PROFILING BIOACTIVE COMPOUNDS IN AVOCADO (*Persea americana* Mill.) CULTIVARS AND FREEZE DRIED FRUIT POWDERS, AND INVESTIGATING ITS POTENTIAL IN CANCER CELL LINES

by

Archana L

(2017-16-009)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN COMMUNITY SCIENCE

(Food Science and Nutrition)

Faculty of Agriculture

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM - 695 522

KERALA, INDIA

2019

DECLARATION

I, hereby declare that this thesis entitled "Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines." 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, fellowship or other similar title, of any other University or Society.

Place: Vellayani
Date: 09/12/2019

ARCHANA L
(2017-16-009)

CERTIFICATE

Certified that this thesis entitled "**Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines.**" is a record of bonafide research work done independently by Ms. ARCHANA L (2017-16-009) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship to her.

Place: Vellayani

Date: 09/12/2019

Dr. ANITHA CHANDRAN.C
Major advisor
Assistant Professor
Department of Community Science
College of Agriculture, Vellayani

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. ARCHANA L (2017-16-009), a candidate for the degree of **Master of Science** in Community Science with major in Food science and Nutrition, agree that this thesis entitled **"Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines."** may be submitted by Ms. Archana L, in partial fulfilment of the requirement for the degree.

Dr. Anitha Chandran.C
(Chairperson, Advisory Committee)
Assistant Professor
Department of Community Science
College of Agriculture, Vellayani
Thiruvananthapuram- 695522

Dr. Suma Divakar
(Member, Advisory Committee)
Professor and Head
Department of Community Science
College of Agriculture, Vellayani
Thiruvananthapuram- 695522

Dr. Bela G. K
Associate Professor
Department of Community Science
College of Agriculture
Vellayani

Dr. Reshma M. V
Senior Scientist
AgroProcessing&Technology Division
CSIR, NIIST, Pappanamcode
Thiruvananthapuram

ACKNOWLEDGEMENT

I thank Lord Almighty for his invisible presence with me in each and every step I take and for working His way through my parents, teachers, friends and each and every person of my life.

Let me place on record of my profound feeling of gratitude and sincere thanks to my chairperson of the advisory committee, **Dr. Anitha Chandran. C,** *Assistant professor for her valuable and affectionate guidance, constant encouragement and unfailing patience through out the course of this research work and in the preparation of the thesis. This work would not have been possible without her help and support.*

I extend my heartfelt thanks to **Dr. Suma Divakar**, *Professor and Head, Depatment of Community Science, for her motivation, guidance and advices during the course of study and writing the thesis. All those advices were extensively helpful, and I would like to thank you Madam, for that.*

I wish to express my sincere gratitude to **Dr. Bela G.K**, *Professor for her keen interest immense help, constructive suggestions and timely support and cooperation rendered throughout the course of this research endeavor.*

I avail this opportunity to pay sincere thanks to **Dr. Reshma M.V**, *Senior Scientist, CSIR, NIIST, Pappanamcode for her keen interest, immense help for freeze drying the avocados, constructive suggestions and timely support and co-operation rendered throughout my research work.*

*I am also grateful to **Dr. Brigit Joseph**, Associate Professor and Head, Department of Agricultural Statistics for his guidance, timely advice, stimulating suggestions and statistical interpretation of the experiment data.*

*I convey my gratitude to **Dr. Priya**, Senior Scientist, CSIR, NIIST, Pappanamcode, for her immense help for the cell line study of avocado, timely support, and co-operation during the course of study.*

*I am truly and deeply grateful to **Dr. Krishnaja,** Assistant Professor, for her suggestions and support for my research work*

*I wish to express my heartfelt thanks to **Dr. Anil Kumar**, Dean, College of Agriculture, Vellayani for providing me all the necessary facilities from the university during the whole course of study.*

*From the depth of my heart I thank my dearest classmate **Meera. M.V,** for her indispensable help, love, moral support and constant encouragement.*

*My loving thanks to my seniors **Subha chechi, Anila chechi, Sowmya chechi, Kavitha chechi** and also my juniors **Abhina, Himabindu** for their support and encouragement for my research work.*

I also thank non-teaching staffs of the department for all their love and support.

*I express my thanks and wholehearted cheers to my friends **Annie** and **Roshna** for their help, love, encouragement and support which made my days more beautiful and joyful.*

Words are failed to express my love and gratitude from my deep heart to my dearest ***Achan, Amma, Ammumma*** *and* ***Chettan****. The love, support, patience and constant encouragement given by them was a real source of inspiration without which I could not complete this research endeavor.*

I thank all those who extended help and support to me during the course of my work.

Archana L

DEDICATED TO MY FAMILY

CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF PLATES

LIST OF ABBREVIATIONS

%	Per cent
CD	Critical Difference
et al.	And other co workers
μg	Microgram
G	Gram
g/100g	Gram per 100g
Mg	Milligram
Fig.	Figure
Viz.	Namely

INTRODUCTION

1. INTRODUCTION

Everybody knows the significance of fruits as a part of a well-balanced diet, but all are not aware about the nutritional facts. Eating a healthy amount of fruit helps as to maintain different body processes. Various fruits contain various nutrients such as vitamins, minerals, antioxidants and phytonutrients, so it is important to east variety of fruits.

Avocado *(Persea americana* Mill.*)* is a tropical ever green climacteric fruit.The climate zone of avocados is from true tropical to warmer parts of the temperate zone, also called as alligator pear fruit of the family Lauraceae. Origin of avocado tree is from Western Hemisphere of Mexico and it is a greenish fruit with buttery consistency and a rich nutty taste and flavour. They are often eaten in salads, and adds in different dessert.

Production of avocado in India is very limited and they are not commercial plantation. The agro-climatic conditions prevailing in various parts of the country found to be favourable for bringing more areas under avocado. But in present condition plantations are not well organized and they are scattered. Also, quite a good number of improved varieties are now available in different regions of India with great yield potential. Avocados are grown scattered in south Indian states like Kerala, Tamil Nadu, Karnataka, and Maharashtra (Tripathy *et al.*, 2014).

In Kerala this fruit is introduced in markets by ending of 20^{th} century. The cultivars found in Kerala are Purple hybrid, Fuerte, Kallar round and Pollock and mainly found grown in homesteads of Wayanad and Idukki Districts. A number of varieties of avocados are propagated and distributed to farmers by Regional Agricultural Research Station, Kerala Agricultural University Ambalavayal, Wayanad.

The shelf life qualities of avocado fruits are limited due to its high rate of postharvest respiration. Polyphenol oxidase is an enzyme present in the fruit which makes it easily oxidize, susceptible to browning. Freeze drying of avocado is one of the best methods of processing to reduce its wastage.

Saucedo *et al.* (2014) opined that freeze drying technology is the best and common in foreign countries which is a process of dehydration process to preserve shelf-life and allowing avocado to maintain its sensorial and nutritional characteristics and reported that freeze dried powder is more nutrient rich than fresh avocado.

Avocado fruit as well as its seed and leave had several nutritional factors. The pulp content in different varieties is between 52.9 and 81.3%, relative to fruit mass (Tango *et al.*, 2004).

Tentolouris *et al.* (2008) opined that high lipids and low carbohydrate levels remain in avocado pulp after water removal, thus conferring high dry matter content to the product. Therefore, it is considered one of the few cultured fruits presenting the lipid fraction as the major component, which can reach up to 25% of the fruit portion.

The avocado pulp having moisture content from 67 to 78% , 13.5 to 24 % lipids, 0.8 to 4.8% carbohydrate, 1.0 to 3.0% protein, 0.8 to 1.5% ash, 1.4 to 3.0% (Rainey *et al.*, 1994). The spreadable pulp of avocado is a great source of mono-unsaturated fatty acids like oleic and palmitoleic acids and polyunsaturated fatty acid like linoleic acid.

100 g of fresh pulp of avocado contain an average of 62 mcg of beta-carotene, 28 mcg of beta-cryptoxanthin, 24 mcg of alphacarotene, and 271 mcg of lutein + zeaxanthin, which helps in the prevention of diseases such as cancer, diabetes etc. Sterols are a predominant group present in avocado fruit, and the main component of this group is the β-sitosterol, comprising about 80% of the sterols. The other types of sterols present in avocado are, stigmasterol, campesterol and cholesterol. A seven-carbon sugar present in

avocado pulp that is D-mannoheptulose which enhances the production of insulin by the body and it controls obesity also helps in the destruction of free radicals in the body.

Avocados contain a number of cancer resisting compounds such as bioactive phytochemicals including carotenoids, terpenoids, perseitol A and B, phenols D-mannoheptulose, , and glutathione (Ding *et al.*, 2005). Flagg (1994) opined in his study about the role of avocado in cancer therapy, and he pointed out that avocado's glutathione levels (8.4 mg per 30 g) can prevent breast, laryngeal and mouth cancers. Caroteniod is the component act against breast cancer cells.

Hence the present study "Profiling bioactive compounds in avocado cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines." was undertaken with the objective to ascertain bioactive compounds in fresh fruits and freeze dried powders of avocado (*Persea americana* Mill.) cultivars and to evaluate anti proliferation activities of freeze dried powder of superior cultivar in cancer cell lines.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The relevant literature available on the study entitled "Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines" have been briefly reviewed here.

2.1 Avocado – A Positive Super Fruit

The avocado (*Persea americana* Mill.) is a super positive fruit originated in Mexico, Central or South America, and was first cultivated in Mexico as early as 500 BC (Duester, 2000). The first English language mention of avocado was in 1696. In 1871, avocados were first introduced to the United States, California, with trees from Mexico. In India it was bought during first decade of nineteenth century. In India avocado cultivation mainly going on in Maharashtra, Tamil Nadu, Kerala (wayanaud), Sikkim and Karnataka.

Avocado is the most nutritive among fruits. The avocado contains about 136g of pleasant, creamy, smooth texture edible fruit covered by a thick dark green, purplish black and bumpy skin. The pulp is rich in proteins (up to 4%) and fat (up to 30%), but low in carbohydrates. The avocado seed and skin comprise about 33% of the total whole fruit weight. The fat in fruit pulp is similar to olive oil in composition and is widely used in the preparation of cosmetics. Avocados have the highest energy value (245 cal/100 g) of any fruit besides being a reservoir of several vitamins and minerals. The avocado is rich in copper and iron; two mineral constituents of antioxidant enzymes, avocados again prove their nutritional quality. Potassium is also high in avocados, as it is has one of the highest potassium rates in tropical and non-tropical fruits and vegetables (USDA, 2011).

The avocado is associated with lower blood pressure because and this fruit is high in monounsaturated fat. Almost all amino acids are found in the avocado. Another promising fact is that avocados have been shown to maintain good cholesterol while reducing bad cholesterol. Avocado is mainly used fresh, in sandwich filling or in salads. It can also be used in ice creams and milk shakes and the pulp may be preserved by freezing. Three antifungal compounds have been isolated from the peel extracts of immature fruits of the Green cultivar. The avocados are found, to contain high proteins, their caloric value ranges from 1500to 2250 calories per Kg (i.e. 2.3 pounds) (Ana *et al.*, 2013).

2.1.1 Major avocado varieties grown in different areas

Table: 1

India	Green Type, Purple, TKD-1, Nabal, Linda, Puyevla, Gott-Froid, ,Furete, Pullock, Waldin, Kallar round.
USA	Furerte, Hass, Zutano, Bacon, Reed, Gwen, Pinkerton,Pollock, Simmonds, Nadir, Booth 8, Lula, Hardee, Ruchle, Hall, Hickson, Monroe.
Mexico	Fuerte, Hass, Bacon, Reed, Criollor, Zutano
New zealand	Fuerte, Zutano, Hayes, Hopkins, Hass, Polock, Booth, Simmonds, Reed.
Australia	Zutano, Sharwd, Bacon, Purete, Hass, Green Gold.
Brazil	Fuerte, Hass, Carlsbad, Nabal, Ryan, Edranol, Corona, Solano, Quintal, Fortuna, Ouro Verde
South Africa	Fuerte, Hass, Edranol, Ryan, Hayes, Pollock
Spain	Hass, Bacon, Furete, Reed, Sutano, Gwen

(Tripathi and Karunakaran, 2014)

2.2 Nutritional composition of avocado fruit (100g)

Avocado is a nutrient and phytochemical dense fruit, which contain following nutrients

Table: 2

Nutrients	Quantity	Nutrients	Quantity
Protein	1.7g	Calcium	10.00mg
Fat	26.4g	Chlorine	11.00mg
Carbohydrate	5.1g	Copper	0.45mg
Crude fibre	1.8g	Iron	0.60mg
Vitamin A(carotene)	0.17mg	Magnesium	35.00mg
Ascorbic acid	16.00mg	Manganese	4.21mg
Niacin	1.10mg	Phosphorus	38.00mg
Riboflavin	0.13mg	Sodium	368.00mg
Thiamine	0.06mg	Sulphur	28.50mg
Folic acid	89.0mg	Beta carotene	63.0mg
Monounsaturated fatty acids	9.80g	Polyunsaturated fatty acids	1.82g

(Tripathi and Karunakaran, 2014)

24p

2.3 Uses of other parts of avocado

Avocado seed is underutilized and represents a large portion of the fruit, thus its use can be an alternative to reduce the production cost of edible oil. However, the main problem in the use of avocado seeds is the presence of phenolic compounds that exhibit toxicity (Wright *et al.*, 2007).

Studies by Bergh (1992) have shown that the seeds can be used in feed for monogastric animals after extraction of these substances with ethanol. The extract may present antioxidant activity, once the phenolics levels in seeds vary from 2.3 to 5.7%. In addition to the starch and fiber, there are other non-nitrogenous substances present in seeds, ranging from 5.1 to 13.2%.

Avocado leaves are a pharmaceutical ingredient widely used in extracts for therapeutic purposes, and also used as folk medicine probably due to the diuretic properties (Wright *et al.*, 2007).

Phytochemicals present in leaves are luteolin, rutin, quercetin, and, which can help prevent the progress of various diseases related to oxidative stress (Olatunji *et al.*, 2010).

2.3.1 Avocado based cosmetics and beauty

Skin creams in early times were described as foods for skin and they were said to penetrate and feed the skin, reduce wrinkles and generally make the face look younger. The constituents in skin foods that 'fed' the skin were oils and fats with the better skin food. Because of the fat content we can use avocado to nourish our skin (Roberts *et al.*, 2009).

Batiska *et al.* (1993) opined that one of the suggested uses for avocado oil was cosmetics and initial findings appeared promising. Apart from its greenish colour it compared favourably with lanolin as it had good skin penetration, high vitamin content and excellent keeping qualities. Another advantage is that it also produced finer emulsions when used in skin creams and lotions.

Among the properties of the avocado oil which make it valuable as a cosmetic ingredient are the following:

1. It is one of the most penetrating oils. It serves as a "carrier" for other valuable substances which lack penetrating power. This property makes it of value for use in tissue or nourishing creams, massage creams, massage oils (muscle oils)

2. Manufacturers who use Avocado oil in high quality toilet soaps attribute its superior lathering and cleansing properties to the oil.

3. Avocado is rich in vitamins A, B, E and D (oil soluble). When properly prepared, it presents an advantage over most other vegetable or fruit oils available for cosmetic use (Batiska *et al.*, 1993).

The important fact is that Avocado oil is the highest priced oil used in cosmetics and medicines has proved of benefit to manufacturers who use the product.

Whiley (2000) opined that most avocado oil used in cosmetics in the late twentieth century was refined and bleached with the resulting product being an odourless, yellow oil. The introduction of cold-pressed avocado oil was first investigated in the 1980s but only becoming a commercial reality in the twenty-first century – has opened up new possibilities for the oil, with virgin and extra virgin avocado oil and now being sold as a culinary oil in supermarkets.

2.3.2 Complementary and Transitional Feeding

Birch L.L and Doub (2014), in their study reported that proper nutrition is one of the most influential factors for insuring normal growth and development during a child's first years of life, yet there are currently less research-based dietary recommendations for parents regarding this critical time period (Currently, U.S. dietary guidelines do not differentiate between age groups of children under two years old even though the nutritional requirements of infants and toddlers differ from each other, and from the nutritional requirements of older children, adolescents, and adults).

American Heart Association (AHA), reported that Hass Avocado Board (HAB) conducted a survey (HAB Caregiver Survey) among 338 caregivers of infants between the ages of four and 24 months also shows nearly half of the respondents introduced avocado fruit supplements between four and six months of age, while approximately one-third introduced protein foods (meat, beans, eggs, peanut butter), grains or dairy in that same time period.

By the end of the survey AHA found that infants tripled their body weight between birth and the end of their first year, and much of this growth occurs in the complementary feeding period with avocado based complementary food. In order to accomplish the high rate of growth and development, infants must consume both adequate amounts of energy and essential nutrients. Foods consumed throughout the complementary feeding stage should balance the nutrients from milk or formula, without imposing excess intakes of energy or nutrients. Since complementary foods are initially consumed in very small quantities, caregivers should offer moderately energy-dense foods, rich in multiple nutrients that are key for proper infant health and development (*i.e.*, iron, zinc, calcium, provitamin A/carotenoids, vitamin C, and folate) (Birch L.L and Doub, 2014).

This findings shows avocado is a better option for infants as complementary food.

2.4 Nutritional and physiochemical compounds of avocado

Avocado can be consumed as puree, salads, seasoned with salt, pepper, and other condiments, as well as being used in the preparation of other variety of dishes (Koller, 1992).

In Brazil, the ripe fruit is more acceptable, together with sugar, honey and liqueurs, and consumption is influenced mainly by its sensory and nutritional characteristics (Koller, 1992).

The pulp content in several varieties is between 52.9 and 81.3%, relative to fruit mass (Tango *et al.*, 2004). High lipids and low carbohydrate levels remain in avocado pulp after water removal, thus conferring high dry matter content to the product. Therefore, it is considered one of the few cultured fruits presenting the lipid fraction as the major component which can reach up to 25% of the fruit portion.

The avocado pulp contains from 67 to 78% moisture, 13.5 to 24 % lipids, 0.8 to 4.8% carbohydrate, 1.0 to 3.0% protein, 0.8 to 1.5% ash, 1.4 to 3.0% fiber, and energy density between 140 and 228kcal (USDA, 2011).

Avocado has four times more nutritional value than any other fruit except banana, containing proteins (1 to 3%) and significant levels of fat-soluble vitamins, folic acid, and appreciable amounts of calcium, potassium, magnesium, sodium, phosphorus, sulfur and silicon, and vitamins E, B1, B2, and D.

The avocado fruit stands out on potassium levels (339mg $100g^{-1)}$ when compared to other fruits, which regulates muscle activity and protects the body from cardiovascular diseases. It also represents a source of glutathione, a powerful antioxidant that acts on potentially carcinogenic compounds (Dembitsky *et al.*, 2011).

2.4.1 Minerals

2.4.1.1 Potassium

Clinical evidence suggests that adequate potassium intake may promote blood pressure control in adults. The mean intake of potassium by adults in the United States was approximately 3200 mg per day in men and 2400 mg per day in women, which is lower than the 4700 mg per day recommended intake (USDA, 2011).

Avocados contain about 152 mg and 345 mg of potassium per 30 g and one-half fruit, respectively. Also, avocados are naturally very low in sodium with just 2 mg and 5.5 mg sodium per 30 g and one-half fruit, respectively (USDA, 2011). The health claim for blood pressure identifies foods containing 350 mg potassium and less than 140 mg of sodium per serving as potentially appropriate for this claim (FDA, 2000).

2.4.1. 2 Magnesium

Magnesium acts as a cofactor for many cellular enzymes required in energy metabolism, and it may help support normal vascular tone and insulin sensitivity. Preliminary preclinical and clinical researches suggest that low magnesium may play a role in cardiac ischemia (IOM, 1997).

In the Health Professionals Study by Al-Delaimy *et al.* (2004) the results suggested that the intake of magnesium had a modest inverse association with risk of coronary heart disease in men). Magnesium was shown to inhibit fat absorption to improve postprandial hyperlipidemia in healthy subjects.

Avocados contain about 9 and 20 mg magnesium per 30 g and one-half fruit, noted by (USDA, 2011).

2.4.2 Bioactive compounds and its benefits

In addition to the important major compounds, avocado contains substantial amounts of bioactive compounds such as phytosterols, especially in the lipid fraction, and the main representative is the β-sitosterol (Tripathy and Karunakaran, 2014)

2.4.2.1 Phytosterols

Diets rich in phytosterols can lead to the reduction of the total cholesterol and LDL cholesterol (Bergh, 1992).

Bergh (1992) reported that 17% decrease average in blood cholesterol levels was observed in a study in Mexico with 45 volunteers who consumed avocado once a day for one week.

Phytosterol is a substance of vegetable origin whose structure is very similar to cholesterol. Its mechanism of action in the body involves the inhibition of intestinal cholesterol absorption and decreased hepatic cholesterol synthesis.

According to Bergh (1992) it acts on total plasma cholesterol and LDL cholesterol without affecting HDL and blood triglycerides. The benefit of cholesterol reduction also comes from replacing saturated by unsaturated fats, which promote a decrease in total cholesterol and LDL and an increase in HDL levels.

The β-sitosterol in avocados also has a special effect on immunity, contributing to the treatment of diseases such as cancer, HIV and infections (Bouis, 2002).

Bouis (2002) reported that in relation to cancer, it works by suppressing carcinogenesis and in HIV by strengthening the immune system. This compound enhances lymphocytes proliferation and natural killer cell activity, which inactivates invading microorganisms.

The health effects of sterols and stanols have been the subject of several studies. Some authors have demonstrated a 25% reduction in the risk of coronary heart disease with the consumption of 2g of such compounds per day, which are included in the formulations of margarines, spreads, and vegetable oils by esterification without affecting vitamins solubility. The avocado oil variety Margarida contains a greater diversity of sterols, and β-sitosterol represents 71.8% of the total sterols, besides lower cholesterol levels (0.3%), which can achieve up to 2.3% in other varieties (Bergh, 1992).

Santana *et al.* (2015) investigated the oil from Fortuna avocado extracted with petroleum ether and subjected to drying under forced air (40°C), and found 87.6% β-sitosterol, 12.41% campesterol, and 0.04% stigmasterol of the total phytosterols.

2.4.2.2 Carotenoids

Carotenoids in avocado are a subclass known as xanthophylls, oxygen-containing fat-soluble antioxidants Xanthophylls, such as lutein, are more polar than carotenes (the other carotenoid subclasses including *β*-carotene), so they have a much lower propensity for pro-oxidant activity (USDA, 2011).

Wu *et al.* (2004) reported that avocados have the highest lipophilic total antioxidant capacity among fruits and vegetables. In a relatively healthy population, the DASH diet pattern clinical study reported reduced oxidative stress (blood ORAC and urinary isoprostanes) compared to a typical American diet (Miller *et al.*, 2005), which appears primarily due to the DASH diet providing significantly more serum carotenoids, especially the xanthophyll carotenoids lutein, *β*-cryptoxanthin, and zeaxanthin, as a result of increased fruit and vegetable consumption. Xanthophylls appear to reduce circulating oxidized LDL-C, a preliminary biomarker for the initiation and progression of vascular damage.

The Los Angeles Atherosclerosis Study, a prospective study by Dwyer *et al.* (2004) suggest that higher levels of plasma xanthophylls were inversely related to the progression of carotid intima-media thickness, which may be protective against early atherosclerosis Although this research is encouraging, more clinical studies are needed to understand the cardiovascular health benefits associated with avocado carotenoids.

Intake of avocados can be an important dietary source of xanthophyll carotenoids. Avocado carotenoid levels tend to significantly increase as the harvest season progresses from January to September (Lu *et al.*, 2009).

In avocados, xanthophylls lutein and cryptoxanthin predominate over the carotenes, contributing about 90% of the total carotenoids (Lu *et al.*, 2009). USDA reports lutein and zeaxanthin at 81 μg and 185 μg per 30 g and one half fruit, respectively, and cryptoxanthin at 44 μg and 100 μg per 30 g and one-half fruit, respectively (USDA, 2011). However, a more comprehensive analysis of avocados including xanthophylls has found much higher levels ranging from 350–500 μg per 30 g to 800–1100 μg per one-half fruit at time of harvest . The color of avocado flesh varies from dark green just under the skin to pale green in the middle section of the flesh to yellow near the seed (Lu *et al.*, 2009).

The total carotenoid concentrations were found to be greatest in the dark green flesh close to peel (Lu *et al.*, 2009).

The intestinal absorption of carotenoids associated with the presence of dietary fat to solubilize and release carotenoids for transfer into the gastrointestinal fat micelle and then the circulatory system (Ashton *et al.*, 2006).

Avocado fruit has a unique unsaturated oil and water matrix naturally designed to enhance carotenoid absorption (Unlu *et al.*, 2005).

For salads, a significant source of carotenoids, reduced fat or fat free salad dressings are common in the marketplace and these dressings have been shown to significantly decrease carotenoid absorption compared to full fat dressings (Unlu *et al.*, 2005).

Another clinical research has shown that adding avocado to salad without dressing, or with reduced fat/fat free dressing and serving avocados with salsa increases carotenoid bioavailability by 2–5 times (Unlu *et al.*, 2005).

2.4.2.3 Phenols

Preliminary evidence by Chong *et al.* (2010), suggests beneficial effects of fruit phenolics on reducing CVD risk by reducing oxidative and inflammatory stress, enhancing blood flow and arterial endothelial health, and inhibiting platelet aggregation to help maintain vascular health Avocados contain a moderate level of phenolic compounds contributing 60 mg and 140 mg gallic acid equivalents (GAE) per 30 g and one-half fruit, respectively.

Wu *et al.* (2004), reported that avocado also has a total antioxidant capacity of 600 μmol Trolox Equilvalent (TE) per 30 g or 1350 μmol TE per one-half fruit This places avocados in the mid-range of fruit phenolic levels. Avocados have the highest fruit lipophilic antioxidant capacity, which helps to reduce serum lipid peroxidation and promoting vascular health.

2.4.3 Antioxidant vitamins in avocado

2.4.3.1 Vitamin C and Vitamin E

According to IOM (2000) avocados are one of the few foods that contain significant levels of both vitamins C and E. Vitamin C plays an important role in recycling vitamin E to maintain circulatory antioxidant protection such as potentially slowing the rate of LDL-cholesterol oxidation. Evidence suggests that vitamin C may contribute to vascular health and arterial plaque stabilization.

Honarbakhsh and Schachter, (2009) noted in their recent article that vitamin C might have greater CVD protective effects on specific populations such as smokers, obese, and overweight people; people with elevated cholesterol, hypertension, and type 2 diabetics; and people over 55 years of age.

Avocado fruit contains 2.6 mg and 6.0 mg vitamin C per 30 g and one-half fruit, respectively. Avocados contain 0.59 mg and 1.34 mg vitamin E (α-tocopherol) per 30 g and one-half avocado, respectively (USDA, 2011).

One randomized clinical study by Salonen *et al.* (2003) suggested that a combination of vitamin C and E in avocado may slow atherosclerotic progression in hypercholesterolemic persons.

2.4.3.2 Vitamin K_1 (phylloquinone)

Vitamin K_1 functions as a coenzyme during synthesis of the biologically active form of a number of proteins involved in blood coagulation and bone metabolism (IOM, 2000).

Phylloquinone (K_1) from plant-based foods is considered to be the primary source of vitamin K in the human diet. Vitamin K_1 in its reduced form is a cofactor for the enzymes that facilitate activity for coagulation (McCann and Ames, 2009).

The amount of vitamin K_1 found in avocados is 6.3 μg and 14.3 μg per 30 g and one-half fruit, respectively.

Some people on anticoagulant medications are concerned about vitamin K intake; however, the avocado level of vitamin K_1 per ounce is 150 times lower than the 1000 μg of K_1 expected to potentially interfere with the anticoagulant effect of drugs such as warfarin (Coumadin) (USDA, 2011).

2.4.3.2 B-vitamins

Deficiencies in B-vitamins especially and B-6 may increase homocysteine levels, which could reduce vascular endothelial health and increase CVD risk (FAO, 2014).

Avocados contain 27 μg folate and 0.09 mg vitamin B-6 per 30 g and 61 μg folate, respectively, and 0.20 mg vitamin B-6 per one-half fruit (USDA, 2011).

2.5 Fresh avocado Vs Freeze-dried avocado powder

Freeze - drying or lyophilisation is considered as the best method of water removal to get final products of the highest quality. Low temperatures are required for the process, most of the deterioration reactions and microbiological activities are prevented, which gives a final product of good quality (Morris *et al.*, 2004).

It seems almost mission impossible to turn avocado into powder while keeping all its nutrients. Grinding dried avocados usually results in a viscous thick green mass due to the very high fat content of the avocado (55% of all solids). Usual solutions for this problem have been the addition of high amounts of chemical carriers or extracted fibres or performing defatting of the fresh product prior to drying and grinding (Morris *et al.*, 2004).

According Morris (2004), by freeze drying, all nutrients, smells and colours of the avocado are preserved. The whole process can be defined as raw, as it implies neither heating nor additional processing. The avocado powder is 99% pure with only citric acid and ascorbic acid added. Furthermore, the powder is made from avocados that could not be sold other-wise and would have been thrown out, this way helping reducing food waste.

Freeze-dried avocado powder not only opens up for replacing refrige-rated or frozen avocado paste or fresh fruit thereby making storage and handling easier. It also opens up for new application possibilities e.g. in super food smoothie mixes and in raw food products (Morris *et al.*, 2004).

The pure avocado powder has a long shelf life and does not require refri-geration before opening. Simply by adding cold water, the avocado powder is reconverted into avocado pulp. It can be used in many applications, for instance as a pure natural avocado flavour, as an ingredient for coating of healthy snacks, or as a basis for guacamole (just add your favourite guacamole spices and water) and in dressings (Ana and Sergio, 2013).

2.6 Health benefits of Avocado

2.6.1 Weight management

The availability and consumption of healthy foods, including vegetables and fruits, is associated with lower weight and body mass index (BMI) (Bes-Rastrollo *et al.*, 2008).

Over the last decades, there has been the general thought that consuming foods rich in fat can lead to weight gain, and low-fat diets would more effectively promote weight control and reduce chronic disease risk (Sacks *et al.*, 2009).

However, a key large, randomized, long-term clinical trial found that a moderate fat diet can be an effective part of a weight loss plan and the reduction of chronic disease risk (Sacks *et al.*, 2009).

According to USDA and HHS, (2011), Strong and consistent evidence indicates that dietary patterns that are relatively low in energy density improve weight loss and weight maintenance among adults. Three randomized controlled weight loss trials found that lowering food-based energy density by increasing fruit and/or vegetable intake is associated with significant weight loss (Saquib *et al.*, 2008).

Savage *et al.* (2008), noted that the energy density of an entire dietary pattern is estimated by dividing the total amount of calories by the total weight of food consumed; low, medium, and high energy density diets contain 1.3 kcal, 1.7 kcal, and 2.1 kcal per g, respectively

Avocados have both a medium energy density of 1.7 kcal/g and viscose water, dietary fiber and fruit oil matrix that appears to enhance satiety (Wien *et al.*, 2011). This is consistent with research by Bes-Rastrollo *et al.* (2008), which suggests that avocados support weight control similar to other fruits.

Several preliminary clinical studies suggest that avocados can support weight control. The first trial studied the effect of including one and a half avocados (200g) in a weight loss diet plan. In this study, sixty-one healthy free-living, overweight, and obese subjects were randomly assigned into either a group consuming 200 g/d of avocados (30.6 g fat) substituted for 30 g of mixed fats, such as margarine and oil, or a control group excluding avocados for 6 weeks (Pieterse *et al.*, 2005). Both groups lost similar levels of weight, body mass index (BMI), and percentage of body fat ($p < 0.001$) to confirm that avocados can fit into a weight loss diet plan.

A randomized single blinded, crossover postprandial study of 26 healthy overweight adults suggested that one-half an avocado consumed at lunch significantly reduced self-reported hunger and desire to eat, and increased satiation as compared to the control meal ($p < 0.002$) (Wien *et al.*, 2011).

Additionally, several exploratory trials shown that MUFA rich diets help protect against abdominal fat accumulation and diabetic health complications (Tentolouris *et al.*, 2008).

2.6.2 DNA Damage Protection

Some clinical studies suggest that xanthophylls, similar to those found in avocados, may have antioxidant and DNA protective effects and it also have possible healthy aging protective effects. The study was conducted involving 82 male airline pilots and frequent air travelers who are exposed to high levels of cosmic ionizing radiation known to damage DNA, potentially accelerating the aging process (Yasir *et al.*, 2010).

There was a significant link between the intake of vitamin C, beta-carotene and lutein-zeaxanthin from fruits and vegetables and the frequency of chromosome translocation, a biomarker of cumulative DNA damage ($p < 0.05$). In another trial, lipid peroxidation (8-epiprostaglandin F2a) was correlated inversely with plasma xanthophyll levels (*Haegele et al.*, 2010).

In another study conducted by Hughes *et al.* (2009); Thomson *et al.*(2007), inverse correlations were found between lutein and oxidative DNA damage as measured by the comet assay, and in contrast to beta-carotene.

2.6.3 Osteoarthritis

Osteoarthritis (OA) is defined as the gradual deterioration of joint cartilage and function with associated impairment, and this affects most people as they age or become overweight or obese (Dinubile, 2010). This joint deterioration may be enhanced by oxidative and inflammation stress, which can cause an imbalance in biosynthesis and degradation of the joint extracellular matrix leading to loss of function (Dinubile, 2010; Gabay *et al.*, 2008; Jacques *et al.*, 2006).

Another study reported that fruits and vegetables rich in lutein and zeaxanthin (the primary carotenoids in avocados) are associated with decreased risk of cartilage defects (early indicator of OA) (Wang *et al.*, 2007).

According to Dinubile (2010), avocado and soy unsaponifiables (ASU) is a mixture of fat soluble extracts in a ratio of about 1(avocado):2(soy). The major components of ASU are considered anti-inflammatory compounds with both antioxidant and analgesic activities (Ernst, 2003). In vitro studies found that pretreatment of chondrocytes with ASU blocked the activation of COX-2 transcripts and secretion of prostaglandin E_2 (PGE_2) to baseline levels after activation with lipopolysaccharide (LPS). Further study revealed that ASU can also block tumor necrosis factor-α (TNF-α), IL-1β, and iNOS expression to levels similar to those in nonactivated control cultures. Laboratory studies suggest that ASU may facilitate repair of OA cartilage through its effect on osteoblasts.

Clinical support for ASU in the management of hip and knee OA comes from four randomized controlled trials and one meta-analysis.

All these studies used 300 mg per day. The clinical trials were generally positive with three providing OA support and only one study pointing out that there were no joint cartilage improvement (Dinubile, 2010).

2.6.4 Eye Health

Lutein and zeaxanthin are selectively taken up into the macula of the eye (the portion of the eye where light is focused on the lens) (Caepentier *et al.*, 2009).

According to Johnson *et al.* (2010) relative intakes of lutein and zeaxanthin decrease with age and the levels are lower in females than males. Mexican Americans have the highest intake of lutein and zeaxanthin than any other ethnicity and they are among the highest consumers of avocados in the United States. Observational studies show that low dietary intake and plasma concentration of lutein may increase age-related eye dysfunction (Ma *et al.*, 2009; Chong *et al.*, 2009; Wang *et al.*, 2007).

Research from the Women's Health Initiative Observation Study found that MUFA rich diets were protective of age-related eye dysfunction (Chong *et al.*, 2009).

Avocados may contribute to eye health since they contain a combination of MUFA and lutein and help improve carotenoid absorption from other fruits and vegetables. Avocados contain 185 μg of lutein/zeaxanthin per one-half fruit, which is expected to be more highly bioavailable than most other fruit and vegetable sources (Unlu *et al.*, 2005).

2.6.5 Skin Health

Skin shows the first visible indication of aging. Application or consumption of some fruits and vegetables or their extracts such as avocado has been recommended for skin health (Roberts *et al.*, 2009; Morganti *et al.*, 2002).

The facial skin is frequently subjected to ongoing oxidative and inflammatory damage by exposure to ultraviolet (UV) and visible radiation and carotenoids may be able to combat this damage. A clinical study found that the concentration of carotenoids in the skin is directly related to the level of fruit and vegetable intake Avocado's highly bioavailable lutein and zeaxanthin may help to protect the skin from damage from both UV and visible radiation (Roberts *et al.*, 2009).

Several studies suggest that topical or oral lutein can provide photo-protective activity (Palombo *et al.*, 2007; Morganti *et al.*, 2002).

A cross-sectional study examined the relationship between skin anti-aging and diet choices in 716 Japanese women. After controlling for covariates including age, smoking status, BMI, and lifetime sun exposure, the results showed that higher intakes of total dietary fat were significantly associated with more skin elasticity. A higher intake of green and yellow vegetables was significantly associated with fewer wrinkles.

Several preclinical studies suggest that avocado components may protect skin health by enhancing wound healing activity and reducing UV damage (Nayak *et al.*, 2008).

2.6.6 Avocado and cardiovascular health

Major components in avocado helps to prevent cardiac diseases are Omega3 and Omega 6 fatty acids, which are act by improving the condition of blood vessels. Oleic acid in avocado prevents the absorption of cholesterol and triglycerides to our body (Caepentier *et al.*, 2009)

Salonen (2003) tested three different diets, among 45 hyper cholesterolemic adults between the ages of 40 and 70, which all designed to lower cholesterol: a lower-fat diet, consisting of 24 percent fat, and two moderate fat diets, with 34 percent fat and the researchers tested the diets with the participants' baseline measurements; all three diets significantly lowered LDL also known as bad cholesterol as well as total cholesterol. By the ending researchers found that participants experienced a greater reduction in LDL and total cholesterol while on the avocado diet, compared to the other two diets.

2.7 Avocado: A super cancer fighting food

Bioactive compounds and phytochemicals including carotenoids, terpenoids, D-mannoheptulose, persenone A and B, phenols, and glutathione that have been reported to have anti-carcinogenic properties. The concentrations of some of these phytochemicals in the avocado may be potentially efficacious (Ding *et al.*, 2005).

Currently, direct avocado anti-cancer activity is very preliminary with all data based on in vitro studies on human cancer cell lines.

Cancer of the larynx, pharynx, and oral cavity are the primary area of avocado cancer investigation. Glutathione, a tripeptide composed of three amino acids (glutamic acid, cysteine, and glycine) functions as an antioxidant (Flagg *et al.*, 1994).

The National Cancer Institute found that avocado's glutathione levels of 8.4 mg per 30 g or 19 mg per one-half fruit is several folds higher than that of other fruits. Even though the body digests glutathione down to individual amino acids when foods are consumed, a large population-based case controlled study showed a significant correlation between increased glutathione intakes and decreased risk of oral and pharyngeal cancer (Flagg *et al.*, 1994).

One clinical study found that plasma lutein and total xanthophylls but not individual carotenes or total carotenes reduced biomarkers of oxidative stress (urinary concentrations of both total F2-isoprostanes and 8-epi-prostaglandin) in patients with early-stage cancer of larynx, pharynx, or oral cavity. Xanthophyll rich avocado extracts have been shown in preclinical studies to have anti-Helicobacter pylori activity for a potential effect on gastritis ulcers, which may be associated with gastric cancer risk (Hughes *et al.*, 2009)

Dietary carotenoids in avocados show potential breast cancer protective biological activities, including antioxidant activity, induction of apoptosis, and inhibition of mammary cell proliferation (Thomson *et al.*, 2007). Studies examining the role of fruits and vegetables and carotenoid consumption in relation to breast cancer recurrence are limited and report mixed results (Thomson *et al.*, 2007).

In preclinical studies, total carotenoids and lutein appear to reduce oxidative stress, a potential trigger for breast cancer (Ding *et al.*, 2007). In women previously treated for breast cancer, a significant inverse association was found between total plasma carotenoid concentrations and oxidative stress (Thomson *et al.*, 2007), but more clinical research is needed to confirm this finding.

Mammographic density is one of the strongest predictors of breast cancer risk. The association between carotenoids and breast cancer risk as a function of mammographic density was conducted in a nested, case-control study consisting of 604 breast cancer cases and 626 controls with prospectively measured circulating carotenoid levels and mammographic density in the Nurses' Health Study (Thomson *et al.*, 2007).

Overall, circulating total carotenoids were inversely associated with breast cancer risk. Among women in the highest tertile of mammographic density, elevated levels α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin in the blood were associated with a 40–50% reduction in breast cancer risk. In contrast, there was no inverse association between carotenoids and breast cancer risk among women with low-mammographic density. These results suggest that plasma levels of carotenoids may play a role in reducing breast cancer risk, particularly among women with high mammographic density. There are no direct avocado breast cancer clinical studies (Thomson *et al.*, 2007).

Exploratory studies in prostate cancer cell lines suggest antiproliferative and antitumor effects of avocado lipid extracts (Lu *et al.*, 2005). Lutein is one of the active components identified. There are currently no human studies to confirm this potential lutein and prostate cancer relationship.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled "Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines" is a comprehensive study carried out with the objectives to ascertain bioactive compounds in fresh fruits and freeze dried powders of avocado cultivars and to evaluate anti proliferation activities of freeze dried powder in cancer cell lines.

The methodology followed in the present study is explained under the following headings.

3.1 Selection of avocado cultivars.

3.2 Quality evaluation in fruits of avocado cultivars

3.2.1 Assessment of nutritional and bioactive compounds in fresh and freeze dried powders

3.2.1.1 Freeze drying of avocado fruits for quanlitative studies

3.2.1.2 Assessment of phytochemical composition

3.2.1.3 Assessment of mineral compositions

3.2.1.4 Assessment of antioxidant activity

3.3 Selection of superior cultivar for anti proliferation studies

3.4 Anti proliferation study in superior freeze dried avocado powder

3.4.1 Cell viability assay/MTT assay

3.4.2 Percentage survival of cells (Numbers).

3.5 Statistical analysis

3.1 SELECTION OF AVOCADO CULTIVARS

Four cultivars viz., Pollock, Kallar Round, Purple Hybrid and Fuerte were selected for the study. Mature fruits were collected from Regional Agricultural Research Station, Ambalavayal, Wayanad District, for the present investigation. The importance of avocado fruits is largely increasing because of its amazing health benefits. The cultivars selected were presented in Table 3.

Table: 3 Cultivars selected for the study

Sl. No	Name of the cultivars
1.	Pollock
2.	Kallar Round
3.	Purple Hybrid
4.	Fuerte

3.2 QUALITY EVALUATION IN FRUITS OF AVOCADO CULTIVARS

Detailed studies on different quality parameters of selected cultivars of avocado fruits were ascertained. The parameters studied include bioactive compounds, anti oxidant activities and free radical preventing activities or anti-proliferation characters in fresh and freeze dried fruit powder.

The various indicators analyzed with respective to quality parameters are given below.

3.2.1 Assessment of nutritional and bioactive compounds in fresh and freeze dried powder

In the present experiment different nutritional and bioactive compounds in fresh avocado fruit and freeze dried avocado powder were studied using the methods specified below.

3.2.1.1 Freeze drying of avocado fruits for quantitative studies

Freeze-drying is a drying process in which the solvent and the suspension medium is crystallized at a low temperature and thereafter sublimated from the solid state directly into the vapor phase (Liu *et al.*, 2008).

According to Kusakabe and Kamiguchi (2004), freeze drying is the best method for the preservation of bioactive materials.

For freeze drying of avocados, the parameters such as heating plate temperature, time, freezing temperature, vacuum pressure and moisture were assessed. Freeze drying was done at the Agro-processing division of CSIR, NIIST, Pappanamcode.

Step in freeze drying

1. Ripening

After the harvesting process, fruits were kept in the ripening chamber for ripening. Thus ripened cultivars were used for fruit powder development.

2. Washing

Before freeze drying the fruits were properly washed and allowed to get dry for further procedure.

3. Cutting + pulping

The fruits were cut centrally using a knife and the seeds were removed and pulp scooped. After scooping, the pulp was transferred in to cleaned freeze drying trays.

4. Freezing

After transferring the pulp in to trays the trays were placed in shelves and freeze dried. The freezing temperature for the process was -150C to +200 C.

5. Drying in vacuum

After freezing the drying of the fruit in vacuum occurs. The vacuum pressure applied for drying the fruit pulp was 300 torrs.

6. Weighing

After 19 hours of drying and vacuuming the freeze dried flakes were collected and weighed then the flakes powdered.

7. Vacuum packing

After weighing the powder was vacuum packed for storage purpose

3.2.1.2 Assessment of phytochemical composition

Preparation of extract for analysis

The mature and freshly ripened fruits were washed and cut in to two halves. Seeds were removed by pitting with a knife. The edible portions were scooped out with a spoon for further procedures. 25g of edible portion of the fruit was ground well with the help of a mortar and pistle and the extraction of avocado was done using petroleum ether, methanol and distilled water as solvents. Extracts were centrifuged at 5000 rpm for 20 minutes. The supernatant extracts were kept overnight for incubation at room temperature.

Extraction from freeze dried powder was also done following the above mentioned procedure.

Glutatione

Glutathione was estimated by the method proposed by Sreevasthava and Kumar (1994) using spectrophotometer.

Folic acid

Folic acid content was estimated by the method of Sadasivam and Manicakam *et al.* (2008).

Vitamin K

Estimation Vitamin K was done by the method suggested by Sadasivam and Manickam (2008).

Vitamin E

The levels of tocopherol were estimated spectrophotometrically by the method proposed by Sreevasthava and Kumar (1994).

Total carotenoids

Estimation of total carotenoids was done using spectrophotometric method Sadasivam and manickam (2008).

Lutein

Lutein content was estimated using the HPLC method suggested by Sreevasthava and Kumar (1994).

Total phenols

The amount of total phenols in the fruit samples were estimated by the method proposed by Sreevasthava and Kumar (1994).

Total phytosterols

The amount of total phytosterols in the fruit samples were estimated by the method proposed by Sreevasthava and Kumar (1994)

Total flavonoids

Flavonoid was extracted and estimated by the method of Sreevasthava and Kumar (1994).

3.2.1.3 Assessment of mineral composition

Iron

Iron was estimated by the method suggested by Thimmiah (1999).

Calcium

Calcium content of samples was done using EDTA method suggested by Sadasivam and Manickam (2008).

Sodium

Sodium was estimated by the method suggested by AOAC (1995).

Phosphorus

Phosphorus content was estimated through atomic spectroscopy method suggested by AOAC (1995).

Potassium

Potassium was estimated using flame photometer by the method outlined by AOAC (1995).

Copper

Copper estimation was done with the procedure suggested by AOAC (1995).

Magnesium

Magnesium content of samples was done using EDTA method suggested by Sadasivam and Manickam (2008).

Zinc

Zinc estimation was done with the procedure suggested by AOAC (1995).

Selenium

Selenium content in avocado fruit samples were estimated by the method suggested by AOAC (1995).

3.2.1.4 Assessment of antioxidant activity

Total antioxidant activity

Total antioxidant activity was estimated using phosphomolybdic acid method proposed by Prieto *et al.* (1999). The antioxidant capacity was as AAE (Ascorbic acid equivalent) by using standard Ascorbic acid. The avocado extract was dissolved in phosphomolybdate reagent and incubated in water bath for 90 minutes. It was allowed to cool and absorbance was measured at 765nm against the blank.

DPPH radical scavenging activity

Radical scavenging activity of avocado extracts against stable 2,2 diphenyl 2-picryl hydrazyl hydrate (DPPH) was determined with DPPH. This compound reacts with an antioxidant compound which can donate hydrogen and thereby reduce DPPH. The change in color (from deep violet to light yellow) was measured at 515 nm on UV visible light spectrophotometer.

The solution of DPPH in methanol 60μM was prepared fresh before UV measurements. 3.9 ml of this solution was mixed with test solution at various concentrations. These samples were kept in the dark for 15 minutes at room temperature and decrease in absorbance was measured. The experiment was carried out in triplicate. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the concentration (1mg/1000μl). Control sample was prepared containing the volume without any extract and reference ascorbic acid. 95% methanol was used as blank.

Determination of DPPH was carried out using the method proposed by Ribeiro *et al.*, (2008).

The percentage inhibition of DPPH radical was found by comparing the result of the test with control (methanol and 1ml DPPH) using the formula suggested by Schlesier *et al.*, (2002).

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging activity

OH radicals were generated from FeSO4 and hydrogen peroxide and detected by their ability to hydroxylate salicylate and the hydroxylated salicylate complex was measured at 562 nm. Method of estimation was proposed by Smirnoff and Cumbes (1989).

Super oxide radical scavenging activity

The superoxide radical scavenging activity was proposed by the method of Smirnoff and Cumbes (1989).

3.3 SELECTION OF SUPERIOR CULTIVAR FOR ANTI PROFILERATION STUDIES

From four avocado cultivars, superior cultivar in bioactive and nutritional components was selected for further cell line study. Freeze dried powder of superior cultivar; Pollock was selected for cell line study.

3.4 ANTI PROLIFERATION STUDY IN SUPERIOR FREEZE DRIED AVOCADO POWDER

Anti proliferation activities in freeze dried avocado fruit powder of selected cultivar in cell lines was studied using MTT assay.

The extract of avocado used in the cell culture studies was obtained by homogenizing 42.7 g avocado in methanol without adding the antioxidant. The homogenate was then extracted successively with acetone by sonication. Solvent was then concentrated completely to give 7.2 g residue. It was added to cell cultures without chemical modification after sterile filtration through a 0.2 Am Millipore filter.

3.4.1 Cell viability assay/MTT assay

Assays to measure cellular proliferation and cell viability are generally used to monitor the response and health of cells in culture after treatment with various stimuli.

The cell viability assay or MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD (P) H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport.

Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.

Cell proliferation can be used to assess normal cell health, to measure responses to toxic insult, or as a prognostic and diagnostic tool in several cancers. The available markers typically look at DNA levels or synthesis, cellular metabolism, or proliferation-specific proteins.

For the present study, Human colon cancer cell line (HCT 116 cell line) was used for the cell line study. Method of MTT assay was followed by Morgon (1998).

Procedure of MTT assay

1. The anti-cancer property of methanolic extract of freeze avocado powder will be examined on human colon cancer cell line (HCT 116 cell line) using the MTT cytotoxicity assay.
2. Cells are culture in a clear bottom 96-well tissue culture plates.

3. Typical culture medium contains DMEM, 10 % fetal bovine serum, antibiotics (penicillin /streptomycin, gentamycin, etc), amino acids and other nutrients. 10,000 cell per well will be taken.
4. Extract will be added in different concentration from 10 μg, 50 μg, 100 μg, 150 μg, 200 μg to colon cancer cell line and incubated for twenty-four-hours at 37 °C in an atmosphere of 5 % CO2 and 90 % relative humidity in CO_2 incubator for the 24 hours.
5. In sterile condition MTT (5 mg /ml) will prepared and filtered. 200 μl of MTT reagent will add per well and incubated for 4 hours at 37°C. After that 200 μl of the DMSO (dimethyl sulfoxide) solution will be added and mixed gently on an orbital shaker at room temperature. Optical density was measured at 550 nm.

3.4.2 Percentage survival of cells (Numbers).

Cell viability is a determination of living or dead cells, based on a total cell sample. Viability measurements may be used to evaluate the death or life of cancerous cells.

Human colon cancer cell line (HCT 116 cell line) was selected for cell line study. After the cell line study, percentage survival of cancer cells were accessed and shown in microscopic photograph.

3.5 STATISTICAL ANALYSIS

In order to get suitable interpretations, the generated data was subjected to statistical analysis, simple ANOVA test were the main tools adopted. Graphical interpretation of analyzed data also presented.

RESULTS

4. RESULT

The results of the present investigation entitled "Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines" are detailed in this chapter under following headings.

4.1 Selection of avocado cultivars.

4.2 Quality evaluation in fresh and freeze dried avocado powder

4.3 Selection of superior cultivar

4.4 Anti proliferation study in superior freeze dried avocado powder

4.1 SELECTION OF AVOCADO CULTIVARS

Avocado cultivation holds a good potential in India and is found distributed in many states. But in Kerala, it found to be under exploited due to the lack of organization in marketing system, lack of involvement by middle men and lack of value adding activities. For the present study, mature fruits were collected from Regional Agricultural Research Station, Ambalavayal, Wayanad District, for the present investigation. The varieties selected for the study included Purple Hybrid, Fuerte, Kallar Round and Pollock (Table- 1).

Plate 1. Pollock

Plate 2. Kallar Round

Plate 3. Purple Hybrid

Plate 4. Fuerte

4.2 QUALITY EVALUATION IN FRESH AND FREEZE DRIED AVOCADO POWDER

Quality evaluation in fresh and freeze dried avocado powder were ascertained by studying the phytochemicals, mineral composition and antioxidant activity present in fruits of selected four avocado cultivars. Photochemical, antioxidant activity and mineral composition in the fruits were studied for determining its potential as a nutritional as well as a therapeutic fruit.

4.2.1 Assessment of phytochemical composition, mineral composition and antioxidant activities in fresh avocado fruits

The result of the assessment of phytochemicals, antioxidant activity and mineral components in fresh fruits of avocado cultivars, analyzed through different methods were presented below.

4.2.1.1 Assessment of phytochemical composition

Phytochemicals are bioactive plant chemicals that have protective or disease preventive properties. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. Plants produce these chemicals to protect themselves. There are more than thousand known phytochemicals. Some of the important phytochemicals are total phenols, total carotenoids, lycopene, isoflavones and flavanoids etc.

Most foods contain phytochemicals except for some refined foods such as sugar or alcohol. Some foods, such as vegetables, whole grains, beans, fruits and herbs, contain many phytochemicals. The main way to get phytochemicals is to eat more fruits (blueberries, avocados, cranberries, cherries, apple etc.) and vegetables (cauliflower, cabbage, carrots, broccoli etc.) (Liu, 2004).

Phytochemical components analyzed in the present study were glutathione, folic acid, vitamin K, vitamin E, lutein, total carotenoids, total phenols, total phytosterols, and total flavonoids. The phytochemical composition of avocado fruit cultivars were shown in Table No 2,3,7,8.

Table: 4 Phytochemical composition in fresh avocado cultivars

Cultivars	Glutathione (mg)	Folic acid (mg)	Vitamin K (mg)	Vitamin E (mg)	Total carotenoids (mg)
Pollock	18.33^{a}	80.33^{a}	0.019^{a}	2.52^{a}	2.94^{a}
Kallar Round	15.3^{c}	77.86^{b}	0.017^{a}	2.33^{c}	2.85^{ab}
Purple Hybrid	17.33^{b}	74.03^{c}	0.019^{a}	2.04^{d}	2.77^{b}
Fuerte	18.13^{a}	79.30^{ab}	0.013^{a}	2.40^{b}	2.55^{c}
CD(0.05)	0.652	2.245	0.007	0.007	0.141

(Values indicated are mean values of three replicates). Significant @ 5%

Glutathione

Glutathione is an important antioxidant found in plants, animals, and other mico organisms. The main functions of glutathione include roles in biosynthetic pathways, antioxidant biochemistry, detoxification and redox homeostasis. Glutathione is also used for maintaining the defense mechanism of our immune system and fighting metal and drug poisoning. This compound is also capable of preventing damage to important cellular components in humans, caused by reactive oxygen species such as free radicals, peroxides (Noctor *et al.*, 2011).

Glutathione is an effective chemoprotector against cisplatin-induced side effects in patients with ovarian cancer (Schmidinger, 2000).

The glutathione content of selected fresh avocado cultivars revealed that the highest amount was found in Pollock (18.33mg/100g) and which was found on par with Fuerte (18.13mg/100g). Lowest content of glutathione was observed in the cultivar Kallar Round (15.3 mg/100g). The glutathione content in fours cultivars were statistically different at 5 % level of significance.

Folic acid

Folic acid and its derivatives (folate) are essential nutrients in human body and it plays an important role in methylation reactions and nucleotide synthesis. Folic acid is normally found in foods such as dried beans, peas, lentils, oranges, whole-wheat products, liver, asparagus, broccoli etc. Folic acid deficiency may leads to macrocytic anemia and neural tube defects. Rarely a low-folate diet is related with an increased risk of colorectal neoplasia. Folic acid is used in combination with other medications to treat pernicious or normocytic anemia (Jane *et al.*, 2011).

The highest folic acid content among fresh avocado cultivars was noted in Pollock (80.33mg/100g). Folic acid content of Fuerte (79.30mg/100g) was found to be on par with Pollock (80.33mg/100g) and Kallar Round (77.86mg/100g). Lowest content was found in Purple Hybrid (74.03mg/100g). Folic acid contents among fresh avocado cultivars show statistically significant 5 % level of significance.

Vitamin K

Vitamin K has important functions within the human body. It is an anti-calcifying, anticancer, bone-forming and insulin-sensitising molecule. Severe vitamin K deficiency is not common. Antagonists of vitamin K such as warfarin may cause side effects, which may partly be blunted through vitamin K supplementation (DiNicolantonio *et al.*, 2015).

Vitamin K content of fresh avocado cultivars was analyzed and was found to be significantly different at 5 % level. The vitamin K content of the four cultivars to be in the range of 0.013mg/100g to 0.019mg/100g and highest value was found for Pollock (0.019mg/100g) and Purple Hybrid (0.019mg/100g) and these two cultivars were found to be on par with Kallar Round and Fuerte (0.013mg/100g)

Vitamin E

Main sources of vitamin E are vegetable oils, olive oil, sunflower oil, and wheat germ oil. Vitamin E known as a highly efficient antioxidant and it serves as the major lipid soluble chain-breaking antioxidant that prevents lipid peroxidation (Packer and Landvik, 1989).

Vitamin E content of cultivar Pollock (2.52mg/100g) showed the highest value and the least value observed in Purple Hybrid (2.04mg/100g).Vitamin E of the cultivars were found to be significantly different at 5 % level of significance.

Total carotenoids

Carotenoids are a group of polyphenolic compounds present in fruits and vegetables which have anti-inflammatory and antioxidant effects. Carotenoid intake is relatively associated with reduced CVD risk and it is good for eye health. carotenoids also have an important role to slow down the process of ageing.

The Carotenoids include beta-carotene, lycopene, lutein, and zeaxanthin. In part, the beneficial effects of carotenoids are thought to be due to their role as antioxidants. Beta-Carotene has added benefit due its ability to be converted to vitamin A. lutein and zeaxanthin may be protective in eye disease because they absorb damaging blue light that enters the eye. These carotenoids are also available in supplement forms (Woodside *et al.*, 2015).

The carotenoid content of the four avocado cultivars are depicted in the table 4 and the maximum amount of carotenoid content was found in the Pollock (2.94mg/100g). Carotenoid content of Kallar Round (2.85mg/100g) was found on par with Pollock and Purple Hybrid (2.776mg/100g). Carotenoid content of Fuerte was (2.55mg/100g). Statistical analysis highlighted significant difference in the carotenoid content among the four avocado cultivars.

Table: 5 Phytochemical composition in fresh avocado cultivars

Cultivars	Lutein (mg)	Total phenols (mg)	Total phytosterols (mg)	Total flavanoids (mg)
Pollock	0.183^{c}	13.84^{a}	63.14^{b}	20.73^{a}
Kallar Round	0.185^{c}	12.65^{c}	68.60^{a}	18.13^{b}
Purple Hybrid	0.193^{b}	13^{b}	63.23^{b}	21.07^{a}
Fuerte	0.206^{a}	12.24^{d}	62.30^{c}	18.37^{b}
CD(0.05)	0.004	0.130	0.134	1.064

(Values indicated are mean values of three replicates). Significant @ 5 %

Lutein

Lutein is an oxygenated form carotenoid found naturally in vegetables and fruits and known as eye vitamin. The antioxidant properties of lutein help fight against free radical damage caused by blue light or sun exposure, poor diet, and other factors that increase the risk of developing age-related vision loss. Antioxidants like lutein protect healthy cells while halting the growth of malignant cells. Lutein in our diets can help stop the condition from progressing and further damaging and it may help to reduce cardiac problems like arteriosclerosis (Levy, 2019).

Lutein content was found to be maximum in the cultivar Fuerte (0.206mg/100g) followed by Purple Hybrid (0.193mg/100g), Kallar Round (0.185mg/100g) and Pollock (0.183mg/100g).

Total phenol

Highest total phenolic content was observed in the cultivar Pollock (13.84mg/100g) and the lowest content was noted in the cultivar Fuerte (12.24mg/100g). The cultivars, Purple Hybrid and Kallar Round recorded (13mg/100g), (12.65mg/100g) respectively.

Total phytosterols

Phytosterols (known as plant sterol and stanol esters) are a group of naturally occurring compounds present in plant cell membranes. Structurally phytosterols are similar to the body's cholesterol, when they are consumed they compete with cholesterol for absorption in the digestive system.

The consumption of phytosterols helps to reduce dietary cholesterol and regulate metabolic synthesis of cholesterol and dietary phytosterols may offer protection from colon, prostate or breast cancers (Kritchevsky and Chen, 2005).

The total phytosterol content of four avocado cultivars was in the range of 62.30mg/100g -68.60mg/100g. The highest content was noted in the cultivar Kallar Round (68.60mg/100g) and the lowest in the cultivar Fuerte (62.30mg/100g). The analysis found significant at 5 % level.

Total flavonoids

Flavonoid is a type of compound present in many different foods. There are thousand types of flavonoids, which are broken down into six subgroups: chalcones, flavones, flavonols, flavonones, anthocyanins, and isoflavonoids. Fruits and vegetables along with tea and wine are the main sources of flavonoids for humans. Many flavonoids shows antioxidative activity, free-radical scavenging capacity, anticancer activity, and coronary heart disease prevention and some flavonoids show potential for anti–human immunodeficiency virus functions (Yao *et al.*, 2004).

Total flavonoid content was found to be maximum (21.07mg/100g) in the cultivar Purple Hybrid and minimum in Kallar Round (18.13mg/100g). Purple Hybrid was found to be on par with Pollock (20.73mg/100g). Statistically the values were found to be different in four cultivars at 5 % level.

4.2.1.2 Mineral compositions in fresh fruits of avocado cultivars

Minerals are essential nutrients found in different types of plant and animal based foods. Macro-minerals are found in greater amounts; which include calcium, potassium, sodium, phosphorus, magnesium, chloride, and sulfur. Trace minerals or micro-minerals present in smaller amounts in our diet, include iron, zinc, selenium, manganese, copper, iodine, cobalt, and fluoride. Both macro and micro minerals types bones and teeth to keeping your muscles, heart and brain working properly.

The human body needs minerals to convert food into energy, to prevent dehydration and to regulate the function of the heart and skeletal, muscular and nervous systems.

Macro minerals are required for proper fluid balance, nerve transmission, and muscle contraction; prevent blood clotting, regulation of blood pressure and in the protein metabolism.

Micro minerals are essential for growth and development because they are involved in oxygen transport and various metabolic functions of human body. Some micro minerals function as coenzymes. Almost all micro minerals participate in all enzyme reactions in the body and help in the assimilation and use of vitamins and other nutrients.

Table: 6 Mineral composition of fresh fruits of avocado cultivars

Cultivars	Iron (mg)	Calcium (mg)	Sodium (mg)	Phosphorus (mg)	Potassium (mg)
Pollock	0.320^{b}	9.3^{a}	6.12^{a}	34.90^{b}	394.47^{a}
Kallar Round	0.283^{c}	8.65^{b}	6.12^{a}	32.43^{c}	384.76^{b}
Purple Hybrid	0.293^{c}	9.15^{a}	5.87^{b}	36.13^{a}	393^{a}
Fuerte	0.403^{a}	9.15^{a}	5.51^{c}	32.13^{d}	343.33^{c}
CD(0.05)	0.020	0.05	0.152	0.005	5.432

(Values indicated are mean values of three replicates). Significant @ 5 %

Iron

Iron is considered as one of the major and essential nutrients for the proper growth and development of human body. Lack of iron may lead to several iron deficiency diseases like iron deficiency anemia. Iron present in the human body helps in the production of hemoglobin and red blood cells. It is essential for the blood production in the body. Hemoglobin which is produced by iron is used for transferring oxygen from lungs to the tissues in different parts of the body. Iron is also needed for the functioning of our immune system. Main dietary sources of iron are beef, pork, lamb, chicken, green vegetables, tofu, fish, beans, sweet peas, corns, beet, cabbages and much more.

According to Nelson (2009) iron shows some anti cancer properties particularly it acts against colon cancer.

The iron content in the cultivar Fuerte is 0.403mg/100g, which shows the highest value and the least value was noted in the cultivar Kallar Round (0.283mg/100g) and which was found to be on par with the cultivar Purple Hybrid (0.293mg/100g). The iron content of the cultivars found significant at 5 % level.

Calcium

Calcium is a very essential mineral for the whole body. This mineral prevents arthritis and osteoporosis and it reduces the back pain, keeps the bones in proper shape and strengthens the backbone. Calcium ensures the normal contraction and relaxation of our heart muscles. When calcium level goes down, a specific hormone called calcitriol is released which increases the blood pressure by contracting the smooth muscles. Adequate amount of calcium is needed to ensure that the calcium ions are able to transmit electrical charges that keep our heart beating.

Greater amount of calcium among the four fresh avocado cultivars noted in Pollock (9.3mg100g) and which was found on par with the cultivars Purple Hybrid (9.15mg/100g) and Fuerte (9.15mg/100g). Lowest amount of calcium was observed in Kallar Round (8.65mg/100g).

Sodium

Sodium is a mineral that present in small quantities in almost all natural foods. It is a main component in nerves which helps in muscle contraction and it helps to prevent sun stroke. Higher amount of sodium in blood may leads to increased blood pressure.

The sodium content of the four avocado cultivars was studied and the highest amount was noted in the cultivar Pollock (6.12mg/100g) and was found to have no significant difference with the cultivar Kallar Round (6.12mg/100g). Least amount of sodium was noted in the cultivar Fuerte (5.51mg/100g).

Phosphorus

The important function of phosphorus is to build and maintain bones and teeth; it also needed for the formation of genetic materials (DNA and RNA). Phosphorus also plays a key role in metabolism (the conversion of calories and oxygen to energy), muscle contraction, and the transmission of nerve signals. This mineral is considered as a macro mineral (alongside calcium, sodium, magnesium, potassium, chloride, and sulfur) in that more amounts is needed than trace minerals like iron and zinc (Lee and Cho, 2015).

The phosphorus content of the four avocado cultivars depicted in the Table 6 and the maximum amount of phosphorus was found in the cultivar Purple Hybrid (36.13mg/100g) followed by Pollock (34.90mg/100g), Kallar Round (32.43mg/100g) and Fuerte (32.13mg/100g). The analysis found revealed significant differences between the four cultivars at 5 % level of significance.

Potassium

The main role of potassium in the human body is the regulation of fluid balance and controlling the electrical activity of the heart and other muscles. It also reduces the risk of stroke, lowers blood pressure, protects from loss of muscle mass, preserves bone mineral density, and decreases the formation of kidney stones (Cogswell *et al.*, 2012).

The potassium content of the cultivar Pollock was 394.47mg/100g, which possessed the highest value and which was found to be on par with the cultivar Purple Hybrid (393mg/100g). Least value observed in Fuerte (343.33mg/100g).

Table: 7 Mineral composition of fresh fruits of avocado cultivars

Cultivars	Copper (mg)	Magnesium (mg)	Zinc (mg)	Selenium (µg)
Pollock	0.146[a]	22.20[a]	0.473[a]	0.500[a]
Kallar round	0.136[b]	18.50[b]	0.436[b]	0.243[c]
Purple hybrid	0.141[a]	17.30[c]	0.410[b]	0.203[d]
Fuerte	0.146[a]	18.63[b]	0.420[b]	0.300[b]
CD(0.05)	0.004	0.400	0.030	0.024

(Values indicated are mean of three replicates). Significant @ 5 %

Copper

Copper plays an important role in human metabolism as a cofactor of key metabolic enzymes, which involve respiration, neurotransmitting, biosynthesis, radical detoxification, and iron metabolism (Reddy *et al.*, 2003).

The highest copper content among the four avocado cultivars was noted in Pollock (0.146mg/100g) and Fuerte (0.146mg/100g) and these two cultivars were found to be on par with Purple Hybrid (0.141mg/100g). Less amount of copper was observed in the cultivar Kallar Round (0.136mg/100g).The values showed statistical difference with each other.

Magnesium

Magnesium, the fourth most abundant mineral in the intracellular compartment and whole body, respectively, is of great physiologic importance. Magnesium plays a major role in relaxing the nerves, muscle contraction and prevents cholesterol and it prevents heart attacks. It is present in peaches, almonds, apples, figs, brown rice (Allen, 2013).

From the table 4, it was observed that Pollock got the maximum magnesium content of 22.200mg/100g and minimum amount in the cultivar Purple Hybrid (17.300mg/100g). Statistically cultivar was different at 5 % level.

Zinc

Compared to other minerals with similar chemical properties, zinc is relatively harmless. Only exposure to high doses has toxic effects. It has an impact on human growth, neuronal development, and immunity. Zinc deficiency caused by malnutrition and foods with low bioavailability and certain diseases (Laura *et al.*, 2010).

As indicated in the Table 7 maximum zinc content was found in the cultivar Pollock (0.473mg/100g). The zinc content of Kallar Round (0.436mg/100g) was on par with Fuerte (0.420mg/100g) and Purple Hybrid (0.410mg/100g). The cultivar Purple Hybrid has less amount of zinc (410mg/100g).

Selenium

Selenium is an important micronutrient found in animals, in humans it is a trace element nutrient that functions as cofactor for glutathione peroxidases. In plants, sometimes it occurs in toxic amounts as forage, e.g. locoweed. Selenium is a component of the amino acids selenocysteine and selenomethionine.

This particular mineral supports the metabolic function, prevents DNA damage, plays a role in heart health, and resists harmful organisms on skin and in, boosting immunity. It also acts as an antioxidant, boosts thyroid health, resists harmful organisms, promotes normal lung function, helps fertility, supports healthy pregnancy, and supports heart health (Williams, 1978).

On ascertaining the selenium content of fresh avocado cultivars the highest amount was noted in the cultivar Pollock (0.500µg/100g) and the lowest content was noted in the cultivar Purple Hybrid (203µg/100g). The statistical analysis showed significant difference among the four fresh avocado cultivars.

4.2.1.3 Antioxidant activities of fresh avocado cultivars

Table: 8 Total antioxidant activities of fresh avocado cultivars

Cultivars	Total antioxidant activity (mg/100g)
Pollock	234.50[a]
Kallar Round	220.33[b]
Purple Hybrid	230.76[a]
Fuerte	214[b]
CD(0.05)	6.910

(Values indicated are mean of three replicates). Significant @ 5 %

Total antioxidant activity

Total antioxidant activity is the ability of antioxidants in different foods to clean harmful free radicals in the blood and cells. It takes into account the amount of water-based and fat-based antioxidants present in food. Understanding this figure helps individuals decide which foods offer the greatest antioxidant benefit (Roberta *et al.,* 1999).

The total antioxidant activity of fresh avocado cultivars was found to be in the range of 214mg/100g to 234.50mg/100g. The results of the ANOVA table revealed significant differences in the total antioxidant activity of the fresh avocado cultivars. Highest value for total antioxidant activity was observed in the cultivar Pollock (234.50mg/100g) and it was found to be on par with the freeze dried powder of

Purple Hybrid (230.76mg/100g). Least value observed in Fuerte (214mg/100g) and it was found on par with Kallar Round (220.33mg/100g).

Table: 9 DPPH Radical scavenging activity, hydroxyl radical scavenging activity and superoxide radical scavenging activity of fresh fruits of avocado

Cultivars	DPPH Radical Scavenging Activity IC_{50} values (µg/ml)	Hydroxyl Radical Scavenging Activity IC_{50} values (µg/ml)	Superoxide Radical Scavenging Activity IC_{50} values (µg/ml)
Pollock	42.52[a]	38.86[a]	12.54
Kallar Round	38.98[c]	27.03[d]	12.12
Purple Hybrid	34.03[d]	34.50[c]	12.33
Fuerte	40.76[b]	37.72[b]	13.32
CD(0.05)	0.024	0.058	0.006

(Values indicated are mean of three replicates). Significant @ 5 %

DPPH radical scavenging activity

2,2-diphenyl-1-picrylhydrazyl radical (DPPH), is a stable nitrogen synthetic radical, that is used to evaluate the antioxidant capacity of medicinal herbal products and it has a great ability to inhibit free radicals form in human body.

DPPH is a well-known radical and a scavenger for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical scavenging nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals that can be counted from the change in the optical absorption at 520 nm (Alger, 1997).

Table 9 shows the level of DPPH radical scavenging activity in the four avocado cultivars. The findings revealed that the cultivar Purple Hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 34.03μg/ml, followed by Kallar Round (38.98μg/ml), Fuerte (40.76μg/ml) and the lowest DPPH radical scavenging activity was found in the cultivar Pollock (42.52μg/ml).

Hydroxyl radical scavenging activity

The hydroxyl radical, OH, is the neutral form of the hydroxide ion (OH^-) and these radicals are highly reactive (easily becoming hydroxyl groups) and short-lived. They can form an important part of radical chemistry. Most important fact is that hydroxyl radicals are produced from the decomposition of hydroperoxides.

Hydroxyl radicals can reduce disulfide bonds in proteins, specifically fibrinogen, resulting in their unfolding and scrambled refolding into abnormal spatial configurations. Main problems of this reaction are observed in many diseases such as atherosclerosis, cancer and neurological disorders, and can be prevented by the action of non-reducing substances. Moreover, many therapeutic substances, traditionally classified as antioxidants, accept electrons and thus are effective oxidants (Lipinski, 2011).

Regarding the IC_{50} values for hydroxyl radical scavenging activity of fresh avocado cultivars, higher Hydroxyl radical scavenging activity was noted in the in the cultivar Kallar Round (27.03µg/ml) and the lowest Hydroxyl radical scavenging activity was observed in the cultivar Pollock (38.86µg/ml). Statistical analysis showed significant difference at 5 % level.

Super oxide Radical Scavenging Activity

Super oxide radical is a common reactive form of oxygen (O^{2-}) formed when molecular oxygen gains a single electron, Superoxide radical can attack biological targets which includes lipids, proteins and nucleic acids in human body.

Super oxide radical scavenging activity (IC_{50}) was observed to be higher in the cultivar Kallar Round (12.12µg/ml). Lowest value was noted in the cultivar Fuerte (13.32µg/ml). Statistically no difference was found among four fresh avocado cultivars.

Plate 5. Freeze dried form of Pollock

Plate 6. Freeze dried form of Kallar Round

Plate 7. Freeze dried form of Purple Hybrid

Plate 8. Freeze dried form of Fuerte

4.2.2 Assessment of phytochemical composition, mineral composition and antioxidant activities in freeze dried avocado powders

The result of the biochemical and mineral composition in freeze dried avocado powder is depicted as follows

4.2.2.1 Phytochemical composition of freeze dried avocado powders

Table: 10 Phytochemical composition of freeze dried avocado powders

Cultivars	Glutathione (mg)	Folic acid (mg)	Vitamin K (mg)	Vitamin E (mg)	Total Carotenoids (mg)
Pollock	45.50^{a}	197.04^{b}	0.183^{a}	6.53^{a}	8.15^{a}
Kallar Round	39.33^{d}	185.15^{c}	0.179^{a}	6.21^{a}	7.94^{b}
Purple Hybrid	40.50^{c}	180.79^{d}	0.181^{a}	5.83^{b}	8.07^{a}
Fuerte	42.40^{b}	197.92^{a}	0.185^{a}	6.40^{a}	7.70^{c}
CD(0.05)	0.545	0.003	0.007	0.368	0.112

(Values indicated are mean of three replicates). Significant @ 5 %

Glutathione

Glutathione naturally occurs in some foods, including almonds, spinach, walnuts, garlic, tomatoes, broccoli and cucumber. However, it may not be well-absorbed from these dietary sources. Over cooking, storage and farming methods may reduce glutathione content in foods. Healthcare providers give glutathione as a shot (by injection into the muscle) for preventing poisonous side effects of cancer treatment (chemotherapy) and for treating the infertility in males. (Noctor *et al.,* 2011).

Glutathione content of the freeze dried powders of four avocado cultivars found to be significantly different at 5 % level of significance. The highest amount of glutathione was recorded in the freeze dried powder of Pollock (45.50mg/100g) and lowest amount was recorded in Kallar Round (39.33mg/100g).

Folic acid

According to Figueiredo *et al.* (2011) Folic acid helps our body to produce and maintain new cells, and also helps to prevent changes in DNA that may lead to cancer.

Folic acid content in freeze dried powders of four avocado cultivars found in Fuerte (197.92mg/100g), Pollock (197.04mg/100g), Kallar Round (185.15mg/100g) and Purple Hybrid (180.79mg/100g).

Vitamin K

There is a correlation between deficiency of vitamin K and osteoporosis. Some studies show that vitamin K supports the maintenance of strong bones, improves bone density and decreases the risk of bone fractures, but others have shown no improvement in bone density associated with vitamin K. (DiNicolantonio *et al.,* 2015). This vitamin will be absorbed by the body along with the fat content of the food (Murphy, 2018).

From the table 10, it was clear that the freeze dried powder of Fuerte recorded higher value for vitamin K content (0.185mg/100g) which was found to be on par with Pollock (0.183mg/100mg), Purple Hybrid (0.182mg/100g) and Kallar Round (0.176mg/100g). Statistically there were no significant differences observed among the freeze dried powders of four avocado cultivars.

Vitamin E

Advantages of consuming vitamin E rich foods can include treating and preventing diseases of the heart and blood vessels, such as chest pains, high blood pressure, and blocked or hardened arteries.

On ascertaining the vitamin E content of freeze dried avocado powders Pollock had the higher amount (6.53mg/100g) and it was found to be on par with Fuerte (6.40mg/100g) and Kallar Round (6.21mg/100g). Lowest content was found in Purple Hybrid (5.83mg/100g). Statistically freeze dried powders of the four avocado cultivars were found significantly different at 5% level.

Total carotenoids

Carotenoid content of the freeze dried powders of four avocado cultivars was observed in the range of 7.70mg/100g-8.15mg/100g. As indicated in Table 10, the cultivars Pollock (8.15mg/100g) and Purple Hybrid (8.07mg/100g) were found to be having higher carotenoid content. When the data was analysed statistically, it was observed that there was significant difference among the four cultivars. It was also noticed that the cultivars Pollock and Purple Hybrid were on par with each other. The cultivar Fuerte (7.70mg/100g) recorded least content of total carotenoids.

Table: 11 Phytochemical composition of freeze dried avocado powders

Cultivars	Lutein (mg)	Total phenols (mg)	Total phytosterols (mg)	Total flavanoids (mg)
Pollock	0.437^{b}	51.73^{a}	122.27^{c}	45.27^{a}
Kallar round	0.426^{c}	49.04^{b}	173.80^{a}	44.10^{b}
Purple hybrid	0.503^{a}	48^{c}	164.37^{ab}	45^{a}
Fuerte	0.423^{c}	46.4^{d}	149.04^{b}	43.17^{c}
CD(0.05)	0.005	0.873	21.617	0.787

(Values indicated are mean values of three replicates). Significant @ 5%

Lutein

Lutein is one of the most prevalent carotenoids present in nature and in the human diet. Lutein with zeaxanthin, are highly concentrated as a macular pigment in the foveal retina of primates, providing protection from photo-oxidation and enhancing visual performance. Lutein is important for the development and functioning of brain and only primates accumulate lutein within the brain, but little is known about its distribution or physiological role (Erdman *et al.*, 2015).

On interpreting the lutein content of freeze dried powders of four avocado cultivars, it was noted that the cultivar Purple Hybrid (0.503mg/100g) recorded the highest lutein content and the lowest in the cultivar Fuerte (0.423mg/100g). Kallar Round (0.426mg) was found to be on par with Fuerte. Statistically freeze dried powders of the four avocado cultivars are significant at 5 % level.

Total phenols

Natural phenolic compounds play a significant role in human health as evident from their antifungal, antioxidant and anti-cancerous activities (Abad, 2007).

The values indicated higher phenol content among freeze dried powders of Pollock (51.73mg/100g). Least value of total phenol was observed in the freeze dried powder of the cultivar Fuerte (46.4mg/100g). Statistical difference at 5% level of significance was noted.

Total phytosterols

Phytosterols have been shown to enable antitumor responses by means of the stimulation of immune recognition of cancer, influencing hormonal-dependent growth of endocrine tumors (Kritchevsky and Chen, 2005).

Total phytosterol content of the freeze dried powders of avocado cultivars were revealed to be the range of 122.27mg/100g to 173.80/100g. The phytosterol content of the freeze dried powder of Kallar Round (173.80mg/100g) was followed by Purple Hybrid (164.37mg/100g) and Fuerte (149.04mg/100g). Low amount was found in Pollock (122.27mg/100g).

Total flavonoids

Comparing the flavonoid content of avocado cultivars studied, it was observed that cultivar Pollock (45.27mg/100g) and cultivar Purple Hybrid (45 mg/100g) were having higher amount. Cultivar Fuerte (43.17mg/100g) showed the least amount of flavonoid content. Significant difference at 5% level was observed in total flavonoid content of the samples.

4.2.2.2 Mineral compositions of freeze dried avocado fruit powders

Table: 12 Mineral composition of freeze dried avocado fruit powders

Cultivars	Iron (mg)	Calcium (mg)	Sodium (mg)	Phosphorus (mg)	Potassium (mg)
Pollock	2.27^{b}	104^{b}	13.13^{b}	76.07^{a}	622.76^{a}
Kallar round	1.87^{c}	106.5^{b}	13^{b}	70.30^{c}	618.76^{a}
Purple hybrid	1.97^{c}	142.5^{a}	14.40^{a}	73.07^{b}	623^{a}
Fuerte	2.87^{a}	111.5^{b}	12.3^{c}	75.63^{a}	586.33^{b}
CD(0.05)	0.178	15.2	0.448	0.722	5.153

(Values indicated are mean values of three replicates). Significant @ 5%

Iron

Health benefits of iron include the regulation of the body temperature and it control over metabolic and catabolic process in the body. Every individual is different and the same holds true for the absorption capacity of iron. The presence of sufficient iron content regulates this capacity and contributes to the well being of human beings (Nelson, 2009).

The iron content of the freeze dried powder of the cultivar Fuerte was 2.87mg/100g, which found highest among the cultivars. The cultivar Kallar Round had the least value of iron (1.87mg/100g) and which was found on par with the freeze dried powder of Purple Hybrid (1.97mg/100g). The differences in values of iron were significantly different at 5 % level of significance.

Calcium

Natural sources of calcium like milk and other products, fruits, vegetables etc are healthier than artificial supplement of calcium.

The values indicated that high calcium content was found in the freeze dried powder of Purple Hybrid (142.5mg/100g). The calcium content in Pollock (104mg/100g) was noted lower and it was found to be on par with and Kallar Round (106.5mg/100g) and Fuerte (111.5mg/100g). The values were statistically different at 5% level of significance.

Sodium

Sodium content of freeze powders four avocado cultivars was analyzed. Higher amount of sodium was observed in the freeze dried powder of Purple Hybrid (14.40mg/100g) and lower in Fuerte (12.30mg/100g). Sodium content in Pollock (13.13mg/100g) and Kallar Round (13mg/100g) found to have no significant difference at 5 % level.

Phosphorus

As seen in table 12, phosphorus content of freeze dried powders of avocado cultivars was in the range of 70.30mg/100g to 76.07mg/100g. The study showed significant difference in phosphorus levels between the cultivars. Highest value of phosphorus was recorded in Pollock (76.07mg/100g). Lowest value for phosphorus was noted in the freeze dried powder of Kallar Round (70.30mg/100g).

Potassium

Potassium is one of the important macro nutrient found in large amount and it is important for the maintenance of blood pressure and fluid electrolyte balance of human body.

Table 12 represents the potassium level of freeze dried powders of avocado cultivars. Potassium level of Purple Hybrid (623mg/100g) was recorded to be higher and found to be on par with Pollock (622mg/100g) and Kallar Round (618mg/100g). Least value for potassium was found in Fuerte (586.33mg/100g.). Significant difference was found between the cultivars at 5% level of significance.

Table: 13 Mineral composition of freeze dried avocado fruit powders

Cultivars	Copper (mg)	Magnesium (mg)	Zinc (mg)	Selenium (μg)
Pollock	3.13^{b}	42.13^{a}	3.45^{b}	8.90^{a}
Kallar Round	3.07^{c}	39.23^{b}	4.66^{a}	8.07^{d}
Purple Hybrid	3.23^{a}	38.23^{b}	3.14^{c}	8.50^{c}
Fuerte	3^{d}	34.76^{c}	2.11^{d}	8.80^{b}
CD(0.05)	0.009	1.276	0.249	0.007

(Values indicated are mean of three replicates). Significant @ 5 %

Copper

With regard to copper content of freeze dried fruit powders of avocado cultivars, the values ranged from 3mg/100g to 3.23mg/100g. Highest value for copper content was noted in the freeze dried powder of Purple Hybrid (3.23mg/100g) and least value was observed in Fuerte (3mg/100g). Statistical difference among cultivars was found at 5% level of significance.

Magnesium

Statistical analysis showed significant difference in magnesium content of freeze dried powders of four avocado cultivars. Higher magnesium content was observed in Pollock (42.13mg/100g) followed by Kallar Round (39.23mg/100g), Purple Hybrid (38.23mg/100g) and Fuerte (34.76mg/100g). Kallar Round was found to be on par with Purple Hybrid.

Zinc

The level of zinc was analyzed in the freeze dried powder of avocado cultivars and was found to be in the range of 2.11 mg/100g to 4.66 mg/100g. The values obtained for zinc were as follows; Kallar Round (4.73mg/100g), Pollock (3.45mg/100g) and Purple Hybrid (3.14 mg/100g) and Fuerte (2.11mg/100g). Significant difference in values at 5% level was found.

Selenium

Selenium content for the freeze dried powders of avocado cultivars revealed that Pollock (8.90 μg/100g) was having higher amount and Kallar Round (8.07μg/100g) was having least amount. Statistically no significant difference in values was found between the cultivars.

4.2.2.3 Antioxidant activities of freeze dried avocado powders

Table: 14 Total antioxidant activities of freeze dried avocado powders

Cultivars	Total Antioxidant Activity (mg/100g)
Pollock	461.90^{a}
Kallar round	444.23^{b}
Purple hybrid	460.33^{a}
Fuerte	440.33^{b}
CD	4.456

(Values indicated are mean of three replicates). Significant @ 5 %

Total antioxidant activity

The total antioxidant activity of freeze dried powders of four avocado cultivars ranges from 440.33 mg/100g to 461.90 mg/100g. The results revealed significant differences in the total antioxidant activity. Highest value for total antioxidant activity was observed in the cultivar Pollock (461.90mg/100g) and which was found to be on par with Purple Hybrid (460.33mg/100g). Least value was noted in Fuerte (440.33mg/100g) and it was found to be on par with Kallar round (444.23mg/100g).

Table: 15 DPPH radical scavenging activity, hydroxyl radical scavenging activity and super oxide radical scavenging activity of freeze dried avocado fruit powder.

Cultivars	DPPH Radical Scavenging Activity IC_{50} values (µg/ml)	Hydroxyl Radical Scavenging Activity IC_{50} values (µg/ml)	Superoxide Radical Scavenging Activity IC_{50} values (µg/ml)
Pollock	101.66[a]	86.60[a]	44.63[a]
Kallar Round	98.33[b]	75.73[c]	34.93[c]
Purple Hybrid	90.80[c]	83.92[b]	44.53[b]
Fuerte	100.7[a]	69.25[d]	24.47[d]
CD(0.05)	1.080	0.013	0.013

(Values indicated are mean of three replicates). Significant @ 5 %

DPPH radical scavenging activity

Table 15 shows the level of DPPH radical scavenging activity in the freeze dried powders of four avocado cultivars. The findings revealed that the cultivar Purple hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 90.80µg/ml, followed by Kallar Round (98.33µg/ml), Fuerte (100.66µg/ml) and the lowest in the cultivar Pollock (101.66µg/ml).

Hydroxyl radical scavenging activity

Regarding the IC_{50} values for hydroxyl radical scavenging activity of freeze dried avocado cultivars, highest activity was noted in the freeze dried powder of Fuerte (69.25µg/ml). Lowest activity was observed in the freeze dried powder of Pollock (86.60µg/ml).

Super oxide radical scavenging activity

Super oxide Radical Scavenging Activity (IC_{50}) was observed higher in the freeze dried powder of Fuerte (24.47µg/ml) followed by Kallar Round (34.93µg/ml), Purple Hybrid (44.53µg/ml) and the lowest in the cultivar Pollock (44.63µg/ml).

4.2.3 Selection of superior cultivar

Among the freeze dried avocado powders, the cultivar which possessed qualitatively superior in bioactive compounds, nutrient content, and antioxidant activity and which favoured cancer treatment was selected for further studies. The selected cultivar was Pollock. Cell culture study in freeze dried powder of Pollock was evaluated for its potential in cancer treatment.

4.3 Anti proliferation study in superior freeze dried avocado powder

4.3.1 MTT assay

Anti proliferation activities in freeze dried avocado powder from superior cultivar Pollock was studied in cell lines using MTT assay.

The anti-cancer property of methanolic extract of freeze avocado powder was examined on human colon cancer cell line (HCT 116 cell line).Cells were cultured in a clear bottom 96-well tissue culture plated. Typical medium contains DMEM, 10 % fetal bovine serum, antibiotics, amino acids and other nutrients. Ten thousand cell per well were taken. Extract was added in different concentration from 10 µg, 50 µg, 100 µg, 150 µg, and 200 µg to HCT 116 and incubated for twenty four-hours at 37^0 C. In sterile condition MTT (5 mg /ml) was prepared and filtered. 200 µl of MTT reagent was added per well and incubated for 4 hours at 37°C. After that 200 µl of the DMSO (dimethyl sulfoxide) solution was added and mixed gently on an orbital shaker at room temperature. Optical density was measured at 550 nm. A dose-dependent MTT reduction (or color change from yellow to purple) was observed in treated cells. Extract were added in different concentration from 10 µg, 50 µg, 100 µg, 150 µg, 200 µg to colon cancer cell lines. After the incubation period of cells with avocado extract, inhibition of cancer cells observed.

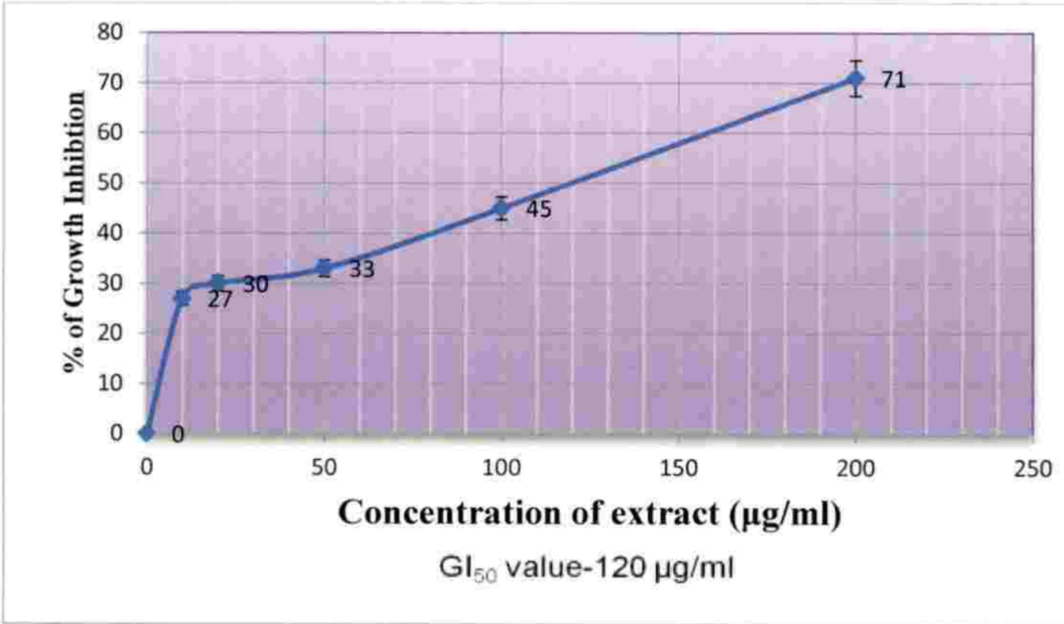
After the incubation period, growth Inhibition of cancer cells were noted 27 %, 30 %, 33%, 45 %, and 71 % against the concentration 1µg, 20µg, 50µg, 100µg, and 200 µg of test sample respectively. Fifty percentage of inhibition (GI_{50}) was shown at 120μg of fruit extract.

4.3.2 Percentage survival of cells

Only 29 percent of cell viability was observed after the exposure of cancer cell lines with avocado extract (200 μg).

Percentage of viable cell showed decrease with increase in extract concentration while percentage cytotoxicity increased with increase in concentration of avocado extract.

Figure. 1: MTT Assay in Colon cancer HCT 116 cells



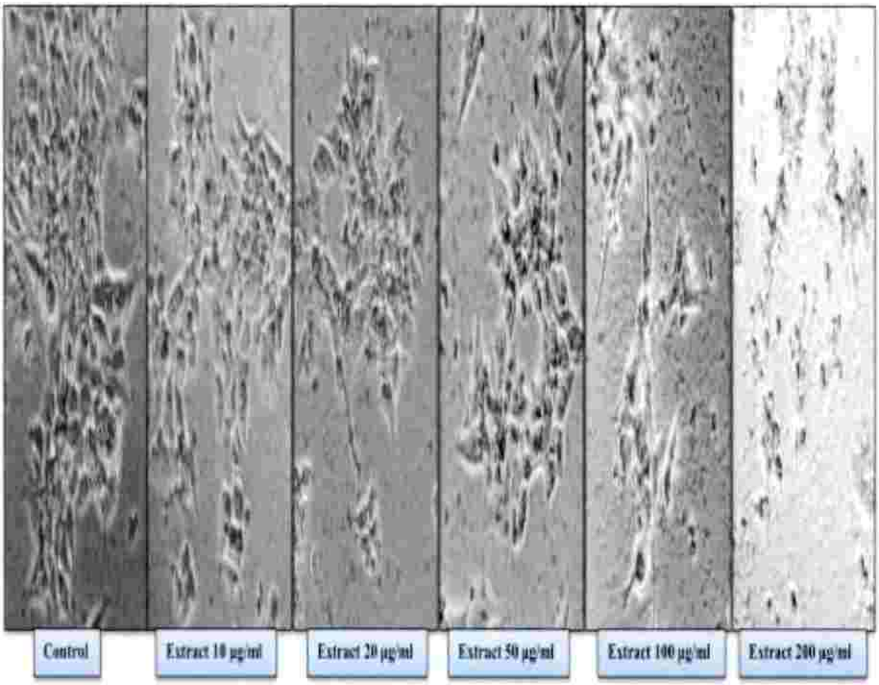


Plate 9. Microscopic pictures of inhibition of cancer cells at different concentrations of avocado extracts

DISCUSSION

5. DISCUSSION

This chapter encompasses a critical appraisal of the salient findings of the study entitled "Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines" and discussion presented under

5.1 Selection of avocado cultivars.

5.2 Quality evaluation in fresh and freeze dried avocado powder

5.3 Anti proliferation studies in fresh fruits and freeze dried avocado powder

5.1 SELECTION OF AVOCADO CULTIVARS

Avocado or butter fruit (*Persea americana* Mill.) is one of the popular tropical ever green climacteric fruit originated from central America and Mexico. This fruit is found distributed in some parts of India. In India Avocados are cultivated in Kerala (Particularly Wayanaud and Idukki), Tamil nadu, Karnataka and Maharashtra.

Avocado is the most nutritive fruit. The pulp is rich in proteins and fat, but low in carbohydrates and it is also a reservoir of several vitamins, minerals, phytochemicals and antioxidant enzymes. Ding *et al.* (2007), reported in their study that avocados should be added to the list of fruits as part of a cancer prevention diet because of the cancer prevention properties provided by phytochemicals and antioxidants present in it.

Avocados can preserve as freeze dried form which is more condensed in nutrients, more convenient for use and shelf stable (Thripathi *et al.*, 2014).

In Kerala, avocados are grown in the homesteads of Wayanaud and Idukki. For the present study fully ripened fruits were selected from Regional Agricultural

Research Station, Kerala Agricultural University, Ambalavayal, Wayanaud District. The selected cultivars are Pollock, Kallar Round, Purple Hybrid and Fuerte.

5.2 Quality evaluation in fresh and freeze dried avocado powder

Quality evaluation in fresh and freeze dried avocado powders were ascertained by studying the nutritional and bioactive compounds that are phytochemical composition, Mineral composition and antioxidant activities

5.2.1 Assessment of phytochemical composition, mineral composition and antioxidant activities in fresh avocado cultivars

Bioactive compounds are extra nutritional constituents that occur in small quantities in foods which are needed for the maintenance of healthy life.

These compounds show protective effects of plant-based diets on cardiovascular disease (CVD) and cancer. Many bioactive compounds include phenols, flavonoids, carotenoids etc shows antioxidant properties (Etherton *et al.*, 2002).

5.2.1.1 Assessment of phytochemical composition of fresh avocado cultivars

Glutathione

The glutathione content of selected fresh avocado cultivars showed that the highest amount was found in Pollock (18.33mg/100g) and it was found to be on par with Fuerte (18.13mg/100g). Lowest content of glutathione was observed in the cultivar Kallar Round (15.3 mg/100g). The fours cultivars were statistically different in the case of glutathione content at 5 % level of significance.

Wank *et al.* (2016) revealed that the glutathione content of Hass avocados ranged from 23.2g/100g to 24.5g/100g of fresh fruit.

Folic acid

The highest folic acid content among fresh avocado cultivars was noted in Pollock (80.33µg/100g). Folic acid content of Fuerte (79.30µg/100g) was found to be on par with Pollock (80.33µg/100g) and Kallar Round (77.86µg/100g). Lowest content was found in Purple Hybrid (74.03µg/100g). Folic acid contents among fresh avocado cultivars were found statistically different at 5 % level of significance. A study conducted by Pacheco *et al.* (2011) revealed that the folic acid content of avocados ranged from 35µg/100g to 89µg/100g of fresh fruit.

Vitamin K

Vitamin K content of fresh avocado cultivars was analyzed and the treatments were found to be significantly different at 5 % level of significance. The Vitamin K content of the four cultivars to be in the range of 0.013mg/100g to 0.019mg/100g and highest value was found for Pollock (0.019mg/100g) and Purple Hybrid (0.019mg/100g) and these two cultivars were found to be on par with Kallar Round and Fuerte (0.013mg/100g). USDA (2011) reported that the amount of vitamin K found in avocados in the range of 0.021mg to 0.023mg per 100g fruit, respectively.

Vitamin E

Vitamin E an important antioxidant helps to prevent the development of neurological complications, and in patients with lesions treatment can arrest or reverse the neuropathy (Traber and Packer, 1995).

A study proved that the composition of vitamin E in avocado fruit is affected by several factors, including the degree of maturation and edaphoclimatic (Arancibia-Avila *et al.*, 2008).

Vitamin E content of cultivar Pollock (2.52mg/100g) showed the highest value and the least value was observed in Purple Hybrid (2.04mg/100g). Vitamin E content of the cultivars was found to be significantly different at 5 % level of significance. USDA, (2011) noted that vitamin E content of Hass avocado was found to be 1.97mg/100g.

Total carotenoids

The consumption of avocados is an important dietary source of carotenoids particularly xanthophyl carotenoids (Lu *et al.*, 2005).

The carotenoid content of the four avocado cultivars are depicted in the table 2 and the maximum amount of carotenoid content was found in the Pollock (2.94mg/100g). Carotenoid content of Kallar Round (2.85mg/100g) was found on par with Pollock and Purple Hybrid (2.77mg/100g). Carotenoid content of Fuerte was (2.55mg/100g).

A study conducted by USDA (2011) reported that total carotenoid content of Hass avocado was found to be 2.45mg/100g.

Lutein

Lutein content was found to be maximum in the cultivar Fuerte (0.206mg/100g) followed by Purple Hybrid (0.193mg/100g), Kallar Round (0.185mg/100g) and Pollock (0.183mg/100g). Unlu *et al.* (2005) reported that lutein content of avocados ranged from 0.194mg/100g to 0.271mg/100g of pulp.

Total Phenols

Highest total phenolic content was observed in the cultivar Pollock (13.8mg/100g) and the lowest content was noted in the cultivar Fuerte (12.24mg/100g). Other two cultivars had the total phenolic content as follows in the values; Purple Hybid (13mg/100g) and Kallar Round (12.6mg/100g).

Avellone *et al.* (2016) opined that the total phenolic content of the cultivar Bacon was found to be 14.6mg/100g.

Total phytosterols

The total phytosterol content of four avocado cultivars was in the range of 62.30mg/100g- 68.60mg/100g respectively. The highest content was noted in the cultivar Kallar Round (68.60mg/100g) and the lowest amount was noted in the cultivar Fuerte (62.30mg/100g). The analysis found significant at 5 % level of significance.

Dreher and Davenport, (2013) reported that the total phytosterol content of various avocado cultivars were ranged from 57mg/100g to 74mg/100g.

Total flavonoids

Total flavonoid content was found to be maximum (21.07mg/100g) in the cultivar Purple Hybrid and minimum in Kallar Round (18.13mg/100g). Purple Hybrid was found to be on par with Pollock (20.73mg/100g). Statistically the values were found to be different in four cultivars at 5 % level. Ana *et al.* (2013) found in their study that Algarvian avocado contains 21.9mg/100g of flavonoid content.

5.2.1.2 Mineral composition of fresh avocado cultivars

Iron

The iron content of the cultivar Fuerte was 0.403mg/100g, which shows the highest value and the least value was noted in the cultivar Kallar Round (0.283mg/100g) and which was found to be on par with the cultivar Purple Hybrid (0.293mg/100g). The iron content of the cultivars found significant at 5 % level of significance. USDA (2011) reported that the iron content of Hass avocado ranged from 0.39mg/100g to 0.61mg/100g of fruit pulp.

Calcium

Greater amount of calcium among the four fresh avocado cultivars noted in Pollock (9.3mg/100g) and which was found to be on par with the cultivars Purple Hybrid (9.15mg/100g) and Fuerte (9.15mg/100g). Lowest amount of calcium was observed in Kallar Round (8.65mg/100g). According to the study conducted by Cutting *et al.* (1992) the calcium content of the avocado cultivar Reed ranged from 8.9mg/100g to 13mg/100g.

Sodium

The sodium content of the four avocado cultivars was studied and the highest amount was noted in the cultivar Pollock (6.12mg/100g) and Pollock was found to have no significant difference with the cultivar Kallar Round (6.12mg/100g). Least amount of sodium was noted in the cultivar Fuerte (5.51 mg/100g). Based on the study conducted by USDA (2011) avocados are very low in sodium with a range of 2 mg to 5.5mg per 100g of fruit.

Phosphorus

The phosphorus content of the four avocado cultivars depicted in the Table 4 and the maximum amount of phosphorus was found in the cultivar Purple Hybrid (36.13mg/100g) followed by Pollock (34.90mg/100g), Kallar Round (32.43mg/100g) and Fuerte (32.13mg/100g). The analysis revealed significant differences between the four cultivars at 5 % level of significance. The phosphorus content of Hass avocado ranged from 40mg/100g to 54mg/100g (IOM, 1997).

Potassium

The potassium content of the cultivar Pollock was 394.47mg/100g, which possessed the highest value and which was found to be on par with the cultivar Purple Hybrid (393mg/100g). Least value observed in Fuerte (343.33mg/100g). Statistical analysis was done at 5 % level. The potassium content of Hass avocado ranged from 405mg/100g - 507mg/100g (USDA, 2011).

Copper

The highest copper content among the four avocado cultivars was noted in Pollock (0.146mg/100g) and Fuerte (0.146mg/100g) and these two cultivars were found to be on par with Purple Hybrid (0.141mg/100g). Less amount of copper was observed in the cultivar Kallar Round (0.136mg/100g). The values showed statistical difference with each other. Hardisson *et al.* (2000) reported that the copper content in the african varity Serpa was 0.172mg/100g.

Magnesium

From the table 5, it was observed that Pollock got the maximum magnesium content of 22.20mg/100g and minimum amount observed in the cultivar Purple Hybrid (17.30mg/100g). Statistically each cultivar was different at 5 % level.

Avocados contain about 16 and 29 mg magnesium per 100 g of fruit pulp based on the study conducted by Batista *et al.* (1993).

Zinc

As noted in the Table 5 maximum zinc content was found in the cultivar Pollock (0.473mg/100g). The zinc content of Kallar Round (0.436mg/100g) was on par with Fuerte (0.420mg/100g) and Purple Hybrid (0.410mg/100g). The cultivar Purple Hybrid has less amount of zinc (410mg/100g). USDA (2010) reported that zinc content of Indian avocados ranged from 0.450mg/100g-0.680mg/100g of fresh fruit pulp.

Selenium

On ascertaining the selenium content of fresh avocado cultivars the highest amount was noted in the cultivar Pollock (0.500µg/100g) and the lowest selenium content was noted in the cultivar Purple Hybrid (0.203µg/100g). The statistical analysis for selenium content showed significant difference among the four fresh avocado cultivars. IOM (2000) opined that the selenium content of avocado was found to be 0.400µg/100g.

5.2.1.3 Assessment of antioxidant activities in fresh avocado cultivars

Total antioxidant activity

The total antioxidant activity of fresh avocado cultivars was found to be in the range of 214mg/100g to 234.50mg/100g respectively. The results of the ANOVA table revealed significant differences in the total antioxidant activity of the fresh avocado cultivars. Highest value for total antioxidant activity was observed in the cultivar Pollock (234.50mg/100g) and it was found to be on par with the freeze dried powder of Purple Hybrid (230.76mg/100g). Least value observed in Fuerte (214mg/100g) and it was found on par with Kallar Round (220.33mg/100g). Ana *et al.* (2013) reported that the total antioxidant activity of the fresh avocado cultivars ranges from 280mg/100g of ascorbic acid to 420mg/100g of ascorbic acid.

DPPH radical scavenging activity

Table 7 depicted the level of DPPH radical scavenging activity in the four avocado cultivars. The findings revealed that the cultivar Purple Hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 34.03µg/ml, followed by Kallar Round (38.98µg/ml), Fuerte (40.76µg/ml) and the lowest DPPH radical scavenging activity was found in the cultivar Pollock (42.52μg/ml). Kosinska *et al.* (2012) reported in their study that DPPH radical scavenging activity in avocados ranges from 32.2μg/ml-39μg/ml.

Hydroxyl radical scavenging activity

On ascertaining the IC_{50} values for hydroxyl radical scavenging activity of fresh avocado cultivars, higher hydroxyl radical scavenging activity was noted in the in the cultivar Kallar Round (27.03μg/ml) and the lowest hydroxyl radical scavenging activity was observed in the cultivar Pollock (38.86μg/ml). Statistical analysis (ANOVA) done at 5 % level of significance. Lee *et al.* (2008) opined that the hydroxyl radical scavenging activity of West Indian Race ranged from 32.5μg/ml-45μg/ml.

Super oxide Radical Scavenging Activity

Super oxide Radical Scavenging Activity (IC_{50}) was observed to be higher in the cultivar Kallar Round (12.12μg/ml). Lowest value for Super oxide radical scavenging activity was noted in the cultivar Fuerte (13.32μg/ml). Statistical analysis was done at 5 % level of significance. Statistically no difference found among four fresh avocado cultivars. Based on the data obtained from the fresh avocados, super oxide radical scavenging activity was found to be 18.56μg/ml (Kosinska *et al.*, 2012).

Figure. 2: Total antioxidant activity of fresh avocado fruit cultivars

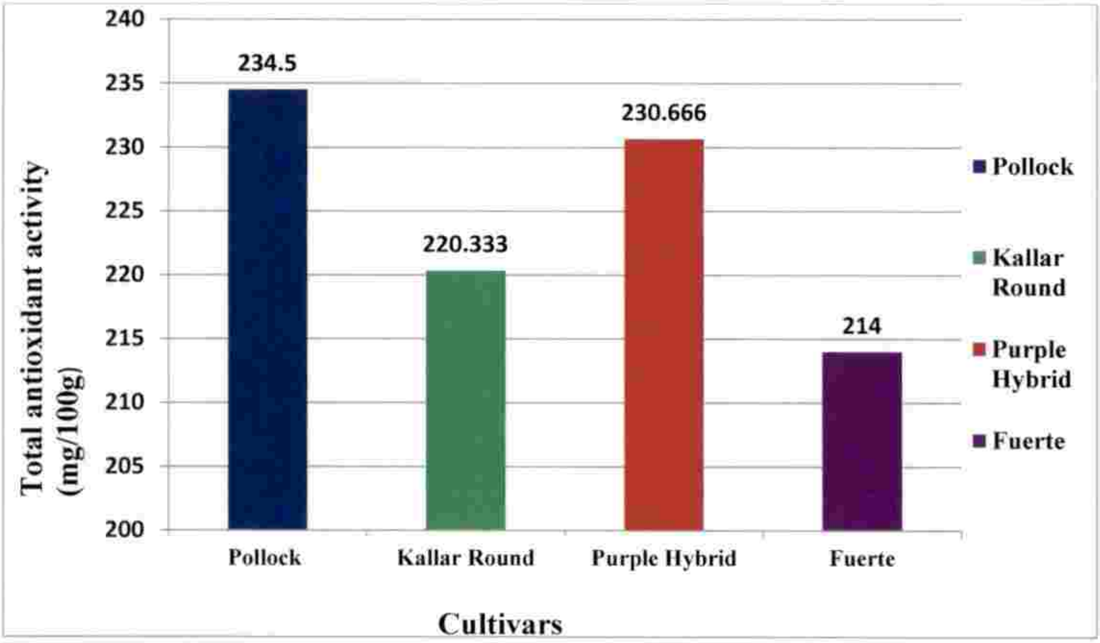


Figure. 3: DPPH radical scavenging activity of fresh avocado fruit cultivars

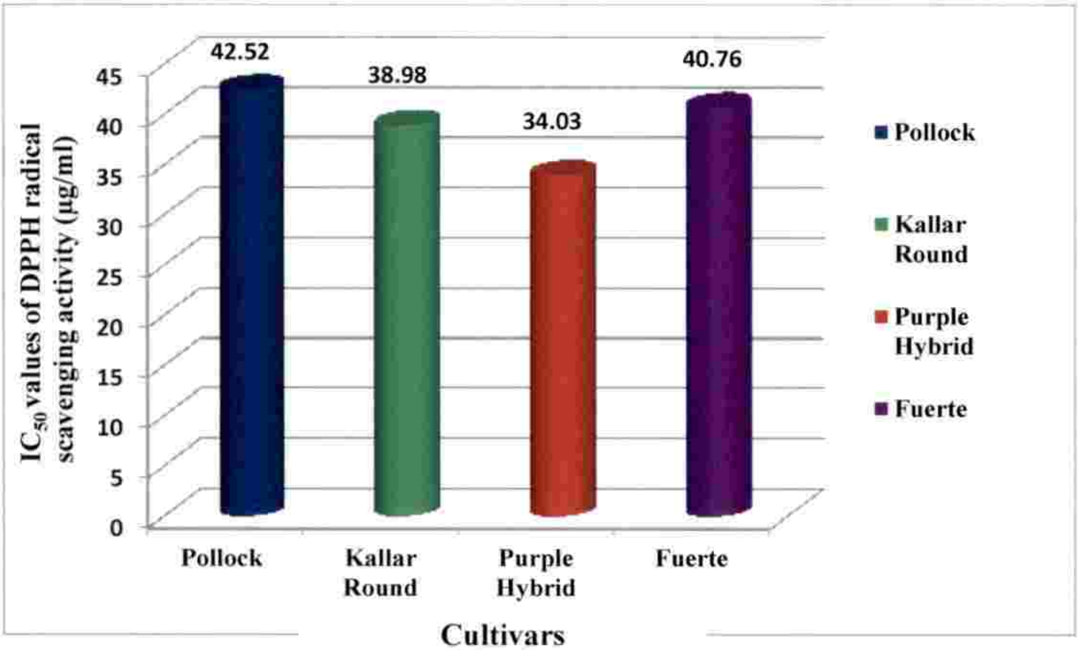


Figure. 4: Hydroxyl radical scavenging activity of fresh avocado fruit cultivars

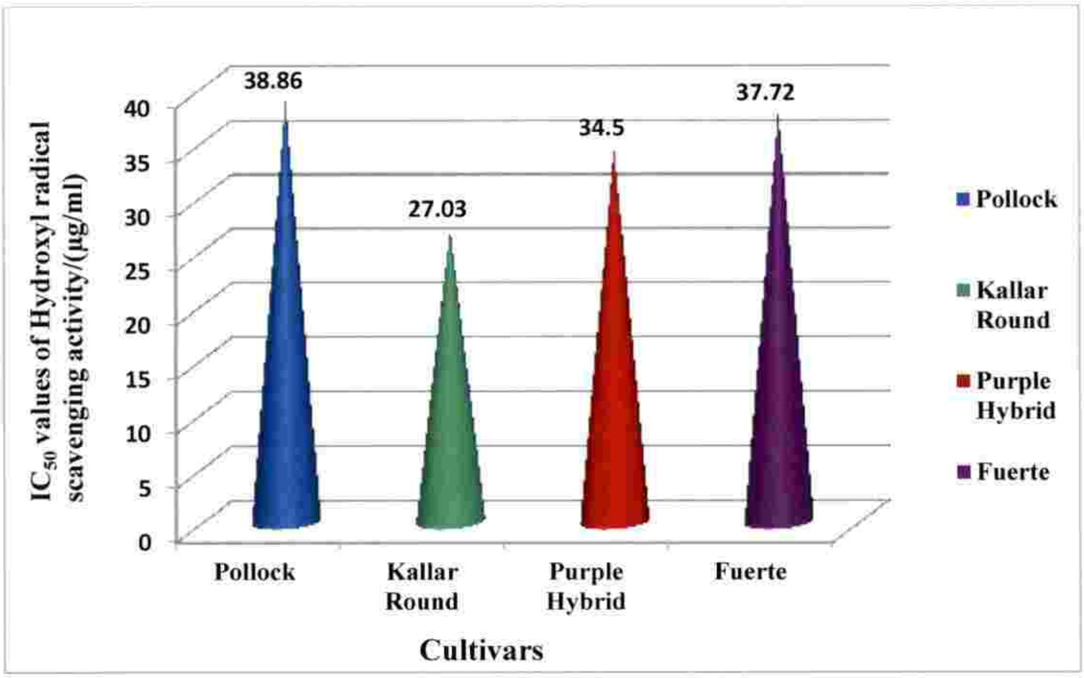
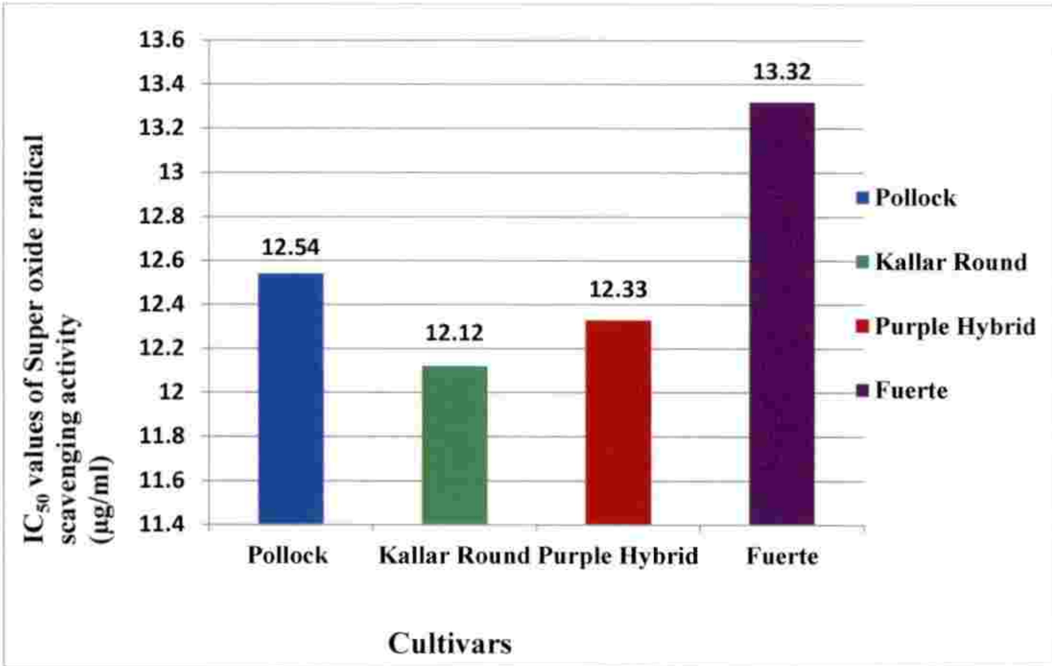


Figure. 5: Superoxide radical scavenging activity of fresh avocado fruit cultivars



5.2.2 Assessment of phytochemical composition, mineral composition and antioxidant activities in freeze dried avocado powders

The results of the study conducted by Shofian *et al.* (2001) showed that freeze-drying can be explored as a viable method for processing tropical and sub tropical fruits retaining the maximum amount of their naturally occurring nutrients. This technique can noticeably affect the composition of some antioxidant components of fruits, but losses of nutrients are minimal.

5.2.2.1 Phytochemical composition of freeze dried avocado powders

Glutathione

Glutathione content of the freeze dried powders of four avocado cultivars found to be statistically different at 5 % level of significance. The highest amount of glutathione was recorded in the freeze dried powder of Pollock (45.50mg/100g) and lowest amount was recorded in Kallar Round (39.33mg/100g). Shyam and Sablani, (2006) reported that the glutathione content of freeze dried strawberry was found to be 22.5mg/100g.

Folic acid

Folic acid content in freeze dried powders of four avocado cultivars was found. Values for folic acid content in freeze dried powders of avocado cultivars as follows as, Fuerte (197.92µg/100g), Pollock (197.04µg/100g), Kallar Round (185.15µg/100g) and Purple Hybrid (180.79µg/100g) respectively. Based on the study conducted by Burdon *et al.* (2007) the folic acid content of freeze dried powder of Hass avocado ranged from 285µg/100g-302µg/100g.

Vitamin K

From the quantitative estimation of vitamin K, it is clear that the freeze dried powder of Fuerte was recorded with higher value for vitamin K content (0.185mg/100g) values was found to be on par with Pollock (0.183mg/100mg), Purple Hybrid (0.182mg/100g) and Kallar Round (0.176mg/100g). Statistically there were no significant differences observed among the freeze dried powders of four avocado cultivars. The vitamin K freeze dried powder of Hass avocado ranged from 0.058mg/100g-0.016mg/100g according to the study conducted by Arancibia and Toledo, (2008).

Vitamin E

In the case of vitamin E content of freeze dried avocado powders, Pollock had the higher amount (6.53mg/100g) and which was on par with Fuerte (6.40mg/100g) and Kallar Round (6.21mg/100g). Lowest content was found in Purple Hybrid (5.83mg/100g). According to the study conducted by Marques (2009) vitamin E content of freeze dried powder of Hass avocado ranged from 12.5mg/100g to 15.4mg/100g.

Total carotenoids

Carotenoid content of the freeze dried powders of four avocado cultivars was observed in the range of 7.70mg/100g to 8.15mg/100g. As indicated in Table 8, the cultivars Pollock (8.15mg/100g) and Purple Hybrid (8.07mg/100g) were found to be having higher carotenoid content. When the data was analysed statistically, it was observed that there was a significant difference among the four cultivars. It was also noticed that the cultivars Pollock and Purple Hybrid were on par with each other. The cultivar Fuerte (7.70mg/100g) recorded least content of carotenoids. Hidalgo *et al.* (2010) reported that total carotenoid content of spray dried Hass avocado ranged from 14.5mg/100g to16.17mg/100g.

Lutein

On comparing the lutein content of freeze dried powders of four avocado cultivars, it was noted that the cultivar Purple Hybrid (0.503mg/100g) recorded the highest lutein content and the lowest in the cultivar Fuerte (0.423mg/100g). Kallar Round (0.426mg) was found to be on par with Fuerte. Statistically freeze dried powders of the four avocado cultivars are significant at 5 % level. A study conducted by Unlu *et al.* (2005) revealed that the lutein content of freeze dried avocado was found to be 2.68mg/100g.

Lutein improves the potential of other bioactive compounds for cancer cell proliferation (Lu *et al.*, 2005).

Total phenols

The values indicated higher phenol content among freeze dried powders of Pollock (51.73mg/100g). Least value of total phenol was observed in the freeze dried powder of the cultivar Fuerte (46.4mg/100g). Statistical difference at 5% level of significance was noted. Total phenolic content of spray dried Hass avocado was observed 79.4mg/100g of avocado powder (Grajales *et al*,. 1999).

Total phytosterols

Total phytosterol content of the freeze dried powders of avocado cultivars were revealed to be in the range of 122.27mg/100g to 173.80/100g. The phytosterol content of the freeze dried powder of Kallar Round (173.80mg/100g) was followed by Purple Hybrid (164.37mg/100g) and Fuerte (149.04mg/100g). Low amount was found in Pollock (122.27mg/100g). Piironen*et al.* (2003) reported that total phytosterol content of freeze dried strawberry ranged from 116mg/100g to 134mg/100g.

Total flavonoids

Comparing the flavonoid content of avocado cultivars studied, it was observed that cultivar Pollock (45.27mg/100g) and cultivar Purple Hybrid (45 mg/100g) were having higher amount. Cultivar Fuerte (43.17mg/100g) showed the least amount of flavonoid content. Significant difference at 5% level was observed in total flavonoid content of the samples. Marinova *et al.* (2005) reported that total flavonoid content of freeze dried cherry was 39.5mg/100g.

5.2.2.2 Mineral compositions of freeze dried avocado fruit powders

Iron

The iron content of the freeze dried powder of the cultivar Fuerte was 2.87mg/100g, which was found to be highest among the cultivars. The cultivar Kallar Round had the least value of iron (1.87mg/100g) and which was found on par with the freeze dried powder of Purple Hybrid (1.97mg/100g). The differences in values of iron were significantly different at 5 % level of significance. Minh *et al.* (2019) found that the iron content of freeze dried avocado ranged from 2.25mg/100g.

Calcium

The values indicated that high calcium content was found in the freeze dried powder of Purple Hybrid (142.5mg/100g). The calcium content in Pollock (104mg/100g) was noted lower and it was found to be on par with and Kallar Round (106.5mg/100g) and Fuerte (111.5mg/100g). The values were statistically different at 5% level of significance. According to the study conducted by Grajales *et al.* (1999) the calcium content of spray dried avocado ranged from 51.36mg/100g-104.6mg/100g.

Sodium

Sodium content of freeze powders four avocado cultivars was analysed. Higher amount of sodium was observed in the freeze dried powder of Purple Hybrid (14.40mg/100g) and lower in Fuerte (12.30mg/100g). Sodium content in Pollock (13.13mg/100g) and Kallar Round (13mg/100g) found to have no significant difference at 5 % level. The sodium content of spray dried avocado pulp was ranged from 0.71mg/100g-4.76mg/100g (Grajales *et al.,* 1999).

Phosphorus

In the present study, phosphorus content of freeze dried powders of avocado cultivars was noted in the range of 70.30mg/100g to 76.07mg/100g. The study showed significant difference in phosphorus levels between the cultivars. Highest value of phosphorus was recorded in Pollock (76.07mg/100g). Lowest value for phosphorus was noted in the freeze dried powder of Kallar round (70.30mg/100g). Ernesto *et al.* (2018) reported that the phosphorus content in freeze dried avocado was ranged from 14mg/100g- 45mg/100g.

Potassium

Potassium level of freeze dried powders of avocado cultivars was analyzed. Potassium level of Purple Hybrid (623mg/100g) was recorded to be higher and found on par with Pollock (622mg/100g) and Kallar Round (618mg/100g). Least value for potassium was found in Fuerte (586.33mg/100g.). Significant difference was found between the cultivars at 5% level of significance. The phosphorus content of freeze dried avocados was ranged from 510mg/100g-1270.92mg/100g (Ernesto *et al.*, 2008).

Copper

With regard to copper content of freeze dried fruit powders of avocado cultivars, the values ranged from 3mg/100g to 3.23mg/100g. Highest value for copper content was noted in the freeze dried powder of Purple Hybrid (3.23mg/100g) and least value was observed in Fuerte (3mg/100g). Statistical difference among the cultivars was found at 5% level of significance. According to study conducted by Marulanda *et al.* (2018) the copper content of spray dried avocado was 0.78mg/100g.

Magnesium

Magnesium content among the four avocado fruit cultivars was analyzed. Higher magnesium content was observed in Pollock (42.13mg/100g) followed by Kallar Round (39.23mg/100g), Purple Hybrid (38.23mg/100g) and Fuerte (34.76mg/100g). Kallar Round was found to be on par with Purple Hybrid. The magnesium content of freeze dried Hass avocado ranges was found to be 67.35mg/100g-88.29mg/100g (Arias *et al.*, 2013).

Zinc

The level of zinc was analyzed in the freeze dried powder of avocado cultivars and found in the range of 2.11 mg/100g to 4.66 mg/100g. The values obtained for zinc were as follows; Kallar Round (4.73mg/100g), Pollock (3.45mg/100g) and Purple Hybrid (3.14 mg/100g) and Fuerte (2.11mg/100g). Significant difference in values at 5% level was found. Based on the study conducted by Chia *et al.*, (2010) the zinc content in freeze dried Hass avocado was 1.69mg/100g.

Selenium

Selenium content for the freeze dried powders of avocado cultivars revealed that Pollock (8.90 µg/100g) was having higher amount and Kallar Round (8.07µg/100g) was having least amount. Statistically no significant difference in values was found between the cultivars. Selenium content of Hass avocado was reported as 6.75µg/100g (Xu *et al.,* 1995).

5.2.2.3 Antioxidant activities of freeze dried avocado powders

Total antioxidant activity

The total antioxidant activity of freeze dried powders of four avocado cultivars ranges from 440.33 mg/100g to 461.90 mg/100g. The results revealed significant differences in the total antioxidant activity. Highest value for total antioxidant activity was observed in the cultivar Pollock (461.90mg/100g) and which was found to be on par with Purple Hybrid (460.33mg/100g). Least value was noted in Fuerte (440.33mg/100g) and which was found to be on par with Kallar Round (444.23mg/100g). According to the study conducted by Darsini *et al.* (2012) the total antioxidant activity of the freeze dried margarinda avocados ranges from 535 to 576mg/100g.

DPPH radical scavenging activity

Table 12 shows the level of DPPH radical scavenging activity in the freeze dried powders of four avocado cultivars. The findings revealed that the cultivar Purple hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 90.80µg/ml, followed by Kallar Round (98.33µg/ml), Fuerte (100.66µg/ml) and the lowest in the cultivar Pollock (101.66µg/ml). Darsini *et al.* (2012) reported in their study that DPPH radical scavenging activity of freeze dried powder of Pisang Awak variety of banana ranged from 167µg/ml-173µg/ml.

Hydroxyl radical scavenging activity

Regarding the IC_{50} values for hydroxyl radical scavenging activity of freeze dried avocado cultivars, highest activity was noted in the freeze dried powder of Fuerte (69.25µg/ml). Lowest activity was observed in the freeze dried powder of Pollock (86.60µg/ml). Based on a study conducted by Lee *et al.* (2006) Hydroxyl radical scavenging activity (IC_{50}) of freeze dried berrys was 95µg/ml.

Super oxide radical scavenging activity

Super oxide Radical Scavenging Activity (IC_{50}) was observed higher in the freeze dried powder of Fuerte (24.47µg/ml) followed by Kallar Round (34.93µg/ml), Purple Hybrid (44.53µg/ml) and the lowest in the cultivar Pollock (44.63µg/ml). Super oxide radical scavenging activity (IC_{50}) of freeze dried powder of berrys ranged from 56µg/ml-65.34µg/ml (Lee *et al.*, 2006).

Figure. 6: Total antioxidant activity of freeze dried avocado fruit powders

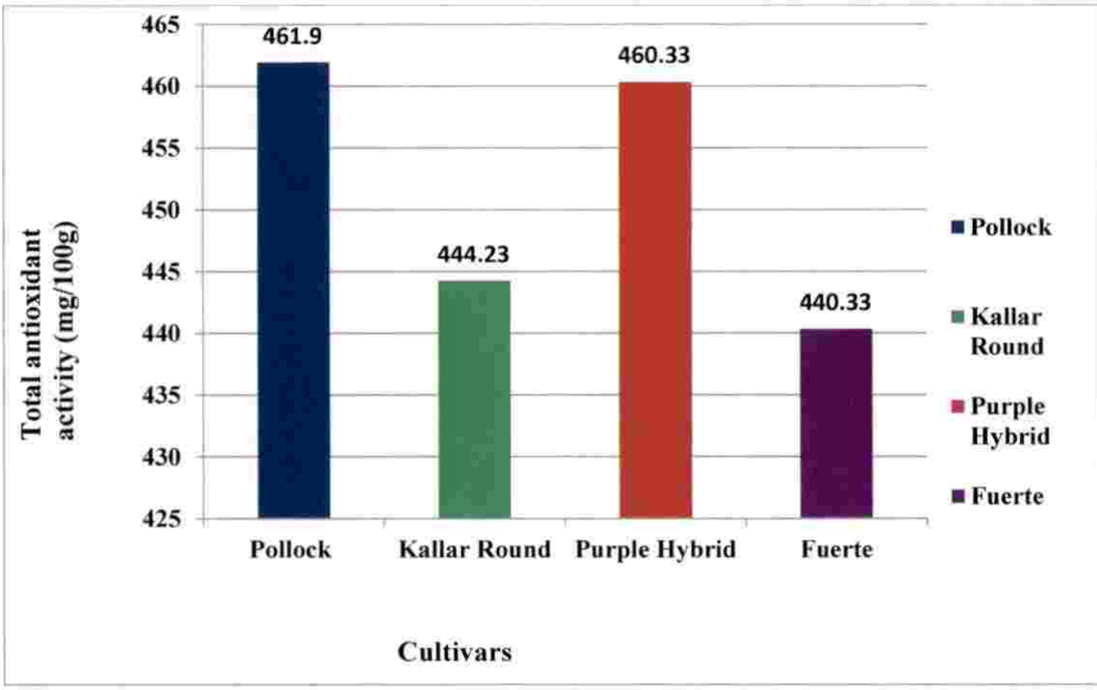


Figure. 7: DPPH radical scavenging activity of freeze dried avocado fruit powders

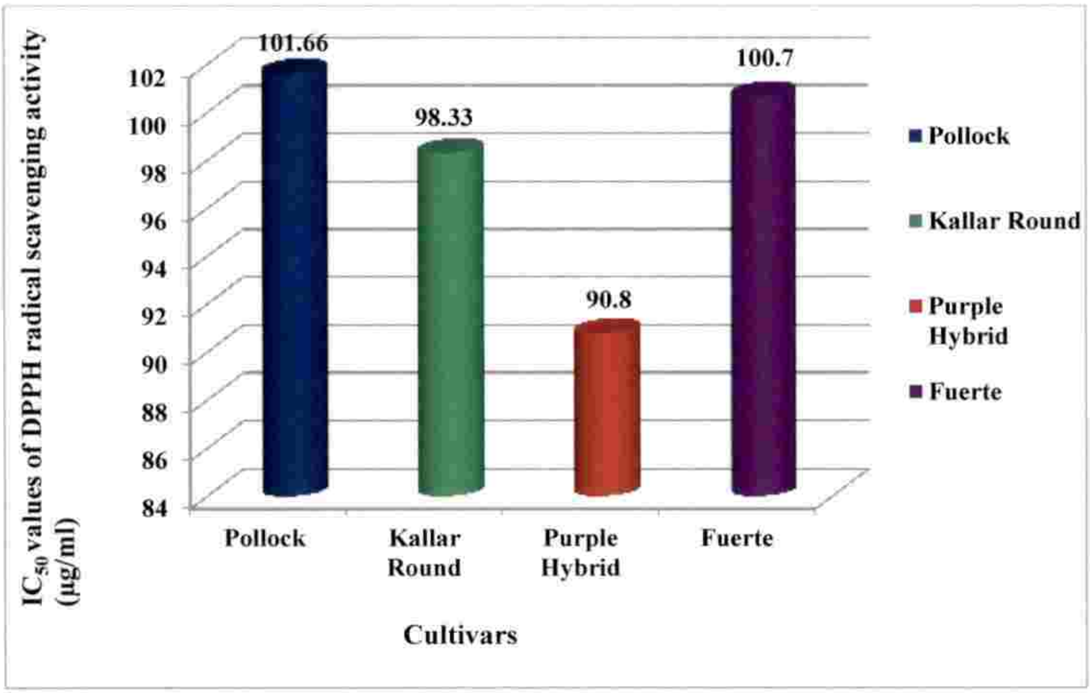


Figure. 8: Hydroxyl radical scavenging activity of freeze dried avocado fruit powders

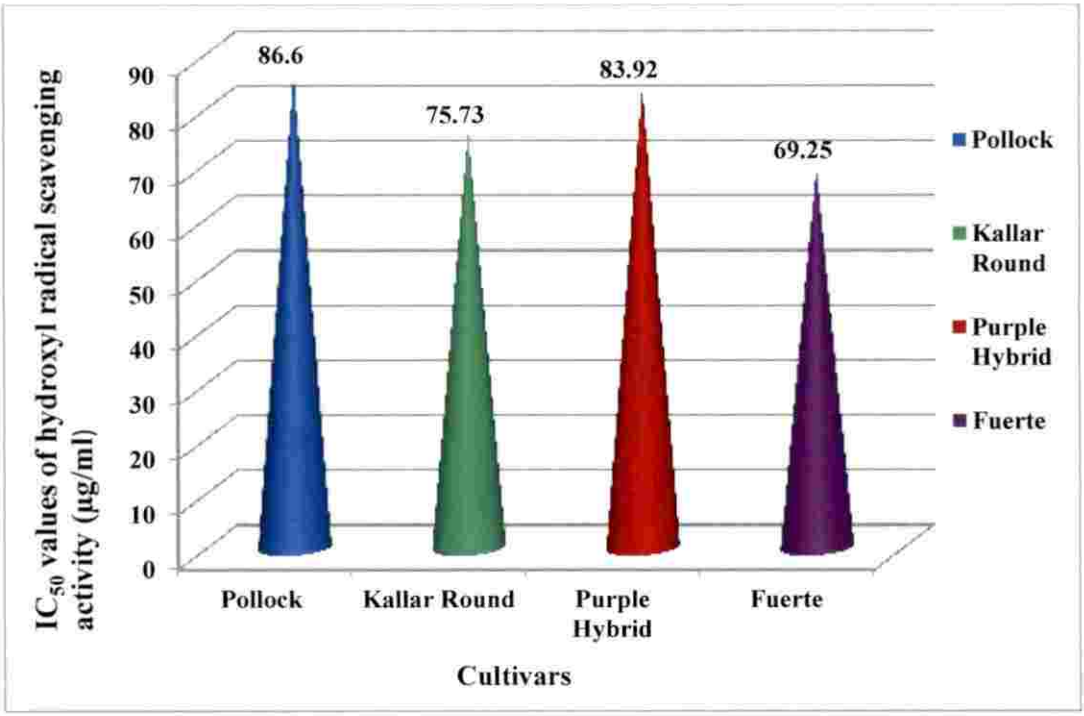
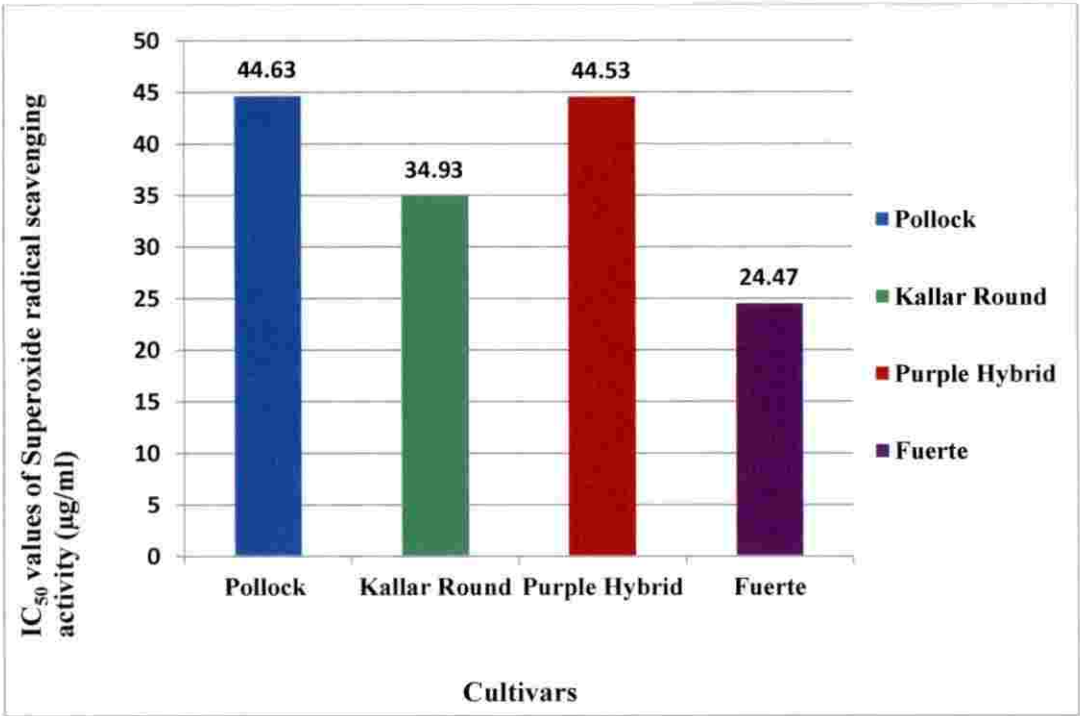


Figure. 9: Superoxide radical scavenging activity of freeze dried avocado fruit powders



5.2.2.4 Comparison of nutrient composition in fresh and freeze dried avocado fruit cultivars

Table: 16 Comparison of phytochemical composition

Phytochemical composition	Fresh avocado fruit cultivars		Freeze dried avocado powders
Glutathione (mg)	Pollock	18.33[a]	45.50[a]
	Kallar Round	15.3[c]	39.33[d]
	Purple Hybrid	17.33[b]	40.50[c]
	Fuerte	18.13[a]	42.40[b]
Folic acid (mg)	Pollock	80.33[a]	197.04[b]
	Kallar Round	77.86[b]	185.15[c]
	Purple Hybrid	74.03[c]	180.79[d]
	Fuerte	79.30[ab]	197.92[a]
Vitamin K (mg)	Pollock	0.019[a]	0.183[a]
	Kallar Round	0.017[a]	0.179[a]
	Purple Hybrid	0.019[a]	0.181[a]
	Fuerte	0.013[a]	0.185[a]

Vitamin E (mg)	Pollock	2.52[a]	6.53[a]
	Kallar Round	2.33[c]	6.21[a]
	Purple Hybrid	2.04[d]	5.83[b]
	Fuerte	2.40[b]	6.40[a]
Total caroteniods (mg)	Pollock	2.94[a]	8.15[a]
	Kallar Round	2.85[ab]	7.94[b]
	Purple Hybrid	2.77[b]	8.07[a]
	Fuerte	2.55[c]	7.70[c]

Table: 17 Comparison of phytochemical composition

Phytochemical composition	Fresh avocado fruit cultivars		Freeze dried avocado powders
Lutein (mg)	Pollock	0.183^{c}	0.437^{b}
	Kallar Round	0.185^{c}	0.426^{c}
	Purple Hybrid	0.193^{b}	0.503^{a}
	Fuerte	0.206^{a}	0.423^{c}
Total phenols (mg)	Pollock	13.84^{a}	51.73^{a}
	Kallar Round	12.65^{c}	49.04^{b}
	Purple Hybrid	13^{b}	48^{c}
	Fuerte	12.24^{d}	46.4^{d}
Total phytosterols (mg)	Pollock	63.14^{b}	122.27^{c}
	Kallar Round	68.60^{a}	173.80^{a}
	Purple Hybrid	63.23^{b}	164.37^{ab}
	Fuerte	62.30^{c}	149.04^{b}

Total flavonoids (mg)	Pollock	20.73^{a}	45.27^{a}
	Kallar Round	18.13^{b}	44.10^{b}
	Purple Hybrid	21.07^{a}	45^{a}
	Fuerte	18.37^{b}	43.17^{c}

Table: 18 Comparison of mineral composition

Mineral composition	Fresh avocado fruit cultivars		Freeze dried avocado powders
Iron (mg)	Pollock	0.320^{b}	2.27^{b}
	Kallar Round	0.283^{c}	1.87^{c}
	Purple Hybrid	0.293^{c}	1.97^{c}
	Fuerte	0.403^{a}	2.87^{a}
Calcium (mg)	Pollock	9.3^{a}	104^{b}
	Kallar Round	8.65^{b}	106.5^{b}
	Purple Hybrid	9.15^{a}	142.5^{a}
	Fuerte	9.15^{a}	111.5^{b}

Sodium (mg)	Pollock	6.12[a]	13.13[b]
	Kallar Round	6.12[a]	13[b]
	Purple Hybrid	5.87[b]	14.40[a]
	Fuerte	5.51[c]	12.3[c]
Phosphorus (mg)	Pollock	34.90[b]	76.07[a]
	Kallar Round	32.43[c]	70.30[c]
	Purple Hybrid	36.13[a]	73.07[b]
	Fuerte	32.13[d]	75.63[a]
Potassium (mg)	Pollock	394.47[a]	622.76[a]
	Kallar Round	384.76[b]	618.76[a]
	Purple Hybrid	393[a]	623[a]
	Fuerte	343.33[c]	586.33[b]

Table: 19 Comparison of mineral composition

Mineral composition	Fresh avocado fruit cultivars		Freeze dried avocado powders
Copper (mg)	Pollock	0.146[a]	3.13[b]
	Kallar Round	0.136[b]	3.07[c]
	Purple Hybrid	0.141[a]	3.23[a]
	Fuerte	0.146[a]	3[d]
Magnesium (mg)	Pollock	22.20[a]	42.13[a]
	Kallar Round	18.50[b]	39.23[b]
	Purple Hybrid	17.30[c]	38.23[b]
	Fuerte	18.63[b]	34.76[c]
Zinc (mg)	Pollock	0.473[a]	3.45[b]
	Kallar Round	0.436[b]	4.66[a]
	Purple Hybrid	0.410[b]	3.14[c]
	Fuerte	0.420[b]	2.11[d]

Selenium (µg)	Pollock	0.500[a]	8.90[a]
	Kallar Round	0.243[c]	8.07[d]
	Purple Hybrid	0.203[d]	8.50[c]
	Fuerte	0.300[b]	8.80[b]

Table: 20 Comparison of antioxidant activities

Antioxidant activities	Fresh avocado fruit cultivars		Freeze dried avocado powders
Total antioxidant activity (mg)	Pollock	234.50[a]	461.90[a]
	Kallar Round	220.33[b]	444.23[b]
	Purple Hybrid	230.76[a]	460.33[a]
	Fuerte	214[b]	440.33[b]
IC_{50} value of DPPH radical scavenging(µg/ml)	Pollock	42.52[a]	101.66[a]
	Kallar Round	38.98[c]	98.33[b]
	Purple Hybrid	34.03[d]	90.80[c]
	Fuerte	40.76[b]	100.7[a]

IC_{50} value of hydroxyl radical scavenging activity (µg/ml)	Pollock	38.86[a]	86.60[a]
	Kallar Round	27.03[d]	75.73[c]
	Purple Hybrid	34.50[c]	83.92[b]
	Fuerte	37.72[b]	69.25[d]
IC_{50} value of super oxide radical scavenging activity (µg/ml)	Pollock	12.54	44.63[a]
	Kallar Round	12.12	34.93[c]
	Purple Hybrid	12.33	44.53[b]
	Fuerte	13.32	24.47[d]

From the above tables, it is clear that nutrient components in freeze dried avocado fruit powders are higher than the nutrient components of fresh avocado fruit cultivars.

Freeze dried fruits lack water and they are highly concentrated, which means they contains more nutrients. Freeze dried fruits are rich in antioxidants, vitamins and minerals (Souza *et al.*, 2015).

5.3 Anti proliferation study in superior freeze dried avocado powder

5.3.1 MTT assay

Anti proliferation activities in freeze dried avocado powder from superior cultivar Pollock was studied in cell lines using MTT assay.

The anti-cancer property of methanolic extract of freeze avocado powder was examined on human colon cancer cell line (HCT 116 cell line).Cells were cultured in a clear bottom 96-well tissue culture plated. Typical medium contains DMEM, 10 % fetal bovine serum, antibiotics, amino acids and other nutrients. Ten thousand cell per well were taken. Extract was added in different concentration from 10 µg, 50 µg, 100 µg, 150 µg, and 200 µg to HCT 116 and incubated for twenty four-hours at 37^0 C. In sterile condition MTT (5 mg /ml) was prepared and filtered. 200 µl of MTT reagent was added per well and incubated for 4 hours at 37°C. After that 200 µl of the DMSO (dimethyl sulfoxide) solution was added and mixed gently on an orbital shaker at room temperature. Optical density was measured at 550 nm. A dose-dependent MTT reduction (or color change from yellow to purple) was observed in treated cells. Extract were added in different concentration from 10 µg, 50 µg, 100 µg, 150 µg, 200 µg to colon cancer cell lines. After the incubation period of cells with avocado extract, inhibition of cancer cells observed.

After the incubation period, growth Inhibition of cancer cells were noted 27 %, 30 %, 33%, 45 %, and 71 % against the concentration 1µg, 20µg, 50µg, 100µg, and 200 µg of test sample respectively. Fifty percentage of inhibition (GI_{50}) was shown at 120µg of fruit extract.

5.3.2 Percentage survival of cells

Only 29 percent of cell viability was observed after the exposure of cancer cell lines with avocado extract (200 µg).

Percentage of viable cell showed decrease with increase in extract concentration while percentage cytotoxicity increased with increase in concentration of avocado extract.

A study was undertaken to examine cytotoxic effect of freeze-dried methanolic extract of Ecballium elaterium fruit on cell lines of human gastric carcinoma (AGS) and human esophageal squamous cell carcinoma (KYSE30). The IC_{50} values were 2.5, 0.7, and 0.7 µg/ml for AGS cell lines after 24, 48, and 72 h, respectively. IC_{50} values for KYSE30 cell line were 500, 150, and 125 µg/ml after 24, 48, and 72 h, respectively. The results of the current study showed that the freeze-dried aqueous extract of Ecballium elaterium fruit has a cytotoxic effect on gastric and esophageal cancer cell lines by means of apoptosis (Bohlooli *et al.*, 2012).

The methanol extract from Morinda citrifolia fruits was studied for cytotoxicity activity on the MTT assay. Cytotoxic changes after exposure to the extract was in a concentration dependent manner (cytotoxicity increases according to the increase in concentration of extract). Human laryngeal carcinoma (Hep2) cells were found to be 2.5, 3 and 5 mg/mL, respectively. A concentration of 0.1 mg/mL of crude extract exhibited cytotoxic activity against breast cancer (MCF7) cell lines at 29% and 36%, respectively (Arpornsuwan and Punjanon, 2006).

A study was conducted by Bakar *et al.* 2015 to investigate the anticancer potential of of Garcinia. dulcis fruit extracts and their possible mechanism of action in liver cancer cell line (HepG2). MTT assay showed that Garcinia. dulcis extracts induced cytotoxicity in HepG2 cell line with IC_{50} value of 38.33 ± 3.51 μg/mL. Findings of the study suggested that the flesh extract of Garcinia. dulcis has its own potential as cancer chemotherapeutic agent against liver cancer cell.

On comparing the cytotoxic effects of other fruits mentioned above with the cytotoxicity of avocado fruit extract, it was clear that avocado extract also showed good anti cancerous properties against cancer cell lines.

The present study suggests that phytochemicals and antioxidants could be responsible for its cytotoxic activity against HCT 116 cell line.

SUMMARY

6. SUMMARY

Avocado (*Persea americana* Mill.) or butter fruit is a worldwide consumed fruit, native from south central Mexico with great interest in pharmaceutical industries. This fruit is used to extract many nutritive and bioactive compounds for the manufacturing of therapeutic drugs because of its high nutritive profile.

The objective of this study was, to ascertain bioactive compounds in fresh fruits and freeze dried powders of avocado (*Persea americana* Mill.) cultivars and to evaluate anti proliferation activities of freeze dried powder of superior cultivar in cancer cell lines.

Four cultivars viz., Pollock, Kallar Round, Purple Hybrid and Fuerte were selected for the study. Mature fruits were collected from Regional Agricultural Research Station, Ambalavayal, Wayanad District, for the present investigation. This study was carried out as two experiments.

On expreriment No.1. Detailed studies on different quality parameters of fresh and freeze dried powders of selected cultivars of avocado fruits were ascertained. The parameters studied include phytochemical composition, mineral composition and antioxidant activities

Phytochemical composition assessed for the study were glutathione, folic acid, vitamin K, vitamin E, total carotenoids, lutein, total phenols, total phytosterols and total flavonoids.

Mineral composition assessed includes iron, calcium, sodium, phosphorus, potassium, copper, magnesium, zinc and selenium.

Antioxidant activities assessed were` total antioxidant activity, DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide radical scavenging activity.

Quantitative estimation of phytochemicals showed that, glutathione content of fresh avocado fruits was in the range of 15.3mg/100g-18.3 mg/100g and was found to be higher in the cultivar Pollock (18.33mg/100g) and which was on par with Fuerte (18.13mg/100g). Folic acid, vitamin E, total carotenoids and total phenol content were observed in fresh fruits of avocado cultivars to be in the range of 79.30mg/100g-80.33mg/100g, 2.04 -2.52mg/100g, 2.55/100g-2.94mg/100g, and 12.24mg/100g -13.84mg/100g respectively. The Cultivar Pollock recorded highest content of folic acid (18.33mg/100g), vitamin E (2.52mg/100g), total carotenoids (2.94mg/100g) and total phenol (13.84mg/100g).Vitamin K and total phytosterols present in the four cultivars were in the range of 0.013mg/100g-0.019mg/100g and 62.30mg/100g-68.60mg/100g respectively. Lutein content was found to be more in the cultivar Fuerte (0.206mg/100g). Purple Hybrid was found to be superior in the case of total flavonoid content (21.07mg/100g).

Mineral analysis of fresh fruits of avocado cultivars revealed that iron content was found to be higher in Fuerte (0.403mg/100g). Calcium, sodium, potassium, magnesium, zinc and selenium contents were found to be more in Pollock (9.3mg/100g, 6.12mg/100g, 394.47 mg/100g, 22.20mg/100g, 0.473mg/100g and 0.500μg/100g respectively). The cultivars Pollock and Fuerte contained the same amount of copper (0.146mg). Phosphorus content was found to be almost the same in all the four cultivars noted in the range of 32.13/100g-34.90mg/100g.

In the case of total antioxidant activity in fresh avocado cultivars, Pollock was found to be superior (234.50mg/100g) and was found to have no significant difference with Purple hybrid (230.66mg). The findings revealed that the cultivar Purple Hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 34.03μg/ml, followed by Kallar Round (38.98μg/ml), Fuerte (40.76μg/ml) and the lowest DPPH radical scavenging activity was found in the cultivar Pollock (42.52μg/ml). Regarding the IC_{50} values for hydroxyl radical scavenging activity of fresh avocado cultivars, higher Hydroxyl radical scavenging activity was noted in the in the cultivar Kalla Round (27.03μg/ml) and the lowest Hydroxyl radical scavenging activity was observed in the cultivar Pollock (38.86μg/ml). Super oxide radical scavenging activity (IC^{50}) was observed to be higher in the cultivar Kallar Round (12.12μg/ml). Lowest value was noted in the cultivar Fuerte (13.32μg/ml). Statistically no difference was found among four fresh avocado cultivars.

Estimation of phytochemicals in freeze dried powders showed more glutathione content in Pollock (45.50mg/100g). Folic acid, vitamin K and lutein content in freeze dried form of the four avocado cultivars ranged from 180.70mg/100g-197.90mg/100g, 0.179mg/100g-0.185mg, and 0.423mg/100g-0.503mg/100g respectively. Total phytosterols was found to be more in Kallar Round (173.80mg/100g) and Total flavonoid content was found higher in Pollock (45.27mg/100g) and Purple Hybrid (45mg/100g).The carotenoid content was observed higher in the cultivar Pollock (8.15mg/100g) and which was found to be on par with Purple Hybrid (8.07mg/100g).

Mineral estimation in the freeze dried form of avocados highlighted the iron content to be higher in Fuerte (2.87mg/100g). Calcium content was in the range of 104mg/100g-142.5mg/100g and was found to be higher for Purple Hybrid and lower for Pollock. Sodium content was found to be higher in Purple Hybrid (14.40mg/100g). Phosphorus and Magnesium contents were found to be more in Pollock (76.07mg/100g and 42.13mg/100g respectively). Potassium content was found to be superior in Purple Hybrid (623mg/100g) and was on par with Pollock (622.76mg/100g) and Kallar Round (618.76mg/100g). Zinc content was found to be higher in the cultivar Kallar Round (4.66mg/100g) and less in Fuerte (2.11mg/100g). Copper and selenium contents were found to be almost same in all the four cultivars and in the range of 3mg/100g-3.23mg/100g and 8.07μg/100g-8.90μg/100g respectively.

Total antioxidant activity was observed to be superior in the cultivar Pollock (461.90mg/100g) and possessed no significant difference with Purple hybrid (460.33mg/100g). The findings revealed that the cultivar Purple hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 90.80µg/ml, followed by Kallar Round (98.33µg/ml), Fuerte (100.66µg/ml) and the lowest in the cultivar Pollock (101.66µg/ml). Regarding the IC_{50} values for hydroxyl radical scavenging activity of freeze dried avocado cultivars, highest activity was noted in the freeze dried powder of Fuerte (69.25µg/ml). Lowest activity was observed in the freeze dried powder of Pollock (86.60µg/ml). Super oxide Radical Scavenging Activity (IC_{50}) was observed higher in the freeze dried powder of Fuerte (24.47µg/ml) followed by Kallar Round (34.93µg/ml), Purple Hybrid (44.53µg/ml) and the lowest in the cultivar Pollock (44.63µg/ml).

From the quality evaluation of fresh avocado fruits and freeze dried avocado fruit powders, all the nutrients founds to be higher in freeze dried avocado powders when compared to the fresh fruits.

Experiment No.2. Anti proliferation activities in freeze dried avocado fruit powder of selected cultivar in cell lines was studied using MTT assay. From four avocado fruit cultivars, superior cultivar in bioactive and nutritional components was selected for further cell line study. Freeze dried powder of superior cultivar; Pollock was selected for cell line study.

Cytotoxicity of freeze dried powder of Pollock was estimated by MTT assay on Human colon cancer cells (HCT 116). Assay was done with different concentrations of avocado extract (10µg, 20µg, 50µg, 100µg, and 200µg). Growth inhibition noted were, 27 %, 30 %, 33%, 45 %, and 71 % for 10µg, 20µg, 50µg, 100µg and 200µg respectively. Percentage of viable cell decreased with increase in extract concentration while percentage cytotoxicity increased with increase in concentration of avocado extract. 50 % growth inhibition (GI_{50}) of cancer cells were shown at 120µg/ml of fruit extract. 29 percent of cells were found to be viable after exposure of extract (200µg).

The study "Profiling bioactive compounds in avocado (*Persea americana* Mill.*)* cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines" revealed that the four avocado fruit cultivars are rich in phytochemical compounds, mineral components and antioxidants. Quality evaluation of freeze dried powders showed a great increase in nutrients compared to fresh avocado fruit cultivars. Among the fresh and freeze dried form of avocado cultivars, Pollock was found to be superior and cell line study in superior cultivar revealed that avocado extract had noticeable anti-carcinogenic properties. Future research work could be concentrated on the application of avocado fruit extract in medicinal field especially for cancer treatment. Freeze dried avocado powder is more suitable for capsulation of drugs with long shelf life.

REFERENCES

7. REFERENCES

Abad, M. J., Ansuategui, M. Bermejo, P. 2007. Active antifungal substances from natural sources. *Food & Nutr.*8: 116-145.

Al-Delaimy, W. K., Cho, E., and Chen, W. Y. 2004. A prospective study of smoking and risk of breast cancer in young adult women. Cancer Epidemiol Biomarkers Prev, 13(3):398-404.

Alger, S. M. 1997. DPPH and oxygen free radicals as pro-oxidant of biomolecules. *Poly. Sci. dict.* p. 152. ISBN 0-412-60870-7.

Allen, L.H. 2013. Magnesium. J. *Nutr.* 12(6):131-135.

Ana, F., Vinha, J. M., and Sergio, V. P. 2013. Phytochemical Composition and Antioxidant Activity of the Algarvian Avocado (Persea americana Mill.). J. Agric. Sci. 5(12): 14-17.

AOAC. 1995. *Official Method of Analysis*. Association of Official Analytical Chemists, ArlintonVA. 16(4): 1-45.

Arancibia, A., Toledo, F., Park, Y. S., Jung, S. T., Kang, S. G., Heo, B. G., Lee, S. H., Sajewicz, M., Kowalska, T., and Gorinstein, S. 2008. Antioxidant properties of durian fruit as influenced by ripening. *LWT– Food Science Technology, 41*, 2118-2125. http://dx.doi.org/10.1016/j.lwt.2007.12.001.

Arias, L.E.H., Gómez, J. H. P. and Salazar, J. A. 2013. Application of the matrixes engineering on the development of minimally processed Hass avocado (*Persea americana* Mill) with additions of vitamin C and calcium. *9*(2), 44–54. Retrived from http://www.redalyc.org/articulo.oa?id=69525875016.

Arpornsuwan , T., and Punjanon, T. 2006. Tumor cell- selective antiproliferative effect of the *Morindacitrifolia* fruit extract. 20(6):515-517. https://doi.org/10.1002/ptr.1902.

Ashton, O. B. O., Wong, M., McGhie, T. K., Vather, R., Wang, Y., RequejoJackman, C., Ramankutty, P. and Woolf, A. B. 2006. Pigments in avocado tissue and oil. *J. Agric. Food Chem.* 54:10151–10158.

Avellone, V. D. S. G., David, B., Serena, I., Roberto, M., and Riccardo, L. B. 2016. Quantitative evaluation of the phenolic profile in fruits of six avocado (*Persea americana*) cultivars by ultra-high-performance liquid chromatography-heated electrospray-mass. *Inter J. Food. Pro.* 7: 1302-1312.

Bakar, M. F. A., Ahmad, N. E., Suleiman, M., Rahmat, A., and Isha, A. 2015. "Garcinia dulcis Fruit Extract Induced Cytotoxicity and Apoptosis in HepG2 Liver Cancer Cell Line," *BioMed Research International*. 10p. https://doi.org/10.1155/2015/916902.

Batista, C. A., Cerezal, M, P., and Funglay, V. 1993. (*persea Americana) Nutritional Composition of Avocado Pear*. pp: 63-69

Bergh, B. 1992. The Avocado and Human Nutrition. .Some Human Health Aspects of The Avocado. Proceedings of Second World Avocado Congress September 28-October 1 Gold Coast, Australia, pp 25-35.

Bes-Rastrollo, M., van Dam, R. M., Martinez-Gonzalez, M. A., Li, T. Y., Sampson, L. L., and Hu, F. B. 2008. Prospective study of dietary energy density and weight gain in women. *Am. J. Clin. Nutr.* 88(3): 769–767.

Birch, L. L., and Doub, A. E. 2014. Learning to eat: birth to age 2 y. *Am. J. Clin. Nutr.* 99 (3): 723S–728S. https://doi.org/10.3945/ajcn.113.069047.

Bohlooli, S., Jafari, N. and Jahed, S. J. 2012.Tumor cell- selective antiproliferative effect of *Morinda citrifolia* fruit. 43: 579. https://doi.org/10.1007/s12029-012-9383-4.

Bouis, H. E. 2002. "Plant breeding: A new tool for fighting micronutrient malnutrition". J. Nutr. 132(3): 491-494.

Burdon, J., Lallu, N., Haynes, G., Francis, K., Boldingh, H., Pak, H. A., and Dixon, J. G. M. 2007. Cutting preliminary studies of physiological and morphological indicators of potential poor quality in late season New Zealand 'Hass' avocados. Proceedings VI World Avocado Congress (Actas VI Congreso Mundial del Aguacate).

Caepentier, S., Knausi, M., and Suhi, M. 2009. Associations between lutein, zeaxanthin, and age-related macular degeneration: An overview. *Crit. Rev. Food Sci. Nutr.* 49: 313–326.

California Avocado Commission. 2011. California Avocado history. Available fromhttp://www.avocado.org/california-avocado-history/ Accessed November 29, 2012.

Chia, T. W. R., and Dykes, G. A. 2010. Antimicrobial activity of crude epicarp and seed extracts from mature avocado fruit (*Persea americana*) of three cultivars. *Pharma. Biol. 49*(9): 723-727.

Chong, E. W. T., Robman, L. D., Simpson, J. A., Hodge, A. M., Aung, K. Z., Dolphin, T. K., English, D. R., Giles, G. G., and Guymer, R. H. 2009. Fat consumption and its association with age-related macular degeneration. *Arch. Ophthalmol.* 127(5): 674–680.

Chong, M. F. F., Macdonald, R. and Lovegrove, J. A. 2010. Fruit polyphenols and CVD risk: A review of human intervention studies. *Br. J. Nutr.* 104: S28–S39.

Cogswell, M. E., Zhang, Z., Carrquiry, A. L., Gunn, J. P., Kuklina, E. V., Saydah, S. H., and Moshfegh, A. J. 2012. Sodium and potassium intakes among U.S. adults: NHANES 2003-2008. *Am. J. Clin. Nutr.* *96*(3), 647-657. https://www.ncbi.nlm.nih.gov/pubmed/22854410

Cutting, J. G. M., Wolstenholme, B. N., and Hardy, J. 2015. Increasing relative maturity alters the base mineral composition and phenolic concentration of avocado fruit. J. Hort. Sci. 67(6): 761-768.

Dantas, D., Pasquali, M. A., Cavalcanti-Mata, M., Duarte, M. E., and Lisboa, H. M. 2018. *Influence of spray drying conditions on the properties of avocado powder drink. Food Chemistry.* 266: 284–291. doi:10.1016/j.foodchem.2018.06.016

Darsini, D., Maheshu, V., Vishnupriya, M., Sasikumar, and Jagathala. 2012). In vitro antioxidant activity of banana (Musa spp. ABB cv. Pisang Awak). *Ind. J.Biochem.* 49:124-9.

Dembitsky, V.M., S. Poovarodom, H. Leontowicz, M. Leontowicz, S. Vearasilp, S. Trakhtenberg and S. Gorinstein. 2011. The multiple nutrition properties of some exotic fruits: Biological activity and active metabolites. *Food. Res. Int.* 44: 1671-1701.

Ding, Q. B., Ainsworth, P., Tucker, G. and Marson, H. 2005. The effect of extrusion conditions on the physicochemical properties and sensory characteristics of rice based expanded snacks. *J. Food Eng.* 66:283-289.

DiNicolantonio, J.J., Bhutani, J.,and O'Keefe, J.H. 2015. The health benefits of vitamin K. *Open Heart* **2:**e000300. doi: 10.1136/openhrt-2015-000300

15

Dinubile, N. A. 2010. A potential role for avocado-and soybean-based nutritional supplementation in the management of osteoarthritis: *A review. Phys. Sportsmen.* 38(2): 71–81.

Dreher, M. L., and Davenport, A. J. 2013. Hass Avocado Composition and Potential Health Effects. J.Food. Sci. Nutr. 53(7): 738-750. https://doi.org/10.1080/10408398.2011.556759.

Duester, K.C. 2000. Avocados a look beyond basic nutrition foroneofnature's whole foods. *Nutr. Today.* 35(4):151–157.

Dwyer, J. H., Navab, M., Dwyer, K. M., Hassan, K., Sun, P., Shircore, A., Levy, S. H., Hough. G., Wang, X., Bairey-Merz, C. N. and Fogelman, A. M. 2001. Oxygenated carotenoid lutein and progression of early atherosclerosis: The Los Angeles atherosclerosis study. *Circulation.* 103: 2922– 2927.

Erdman, J.R., Joshua, W., Smith, Matthew, J., Kuchan, Emily, S., Mohn, Elizabeth, J., Johnson, Stanislav, S., Rubakhin, Lin, W., Jonathan, V., Sweedler, and Martha, N. 2015. Lutein and Brain Function. *4*(4): 547-564. https://doi.org/10.3390/foods4040547

Ernesto, C., Ordoez, A., and Rodriguez, P. 2018. Physicochemical parameters of avocado Persea americana Mill. cv. Hass (Lauraceae) grown in Antioquia (Colombia) for export. Corpoica Cienc Tecnol Agropecuaria. *J. Drying. Tech.* 19(2): 393-402.

Ernst, E. 2003. Avocado-soybean unsaponifiables (ASU) for osteoarthritis—Asystematic review. *Clin. Rheumatol.* 22(4–5):285–288.

Etherton, P. M., Hecker, M. S., Andrea, R. D., Bonanome, M. D., Kirsten,R. D., and Hilpert, B. S. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.*113(9): 71-88.

FAO (Food and Agricultural Organization). 2011. Fao. Available *<http//www.fao.org>*Accessed [16 Aug 2012].

FAO (Food and Agricultural Organization).2014. Available *<http//www.fao.org>*Accessed[16 July 2014].

FDA (Food and Drug Administration). 2000. Potassium and the risk of high blood pressure DocketNo20001582.www.FDA.gov/Food/LabelingNutrition/LabelClaims/FDAModernizationActFDAMAClaims/ucm073606.htm. Accessed November 29, 2012.

Flagg, E. W., Coates, R. J., Jones, D. P., Byers, T. E, Greenberg, R. S., Gridley, G., McLaughlin, J. K., Blot, W. J., Haber, M., and Preston, S. 1994. Dietary glutathione intake and risk of oral and pharyngeal cancer. *Am. J. Epidemiol.* 139(5):453–465

Gabay, O., Gosset, M. and Levy, A. 2008. Stress-induced signaling path ways in hyalin chondrocytes: Inhibition by avocado-soybean unsaponifiables (ASU). *Osteoarthr. Cartilage.* 16(3): 373–384.

Grajales, A., Galindo, H. S. G., Guerrero, J. A. and Monroy-Rivera. 1999. Stability and sensory quality of spray dried avocado paste. *Drying Technology.*17(1-2): 318-326.

Haegele, A. D., Gillette, C., O'Neill, C.,Wolfe, P., Heimendinger, J., Sedlacek,S. and Thompson, H. J. 2000. Plasma xanthophyll carotenoids correlateinversely with indices of oxidative DNA damage and lipid peroxidation. *Cancer Epid. Biomarkers & Prev.* 9:421–425.

Hardisson, A., Rubio,C., and Baez, A. 2000. Comparative assessment of essential and heavy metals in fruits from different geographical origins. *J. Food. Res. Tech.* 213: 225. https://doi.org/10.1007/s002170100292

Harris, E. D., Reddy, M. C., Majumdar, S., and Cantera, M. 2003. Pretranslational control of Menkes disease gene expression. Biometals. 16: 55-61.

Hidalgo, M., Sánchez-Moreno, C. and Pascual-Teresa, S. 2010. Flavonoid-flavonoid interaction and its effect on their antioxidant activity. *Food Chem. 121*(3): 691−696. http://dx.doi.org/10.1016/j.foodchem.

Honarbakhsh, S. and Schachter, M. 2009.Vitamins and cardiovascular disease. *Br. J. Nutr.* 101:1113–1131

Hughes, K. J., Mayne, S. T., Blumberg, J. B., Ribaya-Mercardo, J. D., Johnson, E. J., and Cartmel, B. 2009. Plasma carotenoids and biomarkers of oxidative stress in patients with prior head and neck cancer. *Biomarker Insights*. 4:17–26.

Hughes, K. J., Mayne, S. T., Blumberg, J. B., Ribaya-Mercardo, J. D., Johnson, E. J., and Cartmel, B. 2009. Plasma carotenoids and biomarkers of oxidative stress in patients with prior head and neck cancer. *Biomarker Insights*. 4:17–26.

IOM (Institute of Medicine). (1997). Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. Chapter 6. Magnesium. pp. 186–255. National Academies Press, Washington, DC.

IOM (Institute of Medicine). 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. Chapter 5. Vitamin C. pp. 95–122. National Academies Press, Washington, DC.

Jacques, C., Gosset, M., Berenbaum, F., and Gabay, C. 2006. The role of IL-1 and IL-1Ra in joint inflammation and cartilage degradation. *Vitam. Horm.* 74:371–403.

Jane, C., Figueiredo, Leila, A., Mott, Edward, G., Kana, W., Bernard, C.,Matthew, G.R., Richard Logan, F., and John, A. 2011. Folic Acid and Prevention of Colorectal Adenomas: A Combined Analysis of Randomized Clinical Trials *Int. J. Cancer*. 129(1): 192–203.

Johnson, E., Maras, J. E., Rasmussen, H. M., and Tucker, K. L. 2010. Intake of lutein and zeaxanthin differ with age, sex and ethnicity. *J. Am. Diet. Assoc.* 110:1357–1362

Koller. 1992. Advances in fruit breeding. Purdue University Press, Lafayette: pp. 541-567.

Kosinska, A., Karamac, M., Estrella, I., Hernandez, T., Bartolome, B., and Dykes, G. A. 2012. Phenolic Compound Profiles and Antioxidant Capacity of Persea americana Mill. Peels and Seeds of Two Varieties. *J. Agric. Food. Chem.60* (18): 4613-4619.

Kritchevsky, D.and Chen,C. S. 2005. Phytosterols-health benefits and potential concerns: A review. *J. Nutri.* 25:413-428.

Kusakabe, H., and Kamiguchi, Y. 2004. Chromosomal integrity of freezedried mouse spermatozoa after 137Cs gamma-ray irradiation. Mutat. Res. 556: 163–168.

Laura, M., Plum, Lothar, R., and Hajo, H. 2010. The Essential Toxin: Impact of Zinc on Human. *Int. J. Environ. Res.7*(4):1342-1365. https://doi.org/10.3390/ijerph7041342

Lee, A. and Cho, S. 2015. Association between phosphorus intake and bone health in the NHANES population. *J. Nutr.*14:28.

Lee, K. T., Farid, M., and Nguang, S. K. 2006. The mathematical modelling of the rehydration characteristics of fruits. *J.Food Engi.* 72: 16–23.

Lee, S. G., Yu, M. H., Lee, S. P., and Lee. I. S. 2008. Antioxidant activities and Introduction of Apoptosis by Methanol Extraction from Avocado. J. Kor. Soc. Food. Sci. Nutr. 37(3): 296-275.http:/doi.org/10.3746/jkfn.2008.37.3.269

Levy, C. 2019. Lutein: The Antioxidant That Protects Your Eyes & Skin. *Br. J. Nutr.* 104: 28–39.

Lipinski,B. 2011. Hydroxyl Radical and Its Scavengers in Health and Disease," Oxidative Medicine and Cellular Longevity. *Nutrition.* 21:67–75.

Liu, Y., Zhao Y., and Feng, X. 2008. Exergy analysis for a freeze-drying process. Appl. Thermal Eng. 28: 675–690

Lu, Q., Zhang, Y., Wang, Y., Lee, R. P., Gao, K., Byrns, R., and Heber, D. 2009. California Hass avocado: profiling of carotenoids, tocopherol, fatty acid, and fat content during maturation and from different growing areas. *J. Agri. Food. Chem,* *57*(21). 1040810413. http://dx.doi.org/10.1021/jf901839h

Lu, Q.Y., Arteaga, J. R., Zhang, Q., Huerta, S., Go, V. L., and Heber, D. 2005. Inhibition of prostate cancer cell growth by an avocado extract: Role of lipid-soluble bioactive substances. *J. Nutr. Biochem.* **16**:23–30.

Ma, L., Lin, X. M., Zou, Z. Y., Xu, X. R., Li, Y., and Xu, R. 2009. A 12-weeklutein supplementation improves visual function in Chinese people with long term computer display light exposure. *Br. J. Nutr.* 102:186–190.

Marinova, D., Ribarova, F., and Atanassova, M. 2005. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Chem. Technol. Metallurgy*, 40(3), 255-260. Retrived from http://www.uctm.edu/journal/j2005-3/Marinova.pdf.

Marques, L. G.; Prado, M. M. and Freire, J. T. 2009. Rehydration characteristics of freeze-dried tropical fruits. L W T *Food Sci. Technol.* 42: 1232-1237.

Marulanda, A., Ruiz-Ruiz, M., and Rodriguez, M. C. 2018. Influence of spray drying process on the quality of avocado powder: a functional food with great industrial potential. *J. Drying. Tech.* 25(1): 37-48

McCann, J. C. and Ames, B. N. 2009. Vitamin K, an example of triage theory: Is micronutrient inadequacy linked to disease of aging? *Am. J. Clin. Nutr.* 90:889–907

Miller, E. R., Erlinger, T. P., Sacks, F. M., Svetkey, L. P., Charleston, J., Lin, P.H., and Appel, L. J. 2005. A dietary pattern that lowers oxidative stress increases antibodies to oxidized LDL: Results from a randomized controlled feeding study. *Atherosclerosis*. 183: 175–182.

Minh, N. P., Them, T., Trinh, N.T. L., Linh, N. T. M., Tu, C. N. C., and Tri, L. T. 2019. Several Parameters Influencing to the Production of Avocado (Persea americand) Powder. *J. Pharm. Sci. & Res.*11(2): 289-294

Morgan, D., M., L. 1998. Tetrazolium (MTT) assay for cellular viability and activity. *Methods Mol Biol*. 79: 179-84.

Morganti, P., Bruno, C., Guarneri, F., Cardillo, A., Del Ciotto, P., and Valenzano, F. 2002. Role of topical and nutritional supplement to modify the oxidative stress. *Inter J. Cosmetics Sci.* 24: 331–339.

Morris, A., Barnett, A., and Burrows, O. 2004. Food preservation. Food and Nutrition Resource Manual for the Small-scale Food Processor in the Caribbean 37(3): 7-9.

Murphy, M. J. 2018. Anticoagulant Rodenticides,Veterinary Toxicology: Basic and Clinical . *Biol.* 5(7), 12-16.

Nayak, B. S., Raju, S. S., and Chalapath, A. V. 2008. Wound healing activity of *Persea americana* (avocado) fruit: a preclinical study on rats. *J. Wound Care, 17*(3): 123-126. http://dx.doi.org/10.1155/2013/472382.

Noctor, G., Mhamdi, A., and Chaouch, S. 2011. Glutathione in plants: an integrated overview. 3(4):12-17. https://doi.org/10.1111/j.1365-3040.2011.02400.x

Olatunji, O., Coker, H., and Jaja, S. 2010. Bioactivity of the phytoconstituents of the leaves of Persea americana. *J. Med.Plant Res.* 4:24-36

Pacheco, M., R. Gomez, R. Garciglia, M. Calderon, and R. Muñoz. 2011. FOLATES AND PERSEA AMERICANA MILL. (AVOCADO). *Em. . J. Food .Agri.* 23(3): 4-13. http://www.ejfa.me/index.php/journal/article/view/1161 .

Packer, L.and Landvik, S.1989. Vitamin E: Introduction to Biochemistry and Health Benefits. https://doi.org/10.1111/j.1749-6632.1989.tb14903.x

Palombo, P., Fabrizi, G., Ruocco, V., Ruocco, E., Fluhr, J., Roberts, R., and Morganti, P. 2007. *Skin Pharmacol. Physiol.* 20(4):199–210.

Pieterse, Z., Jerling, J. C. and Oosthuizen, W. 2005. Substitution of high monounsaturated fatty acid avocado for mixed dietary fats during an energy restricted diet: Effects on weight loss, serum lipids, fibrinogen, and vascular function. *Nutrition*. 21:67–75.

Piironen, V., Tovino, J., Pimia, P. and Lampi, M. A. 2003. Plant sterols in vegetables, fruits and berries. *J. Sci. Food. Agric.* 83(4):330 – 337

Prieto, P., Pineda, M., and Anguilar, M. Anal biochem. 1999. 267:339.

Rainey, C., Affleck, M., Bretschger, K., and Roslyn, A.S. 1994. The California avocado: A new look. *Nutr. Today*. 29:23–27.

Ribeiro, S.,M.,R. Barbosa, L., Claudio, Queiroz, J., and Schieber, A. 2008. Phenolic compounds and antioxidant capacity of Brazilian mango (Mangifera indica L.) varieties. *J. Food Chemi*. 110:620-626. 10.1016/j.foodchem.2008.02.067.

Richard, L. and Nelson, M.D.2009. Iron and Colorectal Cancer Risk: Human Studies. *J. Nutr.* 5(59): 140-148. https://doi.org/10.1111/j.1753-4887.2001.tb07002.x

Roberta, R. E., Nicoletta, P., Anna, P., Ananth, P., Min, Y., and Catherine, R. E. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radical. 26(10):1231-1237.

Roberts, R. L., Green, J., and Lewis, B. 2009. Lutein and zeaxanthin in eye and skin health. *Clin. Dermatol.* 27: 195–201.

Sacks, F. M., Bray, G. A., Carey, V. J., Smith, S. R., Ryan, D. H., Anton, S. D., McManus, K., Champagne, C. M., Bishop, L. M., Laranjo, S. D., Leboff, M. S., Rood, J. C., de Jonge, L., Greenway, F. L., Loria, C. M., Obaranek, E., and Williamson, D. A. 2009. Comparison of weight-loss diets with different compositions of fat, protein and carbohydrate. *N. Engl. J. Med.* 360(9): 859–873.

Salonen, R. M., Nyyssonen, K., Kaikkonen, J., Porkkala-Saratabo, E., Voutilainen, S., and Rissanen, T. H. 2003. Six-*year effect of combined vitamin C and E supplementation on atherosclerotic progression. Circulation*. 107: 947–953.

Santana, I., dosReis, L. M., Torres, A. G., Cabral, L and Freitas, S. P. 2015. Avocado (Perseaamericana Mill.) oil produced by microwave drying and expeller pressing exhibits low acidity and high oxidative stability. *Eur. J. Lipid Sci. Technol.* 117: 999-1007.

Saquib, N., Natarajan, L., Rock, C. L., Flatt,S. W., Madlensky, L., Kealey, S. and Pierce, J. P. 2008. The impact of a long-term reduction in dietary energy density on body weight within arandomized diet trial. *Nutr. Cancer.* 60(1):31–38.

Saucedo, C. C., Miramontes, E. H. V., Campos, E. T., Alvarado, A. D., Garcia, A. C. B., Ramirez, M, R. R., and Anaga, J. P. R. 2014. Effect of freeze drying and production. J. Nutr. 3(4): 12-23.

Savage, J. S., Marini, M. and Birch, L. L. 2008. Dietary energy density predicts women's weight change over 6 y. *Am. J. Clin. Nutr.* 88(3): 677–684.

Schmidinger, M., Budinsky, A.C., Wenzel, C., Piribauer, M ., Brix, R ., Kautzky, M ., Oder. W., Locker, G.J., Zielinski, C.C., and Steger, G.G. 2000. Glutathione in the prevention of cisplatin induced toxicities. A prospectively randomized pilot trial in patients with head and neck cancer and non small cell lung cancer. *J. Integr Med.* 112(14):617-623.

Shofian, N. M., Hamid, A. A., Osman, A., Saari, N., Anwar, F., Dek, M. S. P., and Hairuddin, M. R. 2011. Effect of Freeze-Drying on the Antioxidant Compounds and Antioxidant Activity of Selected Tropical Fruits. *Int. J Mol Sci.* 12(7): 4678–4692.

Shyam, S., and Sablani. 2006. Drying of Fruits and Vegetables: Retention of Nutritional/Functional Quality. *Inter. J. Drying Technol.* 24 (2):123-135.

Smirnoff, N., and Cumbes, Q., J. 1989. Hydroxyl radical scavenging activity of compatible solutes. *Phytochem.* 28(4):57-60.

Souza, D. S., Marques, L. G., Gomes, E. D. B., and Narain, N. 2015. Lyophilization of Avocado (*Persea americana* Mill.): Effect of Freezing and Lyophilization Pressure on Antioxidant Activity, Texture, and Browning of Pulp *.J. Drying.Tech.*33(2):194-204. DOI: 10.1080/07373937.2014.943766

Sreevasthava, P. S. and Kumar, S. 1994. *Fruit and Vegetable preservation.* (3rd ed). International book distributing company, India, 114p

Tango, S. T., carvalho, C. R. L., and Soares, N. B. 2004. Physical and chemical characterization of avocado fruits aiming its potential for oil extraction. Revista Brasileira de Fruticultura. Pp. 26.

Tentolouris, N., Arapostathi, C., Perrea, D., Kyriaki, D., Revenas, C., and Katsilambros, N. 2008. Differential effects of two isoenergetic meals rich in saturated or monounsaturated fat on endothelial function in subjects with type 2 diabetes. *Diabetes Care*. 31: 2276–2278

Tentolouris, N., Arapostathi, C., Perrea, D., Kyriaki, D., Revenas, C., and Katsilambros, N. 2008. Differential effects of two isoenergetic meals rich in saturated or monounsaturated fat on endothelial function in subjects with type 2 diabetes. *Diabetes Care*. 31: 2276–2278.

Thimmiah, S. K. 1999. Standard Methods of Biochemical analysis. Kalyani Publishers, New Delhi, 545p.

Thomson, C. A., Stendell, N. R., Rock, C. L., Cussier, E. C., Flatt, S. W., and Pierce, J. P. 2007. Plasma and dietary carotenoids are associated with reduced oxidative stress in women previously treated for breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 16 (10):2008–2015.

Traber, M. G., Packe, L. 1995. Vitamin E: beyond antioxidant function. *Am. J. Clin. Nutr.* 62 (6): 1501S–1509S.

Tripathi, P. C. and Karunakaran, G. 2014. Bharat Mai Navaneet Phal (Avocado) Ki Kheti:Varthaman sthithi evam Sambhavanaye (InHindi) (Avocado cultivation in India: Present status and possibilities) Bhumi Nirman (Bhopal) 16th Jan -15th Feb : 11

Unlu, N. Z., Bohn, T., Clinton, S.K., and Schwartz, S. J. 2005. Carotenoid absorption from salad and salsa by humans is enhanced by the addition of avocado or avocado oil. *J. Nutr. 135*: 431–436.

USDA (U.S. Department of Agriculture). 2011. Avocado, almond, pistachio and walnut Composition. Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 24. U.S. Department of Agriculture. Washington, DC.

USDA and HHS. 2011. Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans (Part B. Section 2: The Total Diet. B2:11). U.S. Department of Agriculture and U.S. Department of Health and Human Services. Washington, DC

Wang,M., Carol, T. K., and Lovatt, J.2016. Developmental differences in antioxidant compounds and systems in normal and small-phenotype fruit of 'Hass' avocado (*Persea americana* Mill.). *Scientia Horticulturae*. 206: 15-23.

Whiley, A. 2000, "Avocado Production in Australia" Food and Agriculture Organization of the United Nations. http://www.fao.org

Wien, M., Haddad, E. and Sabate, J. 2011. Effect of incorporating avocado in meals on satiety in healthy overweight adults. 11th European Nutrition Conference of the Federation of the European Nutrition Societies. October, 27. Madrid, Spain.

Williams, R.J. 1978.New Trends in Bioinorganic Chemistry. Academic Press, New York. 1978: 253-260.

Woodside, J.V., McGrath, A.J., Lyner, N., and McKinley, M.,C. Carotenoids and health in older people. 2015. 80(1):63-8 . https://doi.org/10.1016/j.maturitas.2014.10.012

Wright, C.I. 2007. Herbal medicines as diuretics: A review of the scientific evidence. *J. Ethnopharmacol*. Accessed: [22 Dec. 2015]. v.114: pp.1- 31.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., and Prior, R. L. 2004. Lipophilic and hydrophilic antioxidant capacity of common foods in the U.S. *J. Agric. Food Chem.* 52:4026–4037.

Xu, L. Z., Weber, I. T., Harrison, R. W., Gidh-Jain, M., and Pilkis, S. 1995. *J. Biochem.* 34: 6083-6092

Yao, L.H., Jiang, Ming, Y., Chen, and Shuang. 2004. Flavonoids in Food and Their Health Benefits. *Plant Food For Human Nutr.* (Dordrecht, Netherlands). 59: 13-22.

Yasir, M., Das, S., and Kharya, M. D. 2010. The phytochemical and pharmacological profile of *Persea Americana* Mill., *Pharmacogn Rev.* 4: 77.

ABSTRACT

PROFILING BIOACTIVE COMPOUNDS IN AVOCADO (*Persea americana* Mill.) CULTIVARS AND FREEZE DRIED FRUIT POWDERS, AND INVESTIGATING ITS POTENTIAL IN CANCER CELL LINES

by

Archana L

(2017-16-009)

Abstract of the Thesis

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN COMMUNITY SCIENCE

(Food Science and Nutrition)

Faculty of Agriculture

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695 522

KERALA, INDIA

2019

ABSTRACT

The research work entitled "Profiling bioactive compounds in avocado (*Persea americana* Mill.) cultivars and freeze dried fruit powders, and investigating its potential in cancer cell lines." was conducted at College Of Agriculture, Vellayani during 2017-2019, with the objectives to ascertain bioactive compounds in fresh fruits and freeze dried powders of avocado (*Persea americana* Mill.) cultivars and to investigate anti proliferation activities of freeze dried powder in cancer cell lines. Four cultivars selected for the study were Pollock, Kallar Round, Purple Hybrid and Fuerte.

Quantitative estimation of phytochemicals showed that, glutathione content of fresh avocado fruits was in the range of 15.3mg/100g-18.3 mg/100g and was found to be higher in the cultivar Pollock (18.33mg/100g) and which was on par with Fuerte (18.13mg/100g). Folic acid, vitamin E, total carotenoids and total phenol content were observed in fresh fruits of avocado cultivars to be in the range of 79.30mg/100g-80.33mg/100g, 2.04 -2.52mg/100g, 2.55/100g-2.94mg/100g, and 12.24mg/100g -13.84mg/100g respectively. The Cultivar Pollock recorded highest content of folic acid (18.33mg/100g), vitamin E (2.52mg/100g), total carotenoids (2.94mg/100g) and total phenol (13.84mg/100g).Vitamin K and total phytosterols present in the four cultivars were in the range of 0.013mg/100g-0.019mg/100g and 62.30mg/100g-68.60mg/100g respectively. Lutein content was found to be more in the cultivar Fuerte (0.206mg/100g). Purple Hybrid was found to be superior in the case of total flavonoid content (21.07mg/100g).

Mineral analysis of fresh fruits of avocado cultivars revealed that iron content was found to be higher in Fuerte (0.403mg/100g). Calcium, sodium, potassium, magnesium, zinc and selenium contents were found to be more in Pollock (9.3mg/100g, 6.12mg/100g, 394.47 mg/100g, 22.20mg/100g, 0.473mg/100g and 0.500µg/100g respectively). The cultivars Pollock and Fuerte contained the same

amount of copper (0.146mg). Phosphorus content was found to be almost the same in all the four cultivars noted in the range of 32.13/100g-34.90mg/100g.

In the case of total antioxidant activity in fresh avocado cultivars, Pollock was found to be superior (234.50mg/100g) and was found to have no significant difference with Purple hybrid (230.66mg). The findings revealed that the cultivar Purple Hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 34.03µg/ml. Higher Hydroxyl radical scavenging activity was noted in the in the cultivar Kallar Round (27.03µg/ml) and the lowest Hydroxyl radical scavenging activity was observed in the cultivar Pollock (38.86µg/ml). Super oxide radical scavenging activity (IC_{50}) was observed to be higher in the cultivar Kallar Round (12.12µg/ml).

Estimation of phytochemicals in freeze dried powders showed more glutathione content in Pollock (45.50mg/100g). Folic acid, vitamin K and lutein content in freeze dried form of the four avocado cultivars ranged from 180.70mg/100g-197.90mg/100g, 0.179mg/100g-0.185mg, and 0.423mg/100g-0.503mg/100g respectively. Total phytosterols was found to be more in Kallar Round (173.80mg/100g) and Total flavonoid content was found higher in Pollock (45.27mg/100g) and Purple Hybrid (45mg/100g).The carotenoid content was observed higher in the cultivar Pollock (8.15mg/100g) and which was found to be on par with Purple Hybrid (8.07mg/100g).

Mineral estimation in the freeze dried form of avocados highlighted the iron content to be higher in Fuerte (2.87mg/100g). Calcium content was in the range of 104mg/100g-142.5mg/100g and was found to be higher for Purple Hybrid and lower for Pollock. Sodium content was found to be higher in Purple Hybrid (14.40mg/100g). Phosphorus and Magnesium contents were found to be more in Pollock (76.07mg/100g and 42.13mg/100g respectively). Potassium content was found to be superior in Purple Hybrid (623mg/100g) and was on par with Pollock

(622.76mg/100g) and Kallar Round (618.76mg/100g). Zinc content was found to be higher in the cultivar Kallar Round (4.66mg/100g) and less in Fuerte (2.11mg/100g). Copper and selenium contents were found to be almost same in all the four cultivars and in the range of 3mg/100g-3.23mg/100g and 8.07µg/100g-8.90µg/100g respectively.

Total antioxidant activity was observed to be superior in the freeze dried powder of the cultivar Pollock (461.90mg/100g) and possessed no significant difference with Purple hybrid (460.33mg/100g). The findings revealed that the cultivar Purple hybrid had the highest DPPH radical scavenging activity with an IC_{50} value of 90.80µg/ml. Regarding the IC_{50} values for hydroxyl radical scavenging activity of freeze dried avocado cultivars, highest activity was noted in the freeze dried powder of Fuerte (69.25µg/ml). Lowest activity was observed in the freeze dried powder of Pollock (86.60µg/ml). Super oxide Radical Scavenging Activity (IC_{50}) was observed higher in the freeze dried powder of Fuerte (24.47µg/ml).

Based on the assessment of phytochemicals, minerals and antioxidant activities in fresh and freeze dried avocado powder, Pollock was found to be the superior cultivar. Hence freeze dried fruit powder of the cultivar Pollock was selected for cell line study. Cytotoxicity of freeze dried powder of Pollock was estimated by MTT assay on Human colon cancer cells (HCT 116). Assay was done with different concentrations of avocado extract (10µg, 20µg, 50µg, 100µg, and 200µg). Growth inhibition noted were, 27 %, 30 %, 33%, 45 %, and 71 % for 10 µg, 20 µg, 50 µg, 100 µg and 200 µg respectively. Percentage of viable cell decreased with increase in extract concentration while percentage cytotoxicity increased with increase in concentration of avocado extract. 50 % growth inhibition (GI_{50}) of cancer cells were shown at 120µg/ml of fruit extract. 29 percent of cells were found to be viable after exposure of extract (200µg).

Recordings of the present study revealed that avocado fruit contains many bioactive components such as glutathione, folic Acid, vitamin K, vitamin E, lutein, carotenoids, phenols, phytosterols, and flavonoids which possessed potent antioxidant activities. Among the fresh and freeze dried form of avocado cultivars, Pollock was found to be superior and had noticeable anti-carcinogenic properties

Findings of the present study add to the growing evidence, supporting the promising role of avocado fruit as an anti-cancer agent. Therefore, avocado fruit may represent a valuable therapeutic fruit for the treatment of cancer.