ANTIOXIDANT AND ANTICARCINOGENIC POTENTIAL OF JACKFRUIT BASED READY-TO-COOK (RTC) CURRY MIXES.

by

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(2017-16-005)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Antioxidant and anticarcinogenic potential of jackfruit based Ready-To-Cook (RTC) curry mixes"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any University or Society.

Place: Vellayani
Date: 20-09- 2019

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Certified that this thesis entitled **"Antioxidant and anticarcinogenic potential of jackfruit based Ready-To-Cook (RTC) curry mixes"** is a record of research work done independently by **Ms. Gayathri Mohan (2017-16-005)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

AAE	Ascorbic acid equivalent
AFR	Ascorbate free radical
AOAC	Association of Official Agricultural Chemists
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
CD	Critical Difference
Cfu	Colony Forming Units
CHD	Coronary Heart Disease
CMV	Cytomegalovirus
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picryl hydrazyl
EDTA	Ethylene Diamine Tetra Acetic acid
et al.,	Co-workers
FAO	Food and Agriculture Organization
Fig	Figure
GAE	Galic Acid Equivalent
HIV	Human Immunodeficiency Virus
IC_{50}	Half maximal inhibitory concentration
IOM	Institute of Medicine
IU	International Units
JFL	Jackfruit lectin
KAU	Kerala Agricultural University
LDLC	Low Density Lipoprotein Cholesterol
Mg	Milligram
NADH	Nicotinamide adenine dinucleotide
Nm	Nano meter
NBT	Nitro blue tetrazolium

ROS	Reactive oxygen species
rpm	Rate per minute
RTE	Ready-To-Eat
RTC	Ready-To-Cook
SE_m	Standard error of the mean
SOD	Super oxide dismutase
TCA	Trichloro acetic acid
TCM	Traditional Chinese Medicinal
VZS	Varicella Zoster Virus
WHO	World Health Organization
µg	Micro gram
%	Percentage

Introduction

1. INTRODUCTION

Nature is one of God's beautiful creations, and its vegetation (plants and trees) caters to the nourishment of all living beings. Herbal foods are essential for human nutrition and the promotion of good health, because they offer a range of nutrients and phytochemicals. Nature feeds us with the perfect packages that make human bodies attain their full potential. Vegetables and fruits are considered the most important components of the human diet. It has been found to especially exert a protective effect, due to the presence of phytochemicals.

Kaur and Kapoor (2002) found that diets rich in fruits and vegetables were associated with a lower disease risk, including cancer and cardiovascular diseases. It has also been argued that cooking or processing can improve the health benefits of fruits and vegetables.

Free radicals are involved both in the aging process and in the development of cancer. To fight against free radicals, the body has an effective defense system, that comprises of several enzymes and antioxidants of high and low molecular weight.

Antioxidants are compounds that can delay, or prevent the oxidation process. The natural antioxidants found in fruits and vegetables have attracted the growing attention of food scientists, nutrition experts and consumers, as they reduce the risk of chronic diseases and promote human health. Fruits and vegetables are an important part of the food basket, economical source of energy and good source of phytochemicals and nutrients, such as vitamins and minerals.

Now a day's the daily consumption of fruits and vegetables has increased, due to the realization of their nutritional and therapeutic effects on human health, owing in turn to the presence of phytochemicals and antioxidants. Studies have shown that good nutrition with increased consumption of fruits and vegetables play an important role in the prevention of various lifestyle disorders, such as cancer, strokes, heart disease and diabetes (Willett, 2002, Wright *et al.,* 2008).

Jackfruit fruit is commonly called the "poor man's food," because it is cheap and abundant during season. Jackfruit is believed to have originated in southwestern

India. Jackfruit is not intentionally grown in Kerala, though it grows widely in various parts of the state. Jackfruit has an important contribution to the food supply of the population in the current scenario, when the supply of basic grains has been reduced.

Jackfruit was declared the official fruit of Kerala on 21st March, 2018. Jackfruit is identified with several uses. The young fruits and seeds are used as vegetables. The seeds and meat of the raw fruit of the jackfruit are consumed in the form of curries and boiled forms. During ripening, the color of the fruit changes from yellowish green to yellow due to the conversion of anthocyanins, chlorophylls and carotenoids as pigments. The color of the bulbs can be white, cream, light yellow, yellow, lemon yellow, dark yellow, saffron, light saffron, dark saffron or orange, depending on the variety.

Ripe fruits are used as a table fruit, cooked to desserts, sweet meats and preserved in syrup. The pulp is processed, dehydrated and sold in the form of pulp, juices, cookies, chutneys, jams, jelly, toffee and dried pasta. Each slice of 100 g of ripe fruit contains 18.9 g of carbohydrates, 1.9 g of protein, 0.1 g of fat, 77 per cent of moisture, 1.1 g of fiber and 0.8 g of total mineral matter, 20 mg of calcium, 30 mg of phosphorus, 500 mg of iron, 540 IU of vitamin A, 30 mg of thiamine and a calorific value of 84 calories. Several parts of jacktree, including fruits, leaves and bark, have been widely used in traditional medicine, because of their anticancer, antifungal, antimicrobial, anti-inflammatory, hypoglycemic and healing properties. Jackfruit contains phytonutrients such as carotenoids, which can serve as useful antioxidant compounds. The antioxidant activities of the pulp extracts of the jack fruit are correlated with the total phenolic and flavonoid content. Approximately 70% of the total antioxidant activity of the seeds and pulp of the jackfruit is due to the presence of substantial antioxidant effects of ascorbic acid and phenols. Jackfruit contains many functional compounds that can reduce various diseases such as heart disease, stroke, high blood pressure and bone loss. These compounds can also improve muscle and nerve function by lowering homocysteine levels in the blood.

Jackfruit is usually consumed fresh or in a minimally processed form. Jackfruit consumption has decreased due to the cumbersome processing procedures. Therefore, it is felt apt to market them as convenience foods. Besides, processed vegetables provides the body with more antioxidants such as carotenoids and phenolic compounds, than when they are raw. Dried jackfruit is rich in dietary fiber that nourishes the digestive tract and increases immunity, that protects the body from colds and viruses.

Due to the growing consumer demand for healthy products that can be consumed Ready-To-Eat or Ready-To-Cook, such healthy products have conquered the market. So far, several value-added products based on jackfruit have been standardized and their profiles analyzed for nutrients and chemicals. However, evaluation of their biologically active compounds has not been carried out, when the analysis of these products for their health benefits has become necessary. Hence, this study was conducted with the objective of analysing the antioxidant and anticancer properties of jackfruit based Ready-To-Cook curry mixtures developed at the Department of Community Science.

Review of Literature

2. REVIEW OF LITERATURE

The literature of the current study entitled- 'Antioxidant and anticarcinogenic potential of jackfruit based Ready-To-Cook curry mixes' is presented under the following subheadings:-

2.1 Importance of antioxidants

2.2 Bioactive compounds and phytochemicals in jackfruit

2.3 Therapeutic benefits of jackfruit.

2.4 Picture of utilization of jackfruit

2.5 Dehydrated jackfruit products

2.1 IMPORTANCE OF ANTIOXIDANTS

According to Wong *et al.* (2006), an overproduction of reactive oxygen and nitrogen species may occur due to oxidative stress caused by the imbalance of the body's antioxidant defense system and the formation of free radicals. These reactive species react with biomolecules, causing cell damage and death and also results in the development of cancer.

Now a day's, antioxidants have become the most discussed topic in the world, due to their health benefits. It is a diversified group of chemical substances that protect the body from oxidative damage induced by free radicals and reactive oxygen species (Salvayre *et al.,* 2006). Carocho and Ferreira (2013) reported that the use of synthetic antioxidants such as BHA, BHT and propyl gallate in foods to control lipid peroxidation which has been questioned because of their potential health risk and toxicity.

Li *et al.* (2007) reported a positive linear relationship between the antioxidant and anticancer effects of Traditional Chinese Medicinal (TCM) plants. It was also reported that TCM plants have great potential to prevent and treat various human degenerative diseases, such as cancer, since antioxidants, protect living organisms

from DNA or protein damage and lipid peroxidation, caused by reactive species, such as free radicals.

Many medicinal plants contain large amounts of antioxidants, such as polyphenols, that can play an important role in the adsorption and neutralization of free radicals, deactivation of singlet and triplet oxygen or even the decomposition of peroxides. Free radicals and other reactive oxygen species, known as ROS, are continuously generated through normal physiological processes and also under pathological conditions. ROS plays an important role in the pathogenesis of various diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts and inflammation (Apel and Hirt, 2004).

Dimitrios (2006) noted that natural antioxidants may be phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen-derived compounds, carotenoids, tocopherols or ascorbic acid and their derivatives. Schibmeir *et al.* (2005) stated that antioxidants have gained greater importance due to their positive participation as health promoters in conditions such as cardiovascular problems, atherosclerosis, treatment of many forms of cancer and the process of aging.

Halliwell and Gutteridge (2000) stated that the best known natural antioxidants include hydrophilic compounds, such as vitamin C, thiols and flavonoids, as well as lipophilic compounds, such. as vitamin E, carotenoids and ubiquinoles, that can be obtained by ingestion of vegetable products such as fruits, vegetables, nuts, flours, vegetable oil, beverages and drinks, fresh or processed food stuffs. Nijveldt *et al.* (2001) reported that flavonoids had anti-inflammatory, anti-allergic, antiviral and anti-carcinogenic properties in in vitro experiments.

A study conducted by Thulyathan *et al.* (2012) reported that jackfruit seed lectins showed greater haemagglutination activity and antioxidant activity than testa lectin.

Duchler and Stepnik (2008) found that the combination of three natural compounds consisting of phenethyl ester of caffeic acid, methylglyoxal and

parthenolide exhibited the highest toxicity for leukemic cells, while preserving lymphocytes isolated from healthy donors.

Wu *et al.* (2009) reported that natural isothiocyanates showed an anticancer effect by reducing the activation of carcinogens by increasing their detoxification, as well as by demonstrating antitumor activity. Ginger alcohol extracts were more cytotoxic on Dalton lymphoma tumor cells and human lymphocytes in vitro (Habib *et al.,* 2008)

Podsedek (2007) reported that dietary antioxidants, including vitamin C and phenolic compounds, as well as vitamin E and carotenoids. present in. fruits and vegetables contributed to the first and second most important second line of defense against oxidative stress and protected cells against oxidative damage, thus it could prevent chronic diseases, such as cancer, cardiovascular diseases and diabetes

Consumption of fruits and vegetables containing antioxidants has been shown to provide protection against these diseases. The antioxidant activity of fruits and vegetables is mainly related to their content of polyphenols, carotenoids, vitamins C and vitamin E content (Benzie 2003, Cadenas and Packer, 2002). Of these antioxidants, polyphenols are a broad and complex class of compounds including flavonoids. Interest in flavonoid antioxidants has increased dramatically because of their high ability to trap free radicals associated with various diseases, as evidenced by a large number of in vitro tests. In addition, experimental in vitro data also suggest that flavonoids have anti-inflammatory, anti-allergic, anti-viral and anti-cancer properties (Nijveldt *et al.*, 2001). It has also been reported previously that such natural products have a variety of functions and many of them have interesting and useful biological activity.

The chemopreventive effect of polyphenols, including antiproliferation, oxidation prevention, induction of detoxification enzymes, host immune system regulation and anti-inflammatory activity, has been reported by Garcia-Lafuente *et al.* (2009).

Tannins are water soluble polyphenols found in many plant foods. It was also reported that many tannin molecules reduce the mutagenic activity of several mutagens. Many carcinogens produce oxygen free radicals for interaction with cell macromolecules. The anti-cancer and antimutagenic potentials of tannins was related to their antioxidant property, which is important for the protection of cellular oxidative damage, including lipid peroxidation. Tannins and related compounds inhibit the production of superoxide radicals (Chung *et al.,* 2011).

Beta-carotene is a fat-soluble member of carotenoids found in many fruits, grains, oils and vegetables (Willcox *et al.,* 2004). Beta-carotene is considered a provitamin because it can become active vitamin A. Beta-carotene is converted into retinol, which is essential for vision. It is a powerful antioxidant and the best singlet oxygen extinguisher.

Vitamin C acts as a powerful reducing agent that effectively mitigates the potentially harmful free radicals produced by the body's normal metabolic respiration (Gaziano *et al.,* 2009). The antioxidant property of ascorbic acid is attributed to its ability to reduce potentially harmful ROS, instead it forms a resonance-stabilized and relatively stable ascorbate-free radical (AFR) that serves as an electron donor (Buettner and Schafer, 2001) and are reduced to ascorbate by NADH and NADPH-dependent reductases, with a high affinity for the generated radicals. This mechanism explains a series of cytoprotective functions of vitamin C, including the prevention of oxidation-induced DNA mutation (Lutsenko *et al.,* 2002), the protection of lipids against peroxidation damage (Padayatty *et al.*, 2003) and the repair of oxidized amino acid residues to maintain protein integrity (Banudevi *et al.,* 2004). Since oxidative stress is implicated in the pathogenesis of many disease-related diseases, vitamin C (often used in combination with other antioxidants) has often been used to prevent or treat various diseases due to its antioxidant properties (Heitzer *et al.,* 2001).

The best described property of almost all flavonoid groups is their ability to act as antioxidants and flavones and catechins appear to be the most potent flavonoids for the body's protection against reactive oxygen species (ROS). Valko *et al.* (2004), reported that free radicals and ROS damage, formed during normal oxygen

metabolism, permanently threaten the body's cells and tissues. Quercetin, kaempferol, morina, myricetin and rutin, which act as antioxidants, have had beneficial effects such as anti-inflammatory, anti-allergic, antiviral and anti-cancer activity. Quercetin and silybin, which act as scavengers of free radicals, exert a protective effect against ischemic tissue damage due to hepatic reperfusion (Tapas *et al.,* 2004).

Saponins are naturally occuring steroids found in plants, that play an important role in human nutrition. Several biological effects have been attributed to saponins which include immunostimulatory, hypocholesterolemic and anticancer membranes. It was also observed that these structurally diverse compounds killed protozoa and molluscs, hindered the digestion of proteins and the absorption of vitamins and minerals in the intestine, caused hypoglycemia and acted as antifungal, antioxidant and antiviral agents (Francis *et al.,* 2002).

Selenium is the active site of several antioxidant enzymes, including glutathione peroxidase. Pham-Huy *et al.* (2001) reported that at low dose, selenium had various health benefits as antioxidants, anticancer agents and immunomodulators. Several selenium compounds have antitumor activities in various animal models when administered in amounts greater than those associated with nutritional requirements (Combs, 2001).

2.2 BIOACTIVE COMPOUNDS AND PHYTOCHEMICALS IN JACKFRUIT

"Bioactive compounds" are essential and non-essential compounds that can have an effect on human health (Biesalski, 2009). Nahlar (2013), has defined bioactive compounds as non-nutritional components of foods that are believed to have beneficial effects on health.

The different groups of bioactive compounds are phenolic compounds, alkaloids, flavonoids, saponins, lectins, lignins, prebiotics and tannins. However, a large part of the scientists (Solomon and William, 2003; Liu, 2013) stated that bioactive compounds include vitamins, minerals, fibers, fatty acids, carotenoids along with flavonoids, phytosterols, prebiotics.

Fruits and vegetables contain a series of compounds known as phytochemicals that are considered responsible for their beneficial physiological effects, including vitamins, minerals, carotenoids, polyphenols and alkaloids. The NHB (2014) has defined bioactive compounds as components in foods or food supplements, different from those needed to meet basic human nutritional needs. The recent focus of research is on the detection of such bioactive compounds in foods. Its application ranges from medicinal foods, agriculture, food science to nanoscience. Flavonoid consumption in humans was inversely related to mortality from coronary heart disease (CHD) or incidence of myocardial infarction (Lotito and Frei, 2006) and also lowering of LDL cholesterol and plasma concentrations of total cholesterol (Arai *et al.,* 2000).

Jackfruit is known as the fruit of the poor, it is a highly nutritious seasonal food, rich in carbohydrates, proteins, fats, fibers, calcium, phosphorus, iron, carotene and thiamine. Fructose, glucose and sucrose are the main sugars present in jackfruit. However, their presence varies according to variety, cultural practices and environment.

Bioactive compounds extracted from jackfruit showed antioxidant, antidiabetic, antiatherosclerotic, antibacterial, antiviral, antifungal and anti-inflammatory activities. The potassium in jackfruit helps reduce blood pressure and reverse the effects of sodium, which cause an increase in blood pressure that affects the heart and blood vessels. This in turn helps to prevent heart diseases and strokes. The functional components for this action include its high levels of potassium and B6 (Patel *et al.,* 2019).

One of the main proteins, Jacalin was isolated from the seeds that possessed immunological properties (Silva *et al.,* 2006). Ong *et al.* (2008) reported that jackfruits contained forty-five volatile components of which thirty-two were new. Esters, which give the fruit the desired flavor, have been found in high concentrations (31.9%). Chandrika *et al.* (2004) reported that the jackfruit contains β-carotene, α-carotene, β-zeacarotene, α-zeacarotene, β-carotene -5.6α-epoxide and a dicarboxylic carotenoid and crocetin. Recent studies have also shown that the key carotenoids present in

jackfruit are trans lutein (24–44%), β carotene (24–30%), transneoxanthin (4–19%), 9-cis-neoxanthin (4-9%) and 9-cis-violaxanthin (4-10).

Soong and Barlow (2004) evaluated the antioxidant properties of jackfruit seeds and confirmed that 70% of the total antioxidant activity was due to the phenolic content. Jackfruit has 0.36 mg of GAE / 100 g of total phenol content (Wongsa and Zamaluddien, 2005). It is also said that the jackfruit plant contains artocarpine, artocarpetin, artocarpetin A, cycloheterophylline, artonin A, artonin B, morin, dihydromorin, oxydihydroartocarpine, artocarpine, cincacurin, isoartocarpine and cycloartinone (Lampe and Chang, 2007; Prakash *et al.,* 2009).

Several kinds of flavonoids are abundant in the Jackfruit plant (Lin *et al.,* 2000; Wei *et al.,* 2005). Alkaloids and flavonoids have been characterized in jackfruit seeds. The analysis showed that the seeds of *A. heterophyllus* contained alkaloids; quinine, tomatin and nicotine (Okoye *et al.,* 2012). These alkaloids could be used to eradicate germs that causes disease. The analysis also revealed that the seeds contained myricetin, kaempferol, gossipetin, quercetin and isoliamnetine, as the main types of flavonoids (Okoye, 2016).

The qualitative and quantitative phytochemical analysis of the seeds of *A. heterophyllus* indicated the presence of alkaloids 0.55 ± 0.012%; flavonoids 0.41 ± 0.02%; tannins 0.240 ± 0.001%; saponins 2.74 ± 0.02% and phenols 0.08 ± 0.001% (Ong *et al.,* 2006; Silva *et al.,* 2006; Ajayi, 2008; Baliga *et al.,* 2011). A colorimetric study conducted by Gupta *et al.* (2011) reported that the solvent system methanol (1: 1) was able to extract more bioactive compounds than the acetone solvent system (phenolic content: 1.45 µg; Flavonoid content 290.6 µg). Prasad *et al.* (2012) reported that all extracts of jackfruit plants contained bioactive compounds such as cardiac tannins, alkaloids, carbohydrates and glycosides, that were commonly extracted with water, petroleum ether, hexane and acetone solvents.

Flavonoids and phenolic compounds extracted from jackfruit exhibited high free radical scavenging activities and antioxidants; they had chelation power and iron reduction and prevented the progress of several oxidative stresses (Shanmugapriya *et*

al., 2011). Gupta *et al.* (2011) reported that the alkaloids in jackfruit showed analgesic, antispasmodic and antibacterial activities.

Volatile bioactive compounds such as isopropyl isopentyl butyl isovalerate (25.6%), palmitic acid (8.3%) and ethyl isovalerate (6.2%) were isolated from the jackfruit (Maia *et al.,* 2004). Srinivasan and Kumaravel (2016) isolated a group of volatile bioactive compounds from jackfruit; squalene, campesterol, stigmasterol, lanosterol and γ-sitosterol identified by GC-MS / MS. These compounds belonged to fatty acids, steroids and terpernoid groups and showed pharmacological activities.

All-trans-β-carotene is an important antioxidant, it is observed that it prevents several chronic degenerative diseases such as cancer, inflammation, cardiovascular diseases, cataracts and damaged macular degeneration (Krinsky *et al.,* 2003); (Stahl and Sies, 2005). The main carotenoids found in jackfruit were all trans lutein (37.02 µg / 100 g), all trans β carotene (29.55 µg / 100 g), all trans neocrome (0.88 µg / 100 g), all transluteoxanthin (2.06 µg / 100 g) and cis-luteoxanthin (0.34 µg / 100 g) (Faria *et al.,* 2009).

Jackfruit seeds included 10 to 12 per cent of the total fruit weight. It has been found that these seeds are rich in starch (22 per cent) and dietary fiber (3.9 per cent) that are important for health. Lignans, isoflavones, saponins and all other phytonutrients have anticancer, antihypertensive, antioxidant, anti-aging and anti-aging properties; which have been identified in jackfruit seeds. The seeds can be roasted or boiled to eat them or they can be boiled and stored as a syrup to take by mouth. It has also been reported that the boiled seeds of the fruit contain proteins (31.1 per cent), carbohydrates (66.2 per cent) and raw lipids (1.3 per cent) that are highly nutritious and good enough for human health. It can be prepared in many ways to make a healthy snack (Mukprasirt and Sajjaanantakul, 2004). Jackfruit seeds contain phenolic compounds (Soong and Barlow, 2004). Approximately 6.03 mg / g of non-reducing sugar was extracted from the seeds, that were prebiotic (Nuallaong *et al.,* 2009).

2.3 THERAPEUTIC BENEFITS OF JACKFRUIT

Jackfruit is an ancient fruit that is widely consumed as fresh fruit and the use of different parts of jackfruit has been reported since antiquity for its therapeutic qualities. The beneficial physiological effects also have a protective application in a variety of diseases. This includes the functional, medicinal and physiological properties of jackfruit (Swami *et al.,* 2012). Prakash *et al.* (2009) reported that jackfruit is an important source of compounds such as artocarpin, artocarpesin, betulinic acid, cinomacurin, cyloartocarpin, heterophylol, morin, dihydromorin, isoartocarpin, oxydihydroartocarpesine and artocarpetine which are useful in diuretics,constipation, ophthalmological disorders, snake bites and skin diseases, etc.

2.3.1 Anti-oxidant activity

The pulps and seeds of the jackfruit are very nutritious, rich in vitamins and minerals and can be used as natural antioxidants (Ojwang, *et al.,* 2018). The antioxidant status of human beings could be improved by including *A.heterophyllus* seeds in regular diets (Burci *et al.,* 2015).

Antioxidants are necessary to prevent the formation and counteract the actions of reactive oxygen and nitrogen species, which are generated in vivo and cause damage to DNA, lipids, proteins and other biomolecules (Seifried *et al.,* 2007). Cycloheterophylline in jackfruit inhibited the oxidation of low density lipoproteins (Ko *et al.,* 2000). Young and Woodside (2001), stated that antioxidants prevented tissue damage induced by free radicals by preventing the formation of radicals, eliminating them or promoting decomposition. Lin *et al.* (2005) reported that, prenyl flavonoids, cyclogeracomunin and artoflavanone isolated from jackfruit, showed inhibition of oxidative DNA damage.

Toda and Shirataki (2006) reported that encapsulated flavonoids, isolated from jackfruit showed inhibitory effect. The antioxidant activity of the jackfruit pulp obtained from Western Ghats of India was estimated by the DPPH and ferric reducing capacity, which indicated that the jackfruit is a good source of antioxidants (Jagtap *et al.,* 2010).

Raaman and Sivaraj (2014) conducted a study on phytochemical analysis and antioxidant activities of methanol extract of the leaves of jackfruit to evaluate the phytochemicals, free radical scavenging activities and antioxidant properties. The study affirmed the antioxidant properties of jackfruit leaves. Soong and Barlow (2004) reported the antioxidant properties of jackfruit seeds and the 70 per cent contribution of total antioxidant activity was due to its phenolic content. Munira, (2014) reported that, the total antioxidant capacity of jackfruit seed was 170.75± 0.01 mg/g AAE. Studies have shown that jackfruit extracts inhibited haemoglobin glycation with an IC_{50} value of 56.43 per cent (Agung *et al.,* 2015).

The phytochemical composition and antioxidant activities were higher in the seeds than in the pulp. The flavonoid contents were 0.5 - 0.89 mg / g and 0.18 -0.29 mg / g respectively, while the phenol contents were (17.37 to 18.69 mg / g) and (12.10 to 14.55 mg / g), for jackfruit seeds and pulps.

2.3.2. Anti-carcinogenic property

Cancer rates worldwide have increased mainly due to the aging population and changes in lifestyle in developing countries. Cancer is the leading cause of death in the world. About 12.7 million cancers were diagnosed in 2008 and 7.6 million people died of cancer worldwide. More than half of all cases occur in developing countries (Jemal *et al.,* 2011). The World Health Organization has estimated that if nothing is done, the annual number of cancer deaths worldwide could reach 15 million by 2020 (Rastogi *et al.,* 2004).

Jacalin, one of the main proteins in jackfruit has been used to study the tissue-binding properties of benign and malignant breast and thyroid cancer cell lesions. They are also seen to cause growth inhibition of tumor cell lines MCF7 and H1299 (Zuraidah and Sakinah, 2014). Kabir (1998) and Da-Silva *et al.* (2006) stated that jacalin is mitogenic for human CD4 + T lymphocytes and has been used as a tool to assess the immune status of patients infected with the human immunodeficiency virus (HIV).

Jackfruit acts as laxative, relieves constipation due to high fiber content and cleans up colon thus preventing colon cancer (Rahman *et al.,* 2005). *Artocarpus heterophyllus* extract was one of the strong inhibitors of tyrosinase activity. Artocarpanones, isolated from jackfruit, inhibited fungal tyrosinase activity and melanin production in B16 melanoma cells. This compound is strong enough to treat hyperpigmentation problems in human skin (Arung *et al.*, 2006).

Patel and Patel (2011) conducted a study on methanolic extract of *Artocarpus heterophyllus* seeds, for its cytotoxic activity against A549 and MCF-7 cell lines and it showed excellent toxicity on cancer cells and was seen to be nontoxic to normal cells. Another anti-cancer study was done in diethylether extract of *Artocarpus altilis* wood on human T47D breast cancer cells and observed for its effect on nuclear morphology, cell viability and sub-G1 formation. Thus the study revealed that *Artocarpus altilis* wood extract induced apoptosis and sub-G1 phase formation in breast cancer (T47D) cells, and therefore, had a potential anti-carcinogenic agent (Enos *et al.*, 2009).

A study by Vazhacharickal *et al.* (2015) on methanol extracts obtained from 13 plants against cariogenic bacteria, for its antibacterial activity revealed that the methanol extract of *Artocarpus heterophyllus* showed the most intense activity. The chemoprotective properties of jackfruit with reduced mutagenicity and controlled proliferation of a tumor cell line was reported by Montanez *et al.* (2015).

Nutritional and epidemiological studies have verified that natural bioactive compounds can bring benefits to human health by inhibiting carcinogenic processes and mechanisms of cell death (Nerurkar and Ray, 2010; Wang *et al.*, 2010). The bark extract of *Artocarpus heterophyllus* in 90% ethyl acetate showed cytotoxicity against the tumor lines at an IC_{50} concentration of 3-5.05 ± 0.72 µg / ml against breast adeno-mammary carcinoma cell lines MCF-7 (Rajesh *et al.,* 2011).

Guochan *et al.* (2017) reported that artocarpin showed selective cytotoxicity against human cancer cells. Artocarpin compromised the independent growth capacity of the anchor, suppressed cell growth and induced a cell cycle in the G1phase, followed by apoptotic and autophagic cell death.

2.3.3. Antineoplastic activity

Arung *et al.* (2006) examined the cytotoxic effects of flavonoids substituted with isoprenoids isolated from jackfruit wood and confirmed that flavonoids with an isoprenoid substituent were very effective. Another study by Arung *et al.* (2010) reported that artocarpin showed potent cytotoxic activity in human T47D breast cancer cells, which was cultured in vitro and incubated with artocarpine for 24 hours, that resulted in concentration-dependent cytotoxic effects.

2.3.4 Antidiabetic activity

Diabetes mellitus or simply diabetes is a non-constant metabolism of sugars, lipids and protein digestion: manifesting as hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and occasionally ketonemia, due to a combination or insufficient or complete discharge of insulin activity and or insulin (Das *et al.,* 2012). It is one of the leading causes of death and disabling disease in the world. One hundred and fifty million people suffered from diabetes, which was almost five times higher than the estimates a decade ago, and could double in 2030 (Kannan *et al.,* 2012; Vijay and Vimukta, 2014). It has been observed that *Artocarpus heterophyllus* has significant antiglicative activity and can be used as a traditional medicine in the treatment of chronic diabetes mellitus (Devi *et al.,* 2014).

Osmani *et al.* (2009) reported that the seed powder extract of *Artocarpus heterophyllus* has significant hypoglycaemic activity. The presence of phytochemical components in the jackfruit extract, such as ascorbic acid, β-carotene and lycopene, has antioxidant activity, so the extract can inhibit hemoglobin glycation. Jackfruit's extract has been shown to have the potential of a diabetic agent, since extracts can reduce the level of glycosylated hemoglobin (HbA1c) (Biworo *et al.,* 2015).

Suchithra and Subramanian (2014) studied the antidiabetic activity of the extract of *Artocarpus heterophyllus* in experimental type 2 diabetic mice, induced with low doses of extract and the results of the study clearly indicated that the oral treatment with extract, on diabetic rats increased glycogen content and altered the

activity of glycogen metabolizing enzymes, suggesting the effective use of glucose, which in turn may be due to better insulin sensitivity. The treatment with extract improved the glycemic state, through the modulation of key enzymes or the metabolism of carbohydrates in liver tissues or diabetic rats.

2.3.5. Antimicrobial activity

Theivasanthi and Alagar (2011) revealed that jackfruit seed nanoparticles were successful against *Escherichia coli* and *Bacillus megaterium* microbes. Isoprenyl-artocarpin and artocarpesin flavones separated from jackfruit hindered the development of essential cariogenic microscopic organisms with a convergence of 3.13 - 12.5 µg / ml and also showed the inhibitory developmental impact on the plaque-forming streptococci (Khan *et al.,* 2003). Wetprasit *et al.* (2000) reported that jackfruit lectin was found to have an in vitro inhibitory movement with a cytopathic impact on HSV-2 type herpes simplex infection, Varicella zoster (VZS) and cytomegalovirus (CMV) infection.

The hydrolyzing action of fibrinogen and fibrin in jackfruit concentrate indicated its non-hazardous nature and increased the potential use of seed proteases in the treatment of thrombotic diseases (Gangaraju e*t al.,* 2015). Goncalves *et al.* (2005) and Trindade *et al.* (2006) reported that the jackfruit extract had an antiviral activity of 480 µg / ml, against human rotavirus and the degree of suppression was 99.2 per cent.

Two new lectins that bind to chitin, identified from the seeds of the genus *Artocarpus* limited the development of *Fusarium moniliforme* and *Saccharomyces cerevisiae* introduced haemagglutinating action against human erythrocytes (Trindade *et al.,* 2006).

2.3.6. Antimalarial activity

Quinine was also a type of alkaloid used for the treatment of malaria; 3-Hydroxyquinin, one of the quinine metabolites, had a potential antimalarial activity, which also stimulated insulin secretion and antipyretic activity. Flavonoids replaced

by isoprenoids such as Artocarpin, cudraflavone, 6- prenylapigenin, norartocarpin, albanin A, cudraflavone, brosimone 1 and artocarpanone extracted from jackfruit showed cytotoxicity against melanoma cells (Arung *et al.,* 2006).

2.4. UTILIZATION OF JACKFRUIT

Jackfruit is considered a little used fruit, like many of the fruits. These fruits are wasted due to their short shelf life, ignorance, insufficient processing facilities, lack of technology after the harvest and breaches in the systems of the chain of supply.

The pulp of jackfruit is highly perishable and often suffers loss of flavor, softening of tissues and gilding of the cut surface (Mondal *et al.,* 2013). Due to their high perishability, jackfruits are generally exported as whole fruits, even then more than half of them become edible waste. The inconsistency in the size and shape of the fruit makes the packaging design very complicated (Ramli,2009).

The biggest curse is that it is difficult to collect, cut and peel. The rough skin and latex make them even more difficult to handle. Their large size makes them more unmanageable and perishable in nature. The absence of a strong marketing system is a major obstacle. While there are many indigenous management methods and value-added products available, systematic production to meet demand is lacking. In addition to harvesting, cutting and cleaning the fruit is cumbersome, which makes the fruit neglected.

Harvesting jackfruit in the mature green phase can prevent mechanical damage. Furthermore, adaptation of appropriate post-harvest practices can facilitate export through a prolonged shelf life. Storage of whole jackfruit at 10° C and humidity of 85-90%, can extend the shelf life of the crop by about two weeks (Xu *et al.*, 2018). The conversion of jackfruit waste into value added products would be a better option for spreading the cultivation and consumption of the fruit.

Raw fruit is cooked as a vegetable and stored in pickled or canned forms. The pulp of ripe fruit is consumed fresh and transformed into delights such as halwa, varatty, jam, jelly, etc. Recently, other products such as flavored ice cream, jackfruit

honey, etc. have been launched. A number of factors limits the possible exploitation of this fruit, the main reason being the disorganized management of the supply chain. In Kerala, 50,000 tons of raw fruit are sent to major cities as vegetables, when the state lacks fruits and vegetables. Shreepadre (2015) suggested three 'manthras' to overcome these challenges; Promotion of Ready-To-Cook (RTC) products, Ready-To-Eat (RTE) products and value addition.

2.5 DEHYDRATED JACKFRUIT PRODUCTS

Fruits are undoubtedly very important for nutritional security with a high range of added value and foreign exchange gains. Fruits are now considered an important trading item, since they have acquired enormous market potential. India accounts for 12.5 percent of the world's total population of fruit crops and ranks second with a production of 75 million tonnes in 2013 (FAO, 2014).

Drying is one of the oldest methods for food preservation and an important stage in food processing (Lima *et al.*, 2002). New drying techniques have been developed, such as hot air drying with hygienic and economic considerations. The elimination of water from food is essential to improve the shelf life of fruits and vegetables. Dehydration is one of those techniques, which is used to preserve agricultural products. Dehydration simultaneously combines heat and mass transfer. The fundamental aspect of food dehydration is to reduce the availability of water in food to such an extent that, it is unfavorable for the growth of microorganisms and favorable for minimizing the rates of chemical reactions. Dehydration not only facilitates the reduction of the volume of fresh material, but also facilitates transportation due to the reduction of weight and volume, and also increases the availability of food throughout the year.

Dehydration is an important method used in the food processing industries. Drying aims to remove water from food to a level where microbial spoilage and spoilage reactions are minimized. Today there is a wide range of dehydrated foods available to the consumer in the form of sandwiches, soups or nuts, dried gherkins, etc. The shelf life and energy savings compared to other powdered materials make it

inevitable in the food industry (Krokida and Marolis, 2001.). Food scientists have discovered that reducing the moisture content of food by 10 to 20% prevents bacteria, yeasts, molds and enzymes from ruining it. Aromatic compounds and most nutrients are conserved and concentrated through this method (Dennis, 1999). Dry items are rich sources of energy, assorted vitamins, namely vitamin K and B, minerals, ie, magnesium and phosphorus, antioxidants, ie, iron and folic acid and fibers (soluble fibers). This is mainly due to the concentration of nutrients during processing (Konopacka *et al.,* 2009), which provides the energy for the body needs.

Pardeshi *et al.* (2009) reported that the structure of dry foods depends on the methods and conditions like, temperature, relative humidity, air velocity and the initial physical and chemical characteristics of the product. In addition, the quality of the final product in terms of sensory and physical-chemical factors was significantly influenced by drying conditions (Simal *et al.,* 2005). Dehydrated ripe jackfruit is a nutritious snack made from jackfruit pulp. It is golden, yellow to orange and has a gummy texture with a bitter and sweet taste. Unlike other dehydrated products, it does not have sulfite preservatives, so it will not cause allergic reactions among sensitive consumers (Diamante, 2009). Zuniga *et al.* (2004) developed osmotically dehydrated jackfruit slices using sucrose solutions at 30ºC (40° Brix, 50° Brix and 60° Brix) for three hours. The drying kinetics was carried out using a convection tray dryer at 50 ° C, 60 ° C and 70 ° C (Taib *et al.*, 2013). The results revealed that jackfruit bulbs dehydrated by microwave vacuum obtained a greater capacity for rehydration and a higher score in the sensory evaluation, in terms of color, appearance and aroma. Bhatia *et al.* (1956) recommended the maceration of mature jackfruit bulbs in a 0.1 percent KMS solution for 30 minutes, to improve the quality of dehydrated products. Dehydrated products of good quality (3: 1 drying ratio) were obtained when they were sulfated at a ratio of 16 pounds of sulfur / tonne / fruit / in a space of 1000 cfu (Shanmugam *et al.,* 1992).

Dehydrated jackfruit flakes with a shelf life of one year have been standardized at KAU (2000). The flour prepared with dehydrated jackfruit flakes was suitable for the preparation of chapathis, *pazhampori* and *bajji* replacing wheat flour, maida or bengal gram flour respectively with jackfruit flour. Pua *et al.* (2007) developed drum-

dried jackfruit powder with different concentrations of soybean lecithin and gum arabic. The study revealed that jackfruit puree with soybean incorporations of 2.65% and 10.28% gum arabic was suitable for the production of good quality jackfruit flour. Jackfruit bulbs that are fully ripe or completely raw could be used to prepare jackfruit papad (Bhatia *et al.,* 1956). They discovered that the skin of the jackfruit lasted 4-6 months at room temperature (24-30° C) wrapped in paper.

Ukkuru and Pandey (2005) standardized the jackfruit papad of different flavors from raw jackfruit that were very crispy and tasty, when they were fried. Jackfruit seeds can be converted into flour after inactivating the anti-nutritional factors present by drying. Flour prepared with jackfruit seeds could be used to prepare chappati by mixing with wheat flour (25:75). Jagadeesh *et al.* (2007) reported on the preparation of jackfruit chips, the starch content and the dry matter content of the raw material determines the yield of the processed product. The length of the bulb, total soluble solids and the reducing sugars were important to improve the yield and the quality of jackfruit chips. Giron *et al.* (1975) prepared candied jackfruit by osmotic dehydration that was widely practiced and promoted to extend the utilization of jackfruit. Sliced jackfruit bulbs were immersed in sugar syrup and then it was oven dried at 60° C. Okiya *et al.* (2010) standardized solar dried jackfruit leather and Ukkuru and Pandey (2005) standardized plain and blended jackfruit leather from two varieties of jackfruit. Christina (2018) developed Ready-To-Eat products using jackfruit pulp powder namely jackfruit 'vegetariana', desi jackfruit 'karanji', jackfruit 'appe', jack angel hair and 'jack puda'.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Antioxidant and anticarcinogenic potential of jackfruit based Ready-To-Cook (RTC) curry mixes was conducted as three experiments.

The methodology and procedures adopted for the study have been presented under the following headings.

3.1 Preparation of Ready-to- Cook curry mixes

3.2 Phytochemical screening

3.3 Analysis of antioxidants and antioxidant activity

3.4 Analysis of anticarcinogenic activity

3.5 Statistical Analysis

3.1 PREPARATION OF READY-TO- COOK CURRY MIXES

Three raw jackfruit (Koozha type) based dry mixes which were standardized at the Department of Community Science (Liji,2014), namely 'Avial mix', 'Koottu mix' and 'Ularth mix' were selected for the study.

As per the standardised procedures for the three Ready-To-Cook curry mixes, raw jackfruit of koozha type were collected, washed and the bulbs and seeds were separated. The jackfruit bulbs and seeds were sliced into prescribed dimensions. The sliced jackfruit bulbs were blanched for 3 minutes and immersed in solution with KMS (0.2 per cent) and salt (0.5 per cent) for 30 minutes. The adjuncts used in 'Avial'mix green chillies, garlic, cumin, turmeric powder and curry leaves, whereas in 'Koottu mix' had redchillies, turmeric powder, cumin and curry leaves were blended. The adjuncts used in 'Ularth' mix were crushed green chillies, onion, garlic, turmeric powder and curry leaves. All the formulations were mixed and dehydrated at 65°C till crisp. The details of the formulations of the three mixes are presented in table 1. Raw jackfruit and seeds were taken on the proportion of 70:30.

Ready-To-Cook (RTC) Curry Mixes

Plate:1 'Avial' Mix

Plate:2 'Koottu' mix

Plate:3 'Ularth' mix

Table 1. Proportion of ingredients in the RTC mixes

Sl No	RTC product	Ingredients	Proportion of ingredients in 100g of jackfruit
1	Avial mix	Green chillies + Garlic + Cumin + Turmeric powder + Curry leaves	3:5:1:2:5
2	Kootttu mix	Red chillies + Cumin + Turmeric powder + Curry leaves	3:1:3:5
3	Ularth mix	Crushed green chillies + Onion + Garlic + Turmeric powder + Curry leaves	2:10:5:1:5

3.2 PHYTOCHEMICAL SCREENING

The curry mixes were powdered and extracted using methanol, acetone, ethanol and petroleum ether as solvents. The extracts of the curry mixes were centrifuged at 500 rpm for 20 minutes and the supernatant extracts were kept for overnight incubation.

Phytochemical screening for bioactive compounds such as tannins, flavonoids, phenolic compounds, alkaloids, steroids saponins, cardiac glycosides, phlotobatinins and anthraquinones were done to confirm their presence or absence in the curry mixes. The extracts of jackfruit based curry mixes were subjected to preliminary phytochemical screening, based on the method described by Evans (1996).

3.2.1 Test for Tannins

To 1 ml of the sample extract, an equal volume of ferric chloride was added. Formation of reddish brown precipitate confirmed the presence of tannins.

3.2.2 Test for Flavonoids

To 3 ml of the sample taken in a test tube, 10 ml of ethyl acetate was added and heated over steam bath for 3 minutes. The mixture obtained was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Presence of flavonoids was indicated by the appearance of yellow colouration.

3.2.3 Test for Phenolic compounds

To the sample extract, dilute ammonia solution was added, the formation of reddish or yellow colour confirmed the presence of phenolic compounds.

3.2.4 Test for Alkaloids

One ml of sample extract of curry mix was mixed in 5 ml of dilute hydrochloric acid and steamed over water bath. The mixture was filtered and to 1 ml of the filtrate, 1 ml of Mayer's reagent was added. Absence of cloudiness or slight yellow colour indicated the absence of alkaloids.

3.2.5 Test for Saponins

The presence of saponins in the sample extract was determined by Frothing test, 0.2 ml of the sample extract was mixed with 5 ml of distilled water and was shaken for 20 minutes. Persistance of foams indicated the presence of saponins in the sample.

3.2.6 Test for Steroids

The phytochemical screening for the presence of steroids in the sample extract was done by Liebermann-Burchard's test. For this 0.5 ml of the extract was dissolved in 2 ml acetic anhydride, then 1ml of concentrated H_2SO_4 was added. The formation of blue green ring indicated the presence of steroids.

3.2.7 Test for Cardiac glycosides

Legal's method was used to determine the presence of Cardiac glycosides in the extract, 1 ml of extract was dissolved in 5 ml of pyridine and 2 drops of 2 per cent

sodium nitroprusside and 2 drops of 20 per cent NaOH were added. A deep red colour indicated the presence of cardiac glycoside.

3.2.8 Test for Phlobatannins

Absence of red coloured precipitate, when 2 ml of the sample extract was boiled with 2 ml of 1 per cent aqueous hydrochloric acid, was taken as evidence for the absence of phlobatannins.

3.2.9 Test for Anthraquinones

Borntrager's test was used to determine the presence of anthraquinones in RTC mixes. Two gram of RTC mix extract was dissolved in 10 ml of ethanol and was steamed for 5 minutes. The mixture was filtered and to 2 ml of the filtrate, 2 ml of chloroform was added and shaken thoroughly. The chloroform layer was taken off, 5 ml of distilled water was added and shaken with 5ml of dilute ammonia solution. Presence of red colour in the upper ammonia phase, indicated the presence of anthraquinones.

3.3 ANALYSIS OF ANTIOXIDANTS AND ANTIOXIDANT ACTIVITY

3.3.1 Analysis of antioxidants

Quantitative analysis of antioxidants such as β-carotene, ascorbic acid, saponins, tannins, total phenols, total flavonoids, lectins, alkaloids, selenium, copper, zinc, manganese and iron were conducted to ensure their presence or absence in 'Avial' mix, 'Koottu' mix and 'Ularth' mix.

3.3.1.1 *β carotene*

β carotene was estimated by the method suggested by Sadasivam and Manickam (2008), based on the separation of biologically active carotenoid pigments from total carotenoid pigments.

3.3.1.2 *Ascorbic acid*

Ascorbic acid was estimated titrimetrically by the method of Ranganna (2001), using 2,6 dichloro indophenol dye.

3.3.1.3 Saponins

The method suggested by Obdoni and Ochuko (2001) was used to estimate saponin content in the powdered curry mix samples. Twenty gram of powdered sample was taken in a conical flask and 20 per cent aqueous ethanol of (100 ml) was added. The powdered samples were heated for 4 hours over a water bath at 55° C with continuous stirring and then filtered. The residue was re extracted with ethanol (100 ml of 10 per cent). The extract was reduced to 20 ml over water bath at 90°C.Then the reduced curry mix extract was transferred into 250 ml separating funnel and 20 ml of diethyl ether was added and was shaken vigorously. The ether layer of the mixture was discarded and aqueous layer was recovered. Sixty ml of n-butanol was added after purification and then washed two times, with 10 ml of 5 per cent aqueous sodium chloride. Evaporation was done, the remaining solution was heated using water bath, and the powdered curry mix samples were oven dried to a constant weight. The saponin content was expressed as per cent.

3.3.1.4 Tannins

The method suggested by Sadasivam and Manickam (2008) was used for the estimation of tannins. Estimation of tannin was based on the principle that, in alkaline solution, tannin like compounds reduce phosphotungstomolybdic acid to produce a highly blue coloured solution, and the intensity produced is proportional to the tannin content. Water soluble tannin content was estimated using the standard curve of tannic acid and was expressed as milligrams.

3.3.1.5 Total phenols

The colorimetry method was used to estimate total phenols. In alkaline medium phenols react with phosphomolybdic acid in Folin- Cioalteau reagent and a blue coloured complex was produced. The absorbance of developed blue colour was measured.

3.3.1.6 Total flavonoids

Total flavonoid content was determined by aluminium chloride colorimetric assay. The reaction mixture comprised of 1ml of extract and 4 ml distilled water in a volumetric flask. To the flask, 0.30 ml of sodium nitrite (5 per cent) was added and 0.3 ml of 5 per cent aluminium chloride was added after 5 minutes and mixed well. Two ml of 1M sodium hydroxide was treated and then diluted to 10 ml using distilled water. Morin was used as reference standard and a set of reference solutions (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance of the sample and standard solutions were determined against the blank at 510 nm with the help of spectrophotometer.

3.3.1.7 Lectins

The determination of lectin was done based on the colorimetric assay method, as reported in the manual of food quality (AOAC, 1990). The samples were ground into a slurry and 1g of slurry sample was weighed into a crucible. Ten ml of distilled water was added, followed by the addition of 1 ml concentrated sulphuric acid. The mixture was then allowed to stand for 1 hour. Using distilled water the volume of the prepared solution was made up to 50 ml. From this 5 ml was pipetted out into a test tube and one ml of Schiffs reagent was added to it. The absorbance was measured at 510 nm and the value of lectin in each curry mix sample was estimated from the standard curve of lectin.

3.3.1.8 Alkaloids

One mg of the powdered sample extract was dissolved in dimethyl sulphoxide, to which 2 N of 1 ml HCl was added. The solution was filtered, and then transferred to a separating funnel. 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added to this. The mixture was then taken in 4 test tubes and shaken with chloroform by vigorous shaking and collected in a volumetric flask and diluted using chloroform. Atropine was used as reference and a set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared. The absorbance of test solutions and standard solutions were determined against the blank at 470 nm, using UV/Visible spectrophotometer and the alkaloid content was expressed as milligrams.

3.3.1.9 Selenium

The selenium content in curry mix samples were determined by Atomic absorption spectrophotometer (AOAC, 2005).

3.3.1.10 Copper

For the determination of copper content in the powdered sample, sodium diethyl-dithio-carbamate was made to react with slightly acidic ammonia-cal solution of copper to form a brown colloidal suspension of cupric-diethyl-dithio-carbamate. The suspension was then extracted with an organic solvent and the coloured extract was measured at 440 nm spectrophotometrically (AOAC, 2005).

3.3.1.11 Zinc

Standard flame emission photometer was used to determine the zinc content in the curry mix samples (AOAC, 2005).

3.3.1.12 Manganese

Atomic absorption spectrophotometer was used to determine the manganese content in the powdered sample (AOAC, 2005).

3.3.1.13 Iron

Iron content in the powdered curry mix samples were estimated by spectronic 20 (AOAC, 2005).

3.3.2 Analysis of antioxidant activity

Antioxidants are compounds which are capable of either slowing or preventing the oxidation process. They also act as a defense system against oxidative damage in our bodies and are helpful in avoiding degenerative diseases and the effects of aging (Oliveri, 2000). Recently, it was reported that antioxidants have relation to free radicals, oxidative stress, cancer prophylaxis and therapy (Kalcher *et al.*, 2009).

3.3.2.1 DPPH *radical scavenging activity*

The radical scavenging activity of the powdered curry mix extracts against 2,2 diphenyl 2-picrylhydrazyl hydrate (DPPH) was carried out. The principle of the method is that; the DPPH compound reacts with the antioxidant compound present in the sample, that can donate hydrogen thereby reducing DPPH. The absorbance was measured using UV visible light spectrophotometer at 515 nm. A solution containing DPPH (60 µm) in methanol was made up fresh before absorbance measurements. 3.9 ml of this fresh solution was mixed with various concentrations of test samples (200, 400, 600 and 800 µg) to form 100 µl test solution. Then the curry mix samples were kept at room temperature for 15 minutes and the absorbance was measured. Ascorbic acid was taken as the reference standard and it was dissolved in distilled water to make a stock solution of 1 mg/100 µl concentration. Control sample was prepared excluding the extract and ascorbic acid. Methanol (95%) was used as blank and the radical scavenging activity was calculated by the formula (AOAC,2005).

$$\text{Percentage of inhibition} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} * 100$$

3.3.2.2 *Total antioxidant activity*

Thiocyanate method was used to determine the total antioxidant activity of curry mixes (Oliveri, 2000). Ten mg of ascorbic acid was dissolved in 10 ml of water (stock solution) and 2.5 ml of potassium phosphate buffer (0.04 M, 7.6 pH) was added with 2.5 ml emulsion of linoleic acid. Fifty ml emulsion of linoleic acid contained Tween-20, linoleic acid and potassium phosphate buffer whereas 5 ml of the control contained 2.5 ml of potassium phosphate buffer (0.04 M, 7.6 pH) and 2.5 ml of linoleic acid emulsion. The solutions were then incubated at 37°C in the dark in a glass flask.

After 24 hours incubation, 0.1 ml was taken from the incubated solution and was added to ethanol (44.7 ml of 75% (v/v)) and 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely after 3 minutes 0.1 ml of 0.02 M $FeCl_2$ in 3.5% HCl (w/v) acid was added and the absorbance of the red coloured complex was measured at 500 nm. The inhibition percentage of lipid peroxidation was calculated as; Inhibition % = $[(A_0 - A_1)/A_0 \times 100]$,

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of test solution (AOAC, 2005).

3.3.2.3 *Ferric reducing capacity*

Ferric reducing capacity of the powdered curry mix extracts were determined using potassium ferricyanide-ferric chloride method. 0.2 ml of powdered curry mix extracts at different concentrations (25, 50, 100, 200 µg/ml) were taken and 2.5 ml of phosphate buffer (0.2 M, p^H 6.6), and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$) was added, it was then mixed and incubated at 50°C for 20 minutes to reduce ferricyanide to ferrocyanide. The reaction was stopped by the addition of 2.5 ml of 10% trichloroacetic acid (w/v) followed by centrifugation at 1000 rpm for 10 minutes. Finally, 2.5 ml of the upper layer of the mixture was mixed with distilled water and 0.5 ml of 0.1% $FeCl_3$ and the absorbance was measured at 700 nm (AOAC, 2005).

3.3.2.4 ABTS *radical scavenging activity*

ABTS radical cation was formed by reacting ABTS with potassium per sulphate. A mixture of potassium persulfate (70mM) and ABTS was allowed to stand overnight at room temperature in the dark to form ABTS radical cation, 16 hours before use. 80% methanol was used to dilute ABTS solution, from this diluted solution 100 µl was added to 2 ml of ABTS solution and the absorbance was recorded at 734nm. A standard curve was acquired by using Trolox as standard solution, at various concentrations. The scavenging activity of different concentrations of extracts against ABTS radical was estimated to ascertain IC_{50} (AOAC, 2005).

3.3.2.5 *Nitric oxide scavenging activity*

Sodium nitroprusside was prepared in the phosphate buffered saline and it was then mixed with different concentrations (250 and 500 µg/ml) of the powdered curry mix extracts. The mixture was incubated at 25°C for 30 minutes. A control was taken without the test compound but with equivalent amount of distilled water. Then 1.5 ml of the incubated solution was diluted with 1.5 ml of Griess reagent and the absorbance

was measured at 546 nm and the percentage nitric oxide scavenging activity of the powdered curry mix was calculated with reference to the standard (AOAC, 2005).

3.3.2.6 *Super oxide anion radical scavenging activity*

Super oxide anion radical scavenging activity was determined based on the method described by Robok and Gryglewski (1988). In a PMS-NADH system super oxide radicals were generated via the oxidation of NADH and then it was assayed by the reduction of nitro blue tetrazolium (NBT). The super oxide radicals were generated in a reaction mixture containing 468 µm NADH solution in sodium phosphate buffer in different concentrations of methanolic extracts of the powdered curry mix samples. This was incubated at 25°C for 5 minutes and then the absorbance was recorded at 560 nm.

3.3.2.7 Hydroxyl *radical scavenging activity*

Hydroxyl radical scavenging activity of the extracts of powdered curry mix samples were conducted using the deoxyribose method (Halliwell,1996). The reaction mixture comprised of phosphate buffer (20 mM , p^H 7.4), deoxyribose (10 mM), hydrogen peroxide (1mM), ferric chloride (1.04 mM), and EDTA with different amounts of powdered curry mix samples (2 mM) and ascorbic acid. This solution was incubated at 37°C for 1 hour, after which 17 mM trichloroacetic acid (TCA) was added. Then it was boiled for 15 minutes, ice cooled and the absorbance was recorded at 532 nm. Distilled water was set as blank.

3.4 Analysis of anticarcinogenic activity

Among the three curry mix samples, the sample which showed higher antioxidant potential was taken for studying anticarcinogenic activity.

Cytotoxicity of the powdered curry mix sample was estimated by MTT (3-(4,5-Dimethylthiazol-2-yl- 2,5-Diphenyltetrazolium Bromide) assay on MC7 breast cancer cell lines (Mosmann, 1983). $1x10^5$ml cells were seeded in a 24 well plate, with complete growth medium and allowed to attach. At 60% confluency, the medium was replaced with fresh medium and different concentrations of curry mix extract were added to it (10-100 µg/ml). The cells were further incubated for 48 hours and at the

end of incubation period, the spent medium was replaced with fresh medium containing MTT (100 µl of 0.5% MTT/ml) and incubated for 4hours. Finally, the formazan crystals formed were dissolved in dimethyl sulfoxide and the absorbance was recorded at 570 nm, using UV/Visible spectrophotometer and percentage viability was found using the formula;

$$\%\ Cell\ death = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} * 100$$

3.5 Statistical Analysis

The generated data was statistically analysed using appropriate methods and sufficient replications was maintained for analysis. The data generated from the samples were subjected to Completely Randomized Design (CRD) analysis.

Results

4. RESULTS

Results of the study entitled "Antioxidant and anticarcinogenic potential of jackfruit based Ready-To-Cook curry mixes" is presented under the following headings.

4.1. Phytochemical analysis

4.2. Analysis of antioxidants

4.3. Antioxidant activity

4.4. Anticancerous activity

4.1. PHYTOCHEMICAL ANALYSIS

A number of dietary antioxidants exists beyond the traditional nutrients, commonly known as phytochemicals, which are being increasingly valued for their antioxidant activity (Vermerris and Nicholson, 2006). Phytochemicals are plant chemicals and are defined as "bioactive and non-supplement compounds in vegetables, fruits, grains and other plant foods, that have been reported to control the danger of major chronic diseases" (Liu, 2004). Sun *et al.,* (2002) reported that phytochemical extracts from vegetables and other natural products have immense cancer prevention agents, that are hostile to proliferative impacts and suggested that the phytochemicals in vegetables and fruits are basic to incredible cell reinforcement and anticancer action.

The results of phytochemical screening of the extracts of jackfruit based Ready-To-Cook curry mixes, in different solvents of methanol, ethanol, acetone and petroleum ether are presented in Table 2. The results revealed the presence or absence of tannins, flavonoids, phenols, alkaloids, saponins, steroids, cardiac glycosides, phlobatinnins and antthraquinones.

Table 2. Phytochemical screening of RTC mixes

Phytochemicals	RTC mix	Solvents			
		Methanol	Ethanol	Acetone	Petroleum ether
Tannins	'Avial' mix	+	+	+	–
	'Koottu' mix	+	+	+	–
	'Ularth' mix	+	+	+	–
Flavonoids	'Avial' mix	+	+	+	–
	'Koottu' mix	+	+	+	–
	'Ularth' mix	+	+	+	–
Phenols	'Avial' mix	+	+	–	–
	'Koottu' mix	+	+	–	–
	'Ularth' mix	+	+	–	–
Alkaloids	'Avial' mix	–	–	–	–
	'Koottu' mix	–	–	–	–
	'Ularth' mix	–	–	–	–
Saponins	'Avial' mix	+	+	+	+
	'Koottu' mix	+	+	+	+
	'Ularth' mix	+	+	+	+
Steroids	'Avial' mix	+	+	+	–
	'Koottu' mix	+	+	+	–
	'Ularth' mix	+	+	+	–
Cardiac glycosides	'Avial' mix	+	+	+	–
	'Koottu' mix	+	+	+	–
	'Ularth' mix	+	+	+	–
Phlobatinnins	'Avial' mix	–	–	–	–
	'Koottu' mix	–	–	–	–
	'Ularth' mix	–	–	–	–
Antthraquinones	'Avial' mix	+	+	+	–
	'Koottu' mix	+	+	+	–
	'Ularth' mix	+	+	+	–

(+ presence, - absence)

4.1.1. Tannins

Results of phytochemical screening of Ready-To-Cook curry mixes revealed that tannins were found in methanol, ethanol and acetone extracts, but it was absent in petroleum ether.

4.1.2. Flavonoids

The presence of flavonoids produced a yellow colouration in the extracts of ethanol, methanol, acetone and was absent in the extract of petroleum ether.

4.1.3. Phenolic compounds

Phenols were present in the extracts of methanol, ethanol and were absent in the extracts of acetone and petroleum ether. Presence of phenols produced a reddish colouration in the powdered curry mix extracts.

4.1.4. Alkaloids

Alkaloids were found to be absent in curry mix extracts of methanol, ethanol, acetone and petroleum ether.

4.1.5. Saponin

Presence of saponins were identified in all the solvents of RTC mixes on continuous shaking.

4.1.6. Steroids

The presence of steroids resulted in the formation of blue green ring and was present in all solvents of RTC mixes except petroleum ether.

4.1.7. Cardiac glycosides and Antraquinones

The above results of phytochemical analysis pointed out that cardiac glycosides and anthraquinones were absent in the extracts of petroleum ether and were present in all the other extracts of RTC mixes.

4.1.8. Phlobatinnins

The results revealed the absence of phlobatinnins in all solvents of RTC mixes.

The results of phytochemical analysis indicated the presence of tannins, flavonoids, saponins, steroids, cardiac glycosides and anthraquinones in the three jackfruit based Ready-To-Cook curry mixes; whereas the analysis revealed the absence of alkaloids and phlotobatinins.

After the confirmation of presence of the tannins, flavonoids, phenolic compounds, steroids, saponins, cardiac glycosides and anthraquinones, by preliminary phytochemical tests, the powdered ready to cook curry mixes- 'Avial mix', 'Koottu mix' and 'Ularth mix' were taken for quantitative estimation of antioxidants such as beta carotene, ascorbic acid, saponins, tannins, total phenolic content, total flavonoid content, lectins, alkaloids, selenium, copper, zinc, manganese, copper and iron.

4.2. ANALYSIS OF ANTIOXIDANTS

4.2.1. β carotene

β carotene is a red orange coloured pigment needed for healthy skin, mucus membranes, immune system and good eye health. Diets rich in beta-carotene rich fruits and vegetables or high blood levels of β carotene, are related with reduced risk of cancer at a number of common sites, such as lungs and stomach (Van-poppel, 1996).

Table 3. β carotene content of RTC mixes

Samples	β carotene (mg/100g)
Avial mix	1.16
Koottu mix	0.13
Ularth mix	0.07
SE(m)	0.03
C.D	0.10

SEm : Standard error of the mean

Values are means of triplicates

Table 3 shows β carotene content of 'Avial' mix', 'Koottu mix' and 'Ularth' mix. The results revealed that the β carotene was higher in 'Avial' mix (1.16 mg) and the lowest 'Ularth' mix (0.07 mg), 'Koottu' mix contained 0.13 mg.

4.2.2. Ascorbic acid

Ascorbic acid is fundamental raw material for collagen, carnitine and synapses biosynthesis. Various therapeutic advantages have been attributed to presence of ascorbic acid which has antioxidant, anti-atherogenic, anti-carcinogenic, immunomodulatory and many more healthy features (Naidu, 2003).

Table 4. Ascorbic acid content of RTC mixes

Samples	Ascorbic acid (mg/100g)
Avial mix	38.45
Koottu mix	25.41
Ularth mix	15.34
SE(m)	0.18
C.D	0.59

SEm : Standard error of the mean

Values are means of triplicates

The result showed that the ascorbic acid content was the highest in 'Avial' mix (38.45mg) followed by 'Koottu' mix (25.41 mg). The lowest ascorbic acid content was observed in 'Ularth' mix (15.34 mg). It was found that the differences were statistically significant.

4.2.3. Saponins

Saponins are naturally occurring compounds that are generally distributed in all leguminous plants. Clinical investigations have suggested that the health-promoting components in saponins, help the immune system to secure the human body against malignant growths, and to lower cholesterol levels. A diet rich in saponins can be utilized in the restraint of dental caries and platelet accumulation and in the treatment of hypercalciuria (Shi *et al.*,2004)

Table 5. Saponin content of RTC mixes

Samples	Saponin (%)
Avial mix	6.65
Koottu mix	5.55
Ularth mix	5.00
SE(m)	0.207
C.D.	0.671

SEm : Standard error of the mean

Values are means of triplicates

The data revealed that the saponin content of RTC mixes ranged between 5 to 6.65%. The result showed that the saponin content was found to be higher in 'Avial' mix (6.65%), which was followed by 'Koottu' mix (5.55%), and the lowest content was observed in 'Ularth' mix (5%).

4.2.4. Tannins

Tannins are water soluble polyphenols, present in various plant foods, responsible for reduced food intake, improving growth rate and protein digestibility, as reported from the study on experimental animals (Jackson, 2003).

Table 6. Tannin content of RTC mixes

Samples	Tannin (mg/100g)
Avial mix	11.52
Koottu mix	10.58
Ularth mix	11.57
Sem	0.191
C.D	0.494

SEm : Standard error of the mean

Values are means of triplicates

The tannin content of the RTC mixes are depicted in Table 6. Their levels ranged from 10.58 -11.57 mg/100g. The results showed that the tannin content was higher in 'Ularth' mix (11.57 mg) and was found to be on par with 'Avial' mix (11.52 mg). The lowest content was observed in 'Koottu' mix (10.58 mg).

4.2.5. Total phenols

Plant phenols are products of the phenylpropanoid pathway which includes, benzoic acids, cinnamic acids, flavonoids, lignans and lignins. They are powerful antioxidants and might prevent oxidative harm to biomolecules such as DNA, lipids and proteins which play a role in preventing chronic diseases such as Cancer and Cardiovascular diseases. These plant phenols interfere with malignant growth process, resulting in reduction of Cancer risk (Hollman, 2001).

Table 7. Total phenol content of RTC mixes

Samples	Total phenols (mg/100g)
Avial mix	19.41
Koottu mix	21.53
Ularth mix	13.76
SE(m)	0.138
C.D	0.449

SEm : Standard error of the mean

Values are means of triplicates

Table 7 shows the total phenol content of the curry mixes 'Avial' mix, 'Koottu' mix and 'Ularth' mix. Here, the highest total phenol content was obtained for 'Koottu' mix (21.53 mg) which was followed by 'Avial' mix (19.41 mg). The lowest total phenol content was observed in 'Ularth' mix (13.76 mg), these differences were found to be significant.

4.2.6. Total flavonoids

Flavonoids are low molecular weight and biologically active, secondary metabolites produced by plants. Numerous flavonoids have antioxidant activity, free-radical scavenging capacities, capability to prevent of coronary heart diseases along with anticancer action, while a few flavonoids even showed potential against human immunodeficiency infection (Li *et al.*, 2004).

Table 8. Total flavonoid content of RTC mixes

Samples	Total flavonoids (mg/100g)
Avial mix	1.33
Koottu mix	0.86
Ularth mix	3.25
SE(m)	0.085
C.D	0.299

SEm : Standard error of the mean

Values are means of triplicates

Table 8 displays the total flavonoid content in the Ready-To-Cook curry mixes. The results revealed that 'Ularth' mix had the highest total flavonoid content (3.25mg/100g), which was followed by 'Avial'mix (1.33 mg/100g).'Koottu' mix (0.86 mg/100g) showed least amount of flavonoid content. The difference was found to be statistically significant.

4.2.7. Lectins

Lectins are proteins that bind to carbohydrates and are present in numerous plants. They play significant role in protecting plants from external pathogens, such as fungi and other organisms. Some common food products, such as cereals and legumes, have a relatively high concentration of lectin varieties.

Table 9. Lectin content of RTC mixes

Samples	Lectin (%)
Avial mix	0.35
Koottu mix	0.56
Ularth mix	0.76
SE(m)	0.017
C.D	0.056

SEm : Standard error of the mean

Values are means of triplicates

Table 9 shows the lectin content of curry mixes. It was observed that the lectin content was higher in 'Ularth' mix (0.76 per cent). The lowest lectin content was observed in 'Avial' mix (0.35 per cent). 'Koottu' mix contained 0.56 per cent of lectin.

4.2.8. Alkaloids

Alkaloids are one of the natural, organic substances and active components that are predominantly found in numerous medicinal plants, marine organisms and microorganisms. Because of its evident physiological and therapeutic properties, they have been of great attention to humans from the earliest starting point of development. Alkaloids, especially present in plants were used as a basic and practical cure for various disorders.

Alkaloid content was not detected in 'Avial'mix, 'Koottu' mix and 'Ularth' mix.

4.2.9. Selenium

Selenium content was not detected in the Ready-To-Cook curry mixes.

4.2.10 Copper

Copper is one of the most important minerals required for human health. This element, along with fatty acids and amino acids as well as vitamins is required for normal metabolic process, especially the synthesis of haemoglobin. Since, the body cannot synthesize copper, it has to be supplied through diet.

Table 10. Copper content of RTC mixes

Samples	Copper (µg/100g)
Avial mix	1.79
Koottu mix	1.19
Ularth mix	2.57
SE(m)	0.061
C.D	0.284

SEm : Standard error of the mean

Values are means of triplicates

Table 10 depicts the copper content of the Ready-To-Cook curry mixes. The results showed that the 'Ularth' mix (2.57 µg/100g) had the highest copper content which was followed by 'Avial' mix (1.79 µg/100g). The lowest copper content was obtained for 'Koottu' mix (1.19 µg/100g). The difference was statistically significant.

4.2.11 Zinc

Zinc is an essential component of more than ten enzymes in the body and is found in every single cell. It plays a crucial role in the functioning of immune system and white blood cell depends on zinc for their development and activation, so zinc deficiency can result in diminished amount of white blood cells and thereby leading to reduced ability to fight infections and heal wounds.

Table 11. Zinc content of RTC mixes

Samples	Zinc (µg/100g)
Avial mix	5.80
Koottu mix	6.55
Ularth mix	4.65
SE(m)	0.212
C.D	0.989

SEm : Standard error of the mean

Values are means of triplicates

Data on zinc content (Table 11) of curry mixes revealed that it was highest in 'Koottu'mix (6.55µg/100g), the lowest zinc content was observed in 'Ularth' mix (4.65 µg/100g), 'Avial' mix contained 5.80 µg/100g. The differences in zinc content was found to be statistically significant.

4.2.12 Manganese

Manganese is required for the management of osteoporosis, reducing fatigue and the normal functioning of brain and nervous system.

Table 12. Manganese content of RTC mixes

Samples	Manganese (µg/100g)
Avial mix	2.55
Koottu mix	5.30
Ularth mix	2.51
SE(m)	0.196
C.D	0.913

SEm : Standard error of the mean

Values are means of triplicates

Table 12 displays the manganese content in Ready-To-Cook curry mixes. The result shows that the manganese content was significantly higher in 'Koottu' mix (5.30µg/100g). Manganese content of 'Avial' mix (2.55µg/100g) and 'Ularth' mix (2.51µg/100g) were found to be on par.

4.2.13 Iron

Iron is a key element in body metabolism, brain function, haemoglobin formation, immunity and the regulation of body temperature (Oyarzun *et al.*, 2001).

Table 13. Iron content of RTC mixes

Samples	Iron (µg/100g)
Avial mix	0.84
Koottu mix	0.74
Ularth mix	0.92
SE(m)	0.016
C.D	0.052

SEm : Standard error of the mean

Values are means of triplicates

Table 13 depicts iron content of jackfruit based curry mixes. The results revealed that highest iron content was found in 'Ularth' mix (0.92 µg/100g) followed by 'Avial' mix (0.84 µg/100g). Comparatively, the lowest iron content was observed in 'Koottu' mix (0.74µg/100g).

The study revealed that the higher beta carotene and ascorbic acid content was found in 'Avial' mix (1.16 mg). Similarly, saponin and tannin content was found to be higher in 'Avial mix'. However total phenol content was found to be more in 'Koottu mix' (21.53 mg/100g) and the total flavonoid and flavonoid content were found to be more in 'Koottu mix'. Alkaloids and selenium were found to be absent in all the three RTC mixes.

In case of mineral analyses, zinc and manganese content were higher in 'Koottu mix. Highest iron content was found in 'Ularth mix'. The results revealed that each of the mix was superior in one or other mineral.

4.3. ANTIOXIDANT ACTIVITY

Antioxidants are compounds capable of inhibiting the oxidation process which occur under the influence of reactive oxygen or nitrogen species. Oxidation results in the formation of free radicals, which if continued to propagate will cause degenerative disorders. Antioxidants combat with these free radicals by intervening in the oxidative process. For the body, to remain healthy, there should be a balance between these free radicals and antioxidants, so the antioxidant activity of jackfruit based Ready-To-Cook curry mixes were studied through seven different types of antioxidant analyses.

The concentration of sample that could scavenge 50% free radical (IC_{50}) was used to determine antioxidant capacity of Ready-To-Cook curry mixes. The RTC mixes having lowest IC_{50} had the highest antioxidant activity. According to Blois (1992), 'sample that had $IC_{50} < 50$ ppm, was considered as a very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm as medium antioxidant and weak antioxidant with $IC_{50} > 150$ ppm.

4.3.1 DPPH radical scavenging activity

DPPH is a stable free radical, with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. Free radical scavenging activity is one of the mechanisms by which antioxidants inhibit lipid peroxidation (Blokhina *et al.,* 2003). DPPH scavenging activity has been extensively used for screening antioxidants present in fruits and vegetables (Sanchez, 2002).

Table 14. DPPH radical scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	33.81
Koottu mix	34.50
Ularth mix	36.70
SE(m)	0.305
C.D	0.990

SEm : Standard error of the mean

Values are means of triplicates

In the present study, free radical scavenging activity of Ready-To-Cook curry mixes were studied by the DPPH assay using methanol as solvent. Table 14 illustrates the results of DPPH activity of the jack fruit based Ready-To-Cook curry mixes.

The study revealed that 'Avial mix' had the highest DPPH radical scavenging activity with an IC_{50} value of 33.81 µg/ml, followed by 'Koottu' mix (34.50µg/ml). The lowest DPPH radical scavenging activity was found in 'Ularth' mix (36.70 µg/ml).

4.3.2 Total antioxidant activity

Antioxidants protect the human body from Reactive Oxygen Species (ROS) and the damaging effects of free radicals. They also delay lipid peroxidation and thus the progress of many chronic diseases (Gulcin *et al.*, 2007). On the basis of enzymatic or non-enzymatic species, antioxidant molecules are classified into different categories. They are the main compounds that protect the body by slowing down the oxidation process, by eliminating free radicals produced on many natural occasions. The ultimate goal of antioxidants is to eliminate Reactive Oxygen Species (ROS), by using different mechanisms depending on their structure and site of action. They are also able to act by regulating gene expression that encodes antioxidant enzymes and repairs oxidative damages caused by the radicals and increase the elimination of damaged molecules (Wood *et al.*, 2006).

The total antioxidant activity of RTC mixes is depicted in Table 15.

Table 15. Total antioxidant activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	41.44
Koottu mix	42.41
Ularth mix	43.45
SE(m)	0.133
C.D	0.470

SEm : Standard error of the mean

Values are means of triplicates

The results of above table reveal that antioxidant activity of jackfruit based RTC curry mixes ranged from an IC_{50} values of 41.44 to 43.45 µg/ml. The highest antioxidant capacity was observed in 'Avial' mix (41.44 µg/ml) and the least antioxidant capacity was observed in 'Ularth mix' (43.45 µg/ml).

4.3.3 Ferric reducing capacity

The ferric antioxidant reduction assay measures the antioxidant effect of any substance in the reaction medium for its reducing capacity. The capacity of reduction is considered as the ability of the natural antioxidant to donate electrons. The antioxidant potential of the Ready-To-Cook curry extracts based on jackfruit was estimated for its ability to reduce the ferric complex of 2,4,6-tripyridyltriazine (Fe^{3+} - TPTZ) to colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ). The yellow color of the test solution changed to various shades of blue and green depending on the reduction power of each compound. The presence of antioxidants in the sample causes the reduction of ferricyanide complex to the ferrous form. Therefore, the measurement of Prussian perl blue formation at 700 nm indicated the concentration of Fe^{2+} (Ferreria *et al.*, 2007).

Table 16. Ferric reducing capacity of RTC mixes

Samples	FRAP activity (mg/100)
Avial mix	0.58
Koottu mix	0.13
Ularth mix	0.20
SE(m)	0.034
C.D	0.110

SEm : Standard error of the mean

Values are means of triplicates

Table 16 shows ferric reducing capacity in the jackfruit based curry mixes. The findings revealed that 'Avial' mix (0.58 mg/100g) had the highest ferric reducing capacity followed by 'Ularth' mix (0.20 mg/100g). The lowest ferric reducing capacity was found in 'Koottu' mix (0.13 mg/100g). The difference in values were found to be significant.

4.3.4 ABTS radical scavenging activity

The ABTS assay is one of the widely used methods to evaluate the antioxidant capacities of natural products, based on the quenching of stable colored free radicals. It shows radical elimination capacity of antioxidants, present in complex biological systems, such as plant extracts or food. Antioxidant capacity was measured according to the method of Miller *et al.* (1996), based on the ability of different substances to scavenge the ABTS radical cation compared to a standard antioxidant (Trolox).

Table 17. ABTS radical scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	36.13
Koottu mix	38.04
Ularth mix	45.56
SE(m)	0.872
C.D	2.832

SEm : Standard error of the mean

Values are means of triplicates

Table 17 depicts the level of ABTS radical scavenging activity of the Ready-To-Cook curry mixes. The highest ABTS radical scavenging activity was found in 'Avial' mix with an IC_{50} value of 36.13 µg/ml followed by 'Koottu' mix (38.04 µg/ml). The lowest ABTS radical scavenging activity was found in 'Ularth' mix (45.56 µg/ml).

4.3.5 Nitric oxide scavenging activity

It is known that nitric oxide (NO) plays a crucial role in the pathogenesis of various diseases, caused mainly by inflammation, especially when combined with the superoxide radical to form the peroxynitrite anion (Moncada , 1991). It is generated from sodium nitroprusside in aqueous solution that reacts with oxygen to form nitric ions, that can be estimated by the Griess reagent (Kumaran and Karunakaran, 2007). The nitric oxide or reactive nitrogen species are formed during their reaction with oxygen or superoxides, such as NO_2, N_2O_4, N_3O_4 and NO_3^-, which are very reactive. They are responsible for altering the structural and functional behavior of many cellular components. Plant or vegetable products have the property of counteracting

the effect of NO formation, which in turn avoids the harmful effects of NO generation in the human body.

Table 18. Nitric oxide scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	36.58
Koottu mix	30.92
Ularth mix	14.11
SE(m)	2.233
C.D	7.877

SEm : Standard error of the mean

Values are means of triplicates

Table 18 shows the nitric oxide scavenging activity of jackfruit based Ready-To-Cook curry mixes. The results of the above table revealed that, there was a significant difference in nitric oxide scavenging activity of curry mixes. In case of curry mix samples, highest nitric oxide scavenging activity with an IC_{50} value of 14.11 µg/ml was seen in 'Ularth' mix and the lowest nitric oxide scavenging activity with an IC_{50} value of 36.58 µg/ml was observed in 'Avial'mix.

4.3.6 Superoxide anion radical scavenging activity

The superoxide radical is an oxygen molecule with a biologically unpaired, rather toxic electron, deployed by the immune system to kill the invading pathogen. Although superoxide anion is a weak oxidant, it leads to the generation of powerful and dangerous hydroxyl radicals and singlet oxygen, which contributes to oxidative stress and the generation of numerous degenerative diseases in humans. It is known that the superoxide radical is produced *in vivo* and can cause the formation of H_2O_2 through the dismutation reaction. The conversion of superoxide and H_2O_2 to more reactive species, such as hydroxyl radicals, has been considered one of the unfavorable effects caused by superoxide radicals.

Table 19. Superoxide anion radical scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	48.54
Koottu mix	54.12
Ularth mix	60.73
SE(m)	0.584
C.D	2.059

SEm : Standard error of the mean

Values are means of triplicates

Table 19 shows the superoxide anion radical scavenging activity of curry mixes. The findings reveal that 'Avial' mix (48.54 µg/ml) had the highest superoxide radical scavenging activity followed by 'Koottu' mix (54.12 µg/ml), the lowest superoxide radical scavenging activity was found in 'Ularth' mix (60.73 µg/ml).

4.3.7 Hydroxyl radical scavenging activity

The hydroxyl radical is one of the most reactive free radicals, formed by superoxide anion and hydrogen peroxide, in the presence of metal ions (Li *et al.*, 2008). It is the most reactive ROS and supports the shortest half-life compared to other reactive oxygen species, and causes serious damage to adjacent biomolecules. Hydroxyl radicals have the greatest potential for electron reduction and can react with anything in the living system. The addition of H_2O_2 to cells can lead to oxidative damage of DNA, mediated by ionic radicals, that depend on transition ions (Aljudi and Kamaruddin, 2004). Therefore, the elimination of hydrogen peroxide and superoxide anion is very important for the protection of food and pharmaceutical products (Gulcin *et al.*, 2007).

Table 20. Hydroxyl radical scavenging activity of RTC mixes

Samples	IC_{50} Values (µg/ml)
Avial mix	50.55
Koottu mix	52.55
Ularth mix	51.52
SE(m)	0.181
C.D	0.586

SEm : Standard error of the mean

Values are means of triplicates

Table 20 shows the hydroxyl radical scavenging activity of jackfruit based Ready-To-Cook curry mixes. The findings revealed that 'Avial' mix had the highest hydroxyl radical scavenging activity with an IC_{50} value of 50.55 µg/ml, the lowest hydroxyl radical scavenging activity was found in 'Ularth' mix (51.52 µg/ml).

Among the three jackfruit based Ready-To-cook curry mixes 'Avial' mix was found to have higher DPPH radical scavenging activity, total antioxidant activity, hydroxyl radical scavenging activity, superoxide anion radical scavenging activity and radical scavenging activity and was therefore taken up for studying anticancer property.

4.4. ANTICANCEROUS ACTIVITY

Table 21. Cytotoxicity of 'Avial' mix

Concentration (µg/ml)	% Loss in viability	SD
0	0.00	0.00
12	15.27	3.90
25	15.75	5.00
50	14.60	3.47
75	11.50	5.92
100	17.20	2.07

Table 21 shows the cytotoxicity of 'Avial' mix. Maximum cytotoxicity of 17.20 per cent was observed at 100 µg/ml. However, 'Avial' mix did not show any significant reduction in the viability of Human breast adenocarcinoma (MCF-7) cell lines.

Discussion

5. DISCUSSION

The results obtained from the present study entitled "Antioxidant and anticarcinogenic potential of jackfruit based Ready-To-Cook curry mixes" are discussed in this chapter under the following heads:

5.1 Phytochemical analysis

5.2. Antioxidant properties

5.3. Antioxidant activity

5.4. Anticancerous activity

Jackfruit (*Artocarpus heterophyllus* Lam.), the 'state fruit' of Kerala, originated in the rain forests of Western Ghats of India, South Asia and East Indies. It grows in many household orchards of in South India, especially Kerala. Raw jackfruit is often used for cooking in many countries including India, Bangladesh, Nepal, Sri Lanka, Vietnam and Thailand. However, due to the cumbersome preparation procedures, the use of jackfruit is much limited. Development of processed products would promote its intake by bringing convenience and variety.

Considering the busy life styles of urban societies developing Ready-To-Eat and Ready-To-Cook products will be of great demand among modern customers. So far, various jackfruit based value added products have been standardized and their profile analyzed for nutrients and chemicals. However, their quality assessment with respect to the various bioactive compounds have not been attempted, so far. The analysis of such developed products for their health benefits, adds to customer acceptance. Hence, this study was conducted to analyse the antioxidant and anticarcinogenic properties of jackfruit based curry mixes developed at the Department of Community science.

5.1 PHYTOCHEMICAL ANALYSIS

Phytochemicals are biologically active plant substances that have been associated with the protection of human health from degenerative diseases. The main group of phytochemicals that contribute to the total antioxidant capacity of plant foods includes carotenoids, phenols and antioxidant vitamins such as ascorbic acid, tocopherols etc (Masaki, 2010).

Researchers suggest that phytochemicals work with the nutrients present in natural products and dietary fiber to protect from diseases. They may have superimposed and complementary mechanisms of action in the body, including the antioxidant effect (Dhimati *et al.,* 2003).

Volko *et al.* (2007) reported that oxidative stress plays an important role in the pathogenesis of diseases such as Cancer, Diabetes mellitus, Neurodegenerative disorders and Cardiovascular diseases. ROS includes free radicals such as hydroxyl radical, superoxide and non-radical molecules like singlet oxygen, nitric oxide and hydrogen peroxide etc. These are involved in the pathogenesis of several skin related diseases and cutaneous malignancy (Bickers and Athar, 2006).

Mathai (2000), suggested that phytochemicals reduce the risk of coronary heart diseases by preventing the oxidation of LDL cholesterol, reducing the absorption or synthesis of cholesterol, improving arterial elasticity and normalizing blood pressure.

Phytochemicals are important components of medicinal plants and have been reported to exert a wide range of biochemical activities in the physiological system (Olagunju *et al.,* 2006). In *Artocarpus heterophyllus,* there are various phytochemical compounds present which play a major role in biological activities. Previously it was reported that alkaloids, tannins, phenolic compounds, flavonoids and saponins are present in jackfruit (Shanmugapriya *et al.,*2011). In *Artocarpus heterophyllus* there are various phytochemical compounds present which play a major role in biological activities.

Studies on the pharmaceutical properties of jackfruit extracts revealed the antitumor, antihypertensive and anti-aging ulcer properties. The phytonutrients in jackfruit prevented the formation of cancer cells, reduced blood pressure, stomach ulcers and slowed the process of cell degeneration (Swami *et al.,* 2012). The presence of thousands of phytonutrients in the jackfruit prevented the formation of tumor cells and also provided other health benefits (Ko *et al.,* 2000).

The present study on the three curry mixes revealed the presence of tannins, flavonoids, phenols, saponins, steroids, cardiac glycosides, anthraquinones and absence of alkaloids, phlobatinnins in their extracts. Results of phytochemical screening revealed that tannins were found in the curry mix extracts of methanol, ethanol and acetone. Presence of flavonoids produced a yellow colouration and was present in curry mix extracts of methanol, ethanol and acetone.

Phenols were present in methanol, ethanol and were absent in acetone and petroleum ether extracts of curry mix extracts. The results of the present study pointed out that alkaloids and phlobatinnins were absent in the curry mix extracts of methanol, ethanol, acetone and petroleum ether. Presence of saponins were identified in all the three curry mix extracted solvents. The results of the study revealed the absence of steroids, anthraquinones and cardiac glycosides in petroleum ether solvent and were present in all the other curry mix extracted solvents.

A study conducted by Gupta *et al.* (2011) reported the presence of saponins, steroids, flavonoids and phenolic compounds in jackfruit, similar reports were also reported by Hari *et al.* (2014). The metanolic extract of jackfruit contained steroids, phenols and flavonoids that inhibited the growth of human pathogenic bacteria (Pradhan et al., 2013).

Moke *et al.* (2017) reported that tannins, flavonoids, steroids, phenols, cardiac glycosides and saponins were present in jackfruit extract of different solvents. Methanolic extract of jackfruit contained higher concentrations of flavonoids and phenols (Shafiq *et al.*, 2017). Jackfruit is a good source of phytochemicals that includes phenolic compounds and offers opportunities for value-added nutraceuticals

to improve health benefits (Jagtap *et al.*, 2010). Study by Ojwang *et al.* (2018) found that the phytonutrient content was higher in seeds than in the pulps.

5.2 ANTIOXIDANT PROPERTIES

Plant based antioxidants like carotene, phenolic acid, vitamin C and vitamin E are considered as having the potential to reduce the risk of diseases (Sravani and Paarkash, 2012).

5.2.1 Beta carotene

Carotenoids are synthesized in all photosynthetic organisms and consist of more than 700 species in their family (Britton *et al.*, 2004). The basal structure of carotenoid containing 40 carbon with 3 to 15 conjugated double bonds influences its antioxidant activity (Saura and Goni, 2006).

The results of the present study revealed that among the three jackfruit based curry mixes, beta carotene content was higher in 'Avial mix' (1.16 mg) followed by 'Koottu' mix (0.13mg). The lowest value was obtained for 'Ularth' mix (0.07 mg). According to IOM (Institute of Medicine, 2001), daily consumption of 3-6 mg beta carotene will maintain normal blood levels in the range associated with reducing the risk of chronic diseases.

Rahman *et al.* (2012) reported that dehydrated jackfruit contained 53.02 µg/ 100 g beta-carotene. Another study by Kumar *et al.* (2012) reported that hot air oven dried jackfruit powder contained 150.45 IU beta-carotene. Jahan *et al.* (2011) reported that 4.40 mg beta carotene was present in jackfruit. Study conducted by Bhaskarachary *et al.* (1995) reported that 0.16 mg beta carotene was present in jackfruits. Amaya (1999) reported that carotenoids are sensitive to isomerization and oxidation during food processing and decreases the color and activity of vitamin A carotenoids. This supports the variations reported in various studies.

5.2.2 Ascorbic acid

Ascorbic acid is one of the water soluble antioxidants needed for many physiological functions in human biology. Diet supplemented with fresh fruit, vegetables and synthetic tablets at 100-120 mg / day is suggested to achieve cell saturation and the optimal reduction of the risk of various lifestyle disorders in healthy individuals (Frei and Traber, 2004).

In the present study, the ascorbic acid content was higher in 'Avial' mix (38.45 mg) which was followed by 'Koottu'mix (25.41mg). The lowest content was observed in 'Ularth' mix (15.34 mg). The values of the present study are in accordance with Swaroopa *et al.* (2016) who reported that fresh jack crisps recorded 15.60 mg/100g of ascorbic acid by osmotic dehydration. In a study conducted by Narasimham (1990) it was reported that the amount of ascorbic acid found in fresh jackfruit was 12-14 mg /100g. According to Tanjung *et al.* (2014) jackfruit contained 0.0440±0.012 mg/100 mg ascorbic acid and 0.192±0.021mg/100 ml of beta carotene. Another study conducted by Okudu (2015) reported that jackfruit pulp contained 294.8µg/100g beta carotene and 27.6mg/100g ascorbic acid (27.6mg/100g). Vitamin C is highly heat sensitive hence, the levels decrease with processing.

5.2.3 Saponins

Saponins are secondary metabolites that are extensively distributed in the plants. It was also found to significantly influence growth, food intake and reproduction in animals and extensive research was carried out on the anticancer, immunostimulant and hypocholesteremic properties of saponins (Vinha and Soares, 2012).

In the present study, the highest amount of saponin was found in 'Avial' mix (6.65 per cent) and the lowest content was observed in 'Ularth'mix (5.00 per cent). Gupta *et al.* (2014) reported that saponin content in jackfruit was 2.67±0.17g/100g and high amount of saponins (6.32±0.098g/100g) were found in jackfruit seeds. These observations more or less matches the values obtained in this study.

5.2.4 Tannins

Tannins are polymeric phenol substances that precipitate proteins and have antimicrobial and antioxidant properties (Sumathy *et al.*, 2011). Agniesza and Borowska (2008) found that tannins were responsible for the sensory properties (tart taste) and changes in colour of fruits.

Findings of the present study revealed that the highest tannin content was observed for Ularth' mix (11.57 mg) which was on par with Avial' mix (11.52 mg). The lowest tannin content was noted for 'Koottu' mix (10.58mg). A study by Sirisha *et al.* (2014) reported that, tannin content was higher in *A.incius* 3.36±0.03 followed by *A.integer* 1.19±0.02, *A.hircitus* 1.04±0.03, *A.integrifolia* 0.83±0.03 and the lower tannin content was recorded for *A.heterophyllus* 0.62±0.02 µg of tannic acid equivalents per gram of extract. Nair *et al.* (2012) reported that jackfruit seeds contained 198.38 GAE/100 g of tannin.

Manach *et al.* (2004) reported the environment, genetics, maturity and processing can affect the phenolic compounds in food and their bioavailability. A study by Valverde *et.al* (1994) reported the effect of processing on antinutritional factors of lentils showed increase in tannin content

5.2.5 Total phenols

Phenolic phytochemicals have gained much attention due to their physiological functions which include antioxidant, antitumour and antimutagenic properties (Othman *et al.*, 2007). Plants with phenolic compounds can act as antioxidants thereby preventing the incidence of heart disease (Jin and Mumper, 2010), and reduce the risk of diabetes and cancers (Kusirisin *et al.,* 2009). Based on the number of phenol units in the molecule plant phenols are classified into simple or polyphenols.

In the present study, highest phenol content was observed in 'Koottu' mix (21.53 mg) followed by 'Avial' mix (19.41 mg), the lowest phenol content was observed in 'Ularth' mix (21.53 mg). Yi *et al.* (2016) reported that jackfruit chips contained 1.2 mg $GAEg^{-1}$ of phenolics. Baker *et al.* (2009) found that jackfruit pulp

contained 0.46 mg GAE g^{-1}phenolic content, and jackfruit seeds contained higher amount of total phenolic content (Soong and Barlow, 2004). The phenolic content of plants were influenced by intrinsic (species, genus, cultivar) and extrinsic (environment, storage and handling) factors (Barberan and Espin, 2001).

Study by Talcott *et al.* (2003) reported that polyphenolic compounds are not completely stable during processing. Kader *et al.* (2002) stated that physical and biological factors, such as the increase in temperature and enzymatic activity during processing, cause the destruction of phenolic antioxidants.

5.2.6 Total flavonoids

Flavonoids are polyphenolic compounds with a nuclear structure and are known to possess not only strong antioxidant properties but also anticancer, anti-inflammatory and antiviral properties (Havsteen, 2002). The chemical structure of the flavonoids and the position of the hydroxyl groups affect their ability to eliminate free radicals and antioxidants. It has been said that flavonoids possess a wide range of substances that play an important role in the protection of biological systems from the harmful oxidative process of macromolecules (Atmani *et al.,* 2009).

In the present study, the highest flavonoid content was found in 'Ularth' mix (3.25 mg) followed by 'Avial' mix (1.33 mg). Shrikantha *et al.* (2015) reported that water extract of jackfruit pulp showed more flavonoid content (1.20 mg of rutin equivalents g^{-1}) among underutilized fruits and these results are also in accordance with the results of the study by Basu and Maier (2016). Another study by Shafiq *et al.* (2017) reported that, methanolic extract of ripened jackfruit exhibited high flavonoid content (109.94±2.96 mg QE/100g dry wt). Shin *et al.* (2008) reported that the total concentrations of flavonoids and phenolics and the total antioxidant activity of fruits harvested at the white tip stage were higher than those of the red ripeness stage thus indicating the reason for lower flavonoid on these raw jackfruit based products.

5.2.7 Lectins

The occurrence of lectin, in jackfruit seeds termed as 'jacalin' was first reported by Chatterjee *et al.* (1979). Lectins are structurally oligometric proteins

composed of one or more subunits that carry a sugar binding site (Lis and Sharon, 1998). The function of lectin is based on its ability to recognize or bind the carbohydrate moieties of the glycol conjugates

The present study revealed that lectin content of jackfruit based Ready-To-Cook curry mixes, ranged between 0.35 per cent to 0.76 per cent. Kumar *et al.* (2012) reported that the lectin content in jackfruit seed was 1.2% (by weight).

5.2.8 Alkaloids

Alkaloids are naturally occurring phytochemicals containing heterocyclic nitrogen atoms and have many pharmacological activities.

Alkaloids were found to be absent in all the three jackfruit based curry mixes in this study. A study by Sirisha *et al.* (2014) reported that alkaloid content was higher in *A.heterophyllus* (1.16±0.02 µg) than in *A.integer* (0.34±0.3µg) of boldine equivalents g^{-1} of extract. Onyeka and Nwambekwe (2007) reported a decrease in alkaloid content of vegetables with processing.

5.2.9 Selenium

Selenium is an important trace element that is essential for many bodily processes including healthy immune system and cognitive function. It also contributes to thyroid hormone metabolism and protects from oxidative damage and infection.

The results of the present study revealed the absence of selenium in the three jackfruit based Ready-To-Cook curry mixes and this finding is in agreement with the study conducted by Anila (2018), in different varieties of jackfruits.

5.2.10 Copper

Copper is essential for nerve metabolism, nerve trsansmission, absorption of iron and cardiovascular health (Oyarzun *et al.,* 2001).

The present study showed that 'Ularth' mix contained 2.57 µg of copper which was followed by 'Avial' mix (1.79 µg) and the lowest copper content was observed for 'Koottu' mix (1.19 µg). Sreeletha *et al.* (2017) stated that copper content in jackfruit seed was 0.019 mg/g. Another study by Abedin *et al.* (2012) reported that

copper content in jackfruit seeds ranged from 1.47- 4.13 mg/100g. The comparatively higher content in the mix could be due to the additive effect of copper content in the other ingredients.

5.2.11 Zinc

Zinc is essential for proper functioning of reproductive system (Whitney and Rolfe, 2005). According to the FAO food balance data, (2012) it has been estimated that around 20% of the world population could be at risk of zinc deficiency.

The present study revealed that zinc content was found highest in 'Koottu' mix (6.55 µg) while it was found lowest in 'Ularth' mix (4.65 µg). Amadi *et al.* (2018) reported that jackfruit pulp contained 5.20 mg and jackfruit seed contained 9.28 mg of zinc. Another study by Abedin *et al.* (2012) found that zinc content in jackfruit seeds ranged from 1.5 to 3.1 mg/100g. A study by Goes *et al.* (2002) reported that processing reduced zinc bioavailability.

5.2.12 Manganese

Manganese is a good antioxidant essential for the functioning of nervous system and cardiovascular system. This micronutrient aids in enzymatic reactions and bone formation (Bakhru, 2002). In the present study, highest manganese content was noticed in 'Koottu' mix (5.30 µg) followed by 'Avial' mix (2.55 µg) and 'Ularth' mix (2.51 µg) which were found to be on par. The study is in conformity with the findings of Ocloo *et al.* (2010) who had reported that the jackfruit seed flour contained 1.12 µg of manganese. The additive effect of manganese levels of the other ingredients could be the reason for the higher levels in the mixes, than in the seed flour alone.

5.2.13 Iron

Iron is an essential trace element needed for normal functioning of central nervous system and formation of haemoglobin. In the present study the highest iron content was found in 'Ularth' mix (0.92 µg) and the lowest content was observed in 'Koottu'mix (0.74 µg).

Tejpal and Amrutha (2016) analysed the nutritive value of fresh jackfruit and reported that jackfruit contained 0.42 mg of zinc, 0.197 mg of manganese and 0.60 mg of iron content. Jagtap *et al.* (2010) conducted a study in jackfruits and found that ripe jackfruit pulp contained 500 mg of iron. A study by Paul and Shaha (2004) showed that the nutrient composition of fruits, vitamins and minerals (Fe, Zn, Cu) varies according to weather conditions, soil type and fruit maturity.

The maturity and phytochemical content of processed curry mixes are seen to differ with the value of jackfruit. This variation can be accounted by the effect of processing methods, variety and cultural practices.

5.3. ANTIOXIDANT ACTIVITY

Antioxidants have been found to reduce the harmful effects of free radicals in the body that are produced as by-products of cellular metabolic activities (De beer *et al.*, 2002). The free radicals cause oxidative stress and results in damage to DNA, lipids and proteins (Lobo *et al.*,2010).

Prenylflavones isolated from *Artocarpus heterophyllus* serve as a strong antioxidants against lipid peroxidation (Ko et *al.,* 2000). Carotenoids are also known to have protective function against oxidation (Mezadri *et al.,* 2008). DPPH radical scavenging activity, total antioxidant activity, ferric reducing capacity, ABTS radical scavenging activity, nitric oxide scavenging activity, super oxide anion radical scavenging activity and hydroxyl scavenging activity were carried out for authenticating the antioxidant potential of jackfruit based Ready-To-Cook curry mixes.

5.3.1 DPPH radical scavenging activity

DPPH method is used worldwide for the quantification of free radical scavenging activity (Zhou and Yu, 2004).

In the present study, 'Avial' mix had the highest DPPH activity with an IC_{50} value 33.81 µg/ml, followed by 'Koottu' mix (34.50µg/ml), the lowest DPPH radical scavenging activity was found in 'Ularth' mix (36.70µg/ml). A study by Jagtap *et al.* (2011) stated that jackfruit wine contained 69.44±0.34 per cent of DPPH radical

scavenging activity. Another study by Shafiq *et al.* (2017) reported that the acetone extract of jackfruit showed 89.31±0.78 per cent DPPH radical scavenging activity.

Antioxidant activity is seen to be vary among jackfruit based products, which may due to effect of other ingredients and processing methods.

5.3.2 Total antioxidant activity

During oxygen metabolism, antioxidants are used by aerobic organisms to protect cells from oxidative damage.

The results of the present study revealed that the antioxidant activity of the jackfruit based Ready-To-Cook curry mixes ranged from 41.44 µg/ml to 43.45 µg/ml. The highest antioxidant activity was observed in 'Avial' mix (41.44 µg/ml) and lowest antioxidant capacity was observed in 'Ularth'mix (43.45 µg/ml). A study by Anila (2018) on different varieties of jackfruit reported that the highest antioxidant activity was observed in raw seeds of Koozha (30.35 µg/ml) and the lowest antioxidant activity was observed in raw bulbs of Sindoor (41.75 µg/ml). Another study by Surinut *et al.* (2003) reported jackfruit extract contained lower antioxidant activity (IC_{50} from 50.62 to 110.46 mg/ml). The antioxidant activities of the bulbs reported by Anila (2018) did not differ much with that of curry mixes of this study, which may be because the major components of the mixes are contributed by jackfruit bulbs and seeds.

5.3.3 Ferric reducing capacity

The antioxidant potential of the tested samples were estimated from their ability to reduce TPTZ-Fe (lll) complex to TPTZ-Fe (ll). The reducing ability of methanol extracts of jackfruit based Ready-To-Cook curry mixes were compared with ferrous sulphate standard curve. The reducing power of the curry mix extracts was concentration dependent which increased with increase in concentration.

In the present study, 'Avial' mix showed the highest ferric reducing capacity (0.58 mg) followed by 'Ularth' mix (0.20 mg) and the lowest ferric reducing capacity was found in 'Koottu' mix (0.13 mg). A study by Jagtap *et al.* (2010) found that jackfruit pulp extract showed higher ability to reduce Fe^{3+} to Fe^{2+}(1.7 Mm TEAC g^{-1}

for methanolic extract). Omar *et al.* (2011) studied the ferric reducing capacity of the extract of jackfruit leaf and observed that the percentages of Fe^{++} chelating activity of jackfruit leaf ethanolic extracts were 62, 75, and 78% at concentrations of 0.2, 0.4, and 0.6 mg/mL, respectively.

5.3.4 ABTS radical scavenging activity

The ABTS radicals can easily react with antioxidants because they are relatively unstable than DPPH radicals (Klompong *et al.*, 2009).

In the present study, 'Avial' mix had the highest ABTS radical scavenging activity with an IC50 value of 36.13 µg/ml, the lowest ABTS radical scavenging activity was found in 'Ularth mix (45.56 µg/ml). Shanmugapriya *et al.* (2011) reported that the ABTS radical scavenging activity of jackfruit seed extracts showed IC_{50} value of 51.89% for the ethanolic extract, 52.09% in acetone extract, 50.36% in ethyl acetate and 52.04% for aqueous extract. Here again the combined effect of other ingredients in the mixes could be the reason for the higher values.

Soong and Barlow (2004) conducted a study on the ethanolic extract of defatted jackfruit seeds and in the pulp by ferric reducing antioxidant power assay and ABTS radical scavenging assay. The results of the ABTS radical scavenging assay showed that fresh jackfruit seed contained an antioxidant activity of 7.4 µmol / g AAE and that the edible portion had an antioxidant activity of 3 µmol / g AAE, while the freeze dried sample showed an activity of 25.4 and 11 µmol / g AAE. In the ferric reducing power assay, it was observed that the seed and the pulp contained 6.8 and 2.8 µmol /g of power respectively. The difference in the antioxidant activities could be due to extraction of antioxidants from the adjuncts.

5.3.5 Nitric oxide scavenging activity

Nitric oxide is a potent pleiotropic mediator in several physiological processes and a diffusible radical in pathological conditions, which reacts with superoxide anion and forms a potentially cytotoxic molecule called peroxynitrite. The protonated form of peroxynitrite, the peroxyntric acid, is a strong oxidant (Malinski, 2007).

Hydroxylation or nitration of the aromatic compound, in particular tyrosine, is the main route of damage.

In this study, 'Ularth' mix had the highest nitric oxide scavenging activity with an IC_{50} value of 14.11 µg/ml which was followed by 'Koottu'mix (30.92 µg/ml). The lowest nitric oxide radical scavenging activity was observed in 'Avial' mix (36.58 µg/ml). A study by conducted by Basu and Maier (2016) on in vitro antioxidant activity of seven commercially available fruits reported that jackfruit extract exhibited the highest ABTS radical scavenging activity (35.6 per cent), nitric oxide scavenging activity (87.7 per cent) and superoxide anion radical scavenging activity (55.5 per cent), probably processing had increased the level of nitric oxide scavenging activity, through concentration of components.

5.3.6 Superoxide anion radical scavenging activity

Enzymatic antioxidants such as superoxide dismutase plays an important role in protecting the cells and tissues from oxidative damage caused by reactive oxygen species (Yamaguchy *et al.*,1994). In the present study, it was revealed that 'Avial' mix had the highest superoxide radical scavenging activity with an IC_{50} value of 48.54 µg/ml, the lowest superoxide radical scavenging activity was found in 'Ularth' mix (60.73µg/ml).

A study by Sirisha *et al.* (2014) reported that SOD activity was found to be maximum in *A. integer* (12.3±0.02) followed by *A. heterophyllus* (6.42±0.02) and minimum in *A. hircitus* (5.68±0.02). A study by Zhang *et al.* (2010) reported that thermal processing caused a decrease in superoxide anion radical scavenging activity, which is observed here also.

5.3.7 Hydroxyl scavenging activity

The hydroxyl radical is a reactive oxygen species, responsible for biological damage. It has the ability to cause standard breakage in DNA which is a factor leading to carcinogenesis and mutagenesis. In the present study, 'Avial' mix had the highest hydroxyl radical scavenging activity with an IC_{50} value of 50.55 µg/ml followed by 'Ularth' mix (51.52 µg/ml), the lowest hydroxyl radical scavenging activity was found

in 'Koottu' mix (52.55 µg/ml). Tanjung *et al.* (2014) found that hydroxyl radical scavenging activity of jackfruit extract was 36.66±1.12 per cent.

Sensoy *et al.* (2006) reported that processing on buckwheat decreased antioxidant activity, as is observed in this study also.

5.4. ANTICANCEROUS ACTIVITY

Many studies have reported that all phytonutrients in jackfruit have anticancer benefits. The main role of these phytonutrients is to prevent the harmful free radicals that have been known to develop cancer and many other degenerative diseases. The phytochemicals present in jackfruit prevent the initial stage of tumor cell formation. Among the various phytonutrients, saponins have shown preventive properties for colon cancer. The saponins were able to react with the outer layers of the tumor cells, bind to the tumor cells and prevent the further growth (Sagar et al., 2006). The phytochemicals were also found to induce mitotic arrest in leukemia cells.

In the present study, cytotoxicity of 'Avial 'mix was estimated by MTT assay on Human breast adenocarcinoma cells (MCF-7) and 17 per cent cytotoxicity was observed. A study by Rajesh *et al.* (2011) reported that the bark extract of *Artocarpus heterophyllus* in 90 per cent ethyl acetate exhibited cytotoxicity towards the cancer lines at IC_{50} concentration of 3-5.05±0.72µg/ml against human breast adeno carcinoma MCF-7 cell lines.

The antioxidant activity of all the three products are confirmed in all the seven tests. The propitious product with higher antioxidant activity was taken up for testing its anticarcinogenic potential. Eventhough its direct effect on cancer cell viability was not significant, all other result suggests its possible role in cancer prevention and the study also suggests scope for further studies in cytotoxicity on other cancer cell lines.

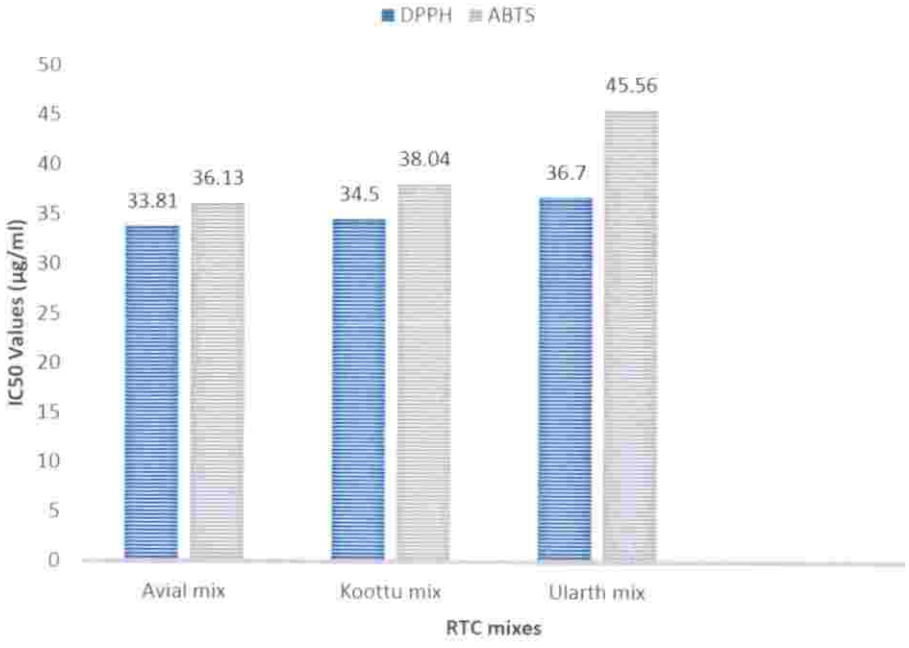


Figure 1. DPPH and ABTS radical scavenging activity of RTC mixes

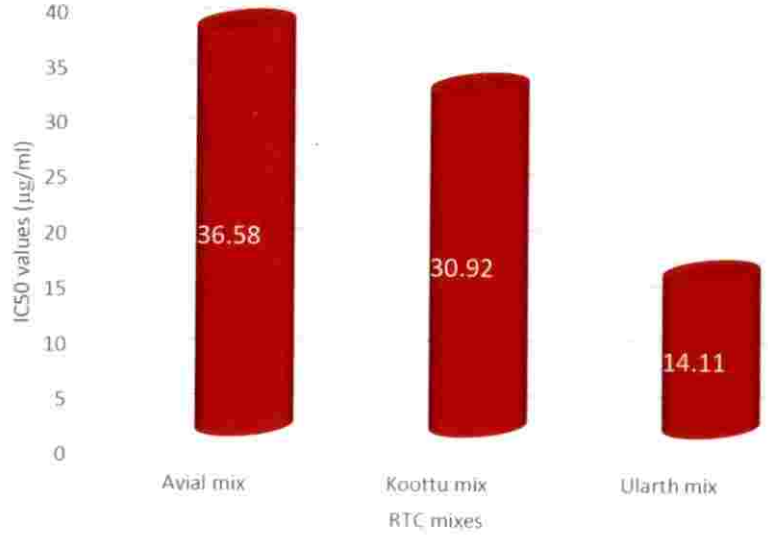


Figure 2. Nitric oxide scavenging activity of RTC mixes

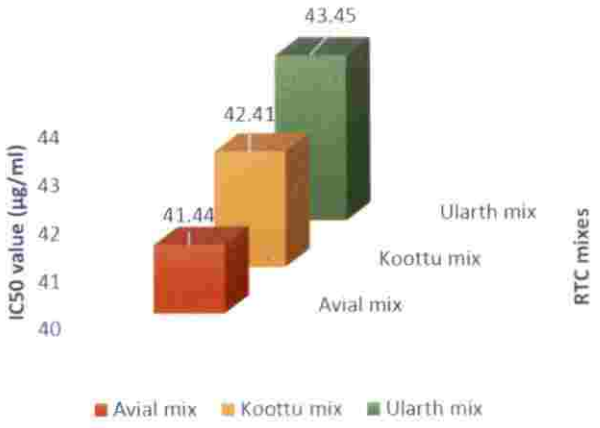


Figure 3. Total antioxidant activity of RTC mixes

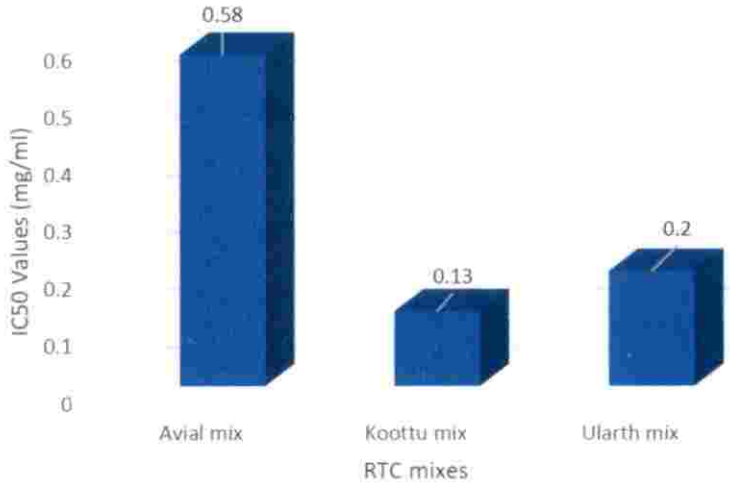


Figure 4. Ferric reducing capacity of RTC mixes

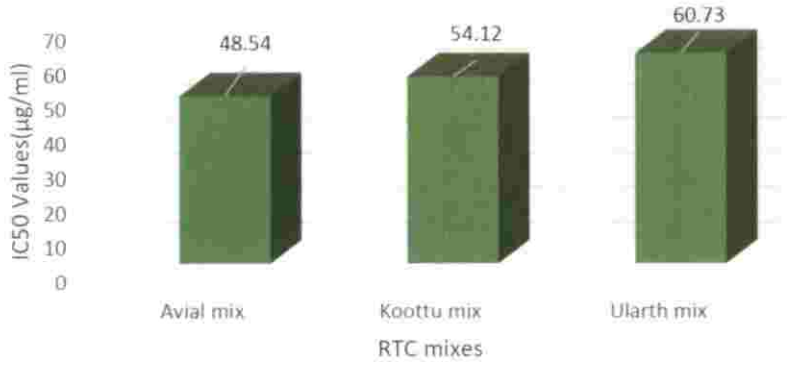
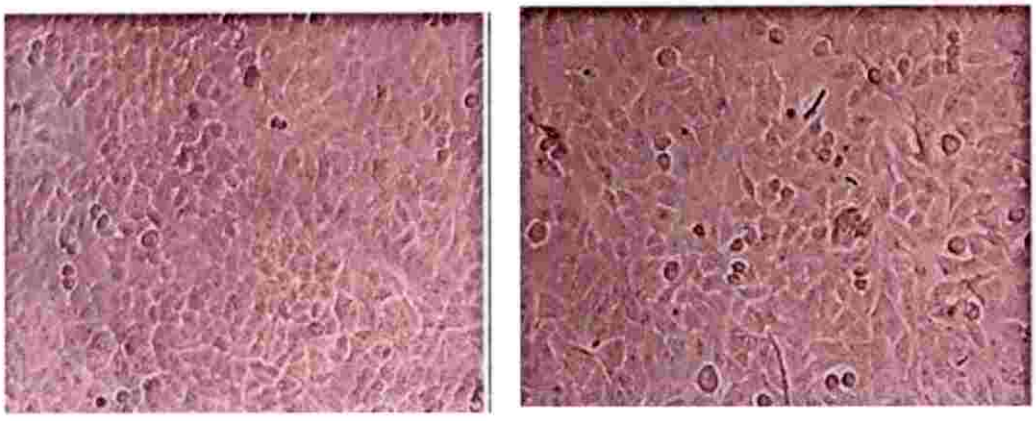


Figure 5. Superoxide anion radical scavenging activity of RTC mixes

Figure 6. Hydroxyl scavenging activity of RTC mixes

Cytotoxicity study report



Control 'Avial' mix

Plate 4 : Effect of 'Avial' mix on the viability of MCF-7 cell lines

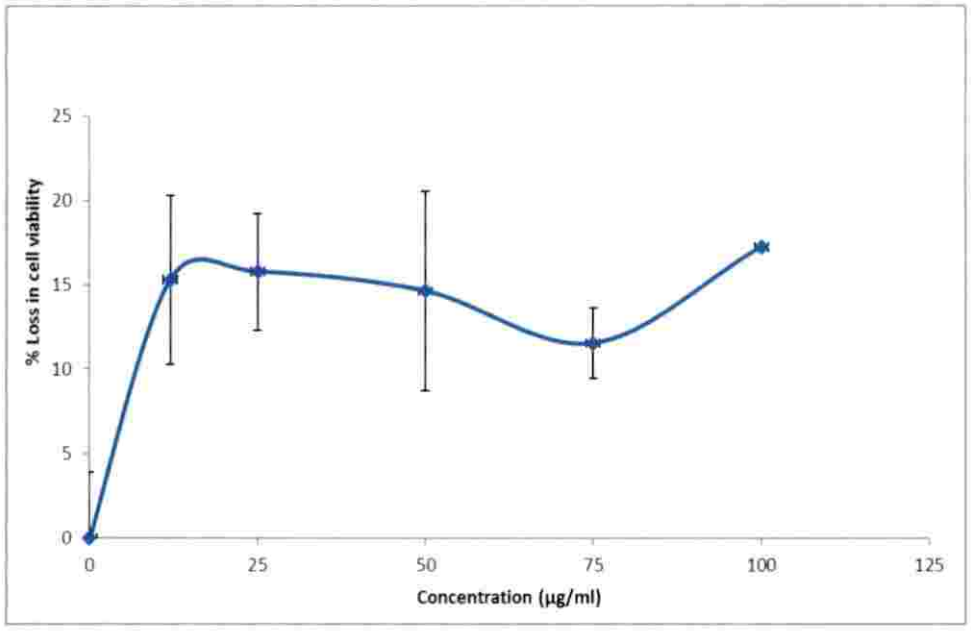


Figure 7. Cytotoxic action of 'Avial' mix

Summary

6. SUMMARY

The present study entitled "Antioxidant and anti-carcinogenic potential of jackfruit based Ready-To-Cook (RTC) curry mixes" was undertaken with the objective to ascertain the antioxidant and anti-carcinogenic properties of the jackfruit based curry mixes.

Three jackfruit based Ready-To-Cook curry mixes ('Avial' mix, 'Koottu' mix and 'Ularth' mix), which were developed earlier at the Department of Community Science, using raw jackfruit (Koozha type) were selected for the study. These curry mixes were based on traditional recipes of Kerala namely, 'Avial', 'Koottu' and 'Ularth'. The adjuncts used in 'Avial'mix were green chillies, garlic, cumin, turmeric powder and curry leaves, whereas in 'Koottu' mix, redchillies, turmeric powder, cumin and curry leaves were blended. The adjuncts used in 'Ularth' mix were crushed green chillies, onion, garlic, turmeric powder and curry leaves. The raw jackfruit bulbs and seeds after pretreatment are blended with the adjuncts as per formulations standardized and dehydrated at 65°C till crisp.

Methanol, ethanol, petroleum ether and acetone were used to extract the bioactive compounds of the powdered mixes for phytochemical screening, antioxidant analysis and verification of anti-carcinogenic property.

Results of phytochemical screening revealed that tannins were found in the curry mix extracts of methanol, ethanol and acetone. Presence of flavonoids produced a yellow colouration and was present in the curry mix extracts of methanol, ethanol and acetone. Phenols were present in methanol and ethanol extracts and were absent in acetone and petroleum ether extracts of curry mix extracts. The results of the present study revealed that alkaloids and phlobatinnins were absent in the curry mix extracts of methanol, ethanol, acetone and petroleum ether. Presence of saponins were identified in all the three curry mix extracted solvents. The results of the study revealed the absence of steroids, anthraquinones and cardiac glycosides in petroleum ether solvent and presence in all the other curry mix extracted solvents.

Quantitative analysis of antioxidants revealed that the beta carotene content of the RTC mixes was in the range of 0.07-1.16 mg/100g. Beta carotene content was found to be more in 'Avial' mix (1.16 mg/100g) and the lowest content was observed in 'Ularth' mix (0.07 mg), while 'Koottu' mix contained 0.13 mg.

Ascorbic acid content was also found to be higher for 'Avial' mix (38.45 mg), the lowest content was found for 'Ularth' mix (15.34 mg) and the difference was found to be statistically significant. The highest amount of saponin was found in 'Avial' mix (6.65 per cent) and the lowest content was observed in 'Ularth'mix (5.00 per cent). Findings of the present study revealed that the highest tannin content was observed for Ularth' mix (11.57 mg) which was on par with Avial' mix (11.52 mg), the lowest tannin content was noted for 'Koottu' mix (10.58 mg).

The phenol content was observed to be highest in 'Koottu' mix (21.53 mg) followed by 'Avial' mix (19.41 mg), the lowest phenol content was observed in 'Ularth' mix (21.53 mg). Flavonoid content of RTC mixes ranged between 0.86-3.25 mg/100g. 'Ularth' mix (3.25 mg/100g) contained highest flavonoid content. Lectin content was found to be high for 'Ularth' mix (0.76 per cent) and 'Avial' mix (0.35 per cent) contained the lowest. Alkaloids and selenium were found to be absent in all the jackfruit based Ready-To-Cook curry mixes.

In case of mineral analysis, copper content was found to be higher in 'Ularth' mix (2.57 µg) and the copper content of 'Avial' mix (1.79 µg) and 'Koottu' mix (1.19 µg) were found to be on par. The zinc content of RTC mixes ranged between 4.65-6.55 µg. The highest zinc content was observed in 'Koottu' mix (6.55 µg) and the lowest was observed for 'Ularth' mix (4.65 µg/100g). Manganese content of 'Avial' mix (2.55µg/100g) and 'Ularth' mix (2.51µg/100g) were found to be on par, and was significantly higher in 'Koottu' mix (5.30 µg/100g). The highest iron content was found in 'Ularth' mix (0.92 µg/100g) followed by 'Avial' mix (0.84 µg/100g), the lowest iron content was observed in 'Koottu' mix (0.74µg/100g).

Antioxidant activity in the present study revealed that 'Avial' mix had the highest DPPH activity with an IC_{50} value of 33.81 µg/ml followed by 'Koottu' mix (34.50 µg/ml). The lowest DPPH radical scavenging activity was observed for

'Ularth' mix (36.70 µg/ml). Total antioxidant activity was found to be more for 'Avial' mix (41.44 µg/ml), followed by 'Koottu' mix (42.41 µg/ml) and 'Ularth' mix (43.45 µg/ml). 'Avial' mix (0.58 mg/100g) showed more ferric reducing capacity while 'Ularth' mix (0.20 mg/100g) had the least capacity in this regard. ABTS radical scavenging activities of RTC mixes ranged between 34.84-46.69 µg/ml. ABTS radical scavenging activity was observed to be higher for 'Avial' mix (34.84 µg/ml) which was followed by 'Koottu' mix (38.04 µg/ml), and lower values were noted for 'Ularth' mix (40.52 µg/ml). Hydroxyl radical scavenging activity of RTC mixes was found to range between 50.55 to 52.55 µg/ml. 'Avial' mix showed higher superoxide radical scavenging activity with an IC_{50} value of 48.54 µg/ml and the lowest superoxide radical scavenging activity was observed in 'Ularth' mix (60.73 µg/ml). However, the highest nitric oxide scavenging activity was observed for 'Ularth' mix (14.11 µg/ml) followed by 'Koottu' mix (30.92 µg/ml) and 'Avial' mix (36.58 µg/ml).

Among the three jackfruit based Ready-To-Cook (RTC) curry mixes 'Avial' mix was found to have higher DPPH radical scavenging activity, higher antioxidant activity, higher ABTS radical scavenging activity, higher superoxide anion radical scavenging activity, higher hydroxyl radical scavenging activity, and was therefore taken up for studying the anti-cancer property. Cytotoxicity of 'Avial' mix was estimated by MTT assay on Human breast adenocarcinoma cells (MCF-7) and 17 per cent of loss in cell viability was observed.

Findings of the present study reveal that the jackfruit based RTC mixes comprised of various health promoting components such as beta carotene, ascorbic acid, saponins, tannins, total phenols, total flavonoids and lectins; they also had potent antioxidant activities. The antioxidant activity of all the three products are confirmed in all the seven tests. The propitious product with higher antioxidant activity was taken up for testing its anticarcinogenic potential, which also revealed reasonable results, suggesting the scope for further studies in cytotoxicity on other cancer cell lines. Thus the curry mixes, in particular the 'Avial' mix can be promoted as functional food which will help to enhance its commercialization.

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7. REFERENCES

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Abstract

ANTIOXIDANT AND ANTICARCINOGENIC POTENTIAL OF JACKFRUIT BASED READY-TO-COOK (RTC) CURRY MIXES

by

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ABSTRACT

The research work entitled, "Antioxidant and anti-carcinogenic potential of jackfruit based Ready-To-Cook (RTC) curry mixes" was conducted at College of Agriculture, Vellayani during 2017-2019, with the objective to ascertain the antioxidant and anti-carcinogenic properties of the jackfruit based curry mixes ('Avial' mix, 'Koottu' mix and 'Ularth' mix) which were developed earlier at the Department of Community Science, using raw jackfruit (Koozha type).

Methanol, ethanol, petroleum ether and acetone were used to extract the bioactive compounds of the powdered mixes for phytochemical screening, antioxidant analysis and verification of anti-carcinogenic property.

The results of the phytochemical analysis indicated the presence of tannins, flavonoids, saponins, steroids, phenolic compounds, cardiac glycosides and anthraquinones in the three mixes; whereas the analyses revealed the absence of alkaloids and phlotobatinins.

Quantitative analysis of antioxidants revealed that the beta carotene content of the RTC mixes was in the range of 0.07-1.16 mg/100g. Beta carotene content was found to be more in 'Avial' mix (1.16mg/100g). Ascorbic acid content was also found to be higher for 'Avial' mix (38.45 mg) and the lowest content was found for 'Ularth' mix (15.34 mg). The saponin content was found to be highest in 'Avial' mix (6.65 per cent) and lowest in 'Ularth' mix (5.00 per cent). The tannin content of 'Ularth' mix (11.57 mg) was observed to be on par with 'Avial' mix (11.52 mg). Total phenol content was found to be more in 'Koottu' mix (21.53 mg/100g) and the total flavonoid content of RTC mixes was seen to be in the range between 0.86-3.25 mg/100g. There was significant difference in the lectin content of 'Avial' mix (0.35%), 'Koottu' mix (0.56%) and 'Ularth' mix (0.75%). Alkaloids and selenium were found to be absent in the RTC mixes.

In case of mineral analyses, Copper content was found to be higher in 'Ularth' mix (2.57 µg); zinc content was in the range of 4.65-6.55 µg and was found

to be higher for 'Koottu' mix (6.55 µg) and lower for 'Ularth' mix (4.65 µg). Manganese content was found to be higher in 'Koottu' mix (5.30 µg) while, it was observed to be on par in 'Avial' mix (2.55 µg) and 'Ularth' mix (2.51 µg). The highest iron content was found in 'Ularth' mix (0.92 µg).

Antioxidant activity in the present study revealed that 'Avial' mix had the highest DPPH activity with an IC_{50} value of 33.81 µg/ml. Total antioxidant activity was found to be more for 'Avial' mix (41.44 per µg/ml), followed by 'Koottu' mix (42.41 µg/ml) and 'Ularth' mix (43.45 µg/ml). 'Avial' mix showed more ferric reducing capacity while 'Ularth' mix had the least capacity in this regard. ABTS radical scavenging activities of RTC mixes ranged between 34.84-46.69 µg/ml. ABTS radical scavenging activity was observed to be higher for 'Avial' mix (34.84 µg/ml) and lower values were noted for 'Ularth' mix (40.52 µg/ml). Hydroxyl radical scavenging activity of RTC mixes was found to range between 50.55-52.55 µg/ml. 'Avial' mix showed higher superoxide radical scavenging activity with an IC_{50} value of 48.54 µg/ml and the lowest superoxide radical scavenging activity was observed in 'Ularth' mix (60.73 µg/ml). However, the highest nitric oxide scavenging activity was observed for 'Ularth' mix (14.11 µg/ml) followed by 'Koottu' mix (30.92 µg/ml) and 'Avial' mix (36.58 µg/ml).

Among the three jackfruit based Ready-To-Cook (RTC) curry mixes 'Avial' mix was found to have higher antioxidant property and was therefore taken up for studying the anti-cancer property. Cytotoxicity of 'Avial' mix was estimated by MTT assay on Human breast adenocarcinoma cells (MCF-7) and 17 per cent of loss in cell viability was observed.

Findings of the present study revealed that the jackfruit based RTC mixes comprised of various health promoting components such as beta carotene, ascorbic acid, saponins, tannins, total phenols, total flavonoids and lectins they also had potent antioxidant activities. Thus the curry mixes, in particular the 'Avial' mix can be promoted as functional food which will help to enhance its commercialization.