, 2010). A study conducted by

Blessing et al. (2010) found out that the amount of beta carotene present in different cucurbitacae varieties ranged from 0.001 mg to 2.477 mg respectively. Another study conducted by Nagarani et al. (2014) reported that 61.8 µg beta carotene was present in the cucurbitacae fruits.

According to Dey et al. (2006), total carotenoid content ranged from 0.205 mg to 3.2 mg with a population mean of 1.6mg/100g of fresh weight in genetically modified bitter gourd varieties. Study carried out by Singh and Sagar (2013) on Pusa hybrid-2 variety bitter gourd reported an amount of 1.19 mg beta carotene in dried samples.

5.1.7 Vitamin C

Vitamin C is a powerful water soluble antioxidant scavenger of ROS (Smirhoff, 2000) to prevent or alleviate deleterious effects caused by ROS. It has the ability to donate electrons in a number of enzymatic and non enzymatic reactions (Thomas et al., 1992). Ascorbic acid plays an important role in minimizing the damage caused by oxidative process. This is performed by its synergistic action with other antioxidants (Athar et al., 2008).

In the present study, highest vitamin C content was noticed in light green big fresh (98.20 mg) and in the case of dried sample, highest vitamin C content was found in light green big (30.42 mg). The result of the present study is in accordance with the study carried out by Gayathri (2014).

A study conducted by Dey et al. (2006) noticed highly significant differences among the genotypes for ascorbic acid and it ranged between 60.20 mg to 122.07 mg/100 g of fresh weight with a population mean of 82.14mg/100g of fresh weight. Vitamin C content is decreased when exposed to direct sunlight and heat due to oxidation and this may be proved by the results of Anju (2013). Singh and Sagar (2013) reported that Pusa hybrid-2 varieties are good source of vitamin C (77.56 mg).

According to Aziz et al. (2011) bitter gourd contains 120.22 mg of vitamin C in flesh and 108.66 mg in skin. A study conducted by Ullah et al. (2011) on different bitter gourd varieties grown in Chittagong hill tracts, Bangladesh had vitamin C content between 9.41 g to 16.20 g respectively.

Vitamin C which prevents the free radical damage that triggers the inflammatory cascade is also associated with reduced severity of inflammatory conditions, such as asthma, osteoarthritis and rheumatoid arthritis (Cohen et al., 2000). Vitamin C is a highly effective antioxidant and a very small daily intake of this vitamin for an adult is required to avoid scurvy. Even in small amounts, it can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants (Caunii et al., 2010).

5.1.8 Folic acid

Folic acid is a hematopoietic vitamin and when deficit in the body leads to anaemia (Robert et al., 2003). Folic acid is a group of organic molecule required in very small quantities in the diet for health, growth and survival. The absence of folic acid or an inadequate intake results in characteristic deficiency signs and ultimately death (Edeoga et al. 2005). Folic acid itself not biologically active, but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver (Bailey and Ayling, 2009).

Folic acid, the synthetic form of folate effectively scavenges oxidizing free radicals and as such can be regarded as an antioxidant (Joshi et al., 2001). Despite its water soluble character, folic acid inhibits lipid peroxidation. Therefore, folic acid can protects bio constituents such as cellular membranes or DNA from free radical damage (Joshi et al., 2001).

The results of the present study showed that lowest folic acid content was found in dark green small and *nei paval* sample both in fresh and dried (0.02µg/ml, 0.42 and 0.40µg/ml) where as it was found maximum in light green big both in fresh and dried bitter gourd samples (0.10µ/ml and 0.87µg/ml). The results are in tune with the studies carried out by Bakare et al. (2010). According to Chunduri (2013), cucurbitacae vegetables seen in India contained fair amount of folic acid and it ranged between 0.23 mg to 1.76 mg.

5.1.9 Minerals

Minerals in the diet are required for proper growth and good health. Those needed in macro, or major quantities are calcium, phosphorus, potassium, sulphur, sodium, and chlorine, and those needed in micro (trace) amounts are iron, iodine, copper, cobalt, chromium, manganese, selenium, zinc, fluorine, and molybdenum. The cucurbitaceae and many other vegetables are excellent sources of minerals, particularly of calcium, phosphorus, magnesium, potassium, iron, sodium, and most of these minerals are present in the available form (Abuye et al., 2003). The trace mineral content of fruits and vegetables depends on the amount present in the soil in which the plant was grown (Aberoumand and Deokule, 2008). The diverse geographical sources of fruits and vegetables and modern systems of transporting produce to market reduce the chance of a low intake. The deficiency of minerals such as potassium, phosphorus, sodium, calcium, and magnesium also influences the capacity of the body to utilize amino acids and proteins (Aberoumand and Deokule, 2008) (Fig 5 & 6).

5.1.10 Calcium

Calcium is an important component of a healthy diet and a mineral necessary for life. The National Osteoporosis Foundation says, “Calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life” (Grant et al., 2005).

Fig 5. Major mineral contents of the bitter gourd types (Fresh)

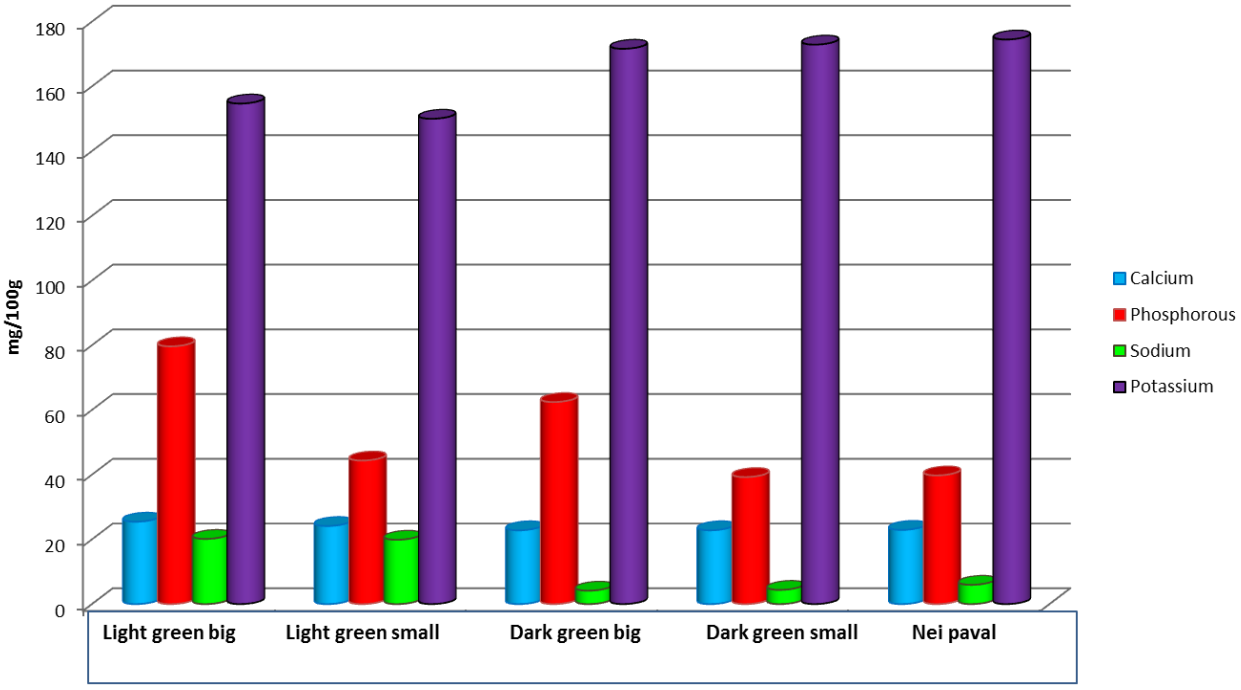
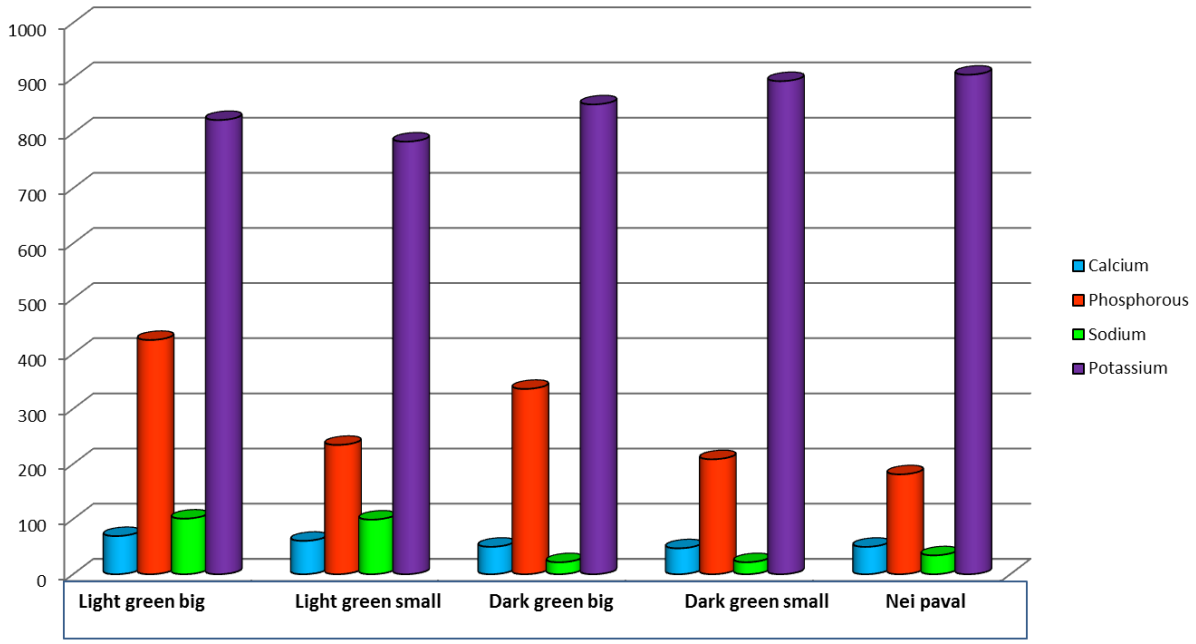


Fig 6. Major mineral contents of the bitter gourd types (Dried)



In the present study highest calcium content was found in light green big (25.44 mg and 69.16 mg) both in the case of fresh and dried samples. The lowest calcium content was found in fresh dark green big (22.78 mg) and fresh dark green small (22.79 mg) in fresh samples and in the case of dried sample lowest calcium content was observed in dried dark green small (46.96 mg). The results are in line with the studies carried out by Bakare et al. (2010). According to Gayathri (2014) dried samples of bitter gourd powder contain 22.4 mg of calcium.

A study conducted by Lucky et al. (2012) on quantitative analysis of different methanol extracts of cucurbitacae family reported an amount of 16.80 mg of calcium. Another study carried out by chunduri (2013) observed calcium content of 62.11 mg in dried bitter gourd samples.

5.1.11 Phosphorus

Phosphorus is integral in reducing muscle weakness, improving bone health, boosting brain function, correcting sexual weakness, and optimizing body metabolism (Wargovich, 2000).

In the present study, highest phosphorus content was noticed in light green big both in the case of fresh and dried samples (79.64 mg and 424.75 mg) and the lowest phosphorus content was found in dried neipaval (181.08 mg). The study supports the findings of Chunduri (2013) who had reported an amount of 17.33 mg of phosphorus in bitter gourd samples analyzed.

5.1.12 Sodium

Sodium being the most abundant mineral found in the fruits. Low sodium diet has been reported to be beneficial in the prevention of high blood pressure (Lichenstein et al., 2006). In the present study sodium content was found highest in light green big both in fresh and dried samples (20.12 mg and 100.76 mg

respectively) while it was found lowest in fresh dark green big (4.13 mg). According to Bakare et al. (2010) bitter gourd is a good source of sodium. The study conducted by Lucky et al. (2012) on quantitative analysis of different methanol extracts of cucurbitacae family coincide with our findings and reported an amount of 16.80 mg of sodium in the samples analyzed.

Sodium content ranged between 2.40 to 120 mg in different cucurbitacae fruis Nagarani et al. (2014). According to Gopalakrishnan and Kalaiarasi (2014), considerable amounts of sodium were present in cucurbitacae fruits.

5.1.13 Potassium

Potassium aids in fluid balance and nerve impulse transmission within the cells (Whitney and Rolfe, 2005). In the present study, potassium content was found highest in *nei paval* both in fresh and dry samples (174.46 mg and 905.76 mg). The lowest amount of potassium was found in light green small both in fresh and dried samples (149.96 mg and 784.47 mg). The results of the present study are in accordance with the study carried out by Nagarani et al. (2014). Bakare et al. (2010) opined that bitter gourd is a good source of potassium. A study conducted by Ali et al. (2008) reported that bitter gourd contained 142 mg of potassium content.

5.1.14 Iron

Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats (Adeyeye and Otokiti, 1999). Iron deficiency causes anaemia, reduced immune function and low resistance to infection, developmental delays, irreversible work performances and adverse frequency outcomes (Naghii and Fouladi, 2006). In the present study, the highest iron content was found in fresh *nei paval* (2.14 mg) and dried *nei paval* (11.26 mg). The results are in agreement with the studies carried out by Blessing et al. (2010) who had observed that the amount of iron present in

cucurbitacae family ranged between 0.001 mg to 0.136 mg. Talukdar et al. (2014) reported that cucurbitacae fruits contained 5.04 mg of iron.

Hussain et al. (2014) conducted a study in different bitter gourd fruits available in Pakistan and found an iron content of 34 mg. Nagarani et al. (2014) had reported 1.8 mg of iron content in cucurbitacae samples. A study conducted by Gayathri (2014) had reported that dried bitter gourd powder contained 0.45 mg of iron.

5.1.15 Manganese

Manganese is essential for normal functioning of the nerve, heartbeat, central nervous system and a good anti-oxidant. It is a micronutrient for bone formation and aids enzymatic actions (Bakhru, 2002). In the present study, highest manganese content was found in fresh light green big (34.57 mg) and lowest content was noticed in fresh *nei paval* (18.41 mg). In the case of dried samples maximum manganese content was noticed in dried light green big (184. 56 mg) and lowest in dried *nei paval* (140.31 mg). The findings are in line with the results of Gopalakrishnan and Kalaiarasi (2014).

5.1.16 Copper

Copper is necessary for nerve metabolism, nerve transmission, many enzyme reactions, blood vessels, fighting inflammation, cholesterol levels, absorption of other minerals such as iron, and cardiovascular health in general (Oyarzun et al., 2001).

The present study showed that bitter gourd contained good amount of copper and was highest in light green big (40.17 mg) and lowest in dark green small (30.10 mg). In the case of bitter gourd dried, highest copper content was found in dried light green big (200.17 mg) and lowest in dried *nei paval* (145.21 mg). The results are in accordance with the study carried out by Ali et al. (2008). A study conducted by Gopalakrishnan and Kalaiarasi (2014) reported that ucurbitacae fruits are good

source of copper. The above results are in accordance with the study carried out by Bakare et al. (2010).

5.1.17 Zinc

Zinc is required for the proper functioning of the reproductive system (Whitney and Rolfe, 2005). According to FAO's food balance data, it has been calculated that about 20 per cent of the world's population could be at risk of zinc deficiency. The average daily intake is less than 70 µg per day (Holt and Brown, 2004).

The present study revealed that zinc content was found highest in light green big both in fresh and dried samples (90.41 mg and 361.13 mg) while it was found lowest in fresh *nei paval* (4.13 mg) and dried neipaval (241.18 mg). The study is in conformity with the findings of Ali et al. (2008) who had reported that cucurbitacae fruits contained good amount of zinc (33 mg) (Fig 7 & 8).

A study conducted by Bello et al. (2014) in different cucurbitacae fruits and the results showed good amount of zinc. The results corroborates with the study carried out by Gopalakrishnan and Kalaiarasi (2014).

To sum up the study revealed that highest protein content was found in Light green big (2.06 g) and in the case of bitter gourd dried, highest protein content was found in light green big dried (30.11 g). The total essential amino acids were highest in light green big dried (157 n moles/ml) and whereas the total non essential amino acids were highest in light green big dried (266 n moles/ml) respectively. Highest carbohydrate content was found in light green small fresh (8.22 g) and in the case of dried type highest carbohydrate content was found in light green small dried (60.13 g). Highest moisture content was found in light green big both in the case of fresh and dried (90.40 per cent and 6.61 per cent/ 100g respectively) bitter gourd types.

Fig 7. Trace mineral contents of the bitter gourd types (Fresh)

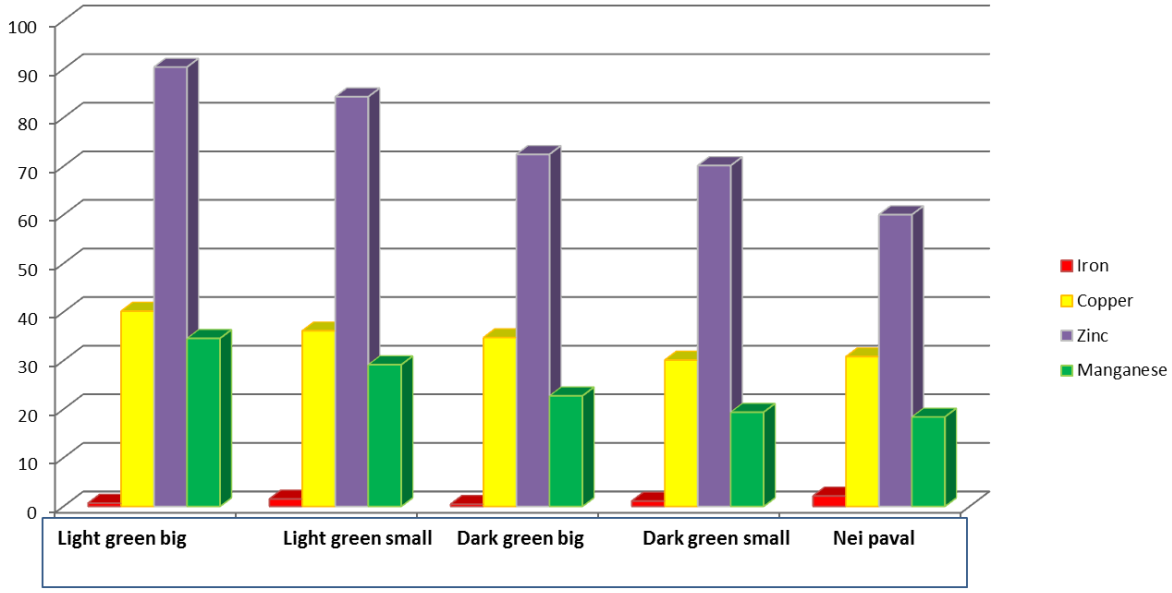
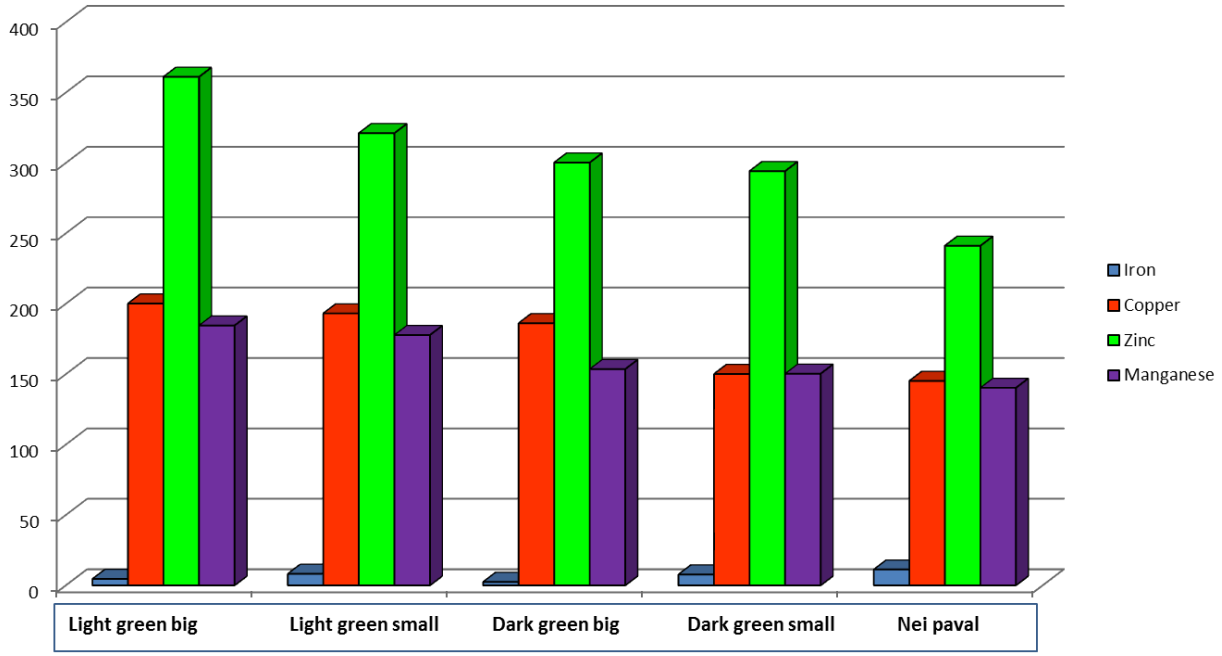


Fig 8. Trace mineral contents of the bitter gourd types (Dried)


400
350
300
250
200
150
100
50
0
Light green big
Light green small
Dark green big
Dark green small
Nei paval
Iron
Copper
Zinc
Manganese

The highest vitamin C content was noticed in light green big both in the case of fresh and dried samples (98.20 mg 97.92 respectively) and was significantly. Folic acid content was found maximum in light green big both in fresh and dried bitter gourd samples (0.10 µg/ ml and 0.87 µg/ ml respectively).

In the case of mineral analysis, highest calcium content was found in light green big (25.44 mg/ 100g and 69.16 mg/100 g respectively) both in the case of fresh and dried samples. Highest phosphorus content was noticed in light green big both in fresh and dry samples (79.64 mg and 424.75 mg respectively). The sodium content was found highest in light green big both in fresh and dry samples (20.12 mg/ 100g and 100.76mg/ 100g respectively). The potassium content was found highest in *nei paval* both in fresh and dry samples (174.46 mg/ 100g and 905.76 mg/ 100g respectively). Highest iron content was found in *nei paval* both in the case of fresh and dried samples (2.14 mg and 11.26 mg) highest iron content was noticed in NP (11.26 mg). Highest manganese content was noticed in light green big both in the case of fresh and dried samples (34.57 mg and 184.56 mg). Copper content was found highest in light green big both in the case of fresh and dried types (40.17 mg and 200.17 mg). The zinc content was found highest in light green big both in fresh and dry samples (90.41 mg/ 100g and 361.13 mg/ 100g respectively).

5.2 PHYTOCHEMICAL SCREENING OF BITTER GOURD (Fig 9 & 10)

Phytochemicals are bioactive substances of plants that have been associated in the protection of human and animal health against chronic degenerative diseases. The major groups of phytochemicals that may contribute to the total antioxidant capacity (TAC) of plant foods include polyphenols, carotenoids and the traditional antioxidant vitamins such as vitamin C and vitamin E (Masaki, 2010).

Fig 9. Phytochemical analysis of the bitter gourd types (Fresh)

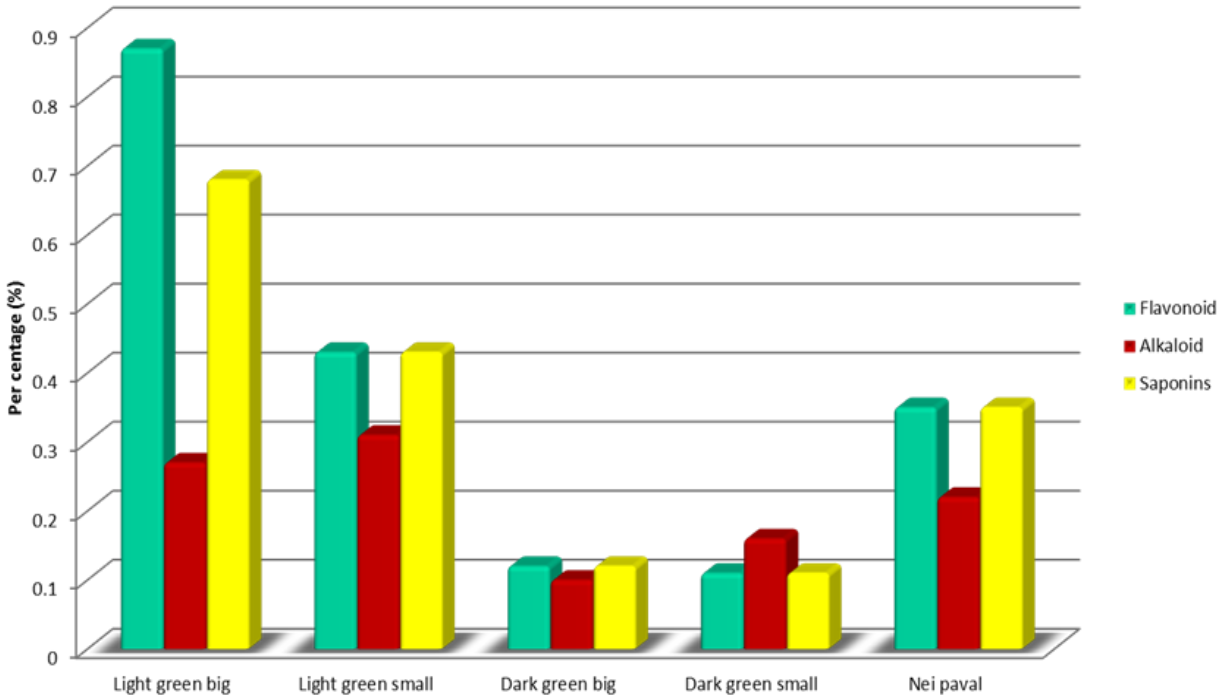
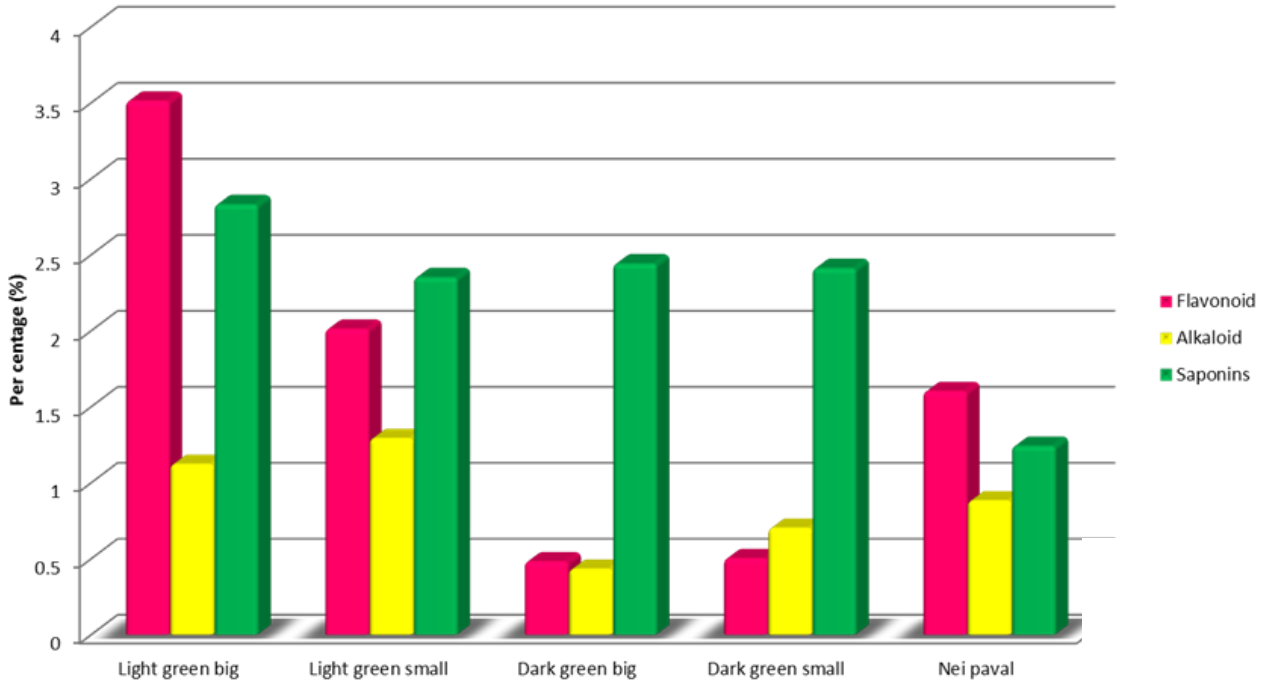


Fig 10. Phytochemical analysis of the bitter gourd types (Dried)



A phytochemical is a natural bioactive compound found in plants foods that works with nutrition and dietary fibre to protect against diseases. Many researchers suggested that phytochemical work together with nutrients found in fruits, vegetables and nuts. They can have complementary and overlapping mechanism of action in the body including antioxidant effect (Dhimati et al., 2003).

Phytochemicals are non nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrated that many phytochemicals can protect human against various diseases (Tupe et al., 2013).

Oxidative stress has been thought to play an important role in the pathogenesis of diseases such as cancer, cardiovascular disease, atherosclerosis, diabetes mellitus, and neurodegenerative disorders (Valko et al., 2007). ROS include free radicals such as superoxide anion ($O2^{-}$), hydroxyl radical (OH), and non-radical molecules like hydrogen peroxide (H_2O_2), singlet oxygen (O_2), nitric oxide (NO), etc. ROS are involved in the pathogenesis of several skin disorders including photosensitivity diseases and some types of cutaneous malignancy (Bickers and Athar, 2006). Additionally, ROS may accelerate aging process and cause uneven pigmentation. Ultra violet radiation has been shown to augment nitric oxide and peroxynitrite formation in keratinocytes (Deliconstmtinos et al., 1996). Phytochemical agents may therefore play a protective role during the development of ROS-mediated skin disorders.

Study findings suggested that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity (Mathai, 2000). Phytochemicals

may detoxify substances that cause cancer. They appear to neutralize free radicals, inhibit enzymes that activate carcinogens and activate enzymes that detoxify carcinogens.

According to Buwa and Staden (2007) various factors including internal biochemical factors, extracted plant part, and external environmental factors such as climate, location, season, and growth conditions all influence the effectiveness of medicinal plants. The medicinal values of bitter melon lies in the bioactive phytochemical constituents that are non nutritive chemicals that produce definite physiological effects on human body and protect them from various diseases. Qualitative phytochemical analysis of *Momordica charantia* confirms the presence of phytochemicals like flavonoids, tannins, saponins, alkaloids, steroids, cardiac glycosides, anthraquinones, polyphenols, phlobatinnins etc (Daniel et al., 2014).

The present study results revealed the presence or absence of tannins, flavonoids, phenols, alkaloids, saponins, steroids, cardiac glycosides, phlobatinnins and anthraquinones in bitter gourd fruit extracts. Results of phytochemical screening revealed that tannin was found in bitter gourd extracts of ethanol, methanol and acetone solvents. Presence of flavonoids produces a yellow colouration and was present in ethanol, methanol, acetone and aqueous solvents of bitter gourd fruit.

Phenols were present in ethanol, methanol and aqueous solvents and were absent in acetone, chloroform and petroleum ether of bitter gourd extracts. Alkaloids were present in all the bitter gourd mixed solvents except petroleum ether. Presence of saponin and steroids were identified in all the bitter gourd extracted solvents. The results of present study pointed out that cardiac glycosides and phlobatinnins were absent in aqueous and petroleum ether and were present in all the other bitter gourd extracted solvents. The results also revealed the absence of anthraquinones in acetone solvent and were present in all the other bitter gourd extracted solvents.

A study conducted by Gupta and Verma (2011) reported that presence of tannin gives a purple colour in bitter gourd fruit extracts. The results are in accordance with the studies carried out by Mir et al. (2012). Another study carried out by Wadood et al. (2013) on momordica fruit extract observed that alkaloids, flavonoids and phlobatannins were present in the ethanol extracts. Gaurav et al. (2014) conducted a study on phytochemical analysis of medicinal plants occurring in local area of Mardan reported the presence of alkaloids, phenol and tannin in bitter gourd extracts.

According to Lucky et al. (2012) tannins, saponins, alkaloids, steroids, cardiac glycosides, flavonoids and phenolics were present in bitter gourd extracts. Another study carried out by Lalhelenmavia et al. (2013) reported that bitter gourd extracts exhibits the presence of alkaloids, flavonoids, saponins, polyphenols and glycosides in qualitative analysis. Thakre et al. (2014) conducted a study on extraction of phytochemical components from the fruit of *Momordica charantia* and evaluation of its antimicrobial activity by using different solvents reported the presence of saponins and flavonoids. The study is in conformity with the findings of Talukdar and Mohammad (2014).

A study conducted by Kumar et al. (2010) on gas chromatography mass spectrometry analysis and phytochemical screening of methanolic fruit extract of bitter gourd fruits revealed that alkaloids, steroids, tannins, flavonoids, saponins, cardiac glycosides and anthraquinones were present in the bitter gourd extract. The results are in accordance with the study carried out by Annapoorani and Manimegalai (2013).

Patel et al. (2011) reported that alkaloids, flavonoids, steroids, saponins, tannins, glycosides and polyphenols were present in bitter gourd extracted with different solvents like petroleum ether, chloroform, alcohol and water. Methanolic

extract of bitter gourd fruit contained higher concentration of active antimicrobial agents such as alkaloids and glycosides, which are found in *Momordica charantia* (Grover and yadhav, 2004).

Phytochemical analysis plays a major resource for information on analytical and instrumental methodology in plant sciences. These phytochemicals are secondary metabolites which are the important constituents of medicinal plant. It has been reported that secondary plant metabolites exert a wide range of biochemical activities on physiological systems (Olagunju et al., 2006). Previously it was reported that the alkaloids, saponins and phenolic constituents are present in bitter gourd (Taylor, 2002). The activities of some phytochemicals with compound nature of flavonoids, phenols and alkaloids as antimicrobial, antioxidant, hypocholesterolemic, cancer protective, antidiabetic and hepato protective activities (Kumar et al., 2010). In *Momordica charantia* there are various phytochemical compounds present which play a major role in the biological activities. Saponins are amphipathic glycosides steroids with a distinctive foaming characteristic which have strong biological activity and antifungal activity and antibacterial properties (Ullah et al., 2011).

The pharmaceuticals studies of fruit extracts of bitter gourd have shown that the plants has antiulcer (Matsuda et al., 1999), anti cancer activity (Sun et al., 2002), anti plasmodial, anti inflammatory and immuno modulatory, antioxidant (Kubola and Siriamornapun, 2008), antimicrobial (Mwambete, 2009) and hypoglycemic and hypolipidemic action.

5.2.1 Polyphenol

Phenolics or polyphenols have received considerable attention because of their physiological function, including antioxidant, antimutagenic, and antitumour activities (Othman et al., 2007). Phenolic compounds are widely distributed in plants

(Li et al., 2006), which have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health (Govindarajan et al., 2007; Li et al., 2006).

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic are hydroxyl group (-OH) containing. plant foods which can act as antioxidants to prevent heart disease (Jin and Mumper, 2010) reduce inflammation (Mohanlal et al., 2012), lower the incidence of cancers and diabetes (Kusirisin et al., 2009), as well as reduce rates of mutagenesis in human cells. The protection afforded by the consumption of plant products such as fruits, vegetables and legumes is mostly associated with the presence of phenolic compounds.

Plant phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Thus, plant phenolics comprise simple phenols, coumarins, lignins, lignans, condensed and hydrolysable tannins, phenolic acids and flavonoids (Slivova et al., 2005). In the present study highest polyphenol content was observed in light green big both in the case of fresh and dried samples (18.76 mg and 74.67 mg respectively). The lowest polyphenol content was observed in fresh dark green small (12.40 mg) and was on par with dark green big (12.66 mg). In the case of dried samples, dried dark green small contained lowest polyphenol (49.53 mg). Budrat and Shotipruk (2008) reported a total phenolic compound in bitter melon (using soxhlet extraction, methanol as solvent) as 4.992 mg Gallic acid equivalent /g and dry weight and (using solvent extraction, methanol as solvent) as 7.743 mg GAE/g dry weight. Horax et al. (2010) and Ghasemi et al. (2011) have investigated the phenolic content of various ethanol extracts from the pericarp and seeds of bitter melon during three stages of maturity (immature, mature and ripe). Extracts of 80 per cent ethanol were shown to be the optimal solvent level for the

extraction of phenols, which was measured by Folin Ciocalteau method, for immature (15.7 ± 0.1 mg GAE/g), mature (14.8 ± 0.3 mg GAE/g) and ripe (14.9 ± 0.2 mg GAE/g) pericarp extracts and also mature (18.0 ± 0.1 mg GAE/g) and ripe (20.9 ± 0.1 mg GAE/g) seed extracts. A study conducted by Gaurav et al. (2014) reported that 11.54 mg GAE/ g of polyphenols were present in bitter gourd.

A study conducted by Hamissou et al. (2013) on antioxidant properties of bitter gourd (*Momordica charantia*) and zucchini (*Cucurbita pepo*) found out an average total phenolic compound in bitter gourd was 13.28 GAE/g fresh weights. The results are in close agreement with Patel et al. (2011) who observed that total phenolic content in the alcoholic extract of *Momordica charantia* extract was found to be 5.61 per cent w/w calculated in terms of gallic acid.

The total phenolic content in oven-dried samples at 40 °C was high (70.25 mg/100 g GAE). Drying temperatures significantly affected the polyphenol content, with different effects according to the class of polyphenols (Del Caro et al. 2004). A study conducted by Islam et al. (2005) on bio active compounds of bitter melon genotypes (*Momordica charantia*) in relation to their physiological functions observed that overall phenolic content in oven dried samples were significantly higher than freeze dried samples. Phenolic content of the oven dried and freeze dried tissues ranged from 5.39-8.94 mg of chlorogenic acid equivalent dry matter and 4.64-8.90 mg/ CAE.g dry matter respectively.

A study conducted by Aminah and Permatasari (2013) on different cooking methods in bitter gourd samples indicated that deep frying had the highest total phenol content at 98.18 mg/100 g GAE, followed by microwave cooking (25.63 mg/100 g GAE). The total phenol content for deep-fried samples was significantly different (p <0.05) from the other cooking methods.

5.2.2 Flavonoid

Flavonoids are polyphenolic compounds with a nuclear structure of C6-C3-C6, which is comprised of two benzene rings linked to a pyrene ring containing oxygen (Ko et al., 2014). Flavonoids occur naturally in plants either linked to sugars (glycosides) or without the sugars (aglycones). Flavonoid aglycones are usually extracted with less polar solvents, such as benzene, chloroform and diethyl ether (Marston and Hostettmann, 2005) while flavonoid glycosides are commonly extracted with more polar solvents, such as acetone, butanol, methanol and ethanol (Shao et al., 2013). Ethanol (Ko et al., 2014), water (Wu and Ng, 2008) and methanol (Kenny et al., 2013) have been used for extracting flavonoids and other bioactive compounds from bitter gourd.

Flavonoids are some of the most common phenolics, widely distributed in plant tissues, and often responsible alongside the carotenoids and chlorophylls for their blue, purple, yellow, orange and red colors. The flavonoid family includes flavones, flavonols, iso-flavonols, anthocyanins, anthocyanidins, pro anthocyanidins and catechins (Ferreria and Pincho, 2012). All flavonoids are derived from the aromatic amino acids, phenylalanine and tyrosine, and have three-ringed structures (Rong, 2010). Variation in flavonoid structure arises from the scale and pattern of hydroxylation, prenylation, alkalinization and glycosylation reactions that alter the basic molecule (Stalikas, 2007).

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham, 1960). The flavonoids appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. Flavonoids are ubiquitous among vascular plants and occur as aglycones, glucosides and methylated derivatives. More than 4000 flavonoids have been described so far within the parts of plants normally

consumed by humans and approximately 650 flavones and1030 flavanols are known (Harborne, 1999). Small amount of aglycones (i.e., flavonoids without attached sugar) are frequently present and occasionally represent a considerably important proportion of the total flavonoid compounds in the plant. The six-membered ring condensed with the benzene ring is either -pyrone (flavones and flavonols) or its dihydroderivative (flavanones and flavan-3-ols).

The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. On the other hand flavonoids such as luteolin and catechins are better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene. Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity (Atmani et al., 2009). Flavonoids constitute a wide range of substances that play important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Atmani et al., 2009).

In the present study, the highest flavonoid content was found in dark green big type both in fresh and dried forms (0.87 per cent and 3.51 per cent respectively). These results are in close agreement with Kale and Laddha (2012) who was observed that different types of cucurbitacae fruits contain 0.47 per cent alkaloids on dry weight basis.

A study conducted by Tan et al. (2014) aimed to extract bitter gourd, using five solvents (ethanol, methanol, n-butanol, acetone and water) before and after the optimal conditions for water were determined in terms of extraction temperature,

time, ratio of water to bitter gourd (mL/g) and number of times the same material was extracted. The total flavonoid content of six varieties of bitter gourd was also determined. Acetone was the best of the five solvents for extracting flavonoids from the Moonlight variety (23.2 mg Rutin Equivalents (RE)/g). Even after increasing the extraction by 88 per cent (1.24 vs 0.66 mg RE/g) using optimized conditions for the aqueous extraction (two extractions at 40˚C for 15 min at a ratio of 100:1 mL/g of bitter melon powder), the flavonoids extracted from the Moonlight variety using water was very little (5.4 per cent) compared to acetone. Furthermore, using acetone, it was shown that the Moonlight variety (23.2 mg RE/g) bought at a local market had higher levels of flavonoids than the greenhouse-grown Jade (15.3 mg RE/g), Niddhi (16.9 mg RE/g), Indra (15.0 mg RE/ g), Hanuman (3.9 mg RE/g) and White (6.9 mg RE/g) varieties. Therefore, acetone was the best solvent for extracting flavonoids from bitter melon and the aqueous extraction could only be improved to extract 5.4 per cent of the flavonoids extracted with acetone from the Moonlight variety, which had the highest total flavonoid of the six varieties of bitter melon. The results are in close agreement with Gaurav et al. (2014) who had observed 4.68 mg CE/g of flavonoids in bitter gourd samples.

According to Patel et al. (2011) flavonoid content of aqueous extract of *Momordica charantia* was 1.77 per cent on dry weight basis. Another study carried out by Agarwal and Kamal (2013) revealed that bitter gourd fruit contained 0.88 mg of flavonoids per 100 g.

5.2.3 Alkaloid

Alkaloids are natural product that contains heterocyclic nitrogen atoms and are basic in character. The word alkaloid was derived from “alkaline” and was used to describe any nitrogen-containing base (Muller et al., 1992). Alkaloids are naturally synthesized by a large number of organisms, including animals, plants, bacteria and fungi. Almost all the alkaloids have a bitter taste. The alkaloid quinine for example is

one of the bitterest tasting substances known and is significantly bitter of $1x10^{-5}$ at a molar concentration (Mishra, 1989). In the present study, alkaloid content of bitter gourd samples (fresh) ranged between 0.10 per cent to 0.27 per cent where as in the case of bitter gourd dried samples, it ranged between 0.90 per cent to 1.01 per cent.

Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrents) and also against other plants by means of allelopathically active chemicals (Molyneue, 1996). The use of alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization. Alkaloids have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine) (Wink et al., 1998). Some alkaloids have stimulant property as caffeine and nicotine, morphine are used as the analgesic and quinine as the antimalarial drug (Dubois and Wagner, 2000).

5.2.4 Tannin

Tannins are phenolic compounds that precipitate protein. They are composed of a very diverse group of oligomers and polymers. The presence and consequent interaction of tannins are proteins in the seeds of cereals and legumes and have been believed to be of the factors involved in reduced protein digestibility. Most berries, such as cranberries and blueberries contain both hydrolysable and blueberries contain both hydrolysable and condensed tannins.

From a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers (Harborne, 1999). It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible

complexes with proteins, mainly polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc. (Schofield et al., 2001). On the basis of their structural characteristics, it is therefore possible to divide the tannins into four major groups-gallotannins, ellagitannins, complex tannins, and condensed tannins.

In the present study, highest tannin content was observed in fresh *nei paval* and was on par with fresh light green small (0.60 mg). In the case of dried bitter gourd samples the highest amount of tannin was found in dried *Nei paval* (1.01 mg). The study is in line with the findings of Gayathri (2014) who had found tannin content of 0.81mg in dried bitter gourd powder.

Tannins are found commonly in vegetables, fruits such as grapes, blueberry, tea, chocolate, legume forages (Serrano et al., 2009). Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established (Serrano, 2009). Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers (Pallavy and Priscilla, 2006). The search for new lead compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been documented by Pallavy and Priscilla, 2006.

Blessing et al. (2010) identified tannin content of different cucurbitacae fruits ranged from 0.017 mg to 0.102 mg/100g dry matter. Another study carried out by Karaye et al. (2013) reported that cucurbitacae seeds tannin content ranged between 1.10 mg to 3.70 mg. Bello et al. (2014) conducted a study on different types of bitter gourd family and the results showed that tannin content of fruits ranged between 6.90 mg/l to 10.4 mg/l respectively.

5.2.5 Saponin

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom. They form a stable foam in aqueous solutions such as soap, hence the name "saponin". Chemically, saponins as a group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives. The main triterpene aglycone is a derivative of oleanane (Bohlmann et al., 1998).

Saponins may be considered as part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phyto protectants (Dubois and Wagner, 2000). Saponin mixtures present in plants and plant products possess diverse biological effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals (Traore et al., 2000). These structurally diverse compounds have also been observed to kill protozoans and mollusks, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycaemia, and to act as antifungal and antiviral agent (Traore et al., 2000).

In the present study, the highest amount of saponin was found in light green big both in the case of fresh and dry samples (0.68 per cent and 2.83 percent). Koneri et al. (2014) found out antidiabetic activity of saponins in bitter gourd powder in an *in vivo* study conducted in mice.

5.2.6 Lectin

Lectins are structurally diverse oligomeric proteins composed of subunits, one or more of which carry a sugar-binding site (Lis and Sharon, 1998). Lectins are

Fig 11. Lectin content in bitter gourd types (Fresh)

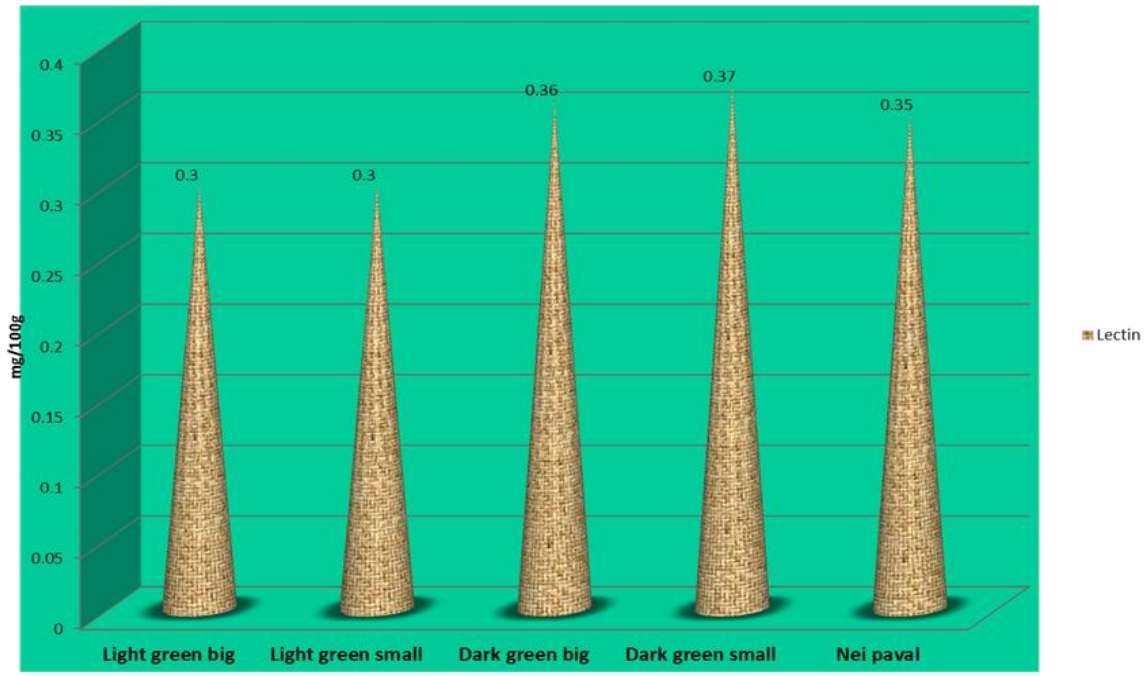
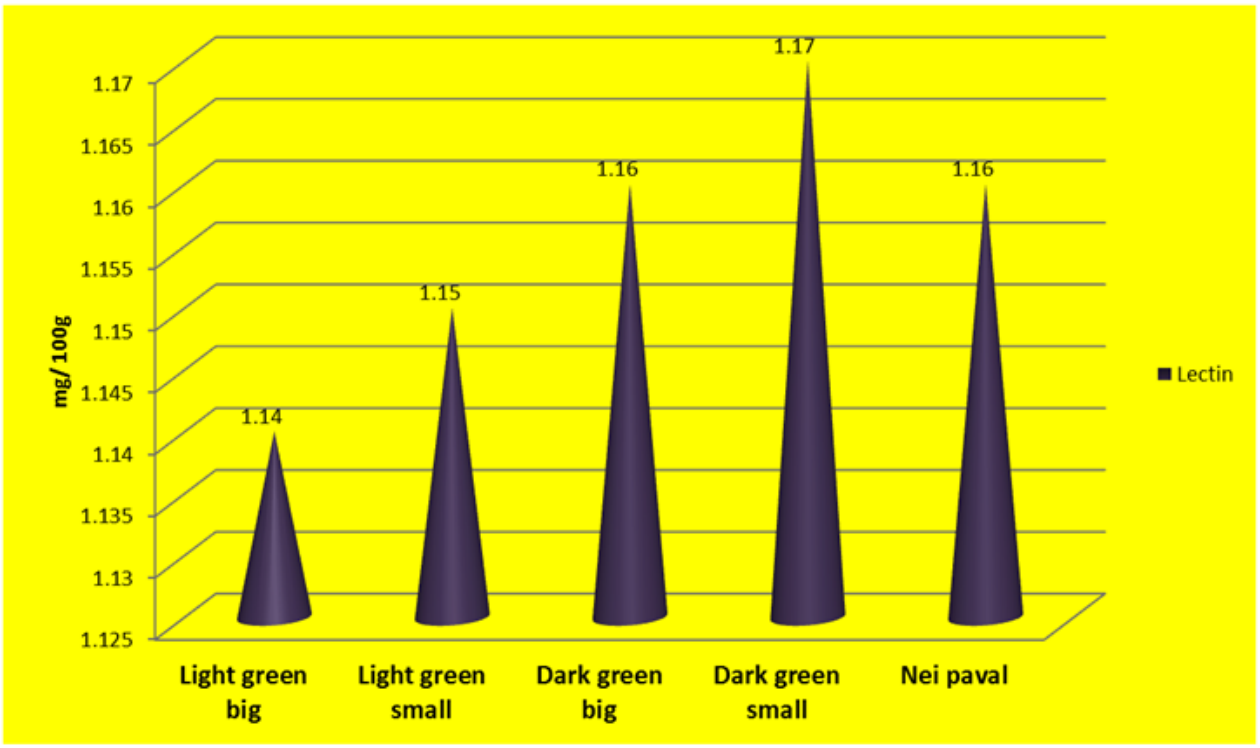


Fig 12. Lectin content in bitter gourd types (Dried)


1.17
1.165
1.16
1.155
1.15
1.145
1.14
1.135
1.13
1.125
mg/100g
1.14
1.15
1.16
1.17
1.16
Light green big
Light green small
Dark green big
Dark green small
Nei paval
Lectin

ubiquitous in nature and are found in plants, animals, microorganisms including fungi and bacteria (Peumans and Damme, 1998). The functions of lectins are extremely diverse and all are based on the ability to bind or recognize the carbohydrate moieties of glycol conjugates.

Lectins are useful as molecular tools for isolating glycoconjugates, mitogenic stimulation of lymphocytes and for cell fractionation, bone marrow transplantation and preferential agglutination of tumor cells (Sharon and Lis, 2003). Some plant lectins play a role in defense mechanisms of the plant. In some legumes, lectins have been shown to mediate plant symbiosis with nitrogen fixing bacteria (Sharon and Lis, 2003). Studies on lectins from other plant families are fewer, and therefore there is a need to investigate them and characterize their physico-chemical and carbohydrate binding properties in detail.

The present study revealed that lectin content of bitter gourd types ranged between 0.30 mg to 0.37 mg in the case of fresh bitter gourd types where as it ranged between 1.14 mg to 1.17mg in the case of dried bitter gourd types (Fig 11 & 12).

Many species from Cucurbitaceae are cultivated for food in different countries, it is of considerable interest to purify and characterize lectins from this family and lectins have been isolated from the seeds and the phloem exudate of several cucurbit species (Sandi and Surolia, 1994). Cucurbit seed lectins are of particular interest in that many of them show structural homology to type-II ribosome inactivating proteins (RIPs). Yet they do not activate ribosomes or do so only weakly. However, only two seed lectins from this family (*Momordica charantia* lectin) (MCL), and Trichosantheskirilowii seed lectin (TKL-I)] were characterized in detail with respect to carbohydrate binding and macromolecular properties (Sultan and Swamy, 2005).

5.2.7 Charantin

Lotlikar and Rao (1996) have isolated a non-nitrogenous substance identified as "charantin." from the fruits of *Momordica charantia*. Charantin is a mixture of sitosteryl-3β-D-Glucoside and 5, 25- stigmastadiene-3 β-ol D-Glucoside. It is a whitish crystalline material melting at 266-268 ^{0}C with decomposition. Charantin is found to have hypoglycemic capacity which is a mixture of two compounds sitosterylglucoside and stigmasterylglucoside (Marderosian, 2001). Charantins, a mixture of steroidal saponins that is abundant in the fruit of bitter gourd, having antihyperglycemic activity of bitter gourd (Harinantenaina et al., 2006).

In the present study, charantin content was found to be highest in light green big both in the case of fresh and dried samples (0.18 µg/ml and 1.33 µg/ml respectively). The charantin content was found to be lowest in dark green small both in fresh and dried bitter gourd samples (0.10 µg/ml and 1.20 µg/ml respectively (Fig 13 & 14). Earlier reports showed that charantin had been isolated from the ethanol/water extracts of leaves and fruits of *Momordica charantia* by HPTLC and TLC (Chanchai, 2003). Patel et al. (2006) separated charantin from the chloroform extract of dried fruits of *Momordica charantia* by HPTLC. Other than *Momordica charantia*, six wild species have been reported from India and those fruits are used as vegetable by the local communities of Tamil Nadu and Kerala respectively (Shanmugapriya and Poornima, 2014).

The sum up the present study revealed that phytochemical screening of the ethanol, methanol, acetone aqueous, petroleum ether, and chloroform fruit extract of *Momordica charantia* revealed the presence/ absence of tannins, flavonoids, phenols, alkaloids, saponins, steroids, cardiac glycosides, phlobatinnins and anthraquinones. Quantitative estimation of phytochemicals revealed that, highest polyphenol content was noticed in light green big both in

Fig 13. Charantin content in bitter gourd types (Fresh)

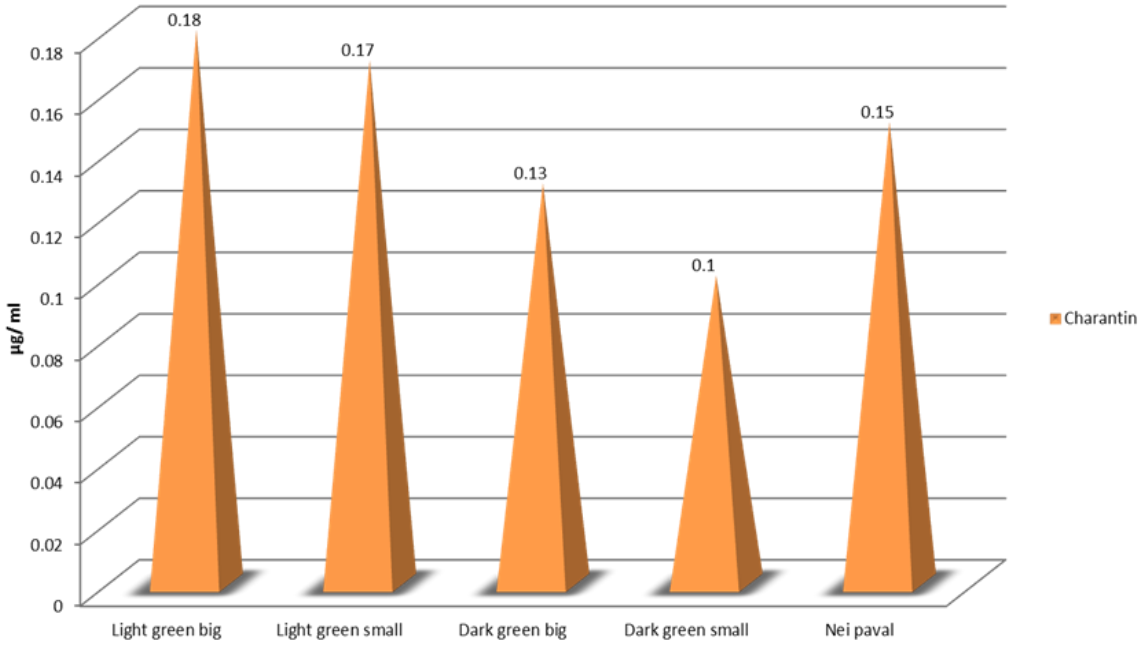
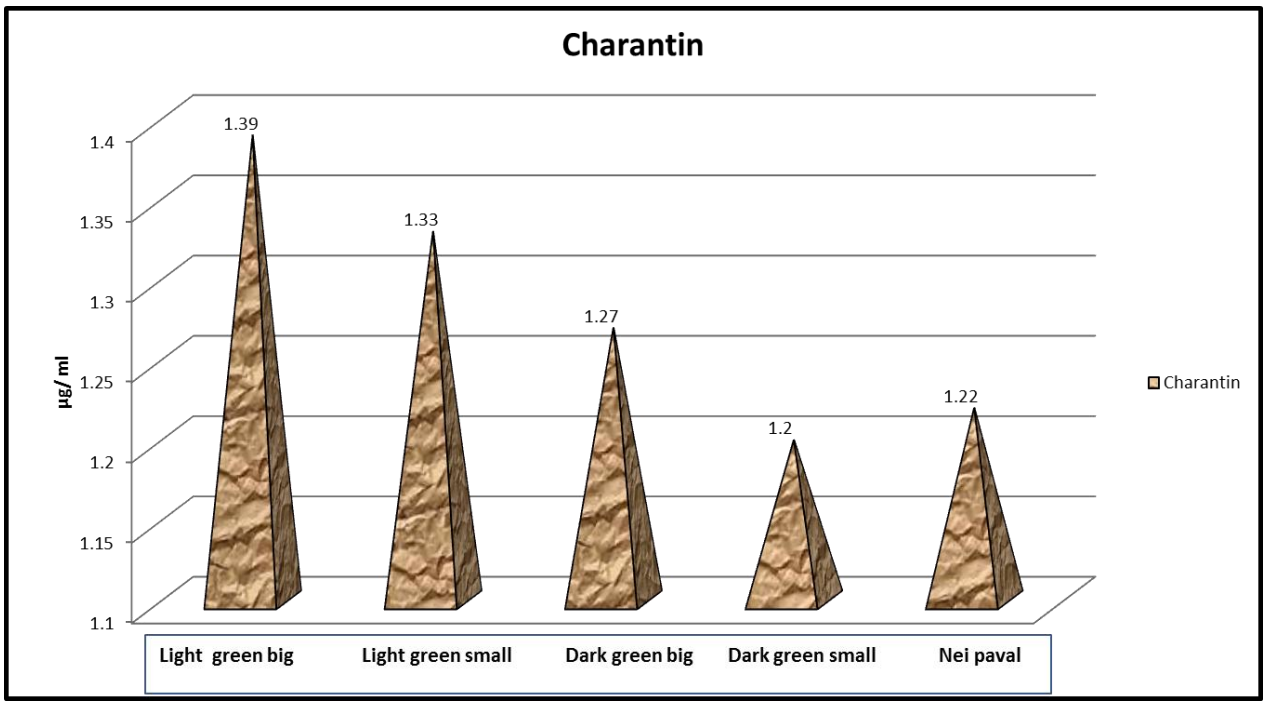


Fig 14. Charatin content in bitter gourd types (Dried)



the fresh and dried forms (18.76 mg and 74.67 mg respectively). The highest flavonoid content was found in dark green type both in the fresh and dried forms (0.87 per cent and 3.51 per cent respectively). The alkaloid content of bitter gourd samples (fresh) ranged between 0.10 to 0.27 per cent where as in the case of bitter gourd dried samples the alkaloid content ranged between 0.90 per cent to 1.01 per cent. In the present study, the highest tannin content was observed in *Nei paval* fresh (0.61 mg/ 100g). Highest amount of saponin was found in light green big both in the case of fresh and dry samples (0.68 per cent and 2.83 per cent respectively). Highest lectin content was observed in dark green small type both in the case of fresh and dried types (0.37mg and 1.17mg) respectively. The charantin content was found to be highest in light green big both in the case of fresh and dried samples (0.18 µg/ ml and1.39 µg/ ml).

5.3 ANTIOXIDANT PROPERTIES

5.3.1 DPPH radical scavenging activity

Several methods have been used to determine antioxidant activity of plants. Our present study, therefore, involved four various established methods to evaluate anti oxidative activity of bitter gourd fruit, namely, total antioxidant capacity, DPPH radical scavenging activity, hydroxyl radical scavenging activity and super oxide anion radical scavenging activity.

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples (Çakmak et al., 2012; Sakanaka et al., 2005). The investigation showed that the radical scavenging activity increased with the increase of the concentration of all the extracts. Rezaeizadeh et al. (2011) found that DPPH radical scavenging activity was higher for fresh whole fruit of *Momordica charantia* methanolic and chloroformic extracts when compared to our results at 500 µg/ml concentration. The radical scavenging values, how-ever, depend on locality and polarity of extraction solvents.

Fig 15. DPPH radical scavenging activity of dried bitter gourd samples (Petroleum ether & Acetone)

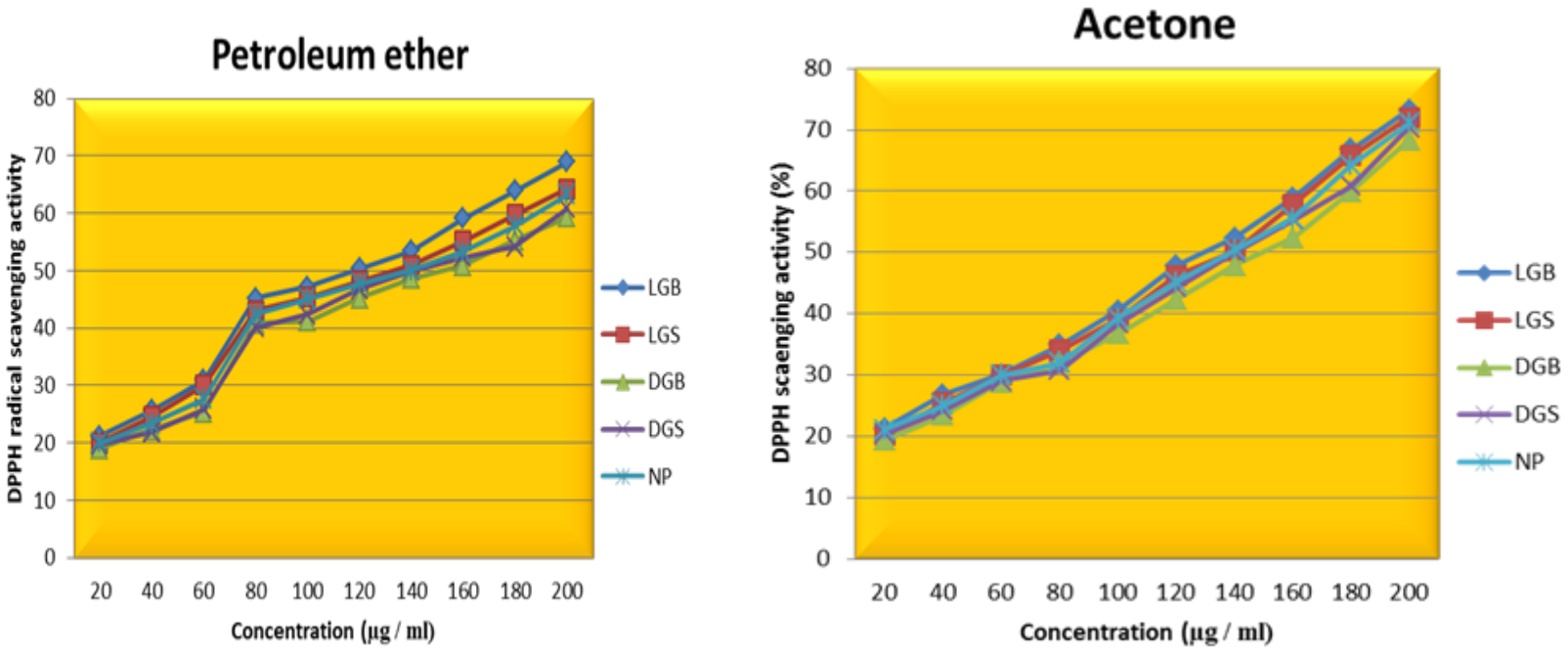
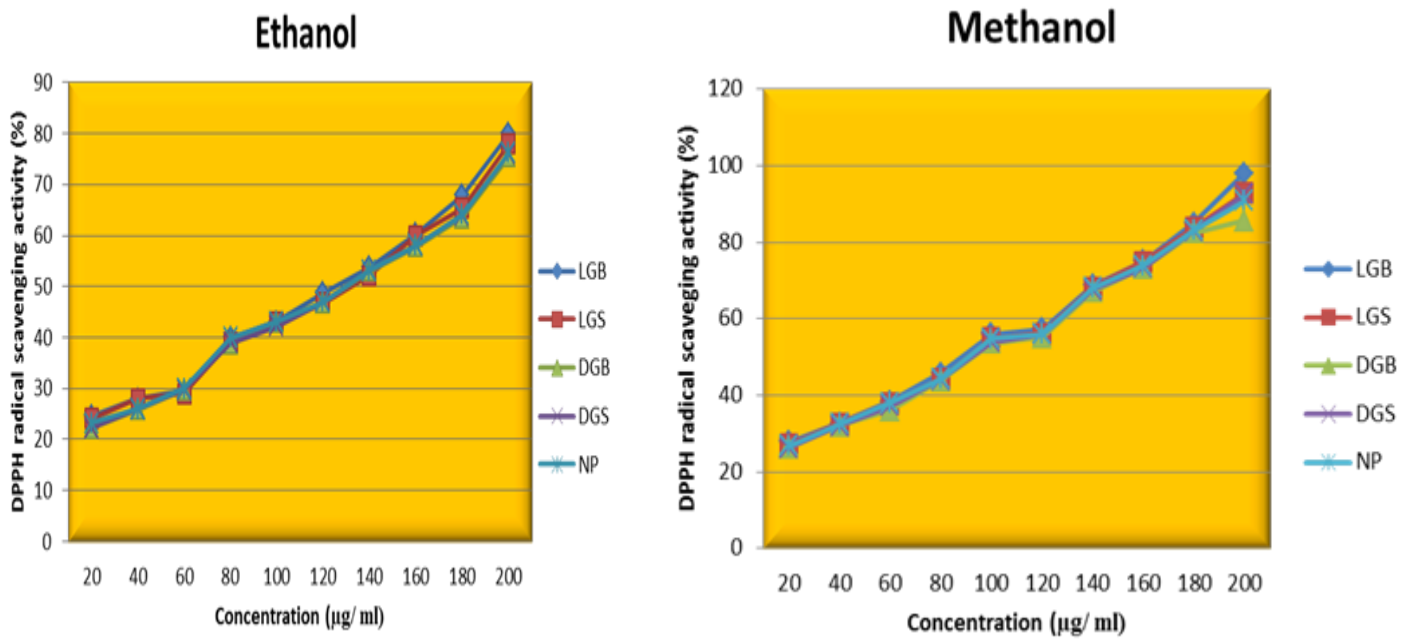


Fig 16. DPPH radical scavenging activity of dried bitter gourd samples (Ethanol & Methanol)



Antioxidant in food or plant samples may be water soluble, fat soluble, insoluble being bound to cell walls hence may not be free to react with DPPH, hence they are react at different rates and follow different kinetics and the reaction will often not reach completion in a reasonable assay time. Therefore the sample size that can lower the initial absorption of DPPH solution by 50 per cent has been chosen as the end point for assessing the antioxidant activity. This change is compared with the change induced by the standards (ascorbic acid, trolex etc.) and the antioxidant activity of the sample is expressed in equivalent of standards (Kedare and Singh, 2011).

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of 1,1-diphenyl-2-picrylhydrazyl(DPPH). The antioxidant activity of the extracts, based on its scavenging activity of the stable 1,1- diphenyl-2-picrylhydrazyl(DPPH) free radical, was determined by the method described by Akuwuoh et al. (2005). IC_{50} value ie. the amount of sample concentration required to produce 50 per cent free radical scavenging activity calculated using L Ascorbic acid as standard. Hence, IC_{50} value is inversely related to the free radical scavenging activity.

This assay has been successfully utilized for investigating the antioxidant properties of vegetables, herbs, edible seed oils, wheat grain and bran and flour in several different solvent system including ethanol, methanol, aqueous acetone, aqueous alcohol and benzene (Kedare and Singh, 2011). Recently the assay has been used to determine antioxidant activity in many plant samples all over the world (Subhasree et al., 2009).

In the present study light green big fresh bitter gourd had the highest DPPH activity with an IC_{50} value of 50.88 µg/ml in methanol solvent. In the case of bitter gourd dried samples, highest DPPH activity with an IC_{50} value of 50.10 µg/ml was

also reported in light green big (Fig 15 & 16). At a concentration of 100µg/ml, the inhibition rate of *Momordica charantia* was found to be 79.2 per cent and the IC_{50} of DPPH (µg/ml) was found to be 10.89µg/ml (Gayathri, 2014).

A study by Kubola and Siriamornapun (2008) on antioxidant activity of various boiled water extracts from unripened bitter melon fruit revealed 53.9 ± 0.73% reduction in the DPPH radical at a concentration of 0.2 mg/g. Patel et al. (2011) on evaluation of antioxidant activity, phenol and flavonoid content of *Momordica charantia* fruit was found to be 120.07 µg/ ml. Hamissou et al. (2013) conducted a study on antioxidant properties of bitter gourd and zucchini and reported that bitter gourd was 82.05 per cent as effective as ascorbic acid in inhibiting the free radical DPPH.

A study conducted by Saeed et al. (2010) on nutritional analysis and antioxidant activity of bitter gourd from Pakistan had reported that the scavenging effect of bitter gourd flakes, seed, peel and standard on the DPPH radical decreased to 74.15, 63.20, 33.05 and 20.10 per cent at the 2mg/ ml concentration respectively. The results are in close agreement with Aminah and Anna (2011) who observed that scavenging activity ranges between 37 per cent to 64.48 per cent. The DPPH of wild bitter gourd ranges between 36.60 per cent to 75.8 per cent (Wu and Ng, 2008). Microwave cooked samples had significantly (p <0.05) higher percentage of DPPH radical scavenging activity (88.54%) (Aminah and Permatasari, 2013) compared to oven drying, frying and boiling methods.

Fig 17. Hydroxyl radical scavenging activity of dried bitter gourd samples (Petroleum ether & Acetone)

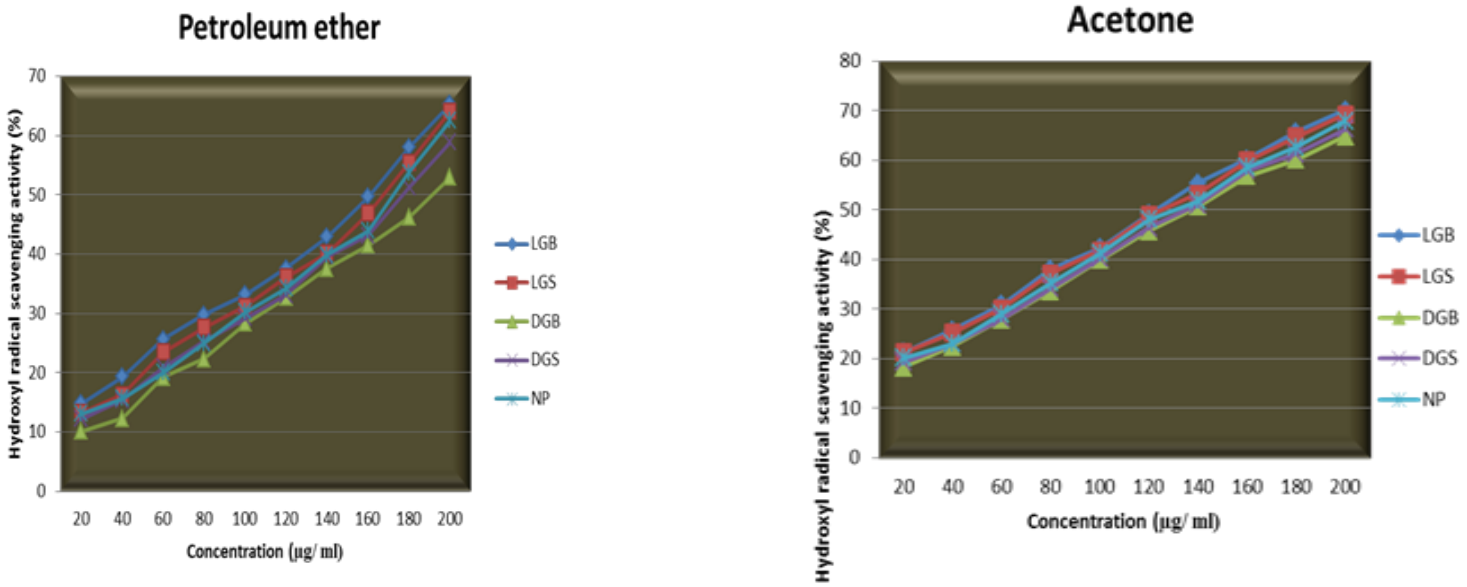
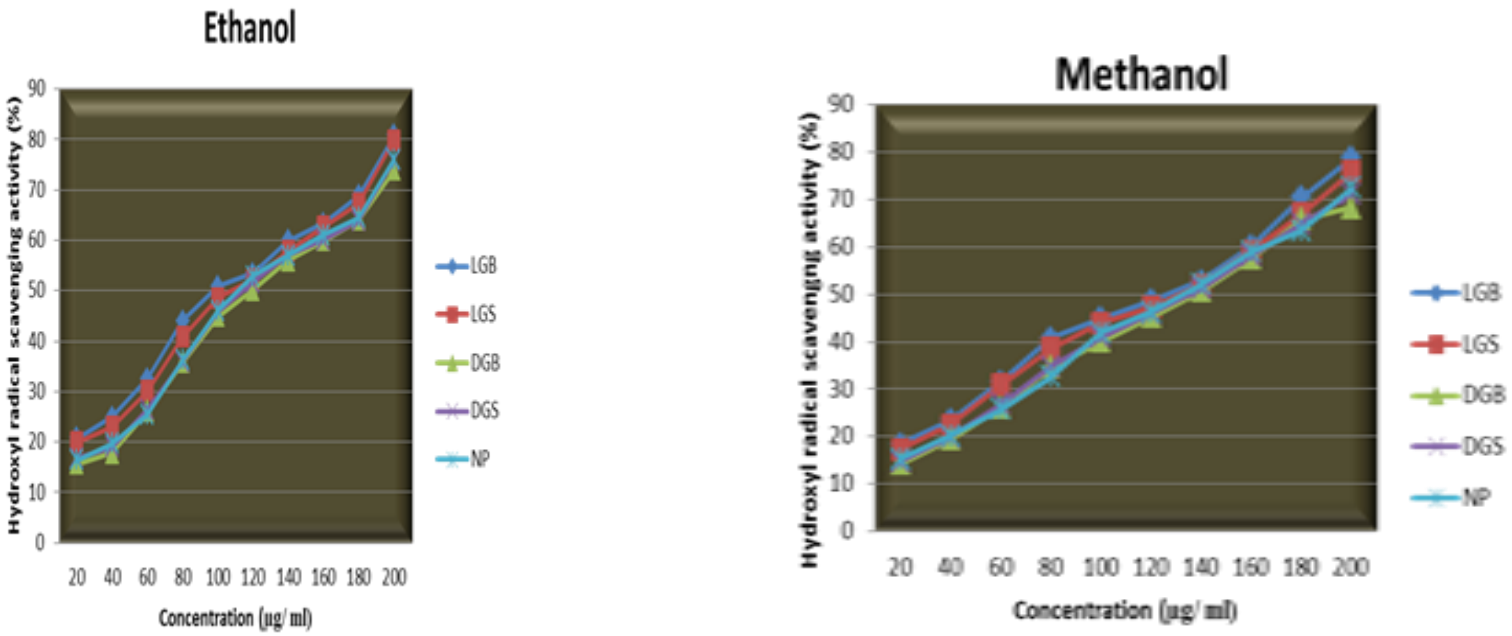


Fig 18. Hydroxyl radical scavenging activity of dried bitter gourd samples (Ethanol & Methanol)



5.3.2 Hydroxyl radical scavenging activity

The hydroxyl radical is a very reactive oxygen species (ROS) with a short half-life, and is considered to be responsible for much of the biological damage inherent to free radical pathology (Hochestein and Atallah, 2008). This radical has the ability to cause standard breakage in DNA, which is a contributing factor to carcinogenesis, mutagenesis and cytotoxicity. Moreover, hydroxyl radicals have been identified as one of the rapid initiation of the lipid peroxidation process, via the abstraction of hydrogen atoms from unsaturated fatty acids (Kappus, 2003).

The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (OH) and the superoxide anion (O_2^-). The hydroxyl radical is particularly unstable and will react rapidly and non specifically with most biological molecules (Rumbaoa et al., 2009). This species is produced from hydrogen peroxide in metal catalyzed redox reactions such as the Fenton reaction. These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA proteins. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, while damage to proteins causes enzyme inhibition, denaturation and protein degradation (Ebrahimzadeh et al., 2008). Oxidative stress thought to the development of a wide range of diseases including alzheimers disease (Gaal, 2006), Parkinsons disease (Lluch et al., 2006), the pathologies caused by diabetes, rheumatoid arthritis, and neuro degeneration in motor neuron diseases.

In the present study hydroxyl radical scavenging activities of light green big was found to be highest both in the case of fresh and dried bitter gourd samples with an IC_{50} values of 50.95 µg/ml and 50.10 µg/ml respectively Fig 17 & 18).The IC_{50} values for hydroxyl radical ranged from 22 to 42 µg/mL. The wild variety and HL-2 were superior to HL-1. Leaf extract of Thai bitter melon possesses hydroxyl-

radical scavenging activity with an IC50 value of 167 ± 0.96 mg/mL (Kubola and Siriamornpun 2008). Liu et al. (2004) demonstrated fruit extract of the most effective white bitter melon cultivar in Taiwan exerted potent hydroxyl-radical scavenging activity with IC_{50} value of 37 μg/mL). These results further supported that leaf and fruit of WBM possess strong hydroxyl-radical scavenging activity.

According to Patel et al. (2011) the IC_{50} values of alcoholic extract in hydrogen peroxide radical scavenging activity was found to be 175.78 μg/ml. Ghaima et al. (2013) conducted a study on extraction and identification of phenol compounds from bitter melon fruits and their role as antioxidants found that activity for hydrogen peroxide radical scavenging activity was about 68.8 per cent in comparison with α – tocopherol 45. 3 per cent, ethanolic extract 52.6 per cent and aqueous extract 36.2 per cent respectively.

5.3.3 Total antioxidants activity

Antioxidant is used by aerobic organisms to protect the cells from oxidative damage by oxidants during oxygen metabolism. It is currently believed that reactive oxygen species (ROS) have an important role in the aetiology of several non communicable diseases. Oxidants and free radicals such as singlet molecular oxygen ($-O_2$), superoxide (-O), hydroxyl (OH) peroxide (O-O-H) and lipid peroxides (LOO) are known to cause tissue damage. Such free radicals also include nitrous oxide radicals that are generated in the gastrointestinal tract. Tissue damage caused by free radicals, when it becomes cumulative, is considered to play an important role in the pathogenesis of several degenerative diseases, for example, cancer, cataract, coronary heart disease, dementia, diabetes mellitus, rheumatic arthritis, muscular degeneration, pulmonary dysfunction and radiation sickness. Lipid peroxidation of membrane lipids, circulating lipoprotein lipids (including cholesterol), oxidation damage of cellular proteins and DNA, and lens protein in the retina are all considered mechanisms by which the oxygen free radical and peroxides lead to these diseases.

These oxidants and free radical species are generated in cells during utilization of oxygen, which is essential for life's sustenance, and they can also be derived from external sources (Narasingha Rao, 2003).

Antioxidants are defense against free radical and oxidative attacks. They act as free radical scavengers and slow down not only oxidation of radical but also the accompanying damaging effects in the body (Aljudi and Kamaruddin, 2004). Fruits and vegetables have conferred on them the status of functional foods. They seem to be capable of delivering health benefits besides fulfilling physiological needs. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer and heart disease (Nabavi et al., 2008). Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Routine or habitual consumption of fruits and vegetables confers significant benefits to human health.

According to Praveen et al. (2007), plants are the potential source of natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants. Carotenoids, flavonoids, cinnamic acid, benzoic acids, folic acid, ascorbic acid, tocopherols, and tocotrienols are some of the antioxidants produced by plant for their sustenance. Beta carotene, ascorbic acid and alpha tocopherols are widely used as antioxidants. Antioxidants are useful for combating degenerative diseases such as cancer, cardiovascular diseases, brain dysfunction and cataracts (Moein, 2007). Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischaemic heart disease, cancer, alzheimer's disease and in the aging process. Antioxidant is one of the most essential ingredients of today's therapy since they reduce in vivo oxidative damages. Plants are the good resources for natural antioxidants (Moein, 2007).

Fig 19. Total antioxidant activity of dried bitter gourd samples (Petroleum ether & Acetone)

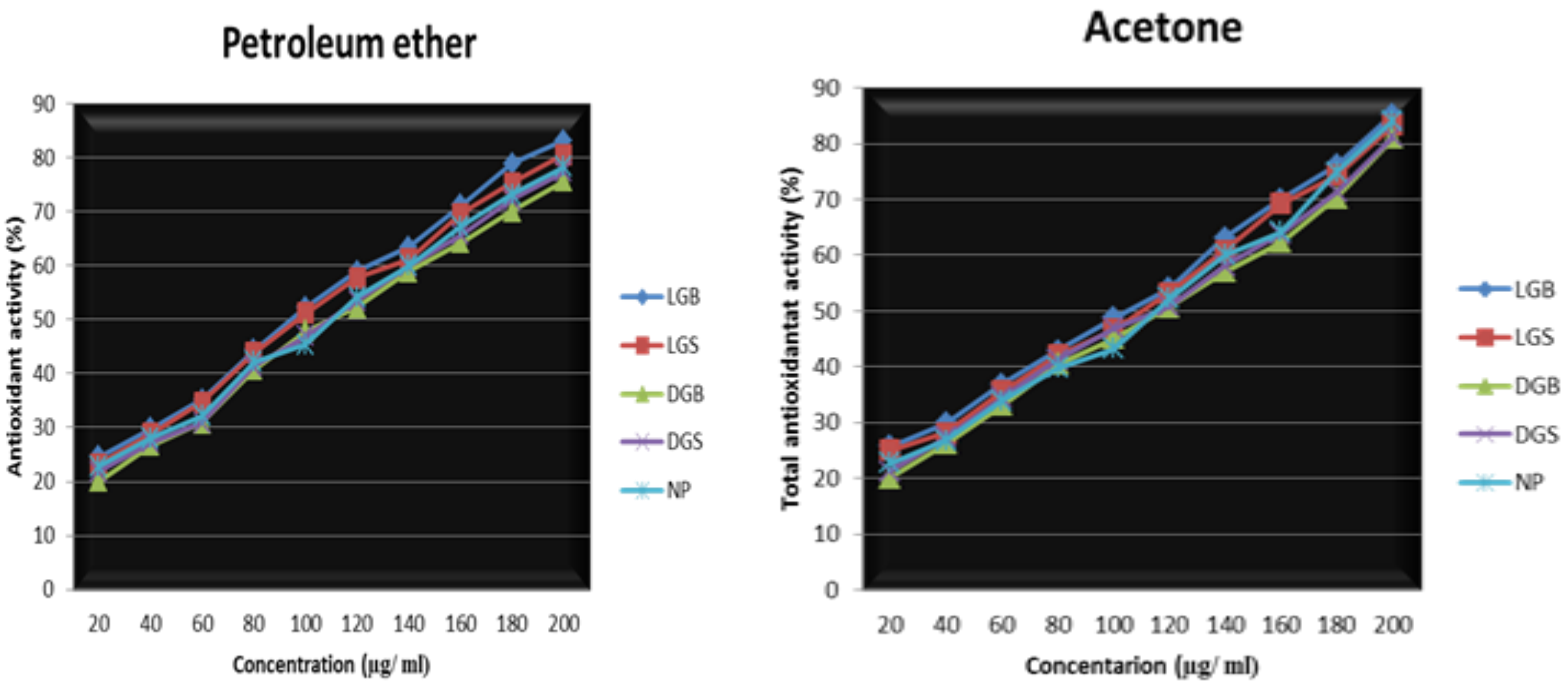
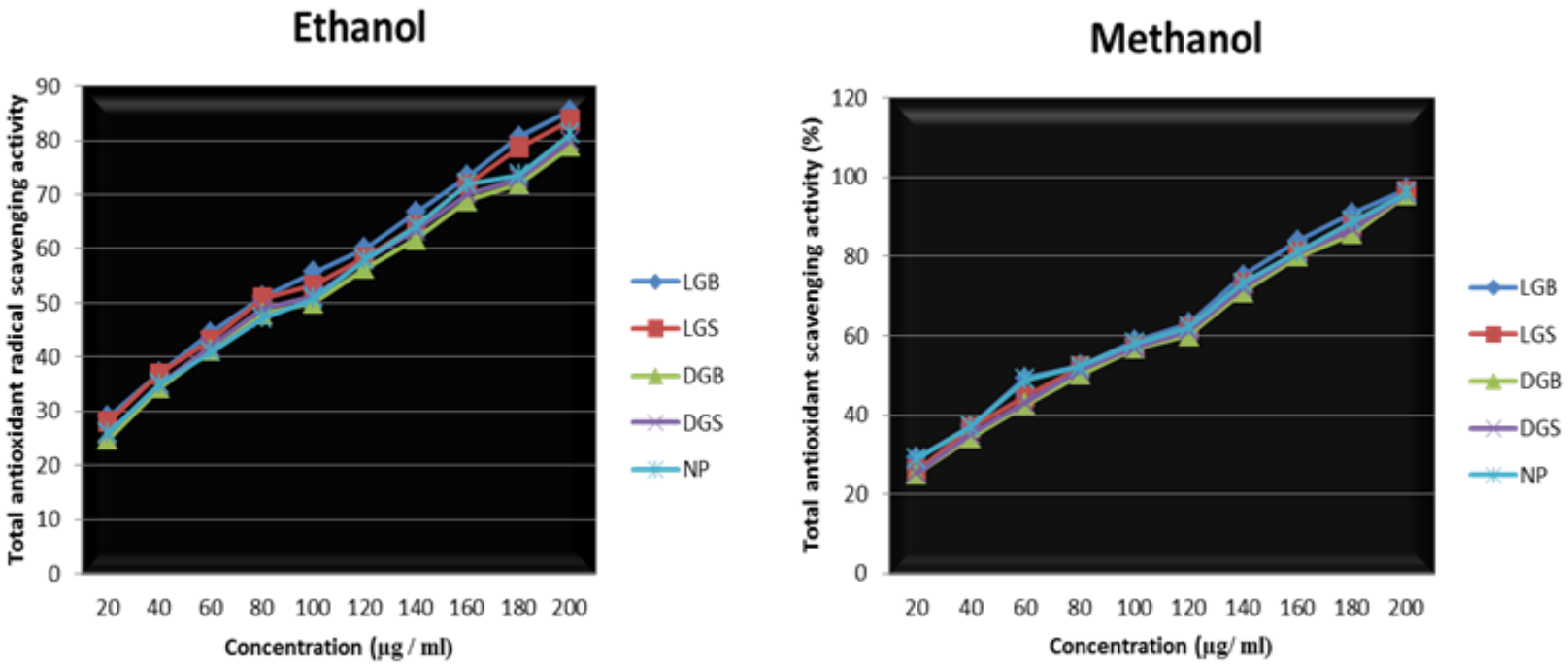


Fig 20. Total antioxidant activity of dried bitter gourd samples (Ethanol & Methanol)



The present study revealed that antioxidant activity ranged with an IC_{50} values of 50.09 µg/ml to 61.90 µg/ml in fresh bitter gourd samples and maximum antioxidant capacity was observed in light green big (50.09 µg/ml). In the case of bitter gourd dried samples, the highest antioxidant activity was observed in light green big (50.07 µg/ml) (Fig 19 & 20). The study is in accordance with the findings of Leelaprakash et al. (2011) who had reported that IC_{50} value of antioxidants activity varies from 53.75 to 56.25. A study conducted by Islam et al. (2011) on bio active compounds of bitter melon genotypes in relation to their physiological functions reported that antioxidant activities of Indian green, Indian white, China green and China white ranged from 79-88, 79-87, 80-86, and 79-87 per cent inhibition, respectively. The antioxidant activities of oven dried samples and freeze dried samples were 79-88 and 79-86 per cent respectively.

A study conducted by Asan and Karacoka, (2013) reported that antioxidant activity of bitter gourd was 39.92 mg. For oven-drying, bitter gourd dried at 40 °C retained the highest antioxidant activities compared to samples dried at 50 or 60 °C. Thus, the best drying temperature to retain antioxidant properties in bitter gourd is at 40 °C while the best cooking method is either microwave or deep fried (Aminah and Permatasari, 2013).

5.3.4 Super oxide anion radical scavenging activity

Enzymatic antioxidants are equally important in protecting organism against free radical build up. Enzymatic antioxidants, such as superoxide dismutase, protect cells and tissues from oxidative damage by reactive oxygen species. Superoxide dismutase (SOD), peroxidases (PO) and catalases are some of the enzymatic antioxidative defense mechanisms (Hamissou et al., 2013). Super oxide assay shows an increase in the antioxidant activity or free radical scavenging. The increase in activity with increase in concentration of the fruit extract and its absorbance are recorded (Mary et al., 2014).

Fig 21. Super oxide radical scavenging activity of dried bitter gourd samples (Petroleum ether & Acetone)

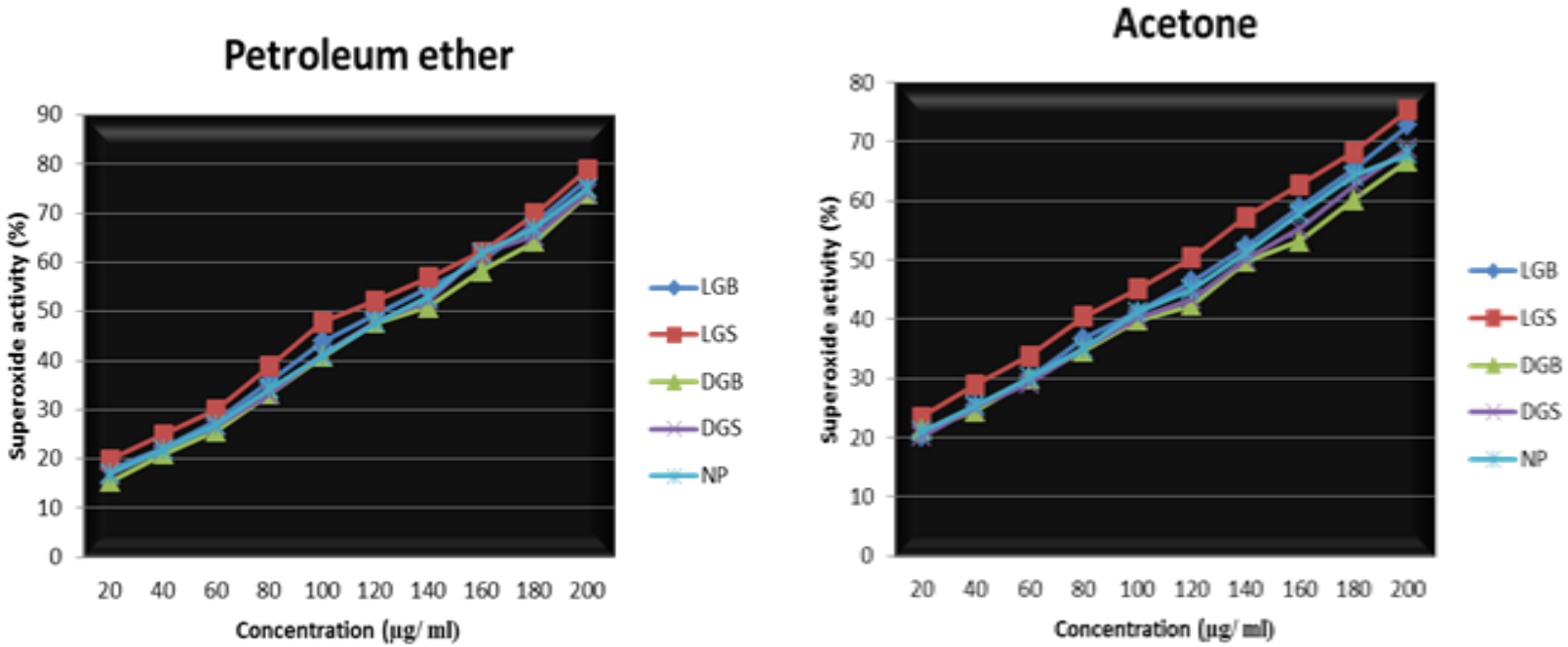
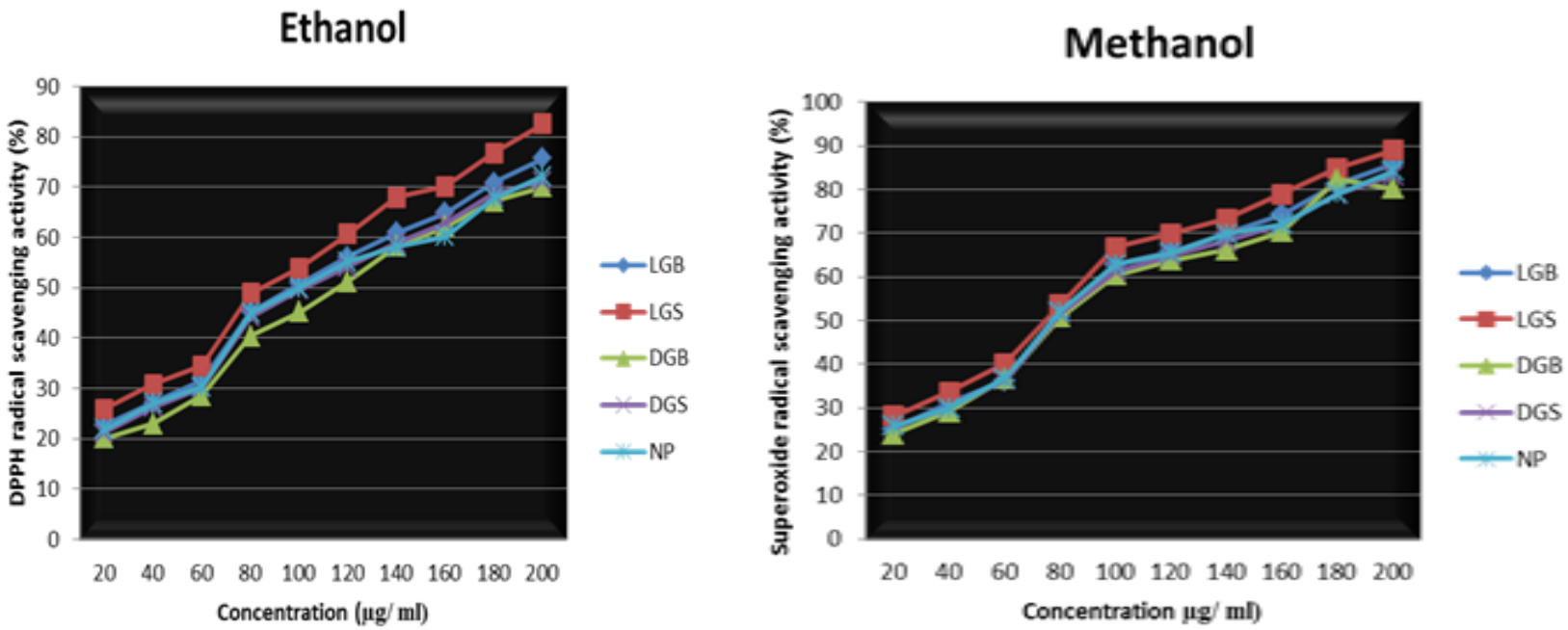


Fig 22. Super oxide radical scavenging activity of dried bitter gourd samples (Ethanol & Methanol)



Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive species (Krishnendu et al., 2011). One risk of the superoxide generation is related to its interaction with nitric oxide to form peroxinitrite (Yamagishi et al., 2001) which is a potent oxidant causes nitrosamine stress in the organ system.

In the present study, light green showed higher superoxide anion radical scavenging activity with an IC_{50} of 50.36 µg/ml in fresh samples and 49.76 µg/ml in dried samples (Fig 21 & 22). According to Hamissou et al. (2013) an average of 1.55 units of SOD activity per µg total proteins was recorded for bitter gourd fruits. A study conducted by Tsai et al. (2014) on antioxidant, cell protective and anti melanogenic activities of leaf extracts from wild bitter melon cultivars reported an activity of 9.12 mg/ml in leaf extracts.

To sum up the study revealed that antioxidant activity in the present study revealed that light green big sample had the highest DPPH activity with an IC_{50} value of 50.88 µg/ ml in methanol solvent. In the case of bitter gourd dried samples, highest DPPH activity with an IC_{50} value of 50.10 µg/ ml was reported in light green big dried type. The hydroxyl radical scavenging activity of light green big was found to be highest both in the case of fresh and dried bitter gourd samples with an IC_{50} values of 50.95 µg/ml and 50.10 µg/ml respectively.

Light green small sample showed higher superoxide anion radical scavenging activity with an IC_{50} value of 50.36 µg/ ml in fresh samples and 49.76 µg/ ml in dried samples, in solvents like petroleum ether and acetone respectively. Antioxidant activity ranged with an IC_{50} values of 50.09 µg/ml to 61.90 µg/ml in fresh bitter gourd samples and maximum antioxidant capacity was observed in light green big (50.09 µg/ ml) whereas dried samples, the highest antioxidant activity was observed in light green dried (50.07 µg/ ml) in acetone solvent.

5.4. SHELF LIFE QUALITY

Shelf life is a guide for the consumer of the period of time that the food can be kept before it starts to deteriorate, provided any stated storage conditions have been followed. The shelf life of a product begins from the time the food is prepared or manufactured. Its length is dependent on many factors including the type of ingredients, manufacturing process, type of packaging and how the food is stored.

Kumar et al. (2001) suggested that there are many ways in which quality and nutrient can be lost. They may not necessarily result in the product being harmful but can mean that it is no longer of an acceptable standard. Moisture gain loss, chemical changes, light induced change, temperature changes, physical damage, spoilage by rodents and insects, flavors and odours from storing food near other strongly smelling products, products tampering etc.

Shelf life study is an objective methodological means to determine how long a food product can reasonably stay safe without any appreciable change in quality. Hence in present investigation moisture, peroxide value and microbial growth were examined periodically up to a period of six months.

5.4.1 Moisture Percentage

Robertson (2000) reported that, the free space volume has an important influence on the rate of oxidation of foods that a large package area and a low bulk density results in greater oxygen transmission.

Moisture is one of the important parameter which determines the shelf life quality of a food product. Low moisture is highly important for longer storage period (Shankar, 2003).

In the present study it was observed that there was no difference of moisture content in powdered bitter gourd. The mean moisture level of bitter gourd powder after six month storage was 6.63 per cent.

Midhila (2013) studied the effect of storage on the dried banana blossom flour. The author also reported that with advancement in the storage period, the moisture level enhanced. But the increase in moisture content did not influence the quality of the developed product because the increase in moisture content was negligible.

Saranya (2012) reported that the moisture content of stored enriched soup mix (ESM) was found to enhance gradually during the storage period. But the increase in moisture content does not influence the quality of the RTC product.

A study conducted by Krishnaja (2014) on development quality assessment and clinical efficacy of functional food supplement for life style diseases and management observed that the moisture content of functional food supplement (FFS) was found to enhance gradually during the period of storage. But the increase in moisture content does not influence the quality of the developed product because the increase in moisture content was negligible.

5.4.2 Peroxide value

The primary products of lipid oxidation are hydro peroxides which are generally present as peroxides. Thus it seemed to determine the concentration of peroxide as a measure of extend of oxidation and thus rancidity. The nature of auto oxidation degradation depends on the extend of un- saturation of lipids. Thus in the present study, peroxide content was not observed in the first four month of the study. The results showed that the bitter gourd powder reported peroxide content in the 5^{th} month (0.10 meq/ 100g) and 6^{th} month (0.12 meq/ 100g) of storage. However it could be noted that the peroxide content in the bitter gourd powder was much minimal than

the permitted limits. This shows that, bitter gourd can be stored for a period of six months without any discriminate changes, thereby giving them a higher shelf life and microbial value.

5.4.3 Assessment of microbial growth in bitter gourd powder

Spoilage causing organisms causes off odour and off taste and lead to economic losses (Rao, 2003). The concepts of spoilage by microorganisms are the primary cause of the end of shelf life and that become reducing initial microbial population is a strategy to extend shelf life (Zygory, 2003).

Serial dilution followed by spread plating was employed to detect the presence of micro organisms. In the present investigation it is evident that during the six months of storage period no actinomycetes were found to be appeared in the bitter gourd powder. But bacterial colonies were observed from the 5th month (1×10^7 cuf/g) and 6th month (2×10^7 cuf/g) and were present only in negligible level. No Fungi and E-coli were detected in bitter gourd powder during the period of six month of storage. It must also brought into the consideration that, among the replicate samples analyzed bacterial colonies was notable only in one sample randomly selected for analysis. So it could be concluded that, it might be due to cross contamination during the period of bulk production of the bitter gourd powder.

Nasheeda (2006) reported that the bacterial population of banana powder packed in poly propylene covers ranged between 6.68- 6.88 10^{-3} cuf/g. Saranya (2012) Reshmi (2012) Krishnaja (2014) also noted no or much less amount of microbes in the products developed by them.

5.5 YEILD RATIO AND PROCESSING LOSS

When bitter gourd was converted into powdered form it was noted that from 1Kg (weight of whole fresh bitter gourd with seed) around 50g (weight of powder) of powder was obtained. The yield ratio was found to be 0.05.

Processing loss was also calculated and the results revealed that the processing loss was found to be 95 per cent.

5.6 COST OF PRODUCTION

Poornima and Dabur (2009) studied that the economic returns from the functional foods can offer improved opportunities for all the members in the food supply chains from raw material producers and processors to retailers. Functional food can be an opportunity for economic growth for many developing countries endowed with rich biodiversity and traditional knowledge of the health effects of certain indigenous plant species.

Hasler (2002) reported that retail price of functional foods are generally higher range between 30 to 500 per centage above the comparable conventional foods. Moreover, demand for functional foods within the developing countries is growing, presenting a lucrative opportunity to develop domestic market.

Functional foods sell at higher prices and contain larger profit margins than conventional foods. This make the food sector attractive for the players of food supply chains including marketing, storage and transportation (Kotilainen et al., 2006). In the present study based on the price of bitter gourd and 10% over head changes it was found that for 1 Kg bitter gourd powder, the cost would be around Rs.100/ 100 g.

On the health point of view, the rate of bitter gourd powder was much less when compared to the hypoglycemic tablets. So bitter gourd powder is a better product and economically viable.

To sum up mean moisture level of bitter gourd powder after six month storage was 6.63 per cent.. The peroxide content was noticed in powder only in 5^{th} month (0.10 meq/100 g) and 6^{th} month (0.12 meq/100 g) of storage. During the storage of six months period, no actinomycetes were found to be appeared in the bitter gourd powder. But bacterial colonies were observed from the 5^{th} month (1×10^7 cuf/g) and 6^{th} month (2×10^7 cuf/g) and were present only in negligible level. When bitter gourd was converted into powdered form it was noticed that from 1Kg fresh bitter gourd with seeds around 50g powder (without seed) was obtained. The yield ratio was found to be 0.05. Processing loss was also calculated and the results revealed that the processing loss was found to be 4 per cent and cost of the product was found that for 1 Kg bitter gourd powder, the cost would be around Rs.100/ 100g.

5.7 CLINICAL EFFICACY OF BITTER GOURD POWDER SUPPLEMENTATION

Ten human subjects (five males and five females) having diabetes in the age group of 45-55 years and not on medication were selected for the case study (Subject A- J). All the respondents were Hindu and were from nuclear family.

The monthly income of the respondents ranged from 20,000 to 90,000. Their educational qualification ranged from plus two to PG, with occupation three subjects were Govt. employee, two business man, two housewives and three respondents worked in private sector.

Duration of the diseases revealed that three respondents were having diabetes for the past 3 years, whereas four respondents were having diabetes for the last one year, one respondent was for the past 6 months, another one having diabetes for the

past 2 years and last one respondent having diabetes for the past four years. Except two respondents, all others were having diabetic history. From the obtained data revealed that five respondents were having hyperlipidemia at present and 5 respondents were having slightly hyperlipidemia. One respondent is having hypertension and another one is having hypotension at present. All the respondents have no exercise habit except subject one and all the respondents were non vegetarian dietary habit except one respondent.

The anthropometric data of the respondents revealed that three respondents were coming under over weight category, with a waist-hip ratio of).97, 0.99 and 0.94 and three respondents were coming under grade 1 obesity category, and two respondents were coming under grade II obesity category and one respondent was under grade III obesity category. Whereas one respondent was having normal BMI category, with a waist hip ratio of 0.92. The waist hip ratio of the respondents ranged between 0.86- 0.99.

Abdominal obesity is defined as a waist-hip ratio of above 0.90 for males and above 0.85 for females. (http://whqlibdoc.who.int.publications /2011/9789241501491_ eng.pdf). Price et al. (2006) is of the opinion that waist hip ratio (WHR) has been found to be a more efficient predictor of mortality than BMI.).

In the present investigation, it can be seen that the male subjects were having waist-hip ratio between 0.92 to 0.98 and the females also were having high waist –hip ratio in the range of 0.86 to 1.03.

Dhar and Sarwate (2013) reported that, there was a linear association existing between adiposity and blood pressure of hypertensive middle aged women. This shows, BMI as an important indicator for hypertension. Similar observations were made Deshmukh et al. (2006), Shahbazpur (2003) and Gus et al. (2004) have also reported earlier significant positive association of BMI with systolic and diastolic pressure.

Mehta (2013) reported that the BMI and Waist Hip Ratio were related to the clinical health parameters and dietary habits of the members of the Marvadi Jain community.

Nande et al. (2010) reported that, waist circumference and hip circumference were found to be directly proportional to weight gain and BMI. Waist circumference gives a better prediction of visceral and total fat and of diabetes risk.

Pradeepa and Beatrice (2013) reported that, mean Waist Hip Ratio of 0.92 of the subjects suggested that the values were above normal and also show a substantial increase in the risk for metabolic complications.

These studies support that, the health and dietary habits of the subjects are one of the foremost causative factors in the development of life style diseases like diabetes, hyperlipidemia, hypertension etc.

The National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) stated that women with waist–hip ratios of more than 0.8, and men with more than 1.0, are at increased health risk because of their fat distribution (Marlowe et al., 2005). The author also stated that a WHR of 0.7 for women and 0.9 for men has been shown to correlate strongly with general health. Women within the 0.7 range have optimal levels of estrogen and are less susceptible to major diseases such as diabetes, cardiovascular disorders and ovarian cancers. Men with WHRs below 0.9, similarly, have been shown to be healthier. In the present study all the subjects were shown to have high waist-hip ratio and this may be one of the reasons for them to be diabetic.

Frequency of consumption of various food groups in the diet revealed that all respondents were included cereals, pulses, vegetables, milk, milk products and tea daily. The average intake of energy as calculated from their diet was ranged between 1598 Kcal – 3100 Kcal respectively. All the respondents were non vegetarian dietary habit except one respondent. The meal frequency pattern of the respondents revealed

that all of them have food 3 to 4 times in a day. All the respondents used different types of cooking oils like coconut oil, sunflower oil and rice bran oil due to health concern.

Blood parameters like Fasting Blood Sugar (FBS) and Post Prandial Blood Sugar of the subjects was monitored before, in between and at the end of the supplementation study to understand the impact of supplementation of bitter gourd powder. A total of ten respondents with hyperglycemia were supplemented with bitter gourd powder accordingly. The respondents having 5g bitter gourd powder in 50 ml water every day early morning and night before food. The respondents opined that the bitterness of the bitter gourd powder is less than that of fresh bitter gourd juice.

The biochemical investigations proved that there was significant variation in the FBS levels of subjects supplemented with bitter gourd over a period of 60 days. The mean initial (Day 0) FBS values for the subjects supplemented with bitter gourd powder were 171 mg/dl, for 30^{th} day 152 mg/dl and for 60^{th} day 132 mg/dl respectively. The investigations also found there is variation in the PPBS levels of subjects supplemented with bitter gourd powder over a period of 60 days. The mean initial (Day 0) PPBS values for the subjects supplemented with bitter gourd powder were 217 mg/dl, for 30^{th} day 189 mg/dl and for 60^{th} day 158 mg/dl respectively. The results of the present study observed that there is a variation in the total cholesterol level of the subjects supplemented with bitter gourd powder over a period of 60 days. The mean initial (Day 0) total cholesterol values for the subjects supplemented with bitter gourd powder were 251 mg/dl, for 30^{th} day 216 mg/dl and for 60^{th} day 180 mg/dl respectively.

A study conducted by Altinternin (2012) on bitter melon and the effects of diabetes disease reported that three different group of constituents found in all parts of *momordica* have clinically demonstrated hypoglycemic properties or other actions of potential benefit against diabetes mellitus. Bitter melon increases the

number of beta cell in the pancreas. Bitter melon has been shown to increase the number of beta cells in the pancreas thereby improving the body's ability to produce insulin (Shetty et al., 2005).

Nerurkar et al. (2008) conducted a study on *momordica charantia* reduces plasma apolipo protein B-100 and increases hepatic insulin receptor substrate and phosphoinositide-3 kinase interactions reported that bitter gourd improve the function of insulin receptors in the liver. It also been widely researched in animal studies for its ability to improve glucose and insulin tolerance.

In rats, oral bitter melon juice has been found to potentiate the glucose lowering effects of the sulfonylurea tolbutamide (Cummings et al., 2004).

A study conducted by Han et al. (2013) on safety and effectiveness of bitter melon as an alternative to traditional hypoglycemic agents for the control of fasting blood sugar in patients with type 2 diabetes mellitus revealed that supplementation of bitter gourd in different forms like bitter gourd juice, tablet and tea reduces the fasting blood glucose and random blood glucose level.

A study conducted by Waheed et al. (2008) conducted a study on clinical investigation of hypoglycemic effect of unripe fruit on *momordica charantia* in type-2 diabetes mellitus reported that the supplementation of powdered bitter gourd for fourteen days revealed that the blood as well as the urinary glucose was reduces significantly.

In one study the treatment of streptozocin diabetic rats with *momordica charantia* fruit extract over a period of 10 weeks returned high blood glucose level to normal (Ahmed et al., 2009). In other study, in the streptozocin diabetic rats, *momordica charantia* improved the oral glucose tolerance causing significant reduction in plasma glucose to 26 per cent at 3.5 hour. *Momordica charantia* extract

(500 mg/kg) caused a 4-5 fold increase in the rate of glycogen synthesis from 4-14 glucose in the liver of normally fed rats (Sarkar et al., 1996).

Lininger et al. (1998) have recommended that a small bitter gourd can be eaten as food or upto 50 ml of fresh juice can be drunk per day or 5 ml bid or tid per day for lowering the blood glucose levels. In another study the juice or tablets from the powder of the fruits of *momordica charantia* showed significant blood glucose lowering effect on patient and the infusion of the drug and crude crystalline substance showed same effect (Khan, 2000).

Hasan and Khatoon (2012) conducted a study on effect of momordica charantia tablets in diabetes mellitus type 1 and type 2 reported that different group of patients were treated by bitter gourd juices and powders have demonstrated potential in lowering blood sugar. Different groups of patients were treated by bitter gourd tablets for 12 weeks. After 12 weeks treatment biochemical parameters from blood serum were analyzed. The significant differences of glucose, cholesterol, HDL, LDL, triglyceride, in bitter gourd tablet group compare to diabetic group were found.

Jaziya (2011) reported that incorporation of five gram dried oyster mushroom powder in the daily diet, reduced blood glucose and blood lipid levels. This might be attributed to the beta glucan content and other phytochemicals of oyster mushroom. Similarly Anju (2013) reported that, incorporation of five gram dried milky mushroom powder in the daily diet, reduced blood glucose and blood lipid levels of the subjects supplemented with it.

Reshmi (2012) inferred that, supplementation of Njavara in the diets of the subjects with diabetes and hyperlipidemia showed significant variation over a period of three months. Another study carried out by Krishnaja (2014) reported that the supplementation of functional food supplement significantly reduced blood glucose and blood lipid level.

The above results strongly suggest that, management of blood sugar could be made possible with bitter gourd powder supplementation with great therapeutic value. In this context, it can be recommended that the developed bitter gourd powder could be added to the daily diet of diabetic patients will help to the proper management of diabetes mellitus.

5.7.1 Glycemic Response of bitter gourd powder (Fig 23, 24 & 25).

The Glycemic Index of the food is affected by various factors. Less gelatinized the starch, slower the rate of digestion; the phytochemicals and polypeptide like compounds found in bitter gourd helps to reduce the blood sugar level (Alibhai et al., 2006). In the present study bitter gourd powder was having a GI of 64 which was around 36 per cent less than that of glucose and results of glycemic load revealed that bitter gourd powder had a glycemic load of 39.

To conclude, the several measures used to correct the imbalances of life style degenerative diseases, do not become healthy alternatives as besides being expensive, produce wide spectrum of adverse effects. There is a wide range of food stuffs that exerts primitive action for counteracting these adverse effects but at present is not used in our daily diet due to ignorance or oversight. Incorporating a food supplement along with medicines creates a more favorable option for the patients in the prevention and management of lifestyle diseases.

Bitter gourd has been used as dietary supplements and ethno medicine throughout centuries for relieving symptoms and conditions related to what we know in modern days as diabetes. To date, bitter gourd has been extensively studied world wide for its medicinal properties to treat a number of diseases. It is described as a versatile plant worthy of treating almost any disease inflicted on mankind. This may be due to the fact that the plant possesses over 225 different medicinal constituents. These different compounds may act either separately or together to exert their

Fig 23. Mean area under the curve of test food and reference food (AUC)

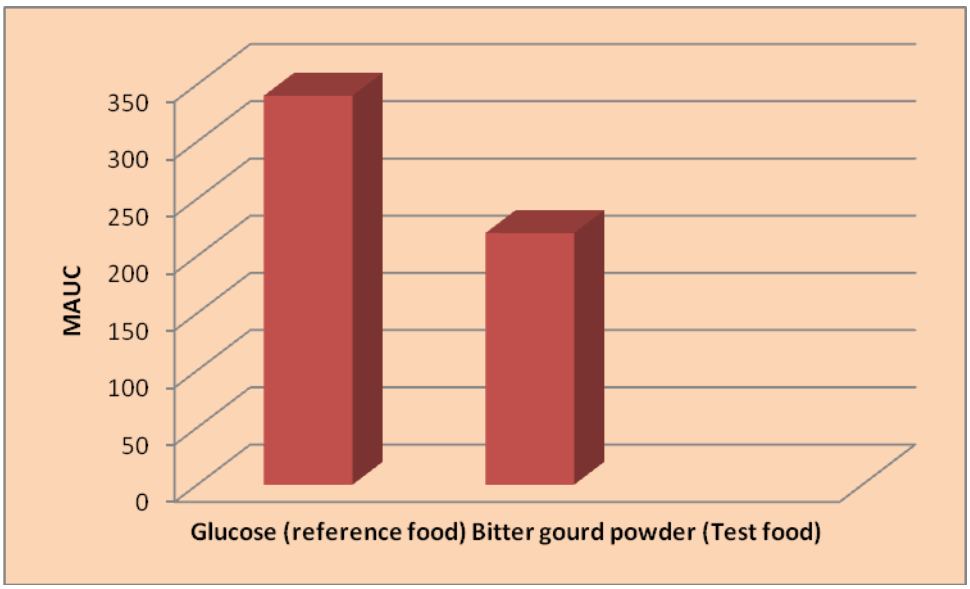
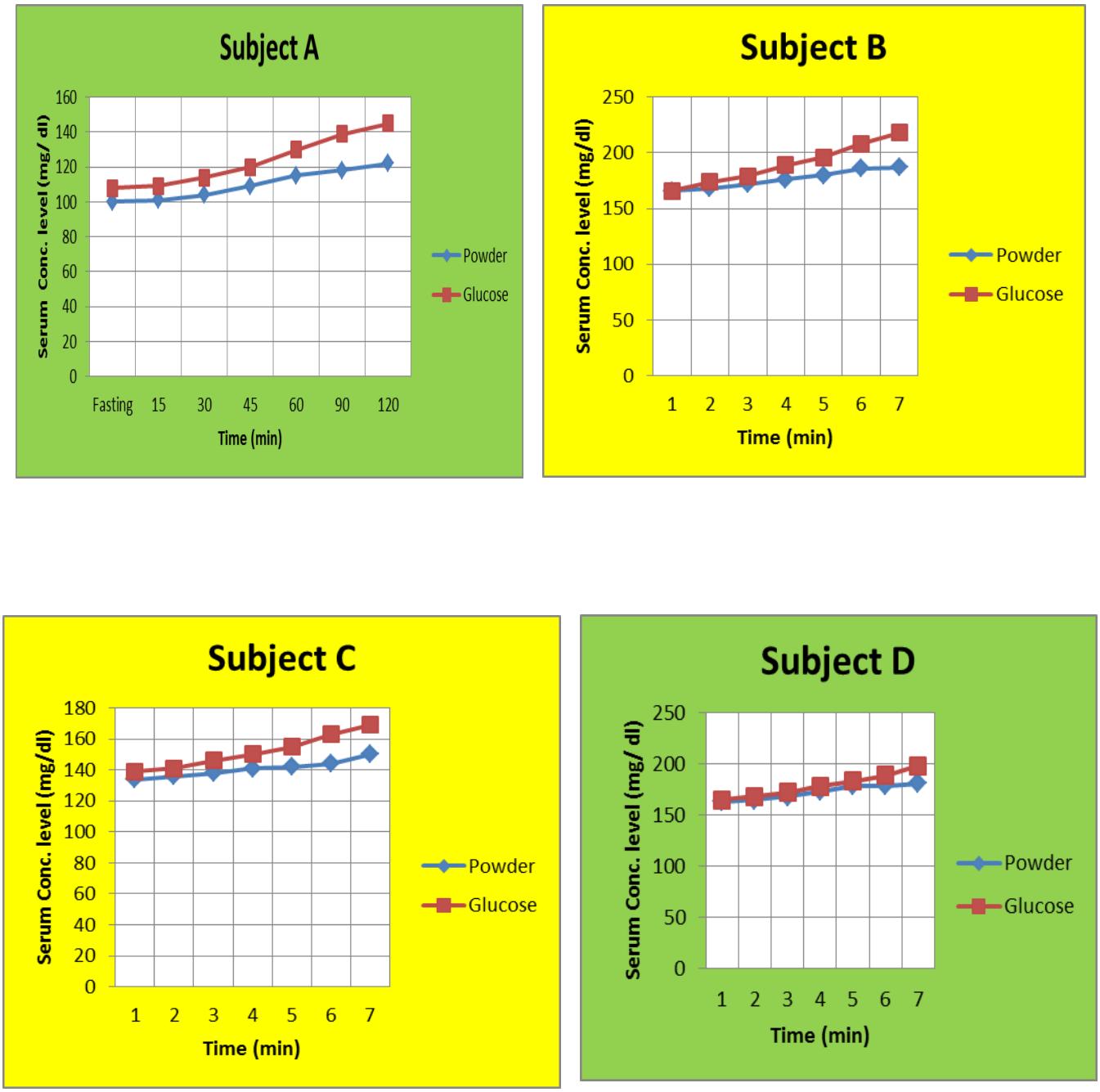
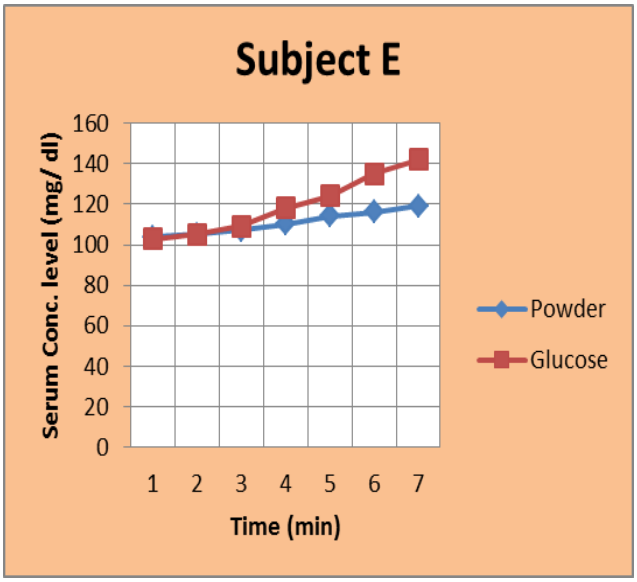
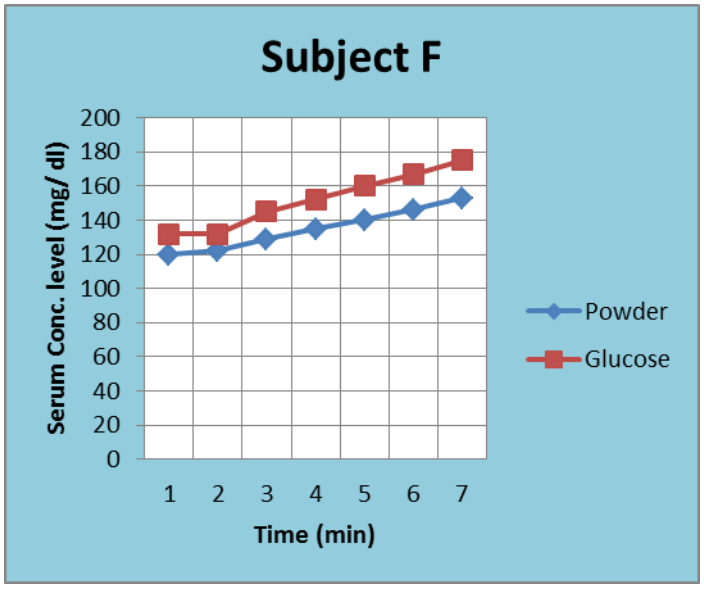
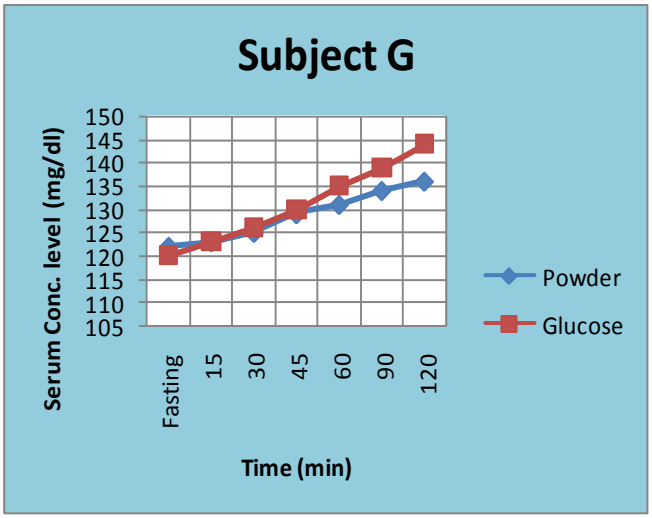


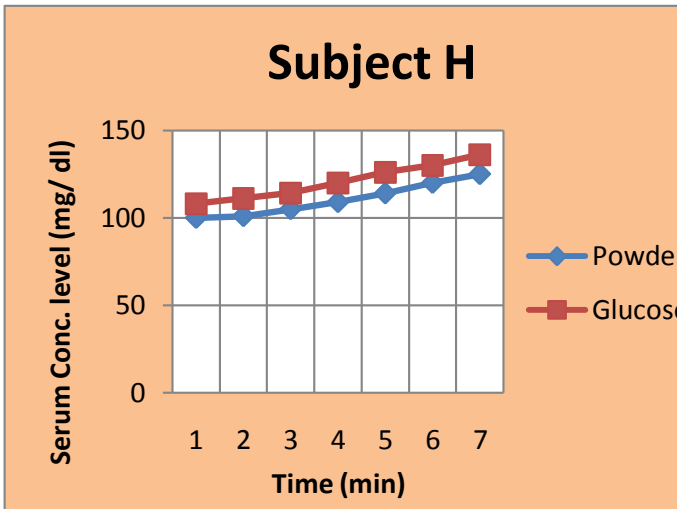
Fig 24. Glycemic Index of ten subjects

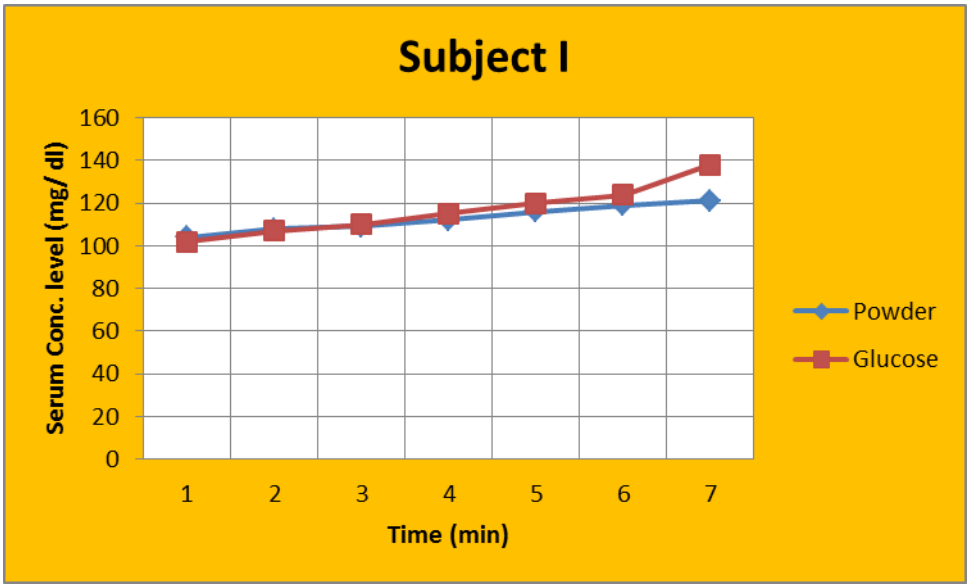



Subject E
Serum Conc. level (mg/ dl)
160
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7
Time (min)
Powder
Glucose


Subject F
Serum Conc. level (mg/ dl)
200
180
160
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7
Time (min)
Powder
Glucose


Subject G
Serum Conc. level (mg/dl)
150
145
140
135
130
125
120
115
110
105
Fasting
15
30
45
60
90
120
Time (min)
Powder
Glucose


Subject H
Serum Conc. level (mg/ dl)
150
100
50
0
1 2 3 4 5 6 7
Time (min)
Powde
Glucos


Subject I
Serum Conc. level (mg/ dl)
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
Time (min)
Powder
Glucose

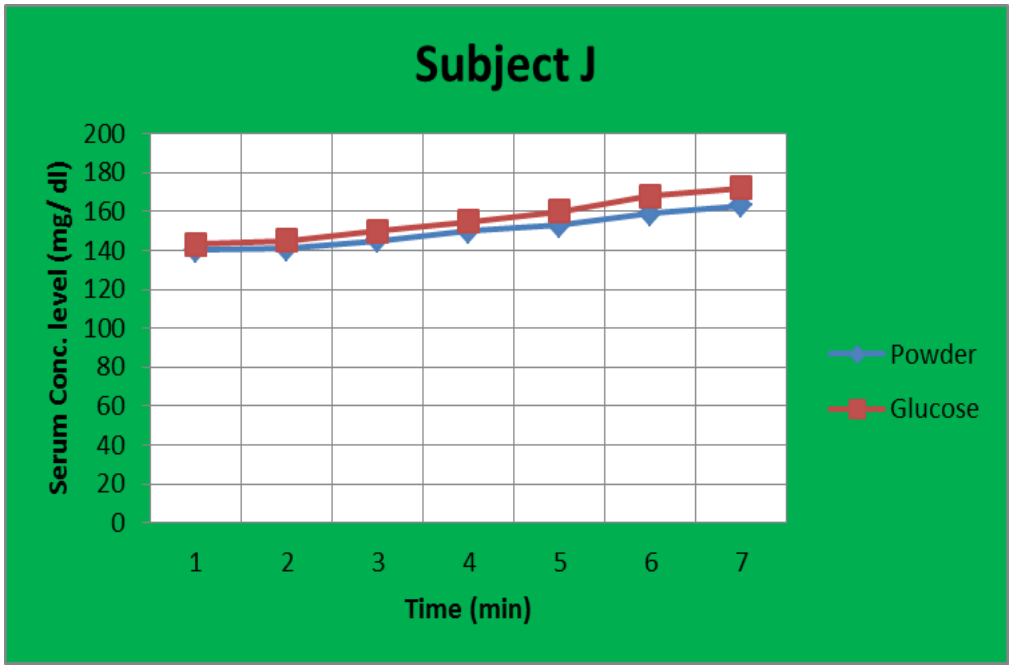
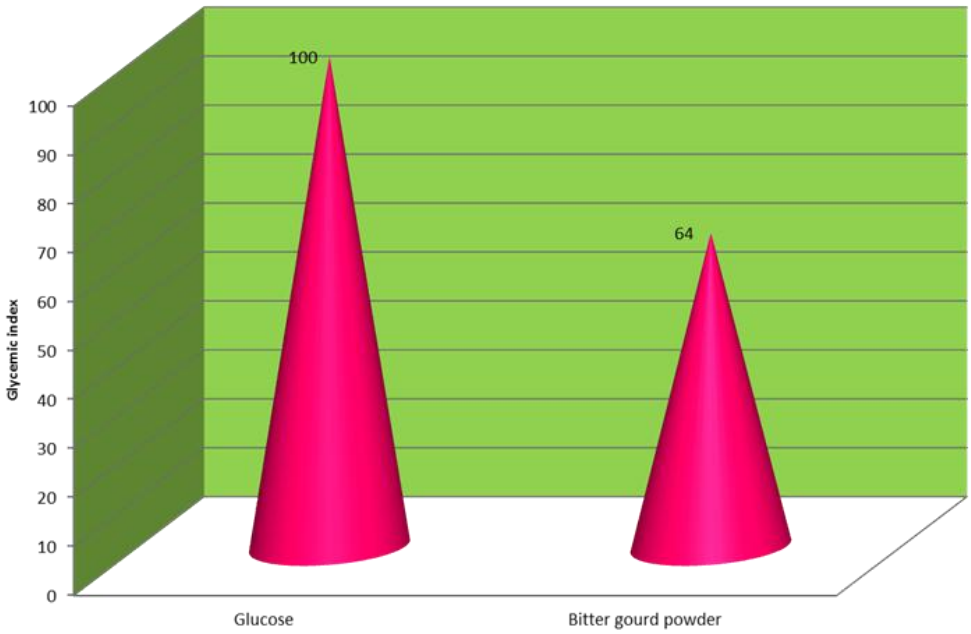

Subject J
Serum Conc. level (mg/ dl)
200
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
Time (min)
Powder
Glucose

Fig 25. Glycemic Index of reference food and test food



medicinal effects. In relation to diabetes, only charantin, insulin like peptide and alkaloid like extracts possess hypoglycemic properties similar to the plant itself or its crude extracts. These different compounds seem to exert their beneficial effects via several mechanisms to control and treat diabetes mellitus.

The sum up the biochemical investigations proved that there was significant variation in the FBS levels of subjects supplemented with bitter gourd powder over a period of 60 days. The mean initial FBS values for the subjects supplemented with bitter gourd powder were 171 mg/dl at 0 day, 152 mg/dl at 30^{th} day and 132 mg/dl at 60^{th} day respectively. The investigations also found that there was variation in the PPBS levels of subjects supplemented with bitter gourd powder over a period of 60 days. The mean initial PPBS value for the subjects supplemented with bitter gourd powder was 217 mg/dl at 0 day, 189 mg/dl at 30^{th} day and 158 mg/dl at 60^{th} day respectively. The results of the present study also observed that there was a variation in the total cholesterol level of the subjects supplemented with bitter gourd powder over a period of 60 days. The mean total cholesterol values for the subjects supplemented with bitter gourd powder were 251 mg/dl at 0 day, 216 mg/dl at 30^{th} day and 180 mg/dl at 60^{th} day respectively. In the present study, bitter gourd powder was having a GI of 64 which was around 36 per cent less than that of glucose. The results of glycemic load revealed that bitter gourd powder had a glycemic load of 39.

SUMMARY

The present investigation entitled “Nutrient composition, antioxidant and hypoglycemic effect of bitter gourd (*Momordica charantia* L.)” was conducted with an objective to study the nutrient/chemical composition, phytochemical analysis, antioxidant activities of the selected bitter gourd types and to assess its hypoglycemic effect on type 2 diabetes mellitus patients.

Four types of commercially cultivated bitter gourd viz. light green small, light green big, dark green small, dark green big along with *Nei paval* were selected for the study. The chemical, nutrient composition phytochemical and antioxidant activities of the selected bitter gourd types were carried out both in fresh and processed (powdered) form and the best bitter gourd type (light green big) was selected to study the clinical efficacy of the bitter gourd powder. Investigations such as shelf life quality, yield ratio, cost of production, glycemic index and glycemic load were also determined.

The highest protein content was found in light green big fresh type (2.06 g) and in the case of bitter gourd dried, highest protein content was found in light green big dried type (30.11 g). The total essential amino acids were highest in light green big dried type (157 n moles/ml) and lowest in *Nei paval* dried (86 n moles/ml) where as the total non essential amino acids were highest in light green big dried (266 n moles/ml) and lowest in dark green small dried type (152 n moles/ml) respectively. Highest carbohydrate content was found in light green small fresh type (8.22 g) and in the case of dried type highest carbohydrate content was found in light green small dried (60.13 g). The carbohydrate content of fresh and dried bitter gourd types was significantly different from each other. The fibre content of bitter gourd both in fresh and dry was found highest in light green small (1.21 g and 20. 31g respectively). Highest moisture content was found in light green big both in the case of fresh and dried (90.40 per cent and 6.61 per cent/ 100g respectively) bitter gourd types. The statistical analysis of the data revealed that there exists a significant difference in beta carotene of all the fresh and dry bitter gourd types. The beta carotene content was found highest in *nei*

paval both in fresh and dry samples (140.03 mcg/ 100g and 98.93 mcg/ 100g respectively).

The highest vitamin C content was noticed in LGB fresh (98.20 mg) and was significantly different from the LGS, DGB, DGS and NP. The vitamin C content of DGS (97.92 mg) and NP (97.91 mg) were on par with each other. In the case of dried bitter gourd samples, the highest vitamin C content was found in light green big dried type (30.42 mg). Folic acid content was found maximum in light green big both in fresh and dried bitter gourd samples (0.10 µg/ ml and 0.87 µg/ ml respectively).

In the case of mineral analysis, highest calcium content was found in light green big (25.44 mg/ 100g and 69.16 mg/100 g respectively) both in the case of fresh and dried samples. There was a significant difference in phosphorus content of bitter gourd fresh and dried samples. Highest phosphorus content was noticed in light green big both in fresh and dry samples (79.64 mg and 424.75 mg respectively). Data on sodium content revealed a significant difference among the bitter gourds types at five per cent level. The sodium content was found highest in light green big both in fresh and dry samples (20.12 mg/ 100g and 100.76mg/ 100g respectively) while it was found lowest in DGB (4.13 mg). The potassium content was found highest in n*ei paval* both in fresh and dry samples (174.46 mg/ 100g and 905.76 mg/ 100g respectively). Highest iron content was found in fresh *nei paval* (2.14 mg) and was significantly different from other types. The lowest iron content was found in DGB (0.45 mg). All the five fresh bitter gourd samples were significantly differ in their iron content. In the case of dried bitter gourd samples, the highest iron content was noticed in NP (11.26 mg) and lowest value in DGB (2.44 mg). Highest manganese content was noticed in light green big both in the case of fresh and dried samples (34.57 mg and 184.56 mg. Copper content was found highest in light green big both in the case of fresh and dried types (40.17 mg and 200.17 mg). Data on zinc content revealed that there was a significant difference among the bitter gourds types at five per cent level. The zinc content was found highest in light green big both in fresh and dry samples (90.41

mg/ 100g and 361.13 mg/ 100g respectively) while it was found lowest in NP (4.13 mg) and NP dried type (241.18 mg).

The result of the phytochemical screening of the ethanol, methanol, acetone aqueous, petroleum ether, and chloroform fruit extract of *Momordica charantia* revealed the presence/ absence of tannins, flavonoids, phenols, alkaloids, saponins, steroids, cardiac glycosides, phlobatinnins and anthraquinones. Quantitative estimation of phytochemicals revealed that, highest polyphenol content was noticed in light green big both in the fresh and dried forms (18.76 mg and 74.67 mg respectively). The highest flavonoid content was found in dark green type both in the fresh and dried forms (0.87 per cent and 3.51 per cent respectively). The quantitative amount of flavonoids in LGS (0.43 per cent) was on par with NP (0.35 per cent) and DGB (0.12 per cent) on par with DGS (0.11 per cent). In the case of dried bitter gourd samples, the lowest flavonoid content was observed in DGS (0.45 per cent) and was on par with DGB (0.45 per cent). The alkaloid content of bitter gourd samples (fresh) ranged between 0.10 to 0.27 per cent where as in the case of bitter gourd dried samples the alkaloid content ranged between 0.90 per cent to 1.01 per cent. In the present study, the highest tannin content was observed in *nei paval* fresh (0.61 mg/ 100g) and was on par with light green small fresh (0.60 mg/ 100g) and in the case of dried bitter gourd samples the highest amount of tannin was found in *nei paval* dried. Highest amount of saponin was found in light green big both in the case of fresh and dry samples (0.68 per cent and 2.83 per cent respectively). The data revealed that lectin content of bitter gourd types ranged between 0.30mg-0.37mg in the case of bitter gourd fresh types where as it ranged between 1.14mg- 1.17mg in the case of bitter gourd dried types. It was observed that light green big bitter gourd had minimum lectin content both in the case of fresh and dried types (0.30mg and1.14mg) respectively. Highest lectin content was observed in dark green small type both in the case of fresh and dried types (0.37mg and 1.17mg) respectively. The charantin content was found to be highest in light green big both in the case

of fresh and dried samples (0.18 µg/ ml and1.39 µg/ ml) followed by light green small fresh type and dried bitter gourd samples (0.17µg/ml and 1.33 µg/ml respectively). The charantin content was found to be minimum in dark green small both in fresh and dried bitter gourd samples (0.10 µg/ ml and 1.20 µg/ ml respectively). The results of ANOVA table revealed that charantin content of bitter gourd samples were significantly different from each other ($P > 0.05$).

Antioxidant activity in the present study revealed that light green big fresh sample had the highest DPPH activity with an IC_{50} value of 50.88 µg/ ml in methanol solvent (i.e. 50.88 µg/ml of light green big fresh type bitter gourd sample is needed to scavenge the free radical of DPPH), followed by dark green small fresh (52.15 µg/ml) in acetone media. The lowest DPPH radical scavenging activity was found in dark green big fresh (59.09 µg/ ml) in methanol solvent. In the case of bitter gourd dried samples, highest DPPH activity with an IC_{50} value of 50.10 µg/ ml was reported in light green big dried and lowest DPPH activity with an IC_{50} value of 57.88 µg/ml was observed in dark green big. The hydroxyl radical scavenging activity of light green big was found to be highest both in the case of fresh and dried bitter gourd samples with an IC_{50} values of 50.95 µg/ml and 50.10 µg/ml respectively, whereas it was found to be minimum in dark green big both in the case of fresh and dried samples with an IC_{50} value of 65.33 µg/ml and 59.56 µg/ml respectively.

Light green small fresh sample showed higher superoxide anion radical scavenging activity with an IC_{50} value of 50.36 µg/ ml in fresh samples and 49.76 µg/ ml in dried samples, in solvents like petroleum ether and acetone respectively. The results revealed that dark green big possessed lowest superoxide anion radical scavenging activity both in the case of fresh and dried samples with an IC_{50} value of 61.22 µg/ ml and 56.90 µg/ ml respectively. Antioxidant activity ranged with an IC_{50} values of 50.09 µg/ml to 61.90 µg/ml in fresh bitter gourd samples and maximum antioxidant capacity was observed in light green big fresh

(50.09 µg/ ml) and minimum antioxidant capacity was observed in dark green big fresh samples (61.90 µg/ ml). In the case of bitter gourd dried samples, the highest antioxidant activity was observed in light green big dried type (50.07 µg/ml) in acetone solvent and lowest antioxidant capacity was noticed in dark green big dried (58.90 µg/ml) sample in petroleum ether as solvent.

Assessment of the shelf life qualities of the bitter gourd powder was assessed by analyzing moisture, microbial infestation and peroxide value. Microbial infestation was found minimum during storage period. The mean moisture level of bitter gourd powder after six month storage was 6.63 per cent. The initial moisture content was 6.59 per cent and after six months of storage it increased to 6.68 per cent. The peroxide content was noticed in powder only in 5^{th} month (0.10 meq/100 g) and 6^{th} month (0.12 meq/100 g) of storage. However it could be noted that the peroxide content in the bitter gourd powder was much minimal than the permitted limits. During the storage of six months period, no actinomycetes were found to be appeared in the bitter gourd powder. But bacterial colonies were observed from the 5^{th} month (1×10^7 cuf/g) and 6^{th} month (2×10^7 cuf/g) and were present only in negligible level. No Fungi and E-coli were detected in bitter gourd powder during the period of six month of storage.

When bitter gourd was converted into powdered form it was noticed that from 1Kg fresh bitter gourd with seeds around 50g powder (without seed) was obtained. The yield ratio was found to be 0.05.

Processing loss was also calculated and the results revealed that the processing loss was found to be 95 per cent.

Along with yield ratio and processing loss, cost was also calculated (based on the price of bitter gourd+ 10% overhead charges) and it was found that for 1 Kg bitter gourd powder, the cost would be around Rs.100/ 100 g.

Efficacy of the bitter gourd powder was tested in among ten selected subjects from Trivandrum city who were initially diabetic and not on medication. The impact of the supplementation (10 g per day) of bitter gourd powder on

selected subjects were closely monitored through clinical parameters like fasting and post prandial blood sugar, HbA_1C, total cholesterol.

The monthly income of the subjects ranged from 20,000 to 90,000. Their educational qualification ranged from plus two to PG, with occupation Govt. employee (subject A, E and I), business (subject B), poojari (subject C), film industry (subject D), teacher (subject F) trainer (subject G) and subject H and J were housewives.

Duration of the disease revealed that subject A, E and I were having diabetes for the past 3 years, whereas subject B, D, F and H too were having diabetes for the last one year, subject C for the past 6 months, subject G having diabetes for the past 2 years and subject J having diabetes for the past four years. Except subject C and I, all others were having diabetic history. The study revealed that subject B, E, F, I and J were having hyperlipidemia at present and subject A, C, D, G and H were having mild hyperlipidemia. Subject I were having hypertension and subject J were having hypotension at present. All the subjects were not having the habit of doing exercise except subject C and all of them were following non vegetarians except subject C.

The anthropometric data of the selected subjects revealed that subject A, E and H were coming under over weight category, with a waist-hip ratio of 0.97, 0.99 and 0.94 respectively. Subject B, D, E and F were coming under grade 1 obesity category, with a waist hip ratio of 0.98, 0.96, 0.95, 0.86 and subject G and I were under grade II obesity category and subject J under grade III obesity category. Whereas subject C were having normal BMI category, with a waist hip ratio of 0.92. The male subjects were having waist-hip ratio in the range of 0.92 to 0.98 and the females were having high waist –hip ratio in the range of 0.86 to 1.03.

Frequency of consumption of various food groups in the diet revealed that all the subjects were included cereals, pulses, vegetables, milk, milk products and tea daily in their diet. The average intake of energy was calculated from their diet

and it was 1598 Kcal for subject A, 2800 Kcal for subject B, 2000 Kcal for subject C, 2200 Kcal for subject D, 3100 Kcal for subject E, 2300 Kcal for subject F, 1710 Kcal for subject G, 2080 Kcal for subject H, 1673 Kcal for subject I and 2173 Kcal for subject J respectively.

The biochemical investigations proved that there was significant variation in the FBS levels of subjects supplemented with bitter gourd powder over a period of 60 days. The mean initial FBS values for the subjects supplemented with bitter gourd powder were 171 mg/dl at 0 day, 152 mg/dl at 30^{th} day and 132 mg/dl at 60^{th} day respectively. The investigations also found that there was variation in the PPBS levels of subjects supplemented with bitter gourd powder over a period of 60 days. The mean initial PPBS value for the subjects supplemented with bitter gourd powder was 217 mg/dl at 0 day, 189 mg/dl at 30^{th} day and 158 mg/dl at 60^{th} day respectively. The results of the present study also observed that there was a variation in the total cholesterol level of the subjects supplemented with bitter gourd powder over a period of 60 days. The mean total cholesterol values for the subjects supplemented with bitter gourd powder were 251 mg/dl at 0 day, 216 mg/dl at 30^{th} day and 180 mg/dl at 60^{th} day respectively.

In the present study, bitter gourd powder was having a GI of 64 which was around 36 per cent less than that of glucose. The results of glycemic load revealed that bitter gourd powder had a glycemic load of 39.

Findings of the present investigation strongly recommended that bitter gourd powder supplementation reduces the blood sugar as well as blood cholesterol level and is efficient in the dietary management of the subjects with diabetes mellitus and hyperlipidemia.

AACC. 2000. Approved methods of 10th ed. The American Association of Cereal Chemists. St. Paul, Minnesota. 1298p.

Aberoumand, A. and Deokule, S.S. 2008. Comparison of compounds of some edible plants of Iran and India, *Pak J. Nutr.* 7 (4), 582-585.

Aboa, K., A. Fred-Jaiyesimi, and Jaiyesimi, A. 2008. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *J. Ethnopharmacol*. 115:67-71.

Abuye, C., Urga, K., Knapp, H., Selmar, D., Omwega, A., Imungi, J. and Winterhalter, P. 2003. A survey of wild, green, leafy vegetables and their potential in combating micronutrient deficiencies in rural populations, *East Afr. Med. J.* 80: 247-252.

Adeyeye, E.I. and Otokiti, M. K. O. 1999. Proximate composition and some nutritionally valuable minerals of two varieties of *Capsicum annum*. (Bell and Cherry peppers). *Disco. Innov.* 11: 75-81.

Agarwal, M. and Kamal, R. 2013 *In Vitro* clonal propagation and phytochemical analysis of and assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.* 25: 94-203.

Ahmed, I., Cummings, E., Sharma, A. K., Adeghate, E. and Singh, J. 2004. Beneficial effect and mechanism of action of *Momordica charantia* fruit juice in the treatment of streptozotocin- induced diabetes mellitus in rats. *Mol cell Biochem.* 261; 63-70.

Ahmed, S. and Beigh, S. H.2009. Ascorbic acid, carotenoids, total phenolic content and antioxidant activity of various genotypes of Brassica Oleracea encephala. *J. Med. Bio. Sci.* 3(1): 1-8.

Aiking, H. 2011. Future protein supply. *Trends. Food. Sci. Technol.* 22: 112-120.

Ajayi, G.O., Olagunju, J.A., Ademuyiwa, O. and Martins, O.C. 2011.Gas chromatography-mass spectrometry analysis and phytochemical screening of ethanolic root extract of *Plumbago zeylanica*, Linn. *J. Med. Plants Res.* 5 (9): 1756-1761.

Akubugwo, I. E., Obasi, N. A., Chinyere, G. C. Ugbogu, A. E. 2007. Nutritional and chemical value of Amaranthus hybride. Leaves from akikpo, Nigeria. *Afr. J. Biotechnol.* 93 (2): 2833-2839.

Akuwuoh, G.A., Ismail, Z., Norhayati, I. and DanSadikun, I. A. 2005. The effect of different extraction solvent of varying polarities on polyphenols of orthosiphon stamineus and evaluation of the free radical-scavenging activity. *Food Chem.* 2:311-317.

Ali, M. A., Sayeed, M. A., Reza, M. S., Yeasmin, S. Khan, A. M. 2008. Characteristics of seed oils and nutritional compositions of seeds from different varieties of *Momordica charantia* Linn. Cultivated in Bangladesh. *Czech J. Food Sci*. 26 (4): 275–283.

Alibhai, Z., Mondor, M., Moresoli, C., Ippersiel, D. and Lamarche, F. 2006. Production of soy protein concentrates/isolates: traditional and membrane technologies. *Desalination. J.* 191 (1): 351-358.

Aljudi, A. M. and Kamaruddin, M. Y. 2004. Evaluation of phenolic contents and antioxidant capacity of two Malaysian floral honeys. *Food Chem.,* 85: 513-518.

Alothman, M. R. Bhat, R. and Karim. 2009. UV radiation induced changes of antioxidants capacity of fresh cut tropical fruits. *Innov. Food Sci. Emerg. Technol*. 10 (4): 512-516.

Altintermim, B. 2012. Bitter melon and the effects of diabetes disease. *J. Agric.*27: 65-69.

Aminah, A. and Anna, P. K. 2011. Influence of ripening stages on physiochemical characteristics and antioxidant properties of bitter gourd. *Int. Food. Res. J*., 18 (3): 863-868.

Aminah, K. and Permatasari, A. 2013. Effect of drying and cooking methods on antioxidant properties of bitter gourd. *J Tropic Agric.* 41(2): 249-256.

Anderson, J. W. Baird, P. Davis, R. H. Ferreri, S. Knudtson, M. and Koraym, A. Waters, V. 2009. Study of different varieties of bitter gourd in regional variation. *Nutr. Rev.* 67(4): 188-205.

Andress, E. C. and Harrison, J. A. 2006. So easy to preserve (Bulletin 989) . Cooperative Extension Service, University of Georgia, Athens.

Anju, R. P. 2013. Evaluation of nutritional quality and health benefits of milky mushroom (*Calocybe indica*). M.Sc. Thesis. Kerala Aricultural University, Thrissur. pp 42-46.

Annapoorani, C. A. and Manimegalai, K. 2013. Screening of medicinal plant momordica charantia leaf for secondary metabolites. *Int. J. Pharm. Res. Dev.* 5(3): 1-3.

Anne, H. (2003). Dietary Fat and the Risk of Clinical Type 2 diabetes *Am. J. Epidemiol.* 159(1): 73-82.

AOAC. 1990. Official methods of analysis. Association of Analytical Chemists. Washington D.C. 15 th edition.1298p.

AOAC. 2000. Official methods of analysis. 17th edition, Association of Official Analytical Chemists, Washington D.C.1212p.

Appleton, J. 2002. Arginine: Clinical potential of a semi essential amino acid. *Altern Med*: 7: 512- 622.

Arrora, A. 1991. The women Elite in India. Sangam Books Ltd., London:40

Arts, I.C. and Hollman, P. C. 2005. Polyphenols and disease risk in epidemiologic studies. *Am. J. Clin. Nutr.* 81: 317-325.

Asan, O. M. and Karakoca, K. 2013. Antimicrobial and antioxidant activities of *Momordica charantia* from Turkey. *Afr. J. Biotech.* 15:44-71.

Association of Analytic Chemists, 1984. Standard Official Methods of Analysis of the Association of Analytical Chemists. 14th Edn, Williams, S. W. (Ed), Washington DC.

Athar, H. U. R., Khan, A. and Ashraf, M. 2008. Exogenously applied ascorbic acid alleviates salt induced oxidative stress in wheat. *Environ. Exp. Bot.*, 63 (1-3): 224-231.

Atmani, D., Nassima, C., Dina, A., Meriem, B.m Nadjet, D. and Hania, B. 2009. Flavonouds in human health, from structure to biological aactivity. Current nutrition and Food science. 5:225-237.

Au, T.K., Collins, R.A., Lam, T.L., Ng, T.B., Fong, W.P. and Wan, D.C. 2000. The plant ribosome inactivating proteins luffin and saponin are potent inhibitors of HIV-1 integrate. FEBS Letters. 471 (2-3): 169–172.

Aziz, M. G. Hierro, A. M. Kulbe, K. D. 2011. Pineapple fruit pulp polysaccharides and their enzymatic liquefaction. *Int. Food Res. J.* 17: 193-203.

Bagchi, and Indrani, 2006. Food for thought: Green 'karela' for Red China". *Times of India*. http://articles.timesofindia.indiatimes.com.

Bailey, S. W. and Ayling, J. E. 2009. The extremely slow and variable activity and reductase in human liver and its implications for high folic acid intake. *Proc. Natl. Acad. Sci.* 106 (36): 15424-15429.

Bakare, R.I., Magbagbeola, O. A., Akinwande, A. I. and Okunowo,O. W. 2010.Nutritional and chemical evaluation of *Momordica charantia. J Med Plants Res*. 4: 2189-2193.

Bakhru, H. K. 2002.Vitamins that heal natural immunity for better health. Orient paperbacks, New York. P345.

Balasubramanian, G., Sarathi, M., Kumar, S.R. and Hameed, A.S.S. 2007 Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. *Aquaculture.* 263 (1): 15–19

Bano, F., Akthar, N. and Naz, H. 2011. Effect of the Aqueous Extract of *Momordica charantia* on body weight of rats. *J. Appl. Sci.* 7: 1-5.

Basch, E, Gabardi, S, and Ulbricht, C. 2003. Bitter melon (*Momordica charantia*): A review of efficacy and safety. *Am. J. Health. Syst. Pharm*. 65: 356–359.

Beckers, H. J. 1988. Microbiology and food hygiene in mass catering. *Cater Health* 10(1) 3-5.

Belinda, O. C. 2003. Herbal supplements in diabetes management. 4: 12-44.

Belinda, O.C., 2000. Diabetes self-management. Herbal supplements in diabetes management.6 : 12-21.

Bello, M. O., Abdul, H. M. and Yekeen, T. A. 2014. Characterization of gourd fruits (*Cucurbitaceae*) for dietary values and anti- nutrient constituents. *Res. J. Pharm. Biol Chem Sci*. 5(4): 232- 226.

Beloin, N., M. Gbeassor, K. Akpagana, J. Hudson, K. de Soussa, K. Koumaglo and Arnason, J. T. 2005. Ethno medicinal uses of *Momordica charantia* in Togo and relation to its phytochemistry and biological activity. *J. Ethanapharmacol*. 96: 49-55.

Bhom and Koupai, R. A. 1994. Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium reticulatum and V. calycinum, *Pac Sci.* 48: 458-463p.

Bickers, D.R. and Athar, M. 2006. Oxidative stress in the pathogenesis of skin disease. *J Invest Dermatol*. 126:2565–2575.

Blessing, A. C., Ugure, M. I. and Oyiga, B. C. 2010. Nutrient evaluation of some Nigerian pumpkins (*Cucurbita* Spp) fruit, vegetable and cereals. Science and biotech global science book. 2: 64-71.

Blois, M. S. 1958. Antioxidants determination by the use of a stable free radical. *Nature*. 181: 119-1200.

Blokhina, O., Virolainen, E. and Fagersledt, K.V. 2003. Antioxidants oxidative damage and oxygen deprivation stress; a review, *Ann. Bot.* 91: 179-194.

Bohlmann, J., Meyer, G. and Croteau, R. 1998. Plant terpenoid synthase, molecular biology and phylogenetic analysis. *Proc Natl Acad Sci*.95:4126-4133.

Bose, T. K., Kabir, J., Maity, T. K., Parthasarathy, V. A. and Som, M. G. 2002. Healthcareherbal.com/Easy ways to get extra calories in your daily diet to gain weight.

Braca, A., Siciliano, T., Arrigo, M.D. and Germano, M.P. 2008. Chemical composition and antimicrobial activity of *Momordica charantia* seed essential oil. *Fitoterapia*. 79 (2): 123-125.

Britton, G. S., Liaeen, J. and Pfander, H. 2004. Carotenoids handbook. Birkhauser Verlag, Boston, USA.

Budrat, P. and Shotipruk, A. 2008. Enhanced recovery of phenolic compounds from bitter melon (*Momordica charantia*) by subcritical water extraction. *Sep. Purif. Technol.* 66 (1): 125-129.

Budrat, P. and Shotipruk, A. 2008. Extraction of phenolic compounds from fruits of bitter melon with subcritical water extraction and antioxidant activities of these extracts, *J. Chaing Mai. Sci*. 35: 123-130.

Buwa, L.V. and Staden, J.2007. Effects of collection time on the antimicrobial activities of *Harpephyllum caffrum* bark. *S. Afr. J. Bot.*73 (2): 242-247.

Cai, R., Hettiarachchy, N. S. and Jalaluddin, M. 2003. High-performance liquid chromatography determination of phenolic constituents in 17 varieties of cowpeas. *J. Agric. Food Chem.* 51 (6):1623-1627.

Çakmak, Y. S., Aktumsek, A. and Duran, A. 2012. Studies on antioxidant activity, volatile compound and fatty acid composition of different parts of *Glycyrrhiza echinata* L. *EXCLI J.* 11:178-187.

Caunii, A., Rodica, C., Andrea, M. Z., Elena, T., Camelia, G. and Seria, S. 2010. Quantitative determination, Metal analysis and antiulcer evaluation of methanol seeds extract of Citrullus lanatus Thunb (Cucurbitaceae) in rats 20(2): 45-48.

Chadha, S. L., Gopinath, N., Katyal, I. and Shekhawat, S. 1995. Dietary profile of adults in an urban and a rural community. *Indian J. Med. Res*. 101: 258-267.

Chanchai, M. 2003. Analysis of charantin from *Momordica charantia* L., MSc thesis in pharmacy, Bangkoke, faculty of graduate studies, Mahidol University.

Chanoine, J. P. 2003. Selenium and thyroid function in infants, children, and adolescents. *Biofactors* 19 (3-4): 137–437.

Chaudhari, B. P., Chaware, V.J., Joshi, Y.R., and Biyani, K.R. 2009. Hepatoprotective activity of hydro alcoholic extract of *Momordica charantia* Linn. leaves against carbon tetrachloride induced hepatopathy in rats. *Int.J. Chem. Tech. Res*. 1 (2): 355-358.

Chen, J. C., Liu, W. Q., Lu, L., Qui, M. H., Zheng, Y. T. and Yang, L. M. 2009. Kuguacins, cucurbitane triterpenoid from *Momordica charantia*. *Phytochem*. 70 (1): 133-140.

Chunduri, J. R. 2013. Antioxidant and nutritional analysis of edible cucurbitacae vegetables of India. *Int. J. Bioassays.* 12 (8): 48-99.

Clifford, A. H. and Cuppett, S. L. 2000. Review : Anthocyanins - nature, occurrence and dietary burden. *J Sci Food Agric*. 80: 1063-1072.

Cohen, B., John, K. L., Subhash, P. and Cherian, L. 2000. Evaluation of the efficacy of bitter gourd as an oral hypoglycemic agent. *Ind J Physiol Pharm*. 47(5): 367-372.

Cousens, G. 2008. There is a cure for diabetes; the tree of life 21 day programme. California: North Atlantic books, 191-192p.

Cummings, E., Hundal, H.S., Wackerhage, H., Hope, M., Belle, M., Adeghate, E. and Singh, J. 2004. *Momordica charantia* fruit juice stimulates glucose and amino acid uptakes in L6 myotubes. *Mol. Cell Biochem*. 261:99–104.

Cummings, J. H. and Stephen, A. M. 2007. Carbohydrate terminology and classification. *Eur J Clin Nutr.* 61: Pp5–18.

Dhar, K. and Sarwate, M. 2013. Extracts of Momordica charantia suppress post prandial hyperglycemia in rats. J Nutr Sci Vitaminol. 53(6): 482-486.

Del Caro, A., Piga, A., Pinna, I., Fenu, P. M. and Agabbio, M. 2004. Effect of drying condition and storage period on polyphenolic content, antioxidant capacity and ascorbic acid of prune. *Agric Food Chem.* 52(15):4780-4784.

Deliconstmtinos, B., Olayi, N. and Adewole, L. 1996. Hypoglycemic and hypotensive effect of bitter gourd. *Cardiovasc J Afr.* 12(5):112-120.

Daniel, P., Supe, U. and Roymon, M. G. 2014. Phytochemical analysis of *Momordica charantia*. *Int J Adv Pharm Biol Chem*.3(1):214-220.

Das, B. and Mishra, P. C. 2011. Antibacterial analysis of crude extracts from the leaves of Tagetes erecta and Cannabis sativa; *Int. J. Environ. Sci.,* 2: (3):1605-09.

Deshmukh. P. R., Gupta, S. S., Dongre, A. R., Bharambe, M. S., Maliye, C., Kaur, S. and Garg, B. S. 2006. Relationship of anthropometric indicators with blood pressure levels in rural Wardha. *Ind. J. Med. Res.* 123 (5): 657-664.

Dey, S. S., Behera, T. K. and Charanjeet, K. 2006. Genetic Variability in Ascorbic Acid and Carotenoids Content in Indian Bitter Gourd (*Momordica charantia* L.) Germplasm. Indian Agricultural Research Institute, New Delhi-110012, India. Cucurbit Genetics Cooperative Report 28-29: 91-93

Dey, S. S., Singh, A.K., Chandel, D., and T. K. Behera. 2006. Genetic diversity of bitter gourd(*Momordica charantia* L.) genotypes revealed by RAPD markers and agronomic traits. *Sci. Hort.* 109:21-28.

Dhimati, K., Gupta, A., Sharma, D. K., Gill, N. S. and Goyal, A. A. 2003. Review on the medicinally important plant of the family cucurbitacae. *Asian. J. Clin. Nutr.* 4: 16-26.

Donovan, J. L., Manach, C., Faulks, R. M. and Kroon, P. A. 2006. Absorption and Metabolism of Dietary Plant Secondary Metabolites. In plant secondary metabolites: occurrence, structure and role in the human diet (Ed: Crozier A, Clifford MN and Ashihara H) Blackwell Publishing, Oxford.

Dubois, M. A. and Wagner, H. 2000. Bioactive saponins from plants, An update. In studies in natural product chemistry, Atta Rahman, ed. Elsevier Science, Amsterdam. 21(4):633-687.

Ebrahimsadeh, K., Stahelin, K. A., Gey, K. and Ludin, E. 2008. Plasma antioxidative vitamins and subsequent cancer mortality in the 12 year follow up of the prospective basel study. Am J Epidemiol. 133(4): 766-775.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*. 4(7): 685-688.

Elia, M. and Cummings, J. H. 2007. Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates. *Eur J Clin Nutr.*, 61:40–74.

Elizabeth, K. and Rao, M.N.A. 1990. Oxygen radical scavenging activity of curcumin. *Int J. Pharm*. 58 (2): 237-240.

Evans, W. C. 1996. Trease and Evans Pharmacognosy, 14th Edition, Bailiere Tindall W. B. Sauders Company Ltd, London. 224: 542-575.

Faruq, U., Sani, A., & Hassan, L., (2002). Composition and distribution of deadly nightshade. *Niger. J. Basic Appl Sci.* 11: 157-164.

Fernandes, N.P., Lagishetty, C.V., Panda, V.S. and Naik, S.R. 2007. An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized *Momordica charantia* fruit extract. BMC Complement *Altern Med* 7:29.

Ferreira, O. Pinho, S.P. 2012. Solubility of flavonoids in pure solvents. *Ind. Eng. Chem. Res. 51*(18): 6586–6590.

Frei, E. C. and Traber, A. R. 2004. Effect of drying method and length of storage on tannin and total phenol concentrations in Pigeon pea seeds, *Food Chem*. 86 (1): 17-23.

Fuangchana, A., Sonthisombata, P., Seubnukarnb, T., Chanouanc, R., Chtochaisuwatd, P. and Sirigulsatiene, V. 2011. Hypoglycemic effect of bitter melon compared with metformin in newly diagnosed type 2 diabetes patients. *J. Ethanopharmacol*. 134: 422-428.

Gadang, V., Gilbert, W., Hettiararchchy, N., Horax, R., Katwa, L., and Devareddy, L. 2011. Dietary bitter melon seed increases peroxisome proliferator-activated receptor-γ gene expression in adipose tissue, down-regulates the nuclear factor-κB expression, and alleviates the symptoms associated with metabolic syndrome. *J. Med. Food*. 14 (1-2): 86–93.

Gaal P. 2006. Antioxidant activity of extract from *Moringa* root, fruit, leaves. *Food Chem*. 94:169-178.

Ganesan, A., Natesan, S., Perumal, P. G., Vellayutham, R., Manickam, K. and Ramasamy, N. 2008. Anxiolytic, Antidepressant and anti inflammatory activities of methanol extract of *Momordica charantia* Linn. Leaves (Cucurbitaceae). *Iran. J. Pharmacol. Therapeutics*. 7: 43.

Gaurav, A., Mondal, D. B. and Vijayakumar, H. 2014. *In vitro* qualitative and quantitative phytochemical analysis of ethanolic extract of *Tinospora cordifolia. Momordica charantia, Cucurbita maxima and Raphanus sativus. Int J Pharm Sci Res.*5(5): 1937-1941.

Gayathri, V. 2014. Analysis on nutritional values and antioxidant properties of powdered *Momordica charantia* and *Colocasia esculenta*. *IJPSBM* 2 (3): 1-4.

Gbeassor, M., Kedjagni, A.Y., Koumaglo, K., De Souza C., Agbo, K., Aklikokou, K. and Amegbo, K.A. 2006. In vitro antimalarial activity of six medicinal plants. *Phytotherapy Research.* 4 (3): 115-117.

Ghaima, P., Alali, F. Q., Mohammad, I. and Elimat, T. 2013. Antioxidant activity and total phenolic content of selected Jordanian bitter melon plant species. *Food Chem.* 104: 1352-1359.

Ghasemi, K., Ghasemi, Y., Ehteshamnia, A., Nabavi, S. M. Nabavi, S. F. Ebrahimzadeh, M. A. and Pourmorad, F. 2011. Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (Juglans regia L.) green husks (Article) *J. Med. Plants Res.* 5: 1128-1133.

Ghosh N., Cakravarthy, D.K. 1990. Predictive analysis of protein quality of *pleurotus citrinopileatus.* J. *Food Sci. Technol*. 27 (4): 236-23

GMA News.TV. March 27, 2007. Ampalaya tablets out soon for diabetics. Game network.com.

Gopalakrishnan, S. B. and Kalairasi T. 2014. Comparative phytochemical screening of the fruits of *cucumis trigonus roxb* and *cucumis sativus Linn. World J Pharm. Pharm Sci.* 3 (4): 1455-1468.

Gopaldas and Sheshadri, S. 1987. Nutrition Monitoring and Assessment. Oxford University Press, Delhi, 140 p.

Govindarajan, R., Singh, D. P. and Rawat, A. K. S. 2007. High-performance liquid chromatographic method for the quantification of phenolics in 'Chyavanprash' a potent *Ayurvedic drug. J. Pharmaceut. Biomed. Anal.* 43 (2): 527-532.

Grant, P., Verma, K. and Lacko, M. 2005. Antidiabetic and adaptogenic properties of *Momordica charantia* extract. *Phytother Res.* 3(1):45-51.

Grover, J. K. and Yadav, S. P. 2004. Pharmacological actions and potential uses of *Momordica charantia*. A review. *J. Ethnopharmacol*. 93 (1): 123-132.

Gry, J., Black, L., Eriksen, F. D., Pilegaard, K., Plumb, J., Rhodes, M., Sheehan, D., Kiely, M. and Kroon, P. 2007. Euro FIR-BASIS – a combined composition and biological activity database for bioactive compounds in plant-based food. *Trends. Fd. Sci. Technol.*, 18 (8): 434-444.

Gulcin, I., M. Oktay, Kufrevioglu and A. Aslan. 2007. Determination of antioxidant activity of lichen *Cetraria islandica Ach. J. Ethanopharmacol*. 79:325-29.

Gupta, S. and Verma, P. 2011. Nutritional and sensory quality of micronutrient rich traditional products incorporated with green leafy vegetables, *Int. Fd. Res. J.* 18: 667-675.

Gurbuz, I., Akyuz, C., Yesilada, E. and Sener, B. 2000. Anti ulcerogenic effect of *Momordica charantia* L. fruits on various ulcer models in rats. *J.Ethanapharmacol*.7:77-82.

Gus, A., Patil, S. A. and Patil, S. B. 2004. Toxicological studies of *Momordica charantia* Linn. Seed extracts in male rats. *Int. J Morphol*. 29(4): 1212-1218.

Haixia, Z., Xiaozuo, Z., Yawei, W., Mancanq, L. and Zhide, H. 2004. Analysis of vicine in bitter melon samples by polyglycol- C8 solid phase with high performance liquid chromatography. *Chin J. Anal. Chem*. 3: 108-408.

Halliwell, B. 1996. Antioxidant (Chap. 60). (In) Present knowledge in Nutrition 7th Ed: 593-603p.

Ham, C. and Wang, J. 2009. Optimization of conditions for charantin extraction in PEG/salt aqueous two- phase systems using response surface methodology. *Open Compl Med J*. 1: 46-50.

Hamissou, M., Smith, A. C., Carter, R. E. and Triplett, K. K. 2013. Antioxidant properties of bitter gourd and zucchini. *Emirates J. Food Agri.*, 25 (9): 641-647.

Han, H., Zuki, A., Goh, Y. M. and Noor, P. 2013. The effects of Momordica charantia on the liver in streptozotocin induced diabetes in neonatal rats. *Afr J Biotechnol*. 9(31): 5004-5009.

Haque, E. M., Alam, B. M. and Hossain, S. 2011. The efficacy of cucurbitane type triterpenoids, glycosides and phenolic compounds isolated from momordica charantia: a review. *Int. J. Pharm. Sci.*,2 (5): 1135-1146.

Harborne, J. B. 1973. Phytochemical methods, London, Chapman and Hall, Ltd. 49-188pp.

Harborne, J. B. 1999. The hand book of natural flavonoids, Chishester, UK,1(2):32-45.

Harinantenaina, L., Tanaka, M., Takaoka, S., Oda, M., Mogami, O., Uchida, M. and Asakawa, Y. 2006. *Momordica charantia* Constituents and Antidiabetic Screening of the Isolated Major Compounds. *Chem Pharm Bull.* 54: 1017-1021.

Hasan, I and Khatoon S. 2012. Effect of *Momordica charatia* tablet in diabetes mellitus- type 1 and type 2. *Prime Res Med.* 2 (2): 72-74.

Hasler, C. M. 2002. A new look at an ancient concept. *Chem. Industry.* Feb. 2: 84-89.

Hellolife, 2008. Polypeptide- p- Anatural treatment for diabetes. The smart living network. http://www.smartlivingnetwork.com/diabetes/polypeptide-p-plant- insulin-a-natural-treatment-for-diabetes.

Hoang, D. M., Trung, T. N., Hein, P. T. T., Ha, D. T., Van Luong, H. and Lee, M. 2010. Screening of protein tyrosine phosphatase 1B inhibitory activity from some Vietnamese medicinal plants. *Nat Prod Sci.* 16 (4): 239-244.

Hochstein, P. and Atallah, A.S. 2008. The nature of oxidants and antioxidant system in the inhibition of mutation and cancer. *Mutat Res.* 202: 363-375.

Hollman, P. C. H. 2001. Evidence for health benefit of plant phenols. *J. Sci. Food. Agric.* 81 (9): 842-852.

Holt, C. and Brown, K. H. 2004. International Zinc Nutrition Consultative Group (IZINCG).

Horax, R., N. Hettiarachchy and Islam, S. 2005. Total phenolic contents and phenolic acid constituents in four varieties of bitter melon and antioxidant activities of their extracts. *J. Food. Sci.* 70 (4): 215-280.

Horax, R., Hettiarachchy, N. and Pengyin, C. 2010. Extraction, quantification and antioxidant activities of phenolic from pericarp and seeds of bitter melons (Momordica charantia) harvested at three maturity stages. *J. Agric. Food Chem.*58 (7) :4428–4433.

Hossain, P., Kawar, B. and El-Nahas, M. 2007. Obesity and diabetes in the developing world - A growing challenge. *New Engl. J.Med.* 356: 213-215.

Hoye, A. T., Davoren, J.E., Wipf, P., Fink, M. P. Kagan, V. E. 2008. Targeting mitochondria. *Acc. Chem. Res. 41*(1): 87–97.

Huang, Y., Han, S., Li Y., Mao Y. and Xie, Y. 2007. Different roles of MTHFR C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. *J Human Genet.* 52(1): 73–85.

Hussain, K., Patel, P, and Doshi, S. 2014. Karella in the treatment of diabetes mellitus. *Ind. J Med Sci.* 22(5):23-32.

Ishida, H., H. Suzuno, N. Sugiyama, S. Innami, T. Todokoro, and Maekawa, A. 2000. Nutritive evaluation of chemical component of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas* poir). *Food Chem*. 68 (3): 359-367.

Islam, S., M. Jalaluddin. 2004. Sweet potato- a potential nutritionally rich multifunctional food crop for Arkansas: *J. Arkansas Agric Rural Dev*. 4:3-7.

Islam, S. M., Jalaluddin, G.O., Garner, M., Yoshimoto, O. and Yamakawa. 2005. Artificial shading and temperature influence on anthocyanin compositions in sweet potato (*Ipomoea batatas* L.) Leaves. H*ort Science.* 40 (1): 176-180.

Jackson, M. 2003. Potential mechanisms of action of bioactive substances found in foods. In: Plants: diet and health. Report of a British Nutrition Foundation Task Force. G Goldberg (Editor). Blackwell Publishing, Oxford.

Jackson, M.L., 1973. Soil chemical analysis. Prentice Hall of India Private Ltd., New Delhi.

Jalaluddin K. 2005. Effects of *Momordica charantia* on insulin resistance and visceral obesity in mice on high fat diet. *Diabetes Res Clin Pract*. 3(2): 139-146.

Jangam, S. V. and Mujumdar, A. S. 2010. Basic concepts and definitions, in drying of foods, vegetables and fruits. Published in Singapore, pp 1-30.

James, W.P.T., Ferro-Luizzi and Waterlow, J.C. 1988. Definition of chronic energy deficiency in adults – Reports of working party of the intervention dietary energy consultation group. *Am. J. Clin. Nutr.* 42:969-981.

Jaszewski, R., Ullah, N. and Misra, S. 2008. High dose folic acid supplementation inhibits recurrence of colorectal adenomas. *World J.Gastroenterology*. 14 (8): 4492-4498.

Javid, H., Najeeb, R. and Abdul, L. K. 2010. Proximate and essential nutrient evaluation of selected vegetables species from khota region, Pakistan. *Pak. J. Bot.* 42(4): 2847-2855.

Jayasooriya, A.P., Sakono, M., Yuxizaki, C., Kawano, M., Yanmoto, K. and Fukuda, N.2000. Effects of *Mormordica charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets. *J Ethnopharmacol*.72 (1-2): 331-136.

Jaziya, S. 2011. Evaluation of nutritional quality and health benefits of oyster mushroom. M.Sc. Thesis. Kerala Agricultural University, Thrissur. pp: 63-71.

Jeevathayaparan, S., Tennekoon, K. H. and Karunanayake, E. H. 1995. A comparative study of the oral hypoglycemic effect of *Momordica charantia* fruit juice and tolbutamine in streptozotocin induced graded severity diabetes in rat. *Int. J. Diabetes*. 3: 99-100.

Jeong, J., Lee, S., Hue, J., Lee, K., Nam, S. Y. and Yun, Y. W. 2008. Effect of bitter melon on antidiabetic activity in C57BL/6J db/db mice. *Korean J. Vet. Res.* 48: 327-336.

Jin, D. and Mumper, R. J. 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Review. *Molecules., 15*(10): 7313–7352.

Jin, X. H., Ohgami, K., Shiratori, K., Suzuki, Y., Koyama, Y., Yoshida, K., Ilieva, I., Tanaka, T., Onoe, K. and Ohno, S. 2006. Effects of blue honeysuckle (*Lonicera caerulea* L.) extract on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. *Exp. Eye Res. 82*: 860–867.

Jiratchariyakul, W., Wiwat, C. and Vongsakul, M. 2001. "HIV inhibitor from Thai bitter gourd". *Planta Med.,* 67: 350–3.

Johansen, J. S., Alex, K. H., David, J., Ryehly, D. J. and Adviye, A. 2007. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. *Cardiovasc Diabetol*. 4 (5): 24- 35.

Johnson, L. F. and Curl, E. A. 1972. Methods for research on the Ecology of soil borne plant pathogens. Burgees Publication Co, New York, 13 p.

Jones, P. J. and Varady, K. A. 2008. Are functional foods redefining nutritional requirements (PDF). *Appl Physiol Nutr Metab.* 33 (1): 118–23.

Joseph, B. and Raj, S. J. 2010. Phytopharmacological properties of Ficus racemosa Linn, An over view. *Int J Pharm Sci Rev Res*. 3: 134-138.

Joseph, B. Jini. D. 2011. Insight into the hypoglycemic effect of traditional Indian herbs used in the treatment of diabetes. *Res. J. Med Plant.* 5:4 352-376.

Joshi, R., Adhikari, B. S., patro, S., Chattopadhyay S. and Mukherjee, T. 2001. Free radical scavenging behavior of folic acid. Evidence for possible antioxidant activity. *Free Radical Biol Med.* 30: 1390-1399.

Kale and Laddha, 2012. Screening and quantitative evaluation of alakaloid content of *Momordica charantia.J Pharm Res*. 6(3):456-464.

Kalia, A.N. 2005. Textbook of Industrial Pharmacognocy, CBS publisher and distributor, New Delhi. 204–208.

Kalaiarasi, B. 2014. Phytochemical screening and properties of different bitter gourd types. *Ind J Dietet Nutr.* 3(1):212-219.

Kamath, S. 1986. Health Assessment. Third edition. Moshy Company Publishers London, 250 p.

Kappus, P. 2003. Hypoglycemic activity of polypeptide from a plant source. *J Nat Prod.* 44: 648-655.

Karakoca, P. 2013. Radical scavenging and reducing properties of wild bitter gourd cultivars. *J. Pharm. Res.* 6: 135-139.

Karaye, I. U., Aliero, A. A., Muhammad, S. and Bilbis, L. S. 2013. Evaluation of nutrient and anti-nutrient contents of selected Nigerian cucurbits Seeds. *J. Pharm. Res.* 4: 137

Katare, D. P., Aeri, V. and Bora, M. 2000. Secondary metabolites and metabolic engineering; *J. Cell. Tissue. Res.* 9 :(3) 2027-2036.

Kaul, S.S. and Nyamongo, K.J. 1990. *Ecology, growth and nutritional status.* Ashis Publishing House, New Delhi, 170 p.

Kedare, S. B. and Singh, R. P. 2011. Genesis and development of DPPH method of antioxidant assay, *J. Fd. Sci. Tech.* 48(4): 412-422.

Keding, G.B. and Krawinkel, M.B. 2006. Bitter gourd (*Momordica charantia*): A dietary approach to hyperglycemia. *Nutr Rev.* 64: 331–7.

Kendler, B. S. 2006. Supplemental conditionally essential nutrients in cardiovascular disease therapy. *J Cardiovasc Nurs*. 21 (1): 9-16.

Kenny, T. J., Smyth, C. M., Hewage and Brunton, N. P. 2013. Antioxidant properties and quantitative UPLC-MS analysis of phenolic compounds

from extracts of fenugreek (*Trigonella foenum*-Graecum) seeds and bitter melon (*Momordica charantia*) Fruit," *Food Chem*. 141 (4): 4295-4302.

Khalid, S. Shazadi, I., Ahmad, I., Ahamad, R., Ashraf, M. and Nisa, V. 2010. Nutritional analysis and antioxidant activity of bitter gourd from Pakistan. *Pharmacologyonline.*,1: 252-260.

Khan, A. and Anderson, R. 2003. Insulin potentiating factor (IPF) present in foods, spices, and natural products. *Pak. J. Nutr*. 2 (4):254-257.

Khan, M. T. H. 2000. Diabetes mellitus. Treatment and complications. *Hamdard Medicus.* XLIII. 3: 71-83.

Kim, K. and Kim, H. Y. 2011. Bitter melon (*Momordica charantia*) extract suppress cytokine induced activation of MAPK and NF in pancreatic beta cells. *Food Sci Biotechnol.* 20(2): 531-535.

Kimura, Yumiko, Akihisa, Toshihiro, Yuasa, Noriko, Ukiya, Motohiko, Suzuki, Takashi, Toriyama, Masaharu, Motohashi, Shigeyasu, Tokuda, and Harukuni, 2005. Cucurbitane-type triterpenoids from the fruit of *Momordica charantia*. *J. Nat. Prod.* 68 (5): 807–809.

Klomann, SD., Mueller, A.S., Pallauf, J. and Krawinkel, M.B. 2010. Antidiabetic effects of bitter gourd extracts in insulin resistant db/db mice. *Brit. J. Nutr*. 10(4): 1613-1620.

Ko, M. J., Cheigh C. I. and Chung, M. S. 2014. Relationship analysis between flavonoids structure and subcritical water extraction (SWE), *Food Chem.*143: pp. 147-155.

Kobori, M., Ohnishi-Kameyama, M., Akimoto, Y., Yukizaki, C. and Yoshida, M. 2008. A-Eleostearic acid and dihydroxy derivative inducing components of bitter gourd.*J. Agric.Food .Chem*. 56 (2) : 10515–10530.

Kohno, H., Yasui, Y., Suzuki, R., Hosokawa, M., Miyashita, K., and Tanaka, T. 2004. Dietary seed oil rich in conjugated linolenic acid from bitter melon inhibits azoxymethane-induced rat colon carcinogenesis through elevation of colonic PPAR γ expression and alteration of lipid composition. *Int. J. Cancer*. 110 (6): 896–901.

Kokate, C.K., Purohit, A.P. and Gokhale, S.B. 2002. Nutraceutical and Cosmaceutical. Pharmacognosy, 21st edition, Pune, India: Nirali Prakashan, p: 542–549.

Koneri, M., Das, P. H. and Darbar, K. 2014. Analysis of phytochemicals in bitter gourd fruits. *Adv Res Pharm Biol.* 2(1): 122-124.

Kotilainen, L. R., Rajalahti, C. Ragasa. And Pehru, E. 2006. Health enhancing food opportunities for strengthening the sector in developing countried. The World Bank Agricultural and Rural Development Discussion Paper 30.

Krishnaja, U. 2014. Development quality assessment and clinical efficacy of functional food supplement for life style management. PhD. Thesis. Kerala Agricultural University, Thrissur. pp 84-97.

Krishnendu, A., Subrata, G. and Gunjan, B. 2011. Comparitive study of antioxidant activity and nitric oxide synthase activation property of different extracts from *Rhododendron arboretum* flower. *Int J. Pharm Tech Res.* 3 (2): 757-762.

Kubola, J. and Siriamornpun, S. 2008. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts in vitro. *Food Chem.* 110:881–890.

Kumar, A. J. K. 2001. Shelf life determinants in dry bakery products. Indian *food Ind.* 20 (3): 69-72.

Kumar, P. J. and Clark, M. 2002. Diabetes mellitus and other disorders of metabolism. Text book of Clinical Medicine. Saunders, London. 1069-1152.

Kumar, D. S., Sharathnath, K. V., Yogeswaran, P., Harani, A., Sudhakar, K., Sudha, P. and Banji, D. A. 2010. A Medical potency of *Momordica charantia*. *Int. J. Pharm. Sci. Rev. Res.* 1 (2): 95-100.

Kusirisin, W., Srichairatanakool, S., Lerttrakarnnon, P., Lailerd, N., Suttajit, M., Jaikang, C. and Chaiyasut, C. 2009. Antioxidative activity, polyphenolic content and anti-glycation effect of some Thai medicinal plants traditionally used in diabetic patients. *Med. Chem.5* (2): 139–147.

Lalhelenmavia, H. Lalremruatal, V. and Mandal, S. C. 2013. Preliminary phytochemical analysis and antioxidant effect of *Momordica charantia. Pak J Sci.* 3(1):564-574.

Lako, J. V., Trenerry, M., Wahlqvist, N., Wattanapenpaiboon, S., Sotheeswaran, R. and Premier. 2007. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem.* 10(4): 1727-1740.

Lancet, L., Birtwhistle, R., Kotecha, J., Hannah, S. and Cuthbertson, S. 2000. Anti-diabetic and hypoglycaemic effects of *Momordica charantia* (bitter melon): a mini review. *Br. J. Nutr*. 102. 1703-1708.

Lean, M.E.J., Han, T.S and Morrison, C.E. 1995. Waist circumference as a measure for indicating need for weight management. *Br. Med. J.* 11: 638-641.

Leelaprakash, G., Carolin, J., Gowtham, B. M., Javvaji, P. K. and Shivaram, A. 2011. In vitro antimicrobial and antioxidant activity of *Momordica charantia* leaves. *Pharamacophore. J.* 2: 244-252.

Leu, J. P. and Zonszein, J. 2010. Diagnostic criteria and classification of diabetes in poretsky, L., (Ed) Principles of diabetes mellitus, second edition. Springer. New York.

Leung, L., Birtwhistle, R., Kotecha, J. and Cuthbertson, S. 2009. Anti diabetic and hypoglycemic effects of *Momordica charantia.* A mini review. *Br. J. Nutr.* 102: 1703.

Levine, M., Rumsey, S. C., Wang, Y., Park, J. B. and Daruwala, R. 2000. Vitamin C. in Stipanuk (ed) "Biochemical and physiological aspects of human nutrition." Philadelphia: W B Saunders. pp541–567.

Li, B. B., Smith, B. and Hossain, M. 2006. Extraction of phenolics from citrus peels I. Solvent extraction method. *Sep. Purif. Technol*. 48 (2): 182-188.

Lichtenstein, A. H., Appel, L. J. Brands, M., Carnethon, M. Daniels, S. Franch, H. A. 2006. Report on cardiovascular related problems. *Arterioscler Thromb Vasc Biol*. 26: 2186-2191.

Lininger, S., Wright, J., Austin, S., Brown, D. and Gaby, A. 1998. The natural pharmacy. Prime health. A division of prima publishing, USA, pp 39-44.

Lis, H. and Sharon, N. 1998. . Lectins: Carbohydrate-specific proteins that mediate cellular recognition. *Chem.Rev.* 98(2): 637-674.

Liu, R. H. 2004. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *J. Nutr.* 134: 3479-3485.

Livingstone, A. S., Feng, J. J. and Malleshi, N. G. 1993. Development and nutritional quality evaluation of weaning foods based on malted, popped and roller dried wheat and chick pea. *Int. J. Food. Sci. Tech.* 28: 35-43.

Lluch, D., Conte, D. and Wei, D. 2006. Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional bitter melon. *Food Chem*. 105: 564-571.

Lotlikar, M.M. and Rajaramrao, M.R. 1966. Pharmacology of a hypoglycaemic principal isolated from the fruits of *Momordica charantia* L. *Ind. J. Pharm.* 28:129.

Lucky, I., Okhunorbo, O., Uwaya, O. John, Imafidon, E., Kate, Osarumwense, O., Peter, Omorodion, E. and Jude. 2012. Study of wild melon family. *Asian Pac J Trop Dis.*44: 167-221.

Malik, Z. A., Singh, M. and Sharma, P. L. 2011. Neuroprotective effect of bitter gourd in global cerebral ischemia and reperfusion induced neuronal damage in diabetic mice. *J. Ethanopharmacol*. 133: 729-734.

Manjamalai, A., Sardar, R., Singh, S., Guruvayoorappan, C. and Grace, V.M.B. 2010. Analysis of phytochemical constituents and anti-microbial activity

of some medicinal plants in Tamil Naidu, India. *Global J. Biotech. Biochem*. 5: 120-128.

Manmbe, M., Takenaka, R., Nakasa, T., Okinaka, O., 2003. Induction of anti inflammatory responses by dietary *Momordica charantia* L. *Biosci. Biotechnol. Biochem*. 67(12):2512-2517.

Marderosian, Q. A. 2001. The review of natural products. Facts and comparisons, St. Louis, MO, Lippincott, Williams and Wilkins publication. 380: 399-509.

Marlowe, F., Apicella, C. and Reed, D. 2005. Men's preferences for women's profile waist to hip ratio in two societies. *Evol. Hum. Behav.* 26 (6): 458-468.

Marston, A. and Hostettmann,K. 2005. "Separation and Quantification of Flavonoids," In: O.M. Andersen and K.R. Markham, Eds., Flavonoids: Chemistry, Biochemistry and Applications, CRC Press, USA, pp. 1-36.

Mary, H. Lakshmi, Florida, T. Jerrine, J. Siddaharth, S. and Sudarsanam. 2014. Phytochemical, antioxidant and cytotoxic properties of the fruit extract from *cucurbita digitata. Int. J. Pharm. Sci.* 6: (4): 975-1491.

Masaki, H. 2010. Role of antioxidants in the skin: anti-aging effects. *J Dermatol Sci.* 58 (2):85–90.

Mathai, K. 2000. Nutrition in the adult years. In Krauses Food, Nutrition and diet therapy. 10th ed. L.K. Mahan and Escott Publisher. 271(2): 274-275.

Matkovic, V. 2007. Nutrition influences skeletal development from childhood to adulthood: a study of hip, spine, and forearm in adolescent females. *J. Nut.* 134 (3): 701–705.

Matsuda, H., Li, Y. and Yoshikwa, M. 1999. Roles of capsaicin sensitive sensory nerves, endogenous nitric oxide, sulfhydryls, and prostaglandins in gastroprotection by momordin I_c, an oleanolic acidoligoglycoside, onethanol induced gastric mucosal lesions in rats. *Life Science.* 65: 27-32.

Mehta, K. 2013. Traditional medicinal plants of Manipur as antidiabetics. *J Med Plants Res.* 5(5): 667-674.

Meege, B. A., Schrader, H. and Ritter, P. R. 2010. Selective amino acid deficiency in patients with impaired glucose tolerance and type 2 diabtes. *Regul Pept.* 83: 541-600.

Microbiome and Me, 2012. Mara Hvistendahl, *Science*. 336: 1248-1250.

Midhila, M. 2013. Developmental and quality evaluation of ready to cook dehydrated product from banana blossom. M.Sc thesis. Kerala Agricultural University. Thrissur. pp: 32-66.

Mir. A. M., Sawhney, S. S. and Jassal, M. M. S. 2013. Qualitative and quantitative analysis of phytochemical of *Taraxacum officinale*. Wudpecker *J. Pharm. Pharmacol.* 2(1): 1-5.

Mishra, S. N. 1989. Analytical methods for analysis of total alkaloids in root of *Withania* spp. All India workshop on MAP, Faizabad, p492-495.

Moein, S. Farzami, B. and Khaghani, S. 2007. Antioxidant properties and protective effect on cellcytotoxicity of *Salvia mirzayanii*. *Pharm. Biol.* 45:458-63.

Mohanlal, S., Parvathy, R., Shalini, V., Mohanan, R., Helen, A. and Jayalekshmy, A. 2012. Chemical indices, Antioxidant activity and anti-inflammatory effect of extracts of the medicinal rice *Njavara* and staple varieties. *J. Food Biochem., 36*, 1–12.

Molyneue, 1996. Alkaloids on their chemical and biological perspective, Pelletier press, Oxford, pp456-472.

Mourya, S.P. and Jaya, N. 1997. Prevalence of malnutrition among tribal children. *Indian. J. Nutr. Diet*. 34: 214-219.

Mudgal, c. 2009. Thin layer drying kinetics of bitter gourd. *J. Food. Sci. Technol.* 46: 236-239.

Muller, H., Mcallan, A. B. Harvey, T. 1992. Tannins, their biochemistry and nutritional properties. Advances in plant cell biochemistry and biotechnology, Jai press Ltd, London.5(4): 151-217.

Mungole, J. A., Awathi, R., Chaturvedi, A. And Zanwan, P. 2010. Preliminary phytochemical screening of *Ipomoea obscura* (L)- A hepato protective medicinal plant. *Int. J. Pharm. Tech. Res.* 2 (4): 2307-2312.

Mwambete, K. D. 2009. The *in vitro* anti microbial activity of fruit and leaf crude extracts of Momordica charantia: A Tanzania medicinal plant. *Afr. Health Sci.* 9(1): 34-39.

Myojin, C. and Enami, N. 2008. Changes in the radical scavenging activity of bitter gourd during freezing and frozen storage with or without blanching. *J.Food. Sci.* 73:120-141.

Nabavi, S. M., Ebrahim, Z. M. and Jafari, M. 2008. Free radical scavenging activity and antioxidant capacity of *Eryngium caucasicum* and *Froripia subpinmata*. *Pharmacology online.* 3: 19-25.

Nacoulma, O.G. 2012. Mutagenic effect, antioxidant and anticancer activities of six medicinal plants from Burkina Faso. *Nat. Prod. Res. 26 (6)*: 575–579.

Nagarani, G., Arumugham, A. Perumal, S. and Siddhu R. 2014. Food prospects and nutraceutical attributes of Momordica species, A potential tropical bioresources- A review. *Food Sci .Hum. Wellness.*

Naghii, M. R. and Fouladi, A. L. 2006. Correct assessment of iron depletion and iron deficiency anaemia. *Nutr Health*. 18(2): 133-139.

Nande, P., Hussain, M. and Vali, S. 2010. Influence of obesity on body measurement and composition in adult women belonging to minority community. *Ind. J. Nutr. Dietet*. 47 (4): 137-151.

Narasingha Rao, 2003. Evaluation of free radical scavenging properties of commercial bitter melon phenol extracts by fast colorimetric method. *Food Chem.* 95:1-6.

Nasheeda, K. 2006. Developing multipurpose convenient mix from selected banana varieties. MSc (FS & N) thesis, Kerala Agricultural University. Thirissur pp 68-70.

Nerurkar, P. V. 2005. Microsomal triglyceride transfer protein gene expression and ApoB secretion are inhibited by Bitter melon in $HepG_2$ cells. *J. Nutr.* April 1:135 (4): 702-706.

Nerurkar, P.V., Lee, Y.K. and Linden, E.H. 2006. Lipid lowering effects of Momordica charantia (Bitter Melon) in HIV-1-protease inhibitor-treated human hepatoma cells, HepG2. *Br. J. Pharmacol.* 148 : 1156–64.

Nerurkar, P.V., Lee, Y.K., Motosue, M., Adeli, K. and Nerurkar, V.R. 2008. *Momordica charantia* (bitter melon) reduces plasma apolipoprotein B-100 and increases hepatic insulin receptor substrate and phosphoinositide-3 kinase interactions. *Br. J. Nutr.*5 (1-9):751-759.

Nijveldt, R. J., Nood, E., Hoorn, D. E. C., Boelens, P. G., Norren, K. Leeuwen, P. A. M. 2001. Flavonoids : A review of probable mechanisms of action and potential applications. *Am. J. Clin Nutr*.74: 418-425.

NIN, 1995. Carotene content of foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.135p.

Nout, M. J. R. and Ngoddy, P. O. 2001. Technological aspects of preparing affordable fermented complementary foods. Food Control. 8: 279-287.

Obdoni, B.O. and Ochuko, P.O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta states of Nigeria. *Global J. Pure Appl. Sci.* 8: 203-208.

Okuda, T., Kimura, Y., Yoshida, T., Hatano, T., Okuda, H. and Arichi, S. 2003. Studies on the activity and related compounds from medicinal plants and drugs. Inhibitory effects on lipid peroxidation on mitochondria and microsomes of liver. *Chem. Pharm. Bull.* 31: 1625-1631.

Olagunju, I. A., Fagbohunka, B. S., Oywdapo, O. O. and Abdul, A. I. A. 2006. Effects of an ethanolic root extracts of plumbago zeylanica Linn. On some serum parameters of the rats. RPMP- *Drug Dev. Mol.* 11: 268-276.

Oliveri, C.S. 2000. Nutraceuticals, Phytochemicals, and antioxidants—What are they all about? South Centers, Ohio State University Extension. SS-210-03.

Olson, J. A. 1999. Carotenoids. Modern Nutrition in Health and Disease, 9th edition. Baltimore, MD: Williams and Wilkins. pp. 525–541.

Oser, B.L. 1995. An integrated essential amino acid index for predicting the biological value of proteins, in protein and amino acid nutrition by Albanase AA, (ed) Academic press, Newyork, 1959, 281.

Othman, A., Ismail, A., Ghani, N. A. and Adenan, I. 2007. Antioxidant capacity and phenolic content of cocoa beans. *Food Chem*. 100 (4): 1523-1530.

Oyarzun, M. T., Uauy, R. and Olivares, S. 2001. Food-based approaches to improve vitamin and mineral nutrition adequacy. *Archivos Latinoamericanos de Nutricion* (Guatemala).51(1):7–18

Pal, J., Ganguly, S., Tahsin, K. S. and Acharya, K. 2010. In vitro free radical scavenging activity of wild edible mushroom, *Pleurotus squarrosulus* (Mont) Singer, *Ind. J. Exp. Biol.* 48 (12): 1210-1218.

Pallavi, J. and Dipika, M. 2010. Effect of dehydration on the nutritive value of drumstick leaves. *J. Metabol. Sys. Biol.* 1 (1): 5-9.

Pallavy, K. and Priscilla, M. D. 2006. Standardization of selected Indian medicinal herbal raw material containing polyphenols as major constituents. *J Pharm Sci*. 68:506-509.

Panda, S. and Kar, A. 2000. Excess use of *Momordica charantia* extract may not be safe with respect to thyroid function and lipid peroxidation. *Current Science.* 79 (2): 222-224.

Parmar, K., Patel, S., Patel, B., Patel, J. and Patel, M. B. 2011. Effects of bitter gourd (*Momordica charantia)* fruit juice on glucose tolerance and lipid profile in type- II diabetic rats. *Int. J. Drug Dev Res*. 3 (2): 139-146.

Patel, S., Patel, T., Parmar, K., Bhatt, Y., Patel, Y. and Patel, N. M. D. 2006. Isolation, characterization and antimicrobial activity of charantin from *Momordica charantia* Linn. Fruit. *Int. J. Drug Deve Res.* 2(3): 629-634.

Patel, S., Patel, T., Patel, B. and Patel, P. 2011. Evaluation of antioxidant activity, phenol and flavonoid contents of *Momordica charantia* Linn fruit. *Adv. Res. Pharm. Biol.* 1(2):120-129.

Paul, A., Mitter, K., Sen, and Raychaudhuri, S. 2009. Effect of polyamines in in vitro somatic embryogenesis in *Momordica charantia L*. *Plant Cell Tissue Organ Culture.* 97: 303-311.

Paul, A. and Raychaudhuri, S. S. 2010. Medicinal uses and molecular identification of two *Momordica charantia* varieties- a review. *Electronic J. Bio.* 6: 43-51.

Paul, A., Bandyopadhyay, S., Acharyya, P. and Raychaudhuri, S. S. 2010. Studies on genetic diversity of twelve accessions of *Momordica charantia* L.

using morphological, RAPD and SCAR markers. *Asian. J. Plant. Sci*. 9: 471.

Pearson, D. 1976. The Chemical Analysis of Foods. 7th ed. Churchill Living stone, London.

Percival, S. S., Talcott, S. T., Chin, S. T., Mallak, A. C., Lound, C. and Pettit-Moore, J. 2006. Neoplastic transformation of BALB/3T3 cells and cell cycle of HL-60 cells are inhibited by mango (*Mangifera indica* L.) juice and mango juice extract. *J. Nutr*. 136 (5): 1300-1304.

Perkinz, P. M., Collins, J. K. and Robert W. 2005. Screening carotenoid content in seeded and seedless watermelon fruit. *J Hort Sci*; 39(4): 830.

Peumans and Damme, K. 1998. Analysis and standardization of lectin ligands from cucurbita fruits. *J Nutr*. 139 (7): 1222-1227.

Pitiphanpong, J., Chitprasert, S., Goto, M., Jiratchariyakul, W., Sasaki, M. and Shotipruk, A. 2007. New approach for extraction of charantin from *Momordica charantia* with pressurized liquid extraction. *Sep Purif Technol*. 52: 416-422.

Poornima, K. and, M and Dabur. 2009. The Hindu survey of Indian Agriculture pp:165-167

Pratheepa, R. and Beatrice, A. D. 2013. Glycemic response of Whey water incorporated idli. *Ind. J. Nutr. Dietet.* 50 (11): 465-472.

Pratibha, V.N., Lee, Y.K., Linden, E.H., Lim, S., Pearson, L., Frank, J. and Nerurkar, V.R. 2006. Lipid lowering effects of *Momordica charantia* (Bitter Melon) in HIV-1-protease inhibitor-treated human hepatoma cells, HepG2. *Brit. J. Pharmacol*.148: 1156–1164.

Praveen, R. L., Rakesh, P., Jeevan, D. and Rittu, P. 2007. Antioxidative properties of different bitter gourd cultivated in Malaysia. *Ind. J Nutr Dietet.* 72(14): 320-322.

Price, G. M., Uauy, R., Breeze, E., Bulpitt, C. J. and Fietcher, A. E. 2006. Weight, shape, and mortality risk in older persons: elevated waist hip ratio, not high body mass index, is associated with a greater risk of death. *Am J. Clin. Nutr*. 84 (2): 449- 460.

Pridham, J. B. 1960. Phenolics in plants in health and disease, Pergamon press, New York, pp34-36.

Pugalanthal, M., Vadivel, V., Gurumoorthi, P. and Janardhanam, K. 2004. Comparative nutritional evaluation of little known legumes *Tamarandus indica, Erythrina indica, Sesbania bispinosa .Trop Subtro Agroecosyst*. 4: 107-123.

Puri, M., Kaur, I., Kanwar, R. K., Gupta, R. C., Chauhan, A. and Kanwar, J. R. 2009. Ribosome inactivating proteins (RIPs) from *Momordica charantia* for anti viral therapy. *Curr. Mol Med*. 9(9): 1080-1094.

Radford, A., H. Ahles, and Bell, C. 1968. Manual of the vascular flora of the Carolinas. Guide to standard flora of the world. 120-125p.

Ragasa, C. Y., Alimboyoguen, A. B., Shen, C. C., Del Fierro, R. S. and Raga, D. D. 2011. Hypoglycemic effects of tea extracts and sterols from *Momordica charantia*. *J. Nat. Remedies*. 11(1): 44-53.

Raghuramalu, N., Nair, M. K. and Kalyansundaram, S. 1983. In a Mannual of laboratory techniques, NIN, ICMR, Hyderabad, India.

Rang, H. P., Dale, M. M. and Ritte, J. M. 2003. Pharmacology. 4th Ed. Churchil livingstone. Melbourne. 385-340.

Ranganna, S. 2001. Hand book of analysis and quality of fruit and vegetable products. Second edition. Tata Mc Graw Hill, publishing compony Ltd, India, p. 112.

Rani, B. 2008. Acceptability study of bitter gourd. *J.Food. Sci.* 2:22-30.

Rao, D. H. and Vijayaraghavan. 1996. Protein Energy Malnutrition in South India. Bulletin No.89. World Health Organization. Geneva, 603-605 p.

Rao, P. K. and Das, H. 2003. Fuzzy logic based optimization of ingredients for production of mango bar and its properties. *Ind. J. Food Sci Technol.* 40: 576-581.

Raschka, L. and Daniel, H. 2005. Mechanisms underlying the effects of inulin-type fructans on calcium absorption in the large intestine of rats. *Bone.* 37 (5): 728–35.

Ratna, R. 2010. Possible cancer cure in karela', also known as bitter melon. The Chakra, 26.

Ren, W., Qian, Z., Wang, H., Zhu, L. and Zhang, L. 2003. Flavonoids : Promising anticancer agents. *Med Res Rev* 23: 519-534.

Renuka, L. 2012. Hypogycemic herbs and their action mechanism. *Chin Med.* 4 (1): 11-14.

Reshmi, R. 2012. Quality evaluation of medicinal rice (*Oryza sativa* L.) cv. Njavara for product development and therapeutic value. PhD. Thesis. Kerala Agricultural University, Thrissur. pp 35-42.

Rezaeizadeh, A., Zuki, A. B. Z., Abdollahi, M., Goh, Y. M., Noordin, M. M., Hamid, M. and Azmi, T. I. 2011. Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. *Afr. J. Biotechnol*. 10: 4932-4940.

Riboli, E. and Horel, T. 2003. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr*. 78 (3) 559–569.

Robert, K. M., Daryl, K. G., Peter, A. M. and Victor, W. R. 2003. Harpers illustrated Biochemistry. In benders and mayes (eds) vitamins and minerals. Lange medical books, Medical publishing division, New York, pp 481-497.

Robertson, G. L. 2000. Shelf life of packaged foods, its measurements and prediction. In developing new products for a changing market place 2000 by CRC Press, Inc.

Robok, J. and Gryglewski, R. J. 1988. Flavonoids are scavengers of superoxide anions. *Biochem Pharmacol.* 37: 837-841.

Roger, P., F. Elie, L., Rose, F., Martin, S., Jacop, A. B., Mercy, and Felicite, M. T. 2005. Methods of preparation and nutritional evaluation of Dishes consumed in a malaria endemic zone in Cameroon (Ngali II). *Afr. J. Biotechnol*. 4(3): 273-278.

Rong, T. 2010.Chemistry and biochemistry of dietary polyphenols, Review. *Nutrients*. 2 (12):1231-1246.

Rose, B., Yadav, F., Parida, P. and Haseena, K. 2014. Study of wild bitter melon species in different geographical area. *J Ethanopharmacol.* 97 (6):156-167.

Rumbaoa, K., Auddy, B., Balsina, L. and Lafon, K. 2009. Screening of antioxidant activity of three Indian medicinal plants traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol*. 84: 131-138.

Sadasivam, S. and A. Manickam, 2004. Biochemical methods. 2nd Edn. New Age International Publications, NewDelhi, India.pp 12-34.

Saeed, K. M., Ahmad, I. Ali, S. and Ejaz, N. 2010. Nutritional facts and reducing power activity of bitter gourd tea. *Pak. J. Food. Sci.* 20 (4): 55-58.

Sakanaka, S., Tachibana, Y. and Okada, Y. 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem.* 89: 569-575.

Sanchez – Moreno, C. 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. technol. Intern*. 8(3): 121-137.

Sandi and Surolia H. 1994. Analysis and isolation of lectin compounds from wild vegetables. *Int J Pharm Sci Rev Res.* 4: 345-349.

Saranya, S. 2012. Development and quality evaluation of enriched Moringa based soup mix (ESM). MSc thesis, Kerala Agricultural University, Thrissur. P 49p.

Sarkar, P., Michael, B. and Krawinkel, M. 1996. Bitter gourd, A dietary approach to hyperglycemia. *Nutr Rev.* 64(7): 331-337.

Sathish, K. D., Vamshi, K. S., Yogeswaran, A., Harani, K., Sudhakar, K., Sudha, P. and David, B. 2010. A medicinal potency of Momordica charantia. *Int. J. Pharm. Sci. Rev. Res.* 1(2):95-99.

Sathishekar, D. 2005. Beneficial effects of *Momordica charantia* seeds in the treatment of STZ-induced diabetes in experimental rats. *Biol. Pharm. Bull.* 28(6): 978-983.

Saura. C. F. and Goni, I. 2006. Antioxidant capacity of the Spanish Mediterranean diet. *Food Chem.* 94: 442-447.

Sawadogo, W. R., Maciuk, A., Banzouzi, J.T., Champy, P., Figadere, B., Guissou, I.P. and Nacoulma, .A.G/ 2011 Mutagenic effect, antioxidant and anticancer activities of six medicinal plants from Burkina Faso. *Nat.Prod Res.* 26(6):545-579

Scartezzini, P. and Speroni, E. 2000. Review on some plants of Indian traditional medicine with antioxidant activity. *J. Ethnopharmacol*., 71 (1-2): 23-43.

Schofield, P., Mbugua, D. M. and Pell, A. N. 2001. Analysis of condensed tannins, a review. Animal feed science technology. 91:21-40.

Semiz, A. and Sen, A. 2007. Antioxidant and chemo protective properties of *Momordica charantia L.* (bitter melon) fruit extract". *Afr. J. Biotech.* 6: 273–277.

Sen, S. and Sen, D. P. 2009. Oxidized fatty acid content of heat damaged frying oils and Indian deep fat fried products. *JOTAI*, pp 89-91.

Serrano, J. 2009. Tannins current knowledge of food sources, intake, bioavailability and biological effects. Molecular nutritional food research. 53: 310-329.

Shahbazpur, N. 2003. Prevalence of overweight and obesity and their relation to hypertension in adult male university students in Kerman, Iran. *Int J. Endocrinol Metab*. 2: 55-60.

Shankar, G. 2003. Role of moisture, temperature and humidity during storage of food grains. Third international Food Convention. 20-23 October 2000. Central Food Technology Research Institute, Mysore, pp 11-16.

Shanmughapriya, R. and Poornima, S. 2014. Detection of charantin in the leaves and fruits of *Momoedica tuberosa* (Cogn) Roxb and *Momordica dioca* (Roxb wild) by analytical HPTLC. *Int. J. Sci. Res. Pub.* 4 (6): 149-151.

Shao, H. T., Jin, H. W., Zhu and J. T. and Liu,2013. Optimization of parameters for ethanol extraction of flavone glycosides from ginkgo cell and antioxidant activity *in Vitro*," *Zhongguo Kuangye Daxue Xuebao. J China Univ. Mining Technol.* 42(4):663-669.

Sharon and Lis. 2003. Lectin ligments analysis in different vegetables. *Emirates J Food Agric.*7 (3):56-59.

Sharma, S., Sharma, M. C., Kohlib, D. V. Chaturvedi, S. C. 2009. Formulation, evaluation and wound healing studies of benzene – 95% absolute ethanol extract of leaves. *J. Optoelectronics. Biomed. Met*. 1(4): 375-378.

Shaw, J. E., Sicree, R. A. Zimmet, P. Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract.* 87: 4 -14.

Shetty, A., Suresh Kumar, G. and Veerayya, S. P. 2005. Bitter gourd (*Momordica charantia*) modulates activities of intestinal and renal disaccharidases in streptozotocin-induced diabetic rats. *Mol. Nutr. Food Res*. 49(8): 791–796.

Shetty, A.K., Kumar, G.S., Sambaiah, K. and Salimath, P.V. 2005. "Effect of bitter gourd (*Momordica charantia*) on glycaemic status in streptozotocin induced diabetic rats". *Plant. Food. Hum. Nutr.* 60 (3): 109–12.

Sindhudevi, P. 1994. Differential preference of work by agricultural labourers and their employment and wage pattern in Thiruvananthapuram District. M.Sc (Ag) thesis, Kerala Agricultural University, Thrissur, 246p.

Singh, J., Adeghate, E., Cummings, E., Giannikipolous, C., Sharma, A.K. and Ahmed, I. 2004. Beneficial effects and mechanism of action of *Momordica charantia* juice in the treatment of streptozotocin-induced diabetes mellitus in rat. *Mol Cell Biochem* 261. 63-70.

Singh, R. K., Dhiman, R. C. and Mittal, P. K. 2006. Mosquito larvicidal properties of Momordica charantia Linn. 43:88p. *J. Vec. Borne Dis*. 43: 88p.

Singh, N., Gupta, M., Sirohi, P. and Varsha. 2008. Effects of alcoholic extracts of *Momordica charantia* whole fruit powder on the pancreatic islets of alloxan diabetic albino rats. *J. Environ Biol.* 29(1): 101-106.

Singh, J., Cumming, E., Manoharan, G., Kalasz, H. and Adeghate, E. 2011. Medicinal chemistry of the anti-diabetic effects of *Momordica charantia*: Active constituents and modes of actions. *Open Med.Chem. J* .5(2): 70-77.

Singh, R., Kumar, A., Bhuvaneshwari, K. And Pandey, K. D. 2012. Gas Chromatography – Mass spectrometry analysis and phytochemical screening of methanolic fruit extract *Momordica charantia. J. Rec. Adv. Agri.* 1 (4): 122-127.

Singh, U., Singh, S. and Kochar, A. 2012. Therapeutic potential of antidiabetic neutraceuticals. *Phytopharmacol.* 2(1): 144-169.

Singh, U. and Sagar, V. R. 2013. Quality characteristics of dehydrated leafy vegetables influenced by packaging materials and storage temperature. *J. Sci. Indus. Res.*69: 785-789.

Sing, P. T., Sophie, E., Parks, Costas, E., Stathopoulos, Paul, D. and Roach. 2014. Extraction of flavonoids from bitter melon. *Food Nutr Sci.* 5: 458-465.

Sitasawad, S. L., Yogita, S., Ramesh, B., Shewade, Y. and Bhonde, R. 2000. Role of bitter gourd fruit in STZ- induced diabetic state in vitro. *J. Ethanapharmacol*. 73(1-2) : 71-79.

Slavin, J. L. 2008. Position of the American Dietetic Association: Health implications of dietary fiber. *J Am Diet Assoc.* 108(10):1716–31.

Slivova, V., Zaloga, G., DeMichele, S. J., Mukerji, P., Huang, Y.S., Siddiqui, R., Harvey, K. 2005. Green tea polyphenols modulate secretion of urokinase plasminogen activator (uPA) and inhibit invasive behavior of breast cancer cells. *Nutr.Res.* 52(1):66-73.

Smirhoff, N. 2000. Ascorbic acid, metabolism and functions of multi facetted molecule. *Curr Opin Plant Biol*. 3(2): 229-235.

Sridhar, M.G., Vinayagamoorthi R, Arul Suyambunathan, V., Bobby, Z. and Selvaraj, N. 2008. Bitter gourd (*Momordica charantia*) improves insulin sensitivity by increasing skeletal muscle insulin-stimulated IRS-1 tyrosine phosphorylation in high-fat-fed rats. *Brit. J. Nutr.* 99 (4) : 806–12.

Stalikas, C.D. 2007. Review: Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci.30*(18): 3268–3295.

Subhasree, B., Baskar, R., Laxmi, K., Lijina, S. and Rajasekaran, P. 2009. Evaluation of antioxidant potential in selected green leafy vegetables, *Food Chem.* 115(4): 1213-1220.

Sultan, A. and Swamy, K. 2005. Phytochemical and qualitative analysis of different bitter gourd varieties. *Int J Pharm Sci.* 6(3):321-326.

Sultana, R. S., and Bari, M. A. 2003. *In vitro* propagation of karela from nodal segment and short tip. *J. Bio. Sci*. 3: 1134-1139.

Sun, J., Chu, Y. F, Wu, X. and Liu, R. H. 2002. Antioxidant and antiproliferative activities of fruits. *J Agric Food Chem*. 50 (25):7449–54.

Suresh, S. N. Nagarajan, N. P. 2009. Preliminary phytochemical and antimicrobial activity analysis of Begonia malabarica Lam. *J. Basic Applied Bio.*, 3(1): 59-61.

Swaminathan M. 1993. Principles of Nutrition and Dietetics. The Banglore printing and publishing Co. Limited. Banglore, pp 334.

Talukdar, N. and Mohammad N. Z. 2014. Phytochemical, phyto therapeutical and pharmacological study of *Momordica dioica. Evid based Complem Alt. Med.*,12: 45-86.

Tan, K., Moein, C. and Hasheedh, K. 2014. Estimation of flavonoids from bitter gourd using different solvent techniques. *Int Drug Dev Res*.4:78-86.

Taylor, L. 2002. Technical data report for bitter melon (*Momordica charantia*), Herbal secrets of the rain forest, 2nd ed., Sage Press Inc.

Tayyab, F., Lal, S. S., Mishra, M. and Kumar, U. 2012. A review: Medicinal plants and its impact on diabetes. *World J. Pharm. Res.* 1(4): 1019-1046.

Thakre, A., Deore, V. Gaikwad, S. and Kawade, S. 2014. Extraction of phytochemical components from the fruit of momrdica charantia and evaluation of its antimicrobial activity. Proceedings of IRF International Conference. 30th March, Pune, 31-32.

Thankappan, K. R., Saha, B., Mathur, P. S., Srinivas, G. and Mini, G. K. 2010. Risk factor profile for chronic non communicable diseases: Results of a community- based study in Kerala, India. *Indian J. Med. Res*. 13: 53-63.

Thite, S. V., Chavan, Y. R., Aparadh, V. T. and Kore, B. A. 2013. Preliminary phytochemical screening of some medicinal plants. *IJPCBS*.3(1): 87-90.

Thomas, C. E. Mclean, R. A. Parker, D. and Ohlweiler, F. 1992. Ascorbate and phenolic antioxidant interactions in prevention of liposomal oxidation. *Lipids.* 27(7): 543-550.

Thomas, C. T., Reddy, P. Y. and Devanna, N. 2012. Impact of cooking on charantin estimated from bitter melon fruits using high performance thin layer chromatography. *Int. Res. J. Pharm*. 3: 149-154.

Traore, F., Faure, R., Ollivier, E., Gasquet, M. and Azas, N. 2000. Structural and antiprotozoal activity of triterpenoid saponins from *Glinus oppositifolius* planta medica. 66: 365-371.

Tsai, H. Ching, J. H., Wen, H. W., Jong, H. and Jung, T. 2014. Antioxidant, cell-protective, and anti-melanogenic activities of leaf extracts from wild bitter melon (*Momordica charantia* Linn. var. *abbreviata* Ser.) cultivars *Bot Stud.* 55:78

Tupe, S. B., Patil, p D. Thoke, R. B. and Aparadh, v. T. 2013. Phytochemical screening in some cucurbitaceae members. *Int. J. Pharm. Appl. Sci.* 3 (1): 49-51.

Ullah, M. Showkat, M. Ahmed, N.U. Islam, S. and Absar, N. 2011. Evaluation of *Momordica charantia* L. Fruit extract for analgesic and anti-inflammatory activities using *in vivo* assay. *Res. J. Med. Plant.,* 6: 236-244.

Umesh, C., Yadav, S., Moorthy, K., Najma, Z. and Baquer. 2005. Combined treatment of sodium orthovanadate and *Momordica charantia* fruit extract prevents alterations in lipid profile and lipogenic enzymes in alloxan diabetic rats. *Mol Cell Biochem*. 268: 111-120.

Valachovicova, T. and Sliva, D. 2005. Green tea polyphenols modulate secretion of urokinase plasminogen activator (uPA) and inhibit invasive behavior of breast cancer cells. *Nutr. Cancer*. *52*(1): 66–73.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M. and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 39:44–84

Vermerris, W. and Nicholson R. L. 2006. Phenolic compound biochemistry. Springer, Netherland, 13:p 288.

Vijayaraghavan, K. 1987. Anthropometry for assessment of Nutritional Status. *Ind. J. Pediatr*. 54 (4): 511-520.

Virdi, J., Sivakami, S., Shahani, S., Suthar, A. C., Banavalikar, M. M. and Biyani, M. K. 2003. Antihyperglycemic effects of three extracts from *Momordica charantia. J. Ethanopharmacol*. 88 (1): 107-111.

Visarata, N. and Ungsurungsie, M. 1981. "Extracts from *Momordica charantia L*. *J. Crude Drug Res* .19:75-80.

Wadood, P., Ghufran, M., Babar, J., Naeem, M. Ajmal, R. 2013. Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochem Anal Biochem*.2(1):32-39.

Waheed, A., Maiana, G. A., Tariq, S. and Ahmad, S. I. 2008. Clinical investigation of hypoglycemic effect of unripe fruit on *Momordica charantia* in type 2 diabetes mellitus. *Pak. J. Pharmacol.* 25(1): 7-12.

Wako, P. J., Gumede, B., Smith, P., and Folb, P. I. 2005. The *in vitro* and *in vivo* anti malarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch. Et Thonn. *J. Ethnopharmacol*. 99(1): 137–43.

Walls, P., Tairou, F. and Van Allen M. 2007. Reduction in neural-tube defects after folic acid fortification in Canada. *New Engl J Med.* 357(2):135-42.

Wang, H.X. and Ng, T.B. 2001. Studies on the anti-mitogenic, anti-phage and hypotensive effects of several ribosome inactivating proteins. Comp Biochem Physiol C-*Pharmacol Toxicol.* 128 (3): 359–366.

Wang, 2007. Keeping quality of fresh cut bitter gourd at low temperature of storage. *J. Food. Process. Pres*.31 (5):23-45.

Wargovich, M. J. 2000. Anticancer properties of fruits and vegetables. *Hort Science.* 35:573-575.

Weber, P. 2001. Vitamin K and bone health. *Nutrition*. 17: 880-887.

Wehash, F. E., Ghanema, A. and Salesh, R. M. 2012. Some physiological effects of *Momordica charantia* and *Trigonella foenum-graecum* extracts in diabetic rats as compared with cidophage. *World Acad Sci Eng Technol*. 64: 1206-1214.

Whitney, E. and Rolfe, S. R. 2005. Understanding Nutrition. 13th edition Centage Advantage Books

WHO.2010. Diabetes: Quick Diabetes facts. http://www.who.int/diabetes/en/

Wildman, R. E. C. 2001. Nutraceuticals: A brief review of historical and teleological aspects. In: Handbook of nutraceuticals and functional foods. CRC Press, Florida; pp 2 -12.

Wink, M., Schmekker, T. and Latz, B. 1998. Modes of action of allelochemical alkaloids, interaction with neuroreceptors, DNA and other molecular targets. *J Chem Ecol.* 24(1):1888-1937.

Wood, L. G., Gibson, P. G. and Garg, M. L. 2006. A review of the methodology for assessing *in vivo* antioxidant capacity. *J. Sci. Food Agric.* 86 (13): 2057-2066.

Wu, S. and L. Ng. T.B. 2008. Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. abbreviata Ser.) in Taiwan. LWT-*Food Sci. Technol*. 41:323–330.

www.diabeteselfmanagement.com.National bitter melon council: Cooking With bitter melon.

www.National Institute of Health: Vitamin C. Article reviewed by MER Last updated on: Mar 23, 2010.

Yamagishi, S. I., Edelstein, D., Du, X. L. and Brownlee, M. 2001. Hyperglycemic potentials collagen induced platelet activation through mitochondrial superoxide overproduction, *Diabetes*.50 (6): 1491-1494.

Zhang, Z., Liao, L., Moore, J., Wu, T. and Wang, Z. 2009. Antioxidant phenolic compounds from walnut kernels. *Food Chem.* 113(1): 160-165.

Zhou, Y. C. and Zheng, R. L. 1991. Phenolic compounds and as analog as superoxide anion scavengers and antioxidants, *Biochem Pharmacol*. 42: 1177-1179.

Zygory, D. 2003. Effect of post processing, handling and packaging on microbial population. Post harvest news and information on fresh fruit and vegetable quality and food safety. *Post harvests Biol. Tech*. 15: 313.

APPENDIX-I A

KERALA AGRICULTURAL UNIVERSITY

COLLEGE OF AGRICULTURE, VELLAYANI

DEPARTMENT OF HOME SCIENCE

Questionnaire to elicit information on the Socio economic profile, anthropometric data and health profile of the selected respondents.

1. Name of the respondents :
2. Age :
3. Sex :
4. Religion :
5. Caste :
6. Address :
7. Type of family :
8. Size of family : Nuclear/Joint
9. Educational qualification :
10. Occupation :
11. Monthly income :
12. Monthly expenditure for food :
13. Monthly expenditure for health care :
14. Do you have a family history for :
    a) Diabetes
    b) Hyperlipidemia
    c) Cardiac problem

d) Hypertension

e) Arthritis

f) Any other

15. Do you have any of the above disease :

16. If yes, how long have been affected :

17. Anthropometric parameters

a) Height

b) Weight

c) BMI

d) Waist Hip Ratio

18. What was your blood profile last recorded for

Hyperglycemia
Hypercholestremia
Hypertension

19. Are you under any medicine : Yes/No

20. Do you consume any medicine : Yes/No

APPENDIX-I B

Questionnaire to elicit information on the Life style pattern of the selected respondents

1. Do you have any stress and strain in your day to day life : Yes/No

2. If yes, type
    a) Occupational / family problem
    b) Health problem
    c) Financial problem
    d) Old age problem
    e) Any other

3. Do you practice any relaxation technique : Yes/No

    If yes what is it

4. Do you have the habit of doing exercise : Yes/No

    If yes, specify

    Time :

    Duration :

    Type :

APPENDIX-I C

Questionnaire to elicit information on the Dietary intake of the selected respondents

1. Food habit : Veg /Non veg

2. No. of meals taken/ day

Two times	Three times	Four times

3. Are you following any special diet :Yes/No
   If yes, give details

4. Do you purchase convenience foods Yes/No

   How often Daily/ weekly/ monthly/ occasionally/ rarely

5. Frequency of use of various food items in the diet by the respondents

Food items	Daily	Alternative days	Twice in a week	Once in a week	Occasionally
Cereals					
Pulses					
Vegetables					
Meat					
Fish					
Fruits					
Milk& milkproducts					
Coffee/Tea					
Beverages					

Bakery items					
Oil used for cooking					

APPENDIX II

Individual case study reports of ten respondents

Ten human subjects (five males and five females) in the age group of 45-55 years were selected for the case study (Subject A to J).

Subject A

1) Socio economic profile

Age	:	45 yrs
Gender	:	Male
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 50, 000 - 60, 000
Educational status	:	Degree
Occupation	:	Govt

2) Duration of diseases and other related problems

The patient is having diabetes for the past three years and slight hyperlipidemic. In his family his father is diabetic and his mother is hyperlipidemic. There was no diabetes related diseases found in him

3) Life style pattern

The subject is having a moderate activity, with irregular exercise habit. He is a non smoker and non alcoholic. He is having some stress and strain in his daily life which was related to health and job.

4) Dietary habit

The subject is a non-vegetarian. He include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was included once in a month in the diet. His frequency of meals was three meals per day and the oil used for cooking is sunflower oil. He is having bakery foods and fruits twice in a week. He has an average calorie intake of 1598 Kcal/day.

5) Anthropometric data

Height (cm)	:	179

Weight (kg)	:	80
BMI	:	24.96
Category	:	Over weight
Waist circumference (cm)	:	100
Hip circumference (cm)	:	103
Waist Hip Ratio	:	0.97

Interpretation

The subject is an overweight person, with waist hip ratio 0.97. The subject was given 10 g bitter gourd powder daily for 2 months.

6) Effect of bitter gourd powder supplementation

The subjects were willingly participated in the supplementation study and was not taking any oral hypoglycemic agents for controlling the disease. Bitter gourd supplement prepared in the laboratory were distributed to the subjects for a period of two months. Close observation was made by the investigator and ensured that the subjects were consuming the supplement promptly. The efficacy of the bitter gourd supplement was assessed by monitoring blood sugar (fasting blood sugar, post prandial blood sugar, HbA_1C) and blood cholesterol levels at different intervals, initially, 30^{th} day and 60^{th} day. The blood sample of the subjects were collected and blood profile was analyzed and the details are given in the Table 37 to 46.

Changes in blood glucose and cholesterol level

Monitoring intervals	Fasting blood sugar (FBS) mg/dl	Post Prandial blood sugar (PPBS) mg/dl	Glycosylated Haemoglobin (HbA_1C) (%)	Total cholesterol (TC) mg/dl
Initial	160	210	6.8	250
Intermittent (30^{th} day)	140	180	….	225
Final (60^{th} day)	108	128	5.3	184
Reduction in blood sugar level (%)	**32.5 %**	**39.04 %**	**22.05 %**	**26.4 %**

The result revealed that initial value obtained for fasting blood glucose of subject A was 160 mg/dl, post prandial blood sugar was 210 mg/dl, glycosylated haemoglobin was 6.8 per cent ant total cholesterol was found to be 250mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60 th day, fasting blood sugar level was again monitored and the value were 108 mg/dl, PPBS was 128 mg/dl, HbA_1C was 5.3 per cent and Total cholesterol was found to be 184 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

7) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject B

1) Socio economic profile

Age : 50 yrs

Gender	:	Male
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 70, 000 - 80, 000
Educational status	:	PG
Occupation	:	Business and Politics

2) Duration of diseases and other related problems

The patient is having diabetes for the past one year and hyperlipidemic. In his family his father and mother is diabetic and his father is hyperlipidemic. There was no diabetes related diseases found in him.

3) Life style pattern

The subject is having a moderate activity and no habit of doing exercise. He is a non smoker and non alcoholic. He is having some stress and strain in his daily life which was related to job.

4) Dietary habit

The subject is a non-vegetarian. He include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was included weekly. His frequency of meals was three meals per day and the oil used for cooking is coconut oil. He is having bakery foods and fruits daily especially fried foods. He has a high calorie intake of 2800 Kcal/day.

5) Anthropometric data

Height (cm)	:	173
Weight (kg)	:	78
BMI	:	26.06
Category	:	Obese Grade 1
Waist circumference (cm)	:	108
Hip circumference (cm)	:	110
Waist Hip Ratio	:	0.98

Interpretation

The subject is an obese person, with waist hip ratio 0.98. The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	Fasting blood sugar (FBS) mg/dl	Post Prandial blood sugar (PPBS) mg/dl	Glycosylated Haemoglobin (HbA_1C) (%)	Total cholesterol (TC) mg/dl
Initial	200	240	7.1	300
Intermittent (30^{th} day)	180	215	….	260
Final (60^{th} day)	147	164	6.5	221
Reduction in blood sugar level (%)	**26.5 %**	**31.6 %**	**8.4 %**	**26.3 %**

The result revealed that initial value obtained for fasting blood glucose of subject B was 200 mg/dl, post prandial blood sugar was 240 mg/dl, glycosylated haemoglobin was 7.1 per cent ant total cholesterol was found to be 300 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60^{th} day, fasting blood sugar level was again monitored and the value were 147 mg/dl, PPBS was 164 mg/dl, HbA_1C was 6.5 per cent and total cholesterol was found to be 221 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject C

1) Socio economic profile

Age	:	55 yrs
Gender	:	Male
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 20, 000 - 30, 000
Educational status	:	Pre-degree
Occupation	:	Poojari

2) Duration of diseases and other related problems

The patient is having diabetes for the past six month and slight hyperlipidemic. In his family no one is diabetic and hyperlipidemic. There was no diabetes related diseases found in him.

3) Life style pattern

The subject is having a moderate activity and regular exercise habit. He is a non smoker and non alcoholic. He is having some stress and strain in her daily life which was related to job.

4) Dietary habit

The subject is a vegetarian. He include cereals, pulses, vegetables, milk and milk products and tea in his daily diet. His frequency of meals was three meals per day and the oil used for cooking is mustard oil and rice bran oil. He is having bakery foods and fruits daily especially fried foods. He has an average calorie intake of 2000 Kcal/day.

5) Anthropometric data

Height (cm)	:	153

Weight (kg) : 48

BMI : 20.50

Category : Normal

Waist circumference (cm) : 83

Hip circumference (cm) : 90

Waist Hip Ratio : 0.92

Interpretation

The subject is a normal person, with waist hip ratio 0.92. The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	**Fasting blood sugar (FBS) mg/dl**	**Post Prandial blood sugar (PPBS) mg/dl**	**Glycosylated Haemoglobin (HbA_1C) (%)**	**Total cholesterol (TC) mg/dl**
Initial	175	200	7.5	205
Intermittent (30^{th} day)	160	185	….	180
Final (60^{th} day)	138	169	6.5	143
Reduction in blood sugar level (%)	**21.14 %**	**15.5 %**	**13.33 %**	**30.24 %**

The result revealed that initial value obtained for fasting blood glucose of subject C was 175 mg/dl, post prandial blood sugar was 200 mg/dl, glycosylated haemoglobin was 7.5 per cent ant total cholesterol was found to be 205 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60 th day, fasting blood sugar level was again monitored and the value were 138 mg/dl, PPBS was 169 mg/dl,

HbA_1C was 6.5 per cent and total cholesterol was found to be 143 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject D

1) Socio economic profile

Age	:	45 yrs
Gender	:	Male
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 70, 000 - 90, 000
Educational status	:	PG
Occupation	:	Assistant director

2) Duration of diseases and other related problems

The patient is having diabetes for the past one year and slight hyperlipidemic. In his family only his sister is having diabetes. There was no diabetes related diseases found in him.

3) Life style pattern

The subject is having a moderate activity and no exercise habit. He is a non smoker and non alcoholic. He is having some stress and strain in her daily life which was related to job.

4) Dietary habit

The subject is a non vegetarian. He include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was included thrice in a day in a normal quantity. His frequency of meals was three meals per

day and the oil used for cooking is coconut and rice bran oil. He is having bakery foods and fruits daily. He has an average calorie intake of 2200 Kcal/day.

5) Anthropometric data

Height (cm) : 176

Weight (kg) : 79

BMI : 25.50

Category : Obese Grade 1

Waist circumference (cm) : 112

Hip circumference (cm) : 116

Waist Hip Ratio : 0.96

Interpretation

The subject is an obese person, with waist hip ratio 0.96. The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	**Fasting blood sugar (FBS) mg/dl**	**Post Prandial blood sugar (PPBS) mg/dl**	**Glycosylated Haemoglobin (HbA_1C) (%)**	**Total cholesterol (TC) mg/dl**
Initial	127	159	6.2	219
Intermittent (30^{th} day)	110	131	….	195
Final (60^{th} day)	101	119	5.3	140
Reduction in blood sugar level (%)	**20.47 %**	**25.15 %**	**14.51 %**	**36.07 %**

The result revealed that initial value obtained for fasting blood glucose of subject D was 127 mg/dl, post prandial blood sugar was 159 mg/dl,

glycosylated haemoglobin was 6.2 per cent ant total cholesterol was found to be 219 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60 th day, fasting blood sugar level was again monitored and the value were 101 mg/dl, PPBS was 119 mg/dl, HbA_1C was 5.3 per cent and total cholesterol was found to be 140 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject E

1) Socio economic profile

Age	:	48 yrs
Gender	:	Male
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 30, 000 - 40, 000
Educational status	:	Degree
Occupation	:	Conductor (Govt.)

2) Duration of diseases and other related problems

The patient is having diabetes for the past three year and hyperlipidemic. In his family his father, mother and sister were having diabetes. There was no diabetes related diseases found in him.

3) Life style pattern

The subject is having a moderate activity and no exercise habit. He is a non smoker and non alcoholic. He is having some stress and strain in her daily life which was related to job.

4) Dietary habit

The subject is a non vegetarian. He include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was included weekly. His frequency of meals was three or four meals per day and the oil used for cooking is coconut and sun flower oil. He is having bakery foods daily and use of fruits were weekly. He has a heavy calorie intake of 3100 Kcal/day.

5) Anthropometric data

Height (cm)	:	180
Weight (kg)	:	86
BMI	:	26.54
Category	:	Obese Grade 1
Waist circumference (cm)	:	110
Hip circumference (cm)	:	115
Waist Hip Ratio	:	0.95

Interpretation

The subject is an obese person, with waist hip ratio 0.95. The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	Fasting blood sugar (FBS) mg/dl	Post Prandial blood sugar (PPBS) mg/dl	Glycosylated Haemoglobin (HbA_1C) (%)	Total cholesterol (TC) mg/dl
Initial	196	249	8.7	295
Intermittent (30^{th} day)	180	220	….	240
Final (60^{th} day)	170	200	7.6	219
Reduction in blood sugar level (%)	**13.26 %**	**19.67 %**	**12.6 %**	**25.76 %**

The result revealed that initial value obtained for fasting blood glucose of subject E was 196 mg/dl, post prandial blood sugar was 249 mg/dl, glycosylated haemoglobin was 8.7 per cent ant total cholesterol was found to be 295 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60 th day, fasting blood sugar level was again monitored and the value were 170 mg/dl, PPBS was 200 mg/dl, HbA_1C was 7.6 per cent and total cholesterol was found to be 219 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject F

1) Socio economic profile

Age : 45 yrs

Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 40, 000 - 50, 000
Educational status	:	PG
Occupation	:	Teacher

2) Duration of diseases and other related problems

The patient is having diabetes for the past one year and hyperlipidemic. In her family her father and mother were having diabetes and she has a history of thyroid problem but now on control. There was no diabetes related diseases found in her.

3) Life style pattern

The subject is having a moderate activity and no exercise habit. She is a non smoker and non alcoholic. She is having some stress and strain in her daily life which was related to job and house hold activities.

4) Dietary habit

The subject is a non vegetarian. She include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was included twice a week. Her frequency of meals was three meals per day and the oil used for cooking is coconut and sun flower oil. She is having bakery foods daily and use of fruits were weekly. She has a heavy calorie intake of 2300 Kcal/day.

5) Anthropometric data

Height (cm)	:	150
Weight (kg)	:	60
BMI	:	26.6
Category	:	Obese Grade 1
Waist circumference (cm)	:	95
Hip circumference (cm)	:	110
Waist Hip Ratio	:	0.86

Interpretation

The subject is a obese person, with waist hip ratio 0.86. The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	**Fasting blood sugar (FBS) mg/dl**	**Post Prandial blood sugar (PPBS) mg/dl**	**Glycosylated Haemoglobin (HbA_1C) (%)**	**Total cholesterol (TC) mg/dl**
Initial	170	210	6.9	260
Intermittent (30^{th} day)	151	186	….	217
Final (60^{th} day)	120	144	6	183
Reduction in blood sugar level (%)	**29.41 %**	**31.42 %**	**13.04 %**	**29.61 %**

The result revealed that initial value obtained for fasting blood glucose of subject F was 170 mg/dl, post prandial blood sugar was 210 mg/dl, glycosylated haemoglobin was 6.9 per cent ant total cholesterol was found to be 260 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60^{th} day, fasting blood sugar level was again monitored and the value were 120 mg/dl, PPBS was 144 mg/dl, HbA_1C was 6 per cent and total cholesterol was found to be 183 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject G

1) Socio economic profile

Age	:	45 yrs
Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 30, 000 - 40, 000
Educational status	:	PG Diploma
Occupation	:	Trainer

2) Duration of diseases and other related problems

The patient is having diabetes for the past two year and slight hyperlipidemic. In her family her mother is having diabetes. There was no diabetes related diseases found in her.

3) Life style pattern

The subject is having a moderate activity and irregular exercise habit. She is a non smoker and non alcoholic. She is having some stress and strain in her daily life which was related to job, disease and house hold activities.

4) Dietary habit

The subject is a non vegetarian. She include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was not included. Her frequency of meals was three meals per day and the oil used for cooking is coconut and sun flower oil. She is having bakery foods daily and use of fruits were thrice a week and the habit of having nuts. She has an average calorie intake of 1710 Kcal/day.

5) Anthropometric data

Height (cm) : 156

Weight (kg) : 70

BMI : 28.76

Category : Obese Grade II

Waist circumference (cm) : 98

Hip circumference (cm) : 109

Waist Hip Ratio : 0.89

Interpretation

The subject is a obese person, with waist hip ratio 0.89. The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	**Fasting blood sugar (FBS) mg/dl**	**Post Prandial blood sugar (PPBS) mg/dl**	**Glycosylated Haemoglobin (HbA_1C) (%)**	**Total cholesterol (TC) mg/dl**
Initial	170	225	7.4	212
Intermittent (30^{th} day)	130	190	….	175
Final (60^{th} day)	105	136	6.1	135
Reduction in blood sugar level (%)	**38.23 %**	**39.55 %**	**17.56 %**	**36.32 %**

The result revealed that initial value obtained for fasting blood glucose of subject G was 170 mg/dl, post prandial blood sugar was 225 mg/dl, glycosylated haemoglobin was 7.4 per cent ant total cholesterol was found to be 212 mg/dl. After supplementation for 30 days, blood profile was monitored and

found that the level has reduced. At the end of the 60 th day, fasting blood sugar level was again monitored and the value were 105 mg/dl, PPBS was 136 mg/dl, HbA_1C was 6.1 per cent and total cholesterol was found to be 135 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject H

1) Socio economic profile

Age	:	52 yrs
Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 20, 000 - 30, 000
Educational status	:	Degree
Occupation	:	Housewife

2) Duration of diseases and other related problems

The patient is having diabetes for the past one year after menopause and slight hyperlipidemic. In her family her mother and brother were having diabetes and she has a history of gestational diabetes. There was no diabetes related diseases found in her.

3) Life style pattern

The subject is having a moderate activity and irregular exercise habit. She is having some stress and strain in her daily life which was related to disease and house hold activities.

4) Dietary habit

The subject is a non vegetarian. She include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was used

once in a week. Her frequency of meals was three meals per day and the oil used for cooking is coconut. She is having bakery foods daily and use of fruits were once in a week and the habit of having too much fried foods. She has an high calorie intake of 2080 Kcal/day.

5) Anthropometric data

Height (cm)	:	153
Weight (kg)	:	60
BMI	:	25.63
Category	:	Over weight
Waist circumference (cm)	:	95
Hip circumference (cm)	:	101
Waist Hip Ratio	:	0.94

Interpretation

The subject is a obese person, with waist hip ratio 0.94. The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	Fasting blood sugar (FBS) mg/dl	Post Prandial blood sugar (PPBS) mg/dl	Glycosylated Haemoglobin (HbA_1C) (%)	Total cholesterol (TC) mg/dl
Initial	145	195	6	246
Intermittent (30^{th} day)	140	160	….	217
Final (60^{th} day)	130	155	5.8	180
Reduction in blood sugar level (%)	**10.34 %**	**20.51 %**	**3.33 %**	**26.82 %**

The result revealed that initial value obtained for fasting blood glucose of subject H was 145 mg/dl, post prandial blood sugar was 195 mg/dl, glycosylated haemoglobin was 6 per cent ant total cholesterol was found to be 246 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60 th day, fasting blood sugar level was again monitored and the value were 130 mg/dl, PPBS was 155 mg/dl, HbA_1C was 5.8 per cent and total cholesterol was found to be 180 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject I

1) Socio economic profile

Age	:	52 yrs
Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 30, 000 - 40, 000
Educational status	:	Degree
Occupation	:	Govt job

2) Duration of diseases and other related problems

The patient is having diabetes for the past three year, hyperlipidemic and hypertensive but not on medication and problem of hernia and a history of uterus removal surgery. In her family no one were having diabetes. There was no diabetes related diseases found in her.

3) Life style pattern

The subject is having a moderate activity and irregular exercise habit. She is having some stress and strain in her daily life which was related to disease and family problems.

4) Dietary habit

The subject is a non vegetarian. She include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was used once in a month. Her frequency of meals was three meals per day and the oil used for cooking is coconut oil. She is having bakery foods sometimes and use of fruits were once in a week. She has an average calorie intake of 1673 Kcal/day.

5) Anthropometric data

Height (cm)	:	158
Weight (kg)	:	72
BMI	:	28.84
Category	:	Obese Grade II
Waist circumference (cm)	:	98

Hip circumference (cm) : 95

Waist Hip Ratio : 1.03

Interpretation

The subject is a obese person, with waist hip ratio 1.03.The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	**Fasting blood sugar (FBS) mg/dl**	**Post Prandial blood sugar (PPBS) mg/dl**	**Glycosylated Haemoglobin (HbA_1C) (%)**	**Total cholesterol (TC) mg/dl**
Initial	175	225	6.9	236
Intermittent (30^{th} day)	170	209	….	204
Final (60^{th} day)	160	178	6.2	183
Reduction in blood sugar level (%)	**8.57 %**	**20.88 %**	**10.14 %**	**22.45 %**

The result revealed that initial value obtained for fasting blood glucose of subject I was 175 mg/dl, post prandial blood sugar was 225 mg/dl, glycosylated haemoglobin was 6.9 per cent ant total cholesterol was found to be 236 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60^{th} day, fasting blood sugar level was again monitored and the value were 160 mg/dl, PPBS was 178 mg/dl, HbA_1C was 6.2 per cent and total cholesterol was found to be 183 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

6) Glucose Tolerance Test (GTT)

Fig. shows the GTT of the subject, and the result revealed that for bitter gourd powder the values were lower when compared to reference food.

Subject J

1) Socio economic profile

Age	:	53 yrs
Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (Monthly)	:	Rs. 20, 000 - 40, 000
Educational status	:	Pre degree
Occupation	:	House wife

2) Duration of diseases and other related problems

The patient is having diabetes for the past four year, hyperlipidemic and hypotensive but not on medication. In her family her father is diabetic and mother is hypotensive and she is suffering from joint pain. There was no diabetes related diseases found in her.

3) Life style pattern

The subject is having a moderate activity and irregular exercise habit. She is having some stress and strain in her daily life which was related to house hold activities.

4) Dietary habit

The subject is a non vegetarian. She include cereals, pulses, vegetables, milk and milk products, tea and fish in his daily diet, where as meat was used twice in a month. Her frequency of meals was three meals per day and the oil used for cooking is coconut. She is having bakery foods and fried snacks daily and use sugar also, and use of fruits were once in a week. She has an average calorie intake of 2179 Kcal/day.

5) Anthropometric data

Height (cms) : 155

Weight (Kg) : 77

BMI : 32.04

Category : Obese Grade III

Waist circumference (cm) : 107

Hip circumference (cm) : 112

Waist Hip Ratio : 0.95

Interpretation

The subject is a obese person, with waist hip ratio 0.95.The subject was given 10 g bitter gourd powder daily for 2 months.

Changes in blood glucose and cholesterol level

Monitoring intervals	**Fasting blood sugar (FBS) mg/dl**	**Post Prandial blood sugar (PPBS) mg/dl**	**Glycosylated Haemoglobin (HbA_1C) (%)**	**Total cholesterol (TC) mg/dl**
Initial	200	265	8	294
Intermittent (30^{th} day)	163	223	….	250
Final (60^{th} day)	141	196	6.8	212
Reduction in blood sugar level (%)	**29.5 %**	**26.03 %**	**15 %**	**27.89 %**

The result revealed that initial value obtained for fasting blood glucose of subject J was 200 mg/dl, post prandial blood sugar was 265 mg/dl,

glycosylated haemoglobin was 8 per cent ant total cholesterol was found to be 294 mg/dl. After supplementation for 30 days, blood profile was monitored and found that the level has reduced. At the end of the 60 th day, fasting blood sugar level was again monitored and the value were 141 mg/dl, PPBS was 196 mg/dl, HbA_1C was 6.8 per cent and total cholesterol was found to be 212 mg/dl. A steady decline was observed in the subject studied with regard to blood sugar levels and blood cholesterol level.

APPENDIX III

Oral Glucose tolerance test (GTT)

GTT is carried out after 12 hrs. of overnight fasting. Glucose 75g in adults is orally administered. Before the glucose load and 15 minutes to 120 minutes after the administration of glucose blood samples were collected and glucose levels were estimated.

The diagnostic criteria for diabetes and impaired glucose tolerance, the fasting blood sugar levels vary between 80-110 mg/100ml. The blood sugar levels increase after the glucose load and come down to basal level within 2 hrs.WHO criteria with glucose load 75g for adult or 1.75g/Kg/body wt. (To a maximum of 75g).

	Glucose concentration		
	Venous whole blood	**Capillary whole blood (mg/dl)**	**Venous plasma (mg/dl)**
	Normal	**Diabetes Mellitus**	**Confirmed**
Fasting	120	120	140
2 hours after glucose load	180	200	200
	Impaired glucose tolerance		
Fasting	120	120	140
2 hours after glucose load	120-180	140-200	140-200

(WHO, 1980)

NUTRIENT COMPOSITION, ANTIOXIDANT AND HYPOGLYCEMIC EFFECT OF BITTER GOURD (*Momordica charantia* L.)

by

KRISHNENDU J.R.
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Abstract of the
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ABSTRACT

The present investigation entitled, "Nutrient composition, antioxidant and hypoglycemic effect of bitter gourd (*Momordica charantia* L.)" was conducted in Thiruvanathapuram district during the period of 2012- 2015 with an objective to study the nutrient composition, phytochemical analysis, antioxidant activities of the selected bitter gourd types along with assessing its hypoglycemic effect on type 2 diabetes mellitus patients.

Four types of commercially cultivated bitter gourd viz., light green small, light green big, dark green small, dark green big along with *nei paval* were selected for the study. The chemical/ nutrient composition, phytochemical and antioxidant activities of the selected types were carried out both in fresh and processed (powdered) forms and the best type was selected (large green) to ascertain the clinical efficacy of the bitter gourd powder. Investigations such as shelf life quality, yield ratio, cost of production, glycemic index and glycemic load were also determined.

Significant differences were found in the nutrient content of fresh and dried bitter gourd types. The highest protein, moisture, vitamin C and folic acid were found in light green big both in the case of fresh and dried samples. The total essential and non essential amino acids were also found highest in light green big. Highest carbohydrate content was observed in light green small type both in the case of fresh and dried samples. β carotene content was found to be highest in *neipaval* both in fresh and dry samples (140.03 mcg/ 100g and 98.93 mcg/ 100g respectively).

In the case of mineral analysis, highest calcium, phosphorus, sodium, manganese, copper and zinc content were observed in light green big. The

potassium and iron content was found to be highest in *nei paval* both fresh and dried samples.

Quantitative estimation of phytochemicals revealed that, highest polyphenol content was noticed in light green big type both in the fresh and dried forms (18.76 mg and 74.67 mg respectively). The highest flavonoid content was found in light green big. The alkaloid content of bitter gourd samples (fresh) ranged between 0.10 to 0.27 per cent where as in the case of dried bitter gourd samples it ranged between 0.90 to 1.01 per cent. Tannin content was found higher in *nei paval*. Saponin and charantin content was found highest in light green big while lectin content was observed to be higher in dark green small type.

Antioxidant activity in the present study revealed that fresh light green big type had the highest DPPH activity with an IC_{50} value of 50.88 µg/ ml in methanol solvent. The hydroxyl radical scavenging activity of light green big was found to be higher both in the case of fresh and dried bitter gourd samples with IC_{50} value of 50.95 µg/ ml and 50.10 µg/ ml respectively. Fresh light green small types showed higher superoxide anion radical scavenging activity with an IC_{50} value of 50.36 µg/ ml but in dried samples, the value was 49.76 µg/ ml. Antioxidant activity ranged with an IC_{50} value of 50.09 µg/ ml to 61.90 µg/ ml in fresh bitter gourd samples. Maximum antioxidant capacity was observed in light green big fresh (50.09 µg/ ml) and in the case of dried bitter gourd samples, the highest antioxidant activity was observed in light green big (50.07 µg/ ml).

Assessment of the shelf life qualities of the bitter gourd powder revealed that mean moisture level of bitter gourd powder after six month storage was 6.63 per cent. The peroxide content was noticed in the powder only in 5^{th} month (0.10 meq/100 g) and 6^{th} month (0.12 meq/100 g) of storage. During the storage period of six months, bacterial colonies were observed in the 5^{th} month (1×10^{7}cuf/g) and 6^{th} month (2×10^{7} cuf/g) and were only in negligible amounts.

Clinical efficacy of the bitter gourd powder revealed that during pre-test, the mean fasting blood sugar was 171.8 mg/dl while in the post test it decreased to 132 mg/dl. The mean value for post prandial blood sugar during pre- test was found to be 217.8 mg/ dl while for post test it decreased to 155.9 mg/ dl. The mean value for glycosylated haemoglobin of pre- test was found to be 7.15 per cent while it decreased to 6.21 per cent after supplementation. Total cholesterol revealed a significant difference at 5 per cent level. The mean initial total cholesterol level of the subjects under study was 251.7 mg/ dl. After the supplementation, the total cholesterol level decreased to 180 mg/ dl. In the present study, bitter gourd powder was having a GI of 64 which was around 36 per cent less than that of glucose. The results of glycemic load revealed that bitter gourd powder had a glycemic load of 39.

Findings of the present investigation strongly recommend that bitter gourd powder supplementation reduces the blood sugar as well as blood cholesterol level and is efficient in the dietary management of the subjects with diabetes mellitus.