

**ANTIOXIDANT ANTICANCER POTENTIALS AND SHELF LIFE OF A FUNCTIONAL
READY TO EAT (RTE) MIX**

By

NALLALA AMRUTHA VARSHINI

(16PFN010)

**THESIS SUBMITTED TO
AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND
HIGHER EDUCATION FOR WOMEN
COIMBATORE- 641 043.**

***In Partial Fulfilment of the Requirement for the Degree of*
MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION**

April, 2018

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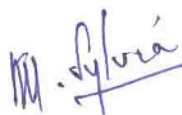
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CERTIFIED AS BONAFIDE RESEARCH WORK



Signature of the Supervisor



Signature of the

Head of the Department

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I.INTRODUCTION

A “Ready-To-Eat” food product is defined as any food product which does not require any elaborate processing procedures on the part of consumer before it is good enough for consumption. It is ready-to-eat as soon as the pack is opened in a form, which is tasty and appetizing. (Collins, 2011)

In India, a variety of chutneys and pickles in large volumes based on vegetables, pulses and spices are consumed along with rice and other breakfast items like chapatti, idly, dosa, and vada. Flaxseed (*Linum usitatissimum* L.) belongs to the family of Linaceae and has recently gained good attention as a functional food due to the presence of omega 3- fatty acid and fibre. The product to be developed is Functional Ready To Eat (FRTE) Mix with Flax seeds, *Annona Squamosa* leaves, and Garlic pods is a functional food adjunct powder which can be consumed along with Dosa, Idli, Rice etc. and can be stored several days.

Flaxseed is mainly considered as oilseed crop. Moreover, the other nutritional parameters than its oil content, make it more favorable choice for food technologist to develop functional foods. Flaxseed contains good amount of α -Linolenic Acid (ALA), a omega-3 fatty acid, protein, dietary fiber, lignan, specifically Secoisolariciresinol diglucoside (SDG). Flaxseeds are consider to be a good source of Alpha linolenic acid (ALA) which is an n-3 polyunsaturated fatty acid has a positive effect on heart health by preventing heart problems like arrhythmias and controlling cholesterol levels in blood vessels of heart and reduces inflammation in arteries and helps in reducing tumor growth and helps in maintaining hormonal balance in the body. Flaxseed is mainly considered as oilseed crop. Moreover, the other nutritional parameters than its oil content, make it more favorable choice for food technologist to develop functional foods. Flaxseed contains good amount of α -Linolenic Acid (ALA), a omega-3 fatty acid, protein, dietary fiber, lignan, specifically Secoisolariciresinol diglucoside (SDG) (Ganorkar 2015).

Flaxseed has greatest quantity of soluble fibre which forms a gel in the presence of water, and this components include gums, pectins and sugars which form mucilage

substances and helps in reducing inflammation and protect the lining of the GI tract. It is a good source of mucilage gum content, a gel-forming fiber that is water-soluble and therefore moves through the gastrointestinal tract undigested. Mucilage from flaxseeds helps food to empty quickly into the small intestine, which can increase nutrient absorption and makes one to feel fuller because the fiber found in flaxseed is not able to be broken down in the digestive tract and stays for long time. Flaxseed dietary fibre helps in preventing constipation by increasing bulk of stool and flush waste from the GI tract due to its gel-like mucilage by maintaining normal bowel movements and promoting and preventing GI tract problems like indigestion, pain during defecation, bloating, flatulence etc. It also helps in maintaining beneficial gut flora even in people with “normal” digestive systems. The soluble fiber content of flaxseed helps in trapping fat and cholesterol in the digestive system and makes it unable to absorb and also traps bile, which is made from cholesterol in the gallbladder, bile is then excreted through the digestive system, forcing the body to make more, using up excess cholesterol in the blood and therefore lowering cholesterol (Williams *et al.*, 2014).

Using flax naturally helps in replacing gluten-containing grains in recipes. Grains, especially those containing gluten are hard to digest, but flax is usually easily metabolized and also anti-inflammatory and reduces inflammation in stomach. Flaxseed protein is effective in lowering plasma cholesterol and triglycerides (TAG) compared to soy protein and casein protein. Flaxseeds are gluten free and have low glycemic effect in the body. One of the greatest benefits of flaxseed is that it contains antioxidants, specifically lignans that are unique fiber-related polyphenols. Lignans are antioxidants that help in reducing free radical damage therefore flax have anti-aging, hormonal-balancing and cellular-regenerating effects. Lignans are considered natural “phytoestrogens,” or plant nutrients that work similarly to the hormone estrogen. Phytoestrogens in flaxseed helps in altering estrogen metabolism, causing either an increase or decrease in estrogen activity depending hormonal status (Oomah *et al.*,2015).

Medicinal plants are widely utilized in traditional medicine. Garlic is among the most important of these plants. For this purpose, garlic has been extensively used

worldwide for centuries. Garlic is reported to be a wonderful medicinal plant, garlic has It contains an abundance of chemical compounds that have been shown to possess beneficial effects to protect against several diseases such as cardiovascular diseases, regulating blood pressure, lowering blood sugar and cholesterol levels, effective against bacterial, viral, fungal, and parasitic infections, enhancing the immune system and having antitumoral and antioxidant features. Garlic has direct influence in preventing atherosclerotic lesions on the artery walls. (Akter *et al.*, 2016).

Garlic helps in boosting the immunity, contains high levels of vitamin C, E, A which help to prevent damage from oxidative agents and free radicals and other vitamins such as B1 and B2, beta-carotene and identified as anticancer food. Potassium in garlic helps in absorbing many essential nutrients, and help avoid digestive problems and fatigue as well, it can also helps in preventing lung, throat problems and prevent inflammation in throat and relieves from pain. Garlic has anti-inflammatory compounds can also benefit musculoskeletal system and respiratory system. Garlic had detoxifying effect and helps in cleansing the toxins from our body. Allicin and selenium are present in garlic that play a integral role in protecting liver and other vital organs from harmful metals and toxins. It contains sulfur compounds (allicin, alliin and a gene), volatile oils, enzymes (allinase, peroxidase and miracynase), carbohydrates (sucrose and glucose), minerals (selenium), amino acids such as cysteine, glutamine, isoleucine and methionine which help to protect cells from the harms of free radicals (Fallahi *et al.*, 2016).

The use of garlic and its preparations includes being used as agents for prevention and treatment of cardiovascular diseases. Consumption of garlic could significantly lower blood pressure, prevent atherosclerosis, reduce serum cholesterol and triglyceride, inhibit platelet aggregation, as well as increase fibrinolytic activity. Dietary inclusion of garlic powder has been reported to protect against gentamycin-induced hepatotoxicity, improve antioxidant status and modulate oxidative stress. Extracts from garlic reduce lipid peroxidation and enhance antioxidant defense system. Allicin present in garlic has therapeutic benefits which help in boosting immunity, maintaining normal blood pressure, fights against cold and flu, lowers cholesterol,

prevent heart diseases, rich in antioxidants and helps in preventing cancer and also helps in curing asthma (Bamidele, 2012).

From ancient times onwards, plants have been one of the essential sources of medicines. It has been quite interest in plant based medicines, health care products, pharmaceuticals, nutrient supplements, and so forth. *Annona squamosa* commonly called as custard apple, is an edible tropical fruit belongs to the family Annonaceae, is said to show varied medicinal effects, including insecticide, anti-tumor, anti-oxidative properties (Vanitha, 2014)

Annona squamosa leaves has typical compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives which reduces oxidative stress and formation of free radicals. Flavonoids, tocals, phospholipids, amino acids and peptides, phytic acid, ascorbic acid, pigments, and sterols. Phenolic antioxidants are primary antioxidants which act as free-radical terminators. The leaves are traditionally used for the treatment of epilepsy is a group of neurological disorders characterized by epileptic seizures is prevented by phenolic compounds in *Annona squamosa* leaves. The plant is attributed with medicinal properties which include antifertility, anti-tumor and antimalarial activities. Annonaceous acetogenins were isolated from Annonaceae plants which exhibited a broad range of biological bioactivities such as cytotoxic, antitumoral, antiparasitic, pesticidal and immunosuppressive activities Yang *et al.*, (2014).

The antioxidants in *Annona squamosa* leaves prevents oxidative damage to cells and also prevents damage from harmful chemicals like hydrogen peroxide which causes cytotoxicity and tissue damage in various diseases is becoming increasingly recognized the antioxidants constituents of plant materials are very important for the maintenance of health and protection against many diseases (e.g. cancer) and raising interest among scientists, food manufacturers and consumers as the trend for the future is toward functional food with specific health effects (Roesler *et al.* 2015).

With this background, the present study was aimed out in formulation of Functional Ready To Eat (FRTE) Mix with the following objectives.

- Prepare a Functional Ready To Eat(FRTE) mix with Flax seeds and Garlic, *Annona squamosa* leaves,
- Evaluate the most acceptable combination through Organoleptic analysis.
- Standardise the acceptable combination of functional food mix powder.
- Assess the antioxidant potentials.
- Determine the anticancer potential on cell line.
- Analyse the shelf life of Functional Ready To Eat (FRTE) mix.

II. REVIEW OF LITERATURE

The present study entitles “**Antioxidant Anticancer Potentials and Shelf life of a Functional Ready To Eat Mix**” has been reviewed under the following headings:

A. Flaxseeds (*Linum usitatissimum*) - A Nutritious Food

B. Flaxseeds in Human Health.

C. Defensive Potentials of *Allium sativum*.

D. *Annona squamosa* Leaves - Source of Antioxidants.

A. FLAXSEEDS – A NUTRITIOUS FOOD

Scientific classification

Kingdom: Plantae

Clade: Angiosperms

Order : Malpighiales

Family : Linaceae

Genus : *Linum*

Species: *L. usitatissimum*

Botanical name: *Linum usitatissimum*

Synonyms: *Linum crepitans*

Hall *et al.*, (2006) studied that traditionally, flaxseed (*Linum usitatissimum*) has been grown for its oil. Flaxseed has been playing a major role in the field of diet and disease research due to its potential health benefits associated with high content of α -Linolenic acid about 57 percent, which is an essential omega-3-fatty acid and also because of a major lignan, namely secoisolariciresinoldiglucoside (SDG). Flaxseed is an oilseed that contains about 38–45 percent oil. ALA content in flax oil is 55-60 percent

and lignan content in flaxseed is up to 13 mg/g flaxseed and he also stated that the lignan SDG is converted by bacteria in the colon of humans and other animals to enterodiol and enterolactone, referred to as mammalian lignans.

Goyal *et al.*, (2013) reported that flax seed is often taken as whole or crushed and the powder is incorporated into food products like breads, muffins, juices, milk, dry pasta, dairy and meat products.

Carter (2011) reported that the texture of flaxseed is crisp and chewy possessing a pleasant nutty taste. Beyond its oilseed crop ability, proximate composition of flaxseed makes it more promising for its utilization in different food products. Flaxseed is one of the richest vegetarian source of α -Linolenic acid (omega 3 fatty acid) and soluble mucilage. In present era, consumer's trend towards functional food has increased significantly as health awareness rose.

1. Source of ALA (Omega-3 fatty acid)

Hurteau (2014) described flaxseeds as a source of omega fats: omega-3 and omega-6 fatty acids. Linolenic acid, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) are three types of omega-3 fatty acids and are nutritionally important. All three fatty acids have been shown to reduce the risk of cardiovascular disease. Flax contains a mixture of fatty acids. It is rich in polyunsaturated fatty acids, particularly ALA, the essential omega-3 fatty acid, and linoleic acid (LA), the essential omega-6 fatty acid. These two polyunsaturated fatty acids are essential for humans – that are present in flaxseeds.

According to Villalobos *et al.* (2009) Addition of flaxseed into processed products is on the rise to extract the beneficial aspects of high α -Linolenic acid content and the lignans present. Incorporation of flaxseed 10, 15, and 20percent to corn tortillas exhibited a high amount of total unsaturated fatty acids between 26.3 and 30.1percent.

Terhi *et al.* (2010) report that addition of flaxseed and flaxseed oil in bread making retains moisture and softness effectively than in control during six days storage at room temperature. Substitution of Flaxseed at 30–50percent for flour greatly

enhanced the nutritional qualities of some nutrients without affecting the overall acceptability of bakery products.

El-Kady (2010) stated that biological value of protein was similar to those of soybean protein. Flaxseed protein was effective in lowering plasma cholesterol and triglycerides compared to soy protein and casein protein

Morris (2010) stated that flaxseeds are gluten-free and can be fearlessly consumed by people with gluten sensitivity.

2. Source of dietary fiber (Mucilage or Gum)

Eastwood (2009) states that dietary fiber is a communal word used to describe a variety of plant substances that are not easily digested by the enzymes responsible for digestion in humans. Morris (2010) reported that flaxseed is a rich source of dietary fiber (accounting 28 percent), both soluble as well as insoluble fibers. High amount of dietary fiber adds bulk to waste products in the gut and increases bile movement in the gastrointestinal movement. It exhibits natural laxative effect of dietary fiber. Flaxseed mucilage associated with hull of flaxseed is a gum like material composed of acidic and neutral polysaccharides. Low glycemic index foods containing soluble fiber not only prevent certain metabolic ramifications of insulin resistance but also reduce insulin resistance. Soluble fiber and other components of flaxseed fraction could potentially affect insulin secretion and its mechanism of action in maintaining plasma glucose homeostasis. Flaxseed was shown to reduce the post prandial blood glucose response in humans

Wang *et al.*, (2010) report that the lignans of flaxseed are phytoestrogens and serve as precursors in the production of mammalian lignans. Flaxseed lignans are converted to mammalian lignans enterolactone and enterodiol by intestinal flora and give protection against many diseases. Kitts *et al.* (2015) reported that enterolactone and enterodiol had greater antioxidant activity, suggesting that the metabolites might be the reason for the health benefits of plant lignans. Prasad (2005) suggested that lignan may act to prevent oxygen radical production, thus effectively reducing atherosclerosis.

Lignans have antioxidant activity and thus may contribute to the anticancer activity of flaxseed. He also suggests that flaxseed lignans may be used as natural antioxidants.

Roseling (2012) states that the recommend daily intake of about 1–2 table spoons, approximately 5–10 mg of hydrogen cyanide is released from flaxseed, which is well below the estimated acute toxic dose for an adult of 50–60 mg inorganic cyanide and below the 30–100 mg/ day humans can detoxify and he also reported that roasting of flaxseed for 5-7 minutes can eliminate the cyanogenic glycosides and also studied that roasting also results in the formation of melanoidins due to Maillard's reaction, that are known to possess pronounced antioxidant properties, thereby improving the oxidative stability of nuts and seeds

B. FLAXSEEDS IN HUMAN HEALTH

Youn *et al.*, (2014) studied that flaxseed and its products have laxative, antidiabetic, cholesterol lowering, antimalarial, antioxidant, anti-arrhythmic, anti-inflammatory, hepatoprotective, anticancer actions and others and also reported that lignans seem to reduce the excess testosterone which plays a key role in the pathogenesis of PCOS. As hyperandrogenemia is an important contributory factor for PCOS, any compound that can reduce androgens will be useful in PCOS.

Shakir (2007) studied that γ -Glutamyl transpeptidase levels are increased abnormally high during inflammation, in alcoholics with abscess and degenerative disease conditions including tumerogenesis. Flaxseed chutney diet showed significant reduction in both γ -glutamyl transpeptidase level (52%) and micro-nuclei formation (47%) in rats.

Oomah (2011) studied the physicochemical characteristics and antioxidant properties of Yield of oil ranged from 9-28% depending on extraction method. It was reported that the crude lipid contained 92.5% neutral lipids, 3.1% phospholipids, 2.4% acidic lipids and 2.1% free fatty acids. Oil extracted from flaxseed hull using supercritical CO₂ showed highest antioxidant capacity and the resultant defatted meal contained the highest (53 mg/g) secoisolariciresinol diglucoside which is an antioxidant phytochemical.

Prasad *et al.*, (2011) studied the antiatherogenic activity of lignans present in flaxseed from the reduction in hypercholesterolemic atherosclerosis due to a decrease in serum total cholesterol and LDL-cholesterol. Vitamin E-deficient diets containing 5 to 20% ground flaxseed or 2.75% ethyl linolenate protected mice against the malaria parasite *Plasmodium yoelii* as shown by decreased parasitemia and enhanced survival.

According to Anand *et al.*, (2011) flax seeds are considered to be extremely beneficial for different types of ailments. Used internally, the seeds are very useful in treatment of constipation and elimination of secretions in the respiratory tract. They are helpful in cases of bronchitis and colds. Daily use of flax oil protects gastric and urinary membrane. It is also said that Flax seeds can act as a helpful prevention against angina pectoris and arteriosclerosis. They can also be helpful in reducing the cholesterol levels. Taken externally, the oil obtained from the seeds is recognized due to its demulcent and emollient properties. It protects and softens the irritated skin.

Christensen *et al.*, (2009) suggests that flaxseeds has a possible antiarrhythmic effect of ALA and omega-3 fatty acids and he also found that antiarrhythmic effects were concentration-dependently enhanced by DHA and EPA, but not by ALA. Higher intake of dietary Linolenic acid might be associated with a reduced risk of abnormally prolonged repolarization in men and women.

C. DEFENSIVE POTENTIALS OF *ALLIUM SATIVUM*

Scientific classification

Kingdom:	Plantae
Clade:	Angiosperms
Order :	Asparagales
Family :	Amaryllidaceae
Subfamily:	Allioideae
Genus :	<i>Allium</i>

Species: *A. sativum*

Botanical name: *Allium sativum*

Synonyms: *Allium arenarium*

Thejasenuo *et al.*, (2017) report that garlic is an important and oldest plant, Garlic (*Allium sativum* L.), cultivated since time immemorial for its use as food and medicine, has many important constituents which requires attention for its many health benefits. It contains sulfur compounds which are responsible for garlic's medicinal effects and pungent odour. Out of the medicinal effects of garlic, one of the main factor or importance of garlic is its potentialities in the treatment of cancer or prevention in cancer development and he also stated that it contains an important organo sulfur compounds which have been attributed in its impact on inhibition of tumour growth, antioxidant properties.

Yadav *et al.*, (2016) state that garlic is a bulbous perennial herb, Garlic contains at least 33 Sulfur compounds, several enzymes (allinase, peroxidase, myrosinase and other), 17 amino acids and minerals such as selenium. Garlic medicinal uses include Anti-atherosclerotic, Anticoagulant, Anti-cancer, Antioxidant, Antibiotic, Anti-parasitic, Anti-inflammatory, Antihypertensive and in Glucose control.

Sanjay *et al.*, (2017) report that *Allium sativum* L. has three important sulphur compounds Allicin, Ajoene (4, 5, 9-trithiadodeca-1, 6, 11-triene-9-oxide) and Alliin. Besides, these sulphur compounds some other chemical compounds such as α -phellandrene, β -phellandrene, citral, linalool, and geraniol are also found so garlic are used in medical biology to prevent and treat cardiovascular diseases by lowering blood pressure and cholesterol, antimicrobial agent, and preventive agent for cancer.

Tsipila *et al.*, (2017) report that Garlic's medicinal effects mainly appear to come from sulfur containing compounds, high trace mineral content, and enzymes. It has synergistic biological effects that either prevents or fight against cancer. Garlic's chemo-preventive activity has been credited to its ability to regulate metabolising enzymes that either activate (cytochrome P450s) or detoxify (glutathione S-transferases) carcinogens.

He also stated that Allicin, being chemically unstable, when cloves are crushed it degrades and arranges to form allyl sulfides and polysulfides which is termed garlic organosulfides have chemo-preventive activities.

Ejaz (2013) conducted experiments on chemo- preventive activity which he accredited to organosulfur compounds, affects on tumour growth inhibition, drug metabolizing enzymes and antioxidant properties. Major compounds like S-allylmercapto-L-cysteine and S-allylcysteine in aged garlic extract were observed to exhibit radical scavenging activity. Some organosulfur compounds, including S-allylcysteine, were also observed to hinder the growth of chemically induced and transplantable tumours in several animal models.

Bhandar (2012) state the common side effect of garlic ingestion includes body odour and bad breath. Excess consumption causes flatulence and gastrointestinal problems. Allergic Dermatitis and blisters have also been reported on the application of garlic. Rarely usual amount of garlic consumption causes health problems but it is considered safe to consume one or two cloves of garlic per day. High dose of garlic consumption is cautioned before surgeries because of its association with prolong bleeding and also spinal epidural hematoma. Due to garlic's antithrombotic properties patients taking anticoagulants are also cautioned.

Amagase (2012) reported that in some rare cases garlic bulb when eaten on empty stomach can cause, diarrhoea, vomiting, and heartburn. Prescribed medicines such as HIV drug saquinavir (Fortovase and Invirase) have also shown to be interfered by garlic. Serum levels are lowered to about 50%. Excess use of garlic for people with stomach ulcers is not recommended. Also people about to undergo surgery, pregnant women and persons using warfarin (Coumadin) are advised not to consume garlic. Bulbs of garlic are sometimes contaminated with a bacterium called *Clostridium botulinum* which can cause toxin in oil goods. Other problems related to garlic occurs when applied to skin such as chemical burns, contact dermatitis etc.

Irshad (2017) conducted a study on effect of *Allium sativum* essential oil on serum triglycerides, total cholesterol, HDLC and LDLC, he stated that *Allium sativum*

protects against coronary artery disease (CAD) through amelioration of LDLc and HDLc cholesterol. An increase in HDLc protects against the CAD by preventing atherosclerosis. ASEO improves the blood lipoproteins with a reduction in bad cholesterol (LDLc) thus inhibiting the atherogenesis. He also stated that the active ingredient of ASE is the “allicin”. Biochemically, the allicin is a “diallyl-disulfide-oxide” and a potent vasodilator. Effects on the alleviation of diastolic blood pressure (DBP) in systemic hypertension and platelet aggregation inhibition in humans

D. ANNONA SQUAMOSA LEAVES – SOURCE OF ANTIOXIDANTS

Scientific classification

Kingdom:	Plantae
Clade:	Angiosperms
Order :	Magnoliales
Family :	Annonaceae
Genus :	<i>Annona</i>
Species:	<i>A. squamosa</i>
Botanical name:	<i>Annona squamosa</i>
Synonyms:	<i>Annona asiatica</i>

According to WHO survey, 80percent populations living in the developing countries rely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extracts (Sandhya *et al.*, 2006). The medicinal plants play an important role in the development of newer drugs because of their effectiveness, less side effects and relatively low cost when compared with synthetic drugs. Suresh *et al.*, (2006) report that *Annona squamosa* (L), belonging to the family *Annonaceae* is a small ever green tree commonly found in India and originates from West Indies and South America. Different parts of *Annona squamosa* (L) are used in folkloric medicine for the treatment of various diseases.

Gajalakshmi *et al.*, (2011) state that *Annona squamosa* leaves are considered beneficial for cardiac disease, diabetes hyperthyroidism and cancer. The root is considered as a drastic purgative. The crushed leaves are sniffed to overcome hysteria and fainting spells and leaf decoction is useful in the case of dysentery.

Patel *et al.*, (2012) state that in India, local empirical knowledge about medicinal properties of plants is the basis for their uses as a home remedies. It is generally accepted by many Indians and elsewhere in the world that beneficial medicinal effects can be obtained by ingesting plant products. Plants have basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has antifertility, antitumor and abortifacient properties.

The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and Tannins etc. These active principles usually remain concentrated in the storage organs of the plants viz., roots, seeds, bark, leaves etc. Considering all these facts, present investigation is designed to find out phytochemical analysis of *A. squamosa* a plant which evokes various therapeutic effects in combination with flaxseeds and garlic.

Bharat *et al.*, (2012) tested for the presence of active principles such as phytosterols, tannins, flavonoids, saponins, alkaloids, glycoside, triterpenoids and proteins in *Annona squamosa* leaves and reported that these leaves are rich sources of Phytochemicals. They also reported that several tribal populations in the northern parts of India use the leaves of the plant to cure diabetes.

Herbal medicine, as the major remedy in traditional medical systems, has been in medical practice for thousands of years. Considering the importance of plants as a source of medicine even today dried powdered leaves of *Annona squamosa* had been chosen to avoid side effects of drugs which are in use traditionally for the treatment of many ailments. Neha *et al.*, (2012).

III. METHODOLOGY

The Methodology pertaining to the study “**Antioxidant Anticancer Potentials and Shelf life of a Functional Ready To Eat (RTE) Mix**” is described under the following headings.

- A. Selection of Ingredients, Formulation and Standardization of Functional Ready To Eat (FRTE) Mix
- B. Organoleptic Analysis and Preparation of Functional Ready To Eat (FRTE) mix.
- C. Analysis of Antioxidant and Anticancer Activity of Functional Ready To Eat (FRTE) Mix.
- D. Shelf life analysis of Functional Ready To Eat (FRTE) Mix.

A. SELECTION OF INGREDIENTS, FORMULATION AND STANDARDISATION OF FUNCTIONAL READY TO EAT (FRTE) MIX

1. Selection of Ingredients:

Natural ingredients are the source of traditional herbal medicine. They are still the primary health care system in many parts of the world. Plants form the basis of many traditional medicines throughout the world since thousands of years and continue to provide new remedies to mankind (Patel, 2010). Natural ingredients such as flaxseeds, custard apple leaves, garlic pods are known to possess several medicinal and functional properties and hence were used to develop a Functional Ready To Eat (FRTE) Mix in the present study.

Flaxseed (*Linum usitatissimum*) is a good source of protein and dietary fibre, accounting for 20 percent and 28 percent of the flaxseed, respectively (Hall, 2013) Flaxseed has immense therapeutic potential, as antioxidant, primarily as hydroxyl radical scavenger, anticancer, antidiabetic antiviral, bactericidal, anti-inflammatory, and anti atherosclerotic food (Zanwar, 2010;) They are rich in essential fatty acids such as poly unsaturated fatty acids (PUFA): Linolenic acid, Linoleic acid, ALA.

Custard apple (*Annona squamosa*) leaves are rich in phytochemicals like glycosides, alkaloids, saponins, flavonoids (Parabia, 2015), These active principles usually remain concentrated in the storage organs of the plant parts viz., roots, leaves, bark, leaves etc.

Garlic (*Allium sativum*) is widely used around the world for its pungent flavour as a seasoning or condiment. Different compounds in garlic are believed to possess euglycemic, anti-tumour and anti-microbial effects and reduce the risk for cardiovascular diseases. They have anti-tumour and anti-microbial effects. There are claims from Alternate medicine about the curative effects of several herbs, leaves, stem, rhizome and roots. Perceiving plant foods as beneficial diet is advised by the folklore of many cultures over centuries. (Piero 2014).

While flax seeds and garlic were purchased from the local market, Custard apple leaves were collected from the home garden of the local population.

2. Formulation of Functional Ready To Eat (FRTE) Mix :

The Functional Ready To Eat (FRTE) Mix was formulated based on the study by Pamidighantam *et al.*, (2013). The composition of food ingredients in FSCP developed by Pamidighantam *et al.*, (2013) contained Flaxseed powder (FSP) 50 parts along with other traditional ingredients and blended in a mixer to obtain Flaxseed Chutney Powder (FSCP). They standardized the (FSCP) recipe in several trials by varying the quantities of flaxseed powder and other ingredients in various proportions (25:75, 30:70, 45:55, 50:50 and 60:40) in order to yield a palatable product The nutrient composition of Flaxseed Powder (FSP) and Flaxseed Chutney Powder(FSCP) developed by Pamidighantam *et al.*, (2015) is shown in Table I.

TABLE I

FUNCTIONAL COMPONENTS IN FLAXSEED POWDER (FSP) AND FLAXSEED CHUTNEY POWDER (FSCP)

Nutrients	FSP	FSCP
Moisture (%)	5.4±0.48	5.7±0.19
Total ash (%)	2.4±0.33	13.7±0.14
Acid insoluble ash (%)	0.11±0.03	0.12±0.02
Crude protein (% N×6.25)	24.2±0.76	23.4±0.85
Crude fat (%)	40.4±1.32	25.4±0.78
Crude fibre (%)	9.2±0.61	7.1±0.62
Carbohydrates (% by difference)	18.4±2.91	24.7±1.08
Energy (Kcal/100 g)	534.0±1.52	420.8±4.73
Calcium (mg/100 g)	41.5±1.34	55.1±0.80
Iron (mg/100 g)	1.5±0.10	1.9±0.20
Phosphorous (mg/100 g)	409.2±3.86	336.0±5.29
Total polyphenols (mg/100 g)	438.5±1.49	522.3±8.06
Fatty acid composition		
16:0	7.1	8.2
18:0	7.8	7.5
Saturated	14.9	15.7
18:1	25.2	28.7
20:1	1.1	0.3
Mono Unsaturated	26.3	29.0
18:2	11.5	17.2
18:3	47.3	38.1
Polyunsaturated	58.8	55.3

From the above ingredients flaxseeds and garlic were selected for the present study to develop Functional Ready To Eat (FRTE) Mix.

3. Standardisation of Functional Ready To Eat (FRTE) Mix:

To prepare the FRTE Mix, the shade dried *Annona squamosa* leaf powder was added in different proportions to TRTE Mix and acceptability trials were carried out before further study. Three variations were used, variation I with 15g of shade dried *Annona squamosa* leaf powder, variation II with 30g of shade dried *Annona squamosa* leaf powder and variation III with 50g of shade dried *Annona squamosa* leaf powder. These three variations were then compared to the traditional recipe in present study. These three variations are prepared and sensory evaluation was done as compared to that of the TRTE mix.

B. ORGANOLEPTIC ANALYSIS AND PREPARATION OF FUNCTIONAL READY TO EAT (FRTE) MIX

1. Organoleptic Analysis:

Standardisation was based on the sensory evaluation of FRTE Mix powder variations with rice, compared to standard recipe using a nine point hedonic scale (Potter 2013) from like extremely (9) to dislike (1). Sensory evaluation was done in the Foods lab, Department of Food Science and Nutrition, Avinashilingam institute for Home Science and Higher Education for Women, Coimbatore, by thirty semi trained personnel.

9- Point Hedonic Scale	
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly

5	Neither Like nor Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

The variation which obtained Maximum scores was chosen for further study.

2. Preparation of Functional Ready To Eat (FRTE) Mix

Variation II with 30g of shade dried *Annona squamosa* leaves obtained the maximum scores and was used for further analysis

Table II shows the Ingredients in the RTE Mixes

TABLE II

INGREDIENTS IN TRADITIONAL RTE (TRTE) MIX AND FUNCTIONAL (FRTE) MIX

Traditional RTE Mix		Functional RTE (FRTE) Mix	
Ingredients	Quantity	Ingredients	Quantity
Flax seeds	100g	Flax seeds	100g
Garlic pods	4no	Garlic pods	4no
Dry red chillies	6no	Dry red chillies	6no
Salt	4tsp	Salt	4tsp
		<i>Annona squamosa</i> leaf powder	30g

Procedure: For Traditional RTE (TRTE) Mix

1. Dry roast flax seeds on a pan for 6-7 minutes.
2. Dry roast red chilly on a pan for 3-4 minutes, Then add all ingredients in a bowl.
3. Blend all the ingredients in a mixer to a fine powder.
4. Store in airtight container for future use.

Procedure: For Functional RTE (FRTE) Mix

1. Dry roast flax seeds on a pan for 6-7 minutes.
2. Dry roast red chilly on a pan for 3-4 minutes.
3. Add shade dried *Annona squamosa* leaf powder.
4. Blend all the ingredients in a mixer to a fine powder.
5. Store in airtight container for future use.

C. ANALYSIS OF ANTIOXIDANT AND ANTICANCER ACTIVITY OF FRTE MIX.

1. Antioxidant Activity of FRTE Mix:

Aqueous Extraction was prepared by adding 2g of sample in 20ml of distilled water in a 250ml conical flask and kept in shaking Incubator at 60-70RPM at 40°C for 24hrs. After incubation, filtration is done by using Whatman Filter paper and the filtrate is collected and used for the study. The extraction was done for FRTE Mix as well as ingredients separately for comparison of their properties in the study.

a. DPPH Free radical scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to determine the free radical scavenging activity of aqueous extract. 1ml of sample was taken in a clean dry

test tube, add 0.1ml of DPPH solution(DPPH solution was prepared using standard procedure) and 0.4ml of 50mM tris HCL(50mml solution was prepared using standard procedure) and mix well. The mixture was incubated in room temperature for 30minutes and OD was taken 517nm. DPPH radical scavenging activity was expressed as inhibition(%), calculated by using the following formula (Gordon *et al.* 2016)

$$\text{Inhibition (\%)} \text{ of DPPH} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

A control

where A is the absorbance

b. Determination of Total Antioxidant Content:

1ml of sample was taken in a clean dry test tube, add 0.5ml of 1mmol reaction mixture (1mmol reaction mixture contains 0.6mol H₂SO₄, 28mmol sodium phosphate and 4mmol ammonium molybdate this was prepared using standard procedure) and mix well. the mixture was incubated at 50°C in water bath for 90 minutes. After the incubation, mixture was allowed to cool then OD is taken at 695nm. (Ragunathan *et al.*, 2017)

c. Determination of Total Phenolic Content:

The Folin-Ciocalteu assay method was used to quantify the total phenolic content in the extract. Take 0.5ml of sample in a clean dry test tube , add 0.5ml of Folin-Ciocalteu reagent . Then 20% sodium carbonate (20% sodium carbonate was prepared using standard procedure) was added and mix well. Then the mixture was incubated at 45°C in water bath for 15-20 minutes and allow to cool and take OD at 765nm. Total phenolic content was determined according to the standard calibration curve of gallic acid solution. The result is expressed as milligram of gallic acid equivalents per gram sample (mg GAE/g) on dry weight basis. (Ragunathan *et al.*, 2017).

2. Anticancer activity of FRTE Mix:

HeLa – cell line was used for the anticancer study. The cell line was procedure from National Centre for Cell Science Pune, India and the cells were sub cultured in DMEM medium with the addition of Na_2CO_3 , glucose and BSA. After the preparation the T Flask was incubated in CO_2 incubator for 24-72 hours in the condition of Co_2 - 5%, at 37°C , pH – 7, Humidity 70-80%. The growth of the culture was identified by color change into yellow and bilayer formation. In 96 well plate Blank (DMEM) , medium (media prepared for primary culture), Sample (next 3 wells sample is added in the series of $5\mu\text{l}$, $10\mu\text{l}$, $15\mu\text{l}$) and cancer cell line used as control without the addition of sample, After this process the plate were incubated in CO_2 incubator in the same condition for 24hours, then the cells are washed using DMSO and trypsin, $20\mu\text{l}$ MTT dye was added to the sample and incubated for 24hours in CO_2 incubator, then OD was taken in ELISA reader at 570nm. The viable cells are calculated using following formula

Cell death was calculated using the following formula,

$$\text{Cell Death} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Cell viability was measured using the formula,

$$\text{Cell Viability} = \frac{\text{Test}}{\text{Control}} \times 100$$

D. SHELF LIFE ANALYSIS OF FRTE MIX

The Nutrient broth was prepared by adding 13g of nutrient broth in 10ml distilled water in 100ml conical flask and sterilize at 121°C for 15minutes. After sterilization take 5ml of nutrient broth was taken in clean dry sterilized test tube and add 0.5g of sample was added and incubated at 37°C for 24hours, then OD was taken at 600. (Ragunathan *et al.*, 2017)

TABLE III

SHELF LIFE ANALYSIS OF FRTE MIX

Day	Sample (g)	Nutrient broth (ml)
0	0.5	5
7	0.5	5
14	0.5	5
21	0.5	5
28	0.5	5
35	0.5	5

Shelf life analysis of a FRTE mix which is stored in glass container was carried out by adding 13g nutrient broth in 10ml distilled water in 100ml conical flask and sterilization was done at 121°C for 15minutes. After sterilization 5ml of nutrient broth was taken in a clean dry sterilized test tube and 0.5g of sample was added and incubated at 37°C for 24hours, then OD was taken at 600 for every 7 days.

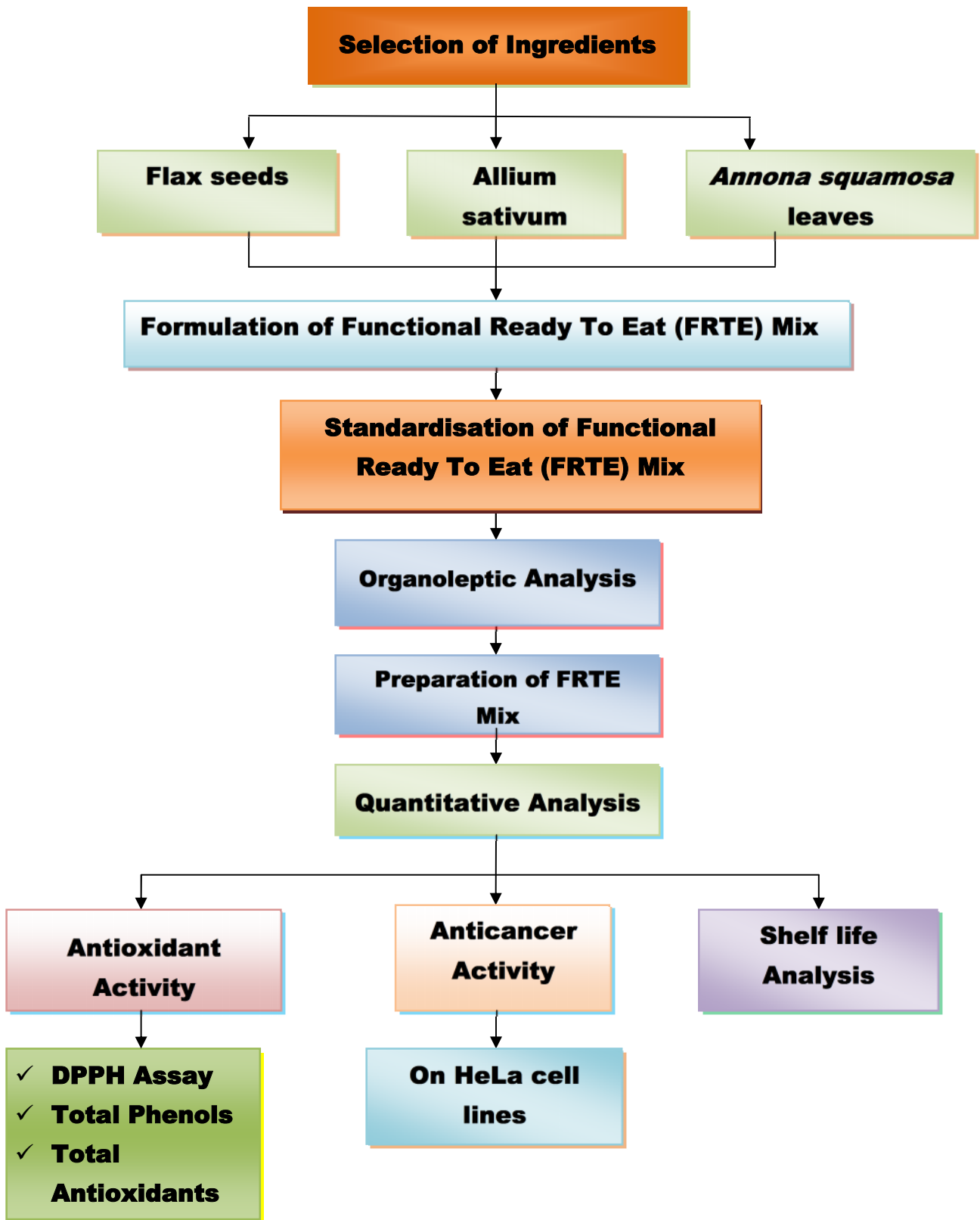


Figure 1 Research Design

IV. RESULTS AND DISCUSSION

The results and discussion pertaining to the study titled “Antioxidant Anticancer Potentials and Shelf life of a Functional ready to eat (RTE) mix” is discussed the under following headings.

- A. Acceptability of Functional Ready To Eat Mix.
- B. Antioxidant Content of Functional Ready To Eat Mix
- C. Anticancer Potentials of Functional Ready To Eat Mix
- D. Shelf life of Functional Ready To Eat Mix

A. ACCEPTABILITY OF FTRE MIX.

Table IV compares the acceptability scores of the three variations (V1, V2 and V3) of the Functional Ready To Eat (FRTE) Mix with rice in comparison with the Traditional Ready To Eat (TRTE) Mix.

Table IV

ACCEPTABILITY OF FRTE MIX

Variations	Appearance	Color	Flavour	Texture	Taste	Overall acceptability
TRTE	8.26±0.52	8.23±0.50	8.06±0.73	8.20±0.16	8.30±0.87	8.16±0.83
V 1	7.43±0.56	7.46±0.68	7.36±0.80	8.23±0.43	8.16±0.79	6.96±0.76
V 2	8.26±0.44	8.30±0.46	8.23±0.43	8.23±0.43	8.16±0.79	8.13±0.43
V 3	6.83±0.53	7.20±0.76	7.06±0.82	7.10±1.21	6.63±1.03	6.56±0.77
One way ANOVA (P= 0.05)	F=8.773** P=0.001	F=8.908** P=0.001	F=4.414** P=0.01	F=10.112** P=0.001	F=2.874** P=0.055	F=8.083** P=0.006

*Significance at 5% level, NS- Not significance

Table IV depicts the sensory evaluation of Functional Ready To Eat (FRTE) Mix variations with Traditional Ready To Eat (TRTE) Mix. V1, V2, V3 were scored for appearance, color, flavour, texture, taste and overall acceptability. Among the three variations, V2 was found to be significantly ($P < 0.05$) superior to the other two variations (V1 and V2) in appearance, color, flavour texture and taste. Moreover it was found to be on par with the Traditional (TRTE) Mix in terms of appearance and overall acceptability. Among the three variations V2 was most acceptable and hence it was chosen for analysis study as the Functional Ready To eat (FRTE) Mix.



Plate 1a – Functional Ready To Eat (FRTE) Mix



Plate 1b – FRTE Mix for sensory evaluation



Plate 1c- Sensory evaluation by Semi trained personnel

Plate 1 – Organoleptic Analysis

B. ANTIOXIDANT CONTENT OF FUNCTIONAL READY TO EAT (FTRE) MIX

1. **DPPH Radical scavenging Activity:** DPPH(1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity is calculated for aqueous extracts of Functional RTE Mix as well as ingredients separately for comparison of their properties is shown in Table V and Figure 2.

TABLE V

DPPH RADICAL SCAVENGING ACTIVITY (%)

Ingredients/ FRTE Mix	DPPH scavenging activity %
Flaxseeds	58%
<i>Annona squamosa</i> leaf powder	71%
Garlic	55%
FRTE mix	85%

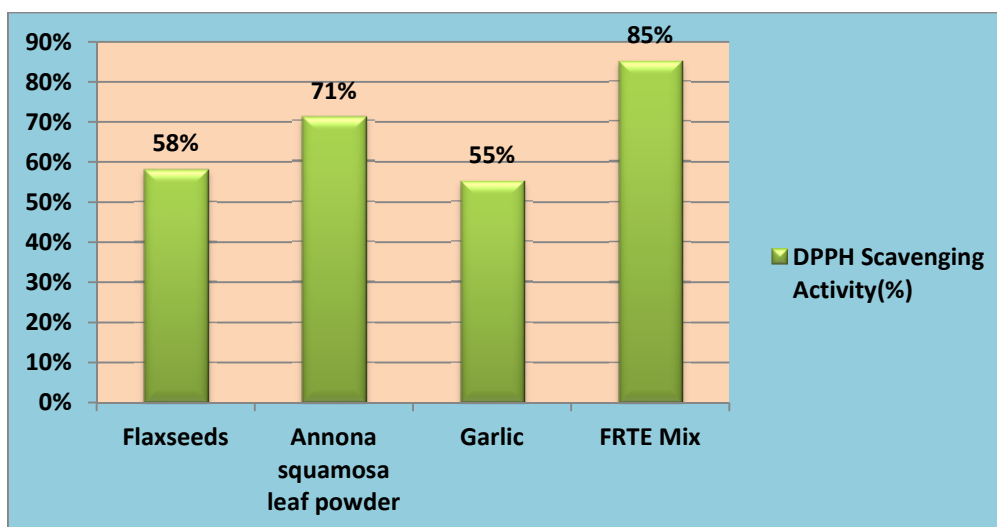


Figure 2 - DPPH Radical Scavenging Activity

The aqueous extract of Flaxseeds exhibited 58 percent of DPPH Scavenging while *Annona squamosa* leaf powder extract had 71percent, Garlic extract had 55 percent and the overall DPPH Scavenging power of the FRTE mix was 85 percent of radical scavenging action. Among the three ingredients, *Annona squamosa* leaf powder exhibited highest inhibition rate of 71percent.

This finding is on par with the results of Abdalbasit *et al.*, (2012) in which they conducted study on alcoholic extract of *Annona squamosa* Linn. Leaves and reported 88.18% of scavenging activity at maximum concentration of 500µg/ml. The higher activity was more probably because their study was on alcoholic extract of *Annona squamosa* leaves.

2. Total Antioxidant Content: Total Antioxidant is calculated for aqueous extracts of Functional RTE Mix as well as ingredients separately for comparison of their properties is shown in Table VI and Figure 3.

TABLE VI

TOTAL ANTIOXIDANT (%)

Ingredients/ FRTE Mix	Total Antioxidant (%)
Flaxseeds	58%
<i>Annona squamosa</i> leaf powder	70%
Garlic	55%
FRTE Mix	75%

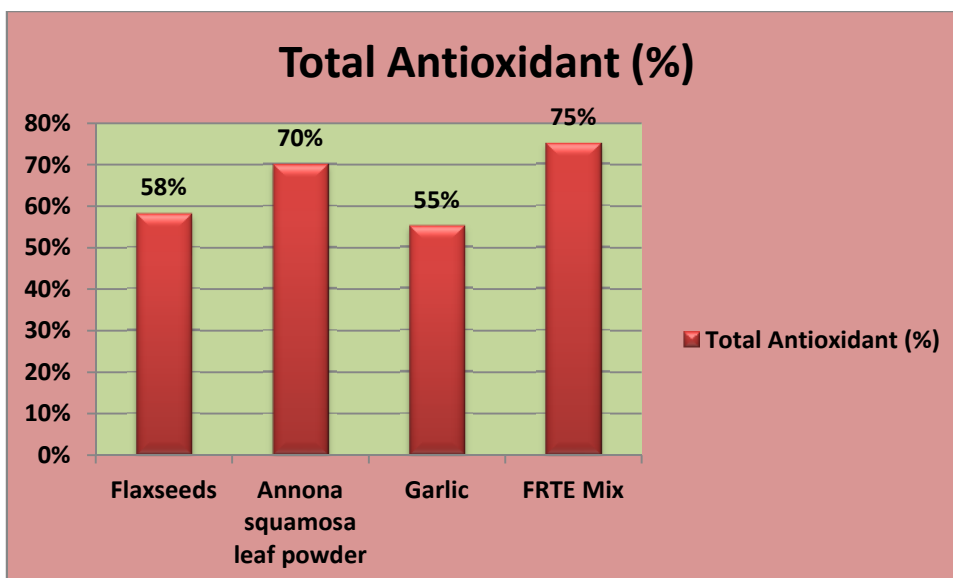


Figure 3 - Total Antioxidant Content (%)

Annona squamosa leaf powder had 70 percent, flaxseeds had 58 percent, garlic had 55 percent of antioxidants. When these ingredients were mixed with *Annona squamosa* leaf powder, developed FRTE mix showed 75 percent of total antioxidants

This finding is on par with the results of Adriana *et al.*,(2015) in which they conducted study on Ferulic acid (FA), butylated hydroxyl toluene (BHT), Secoisolariciresinol (SECO) extract of flaxseed and reported that butylated hydroxyl toluene (BHT) showed much higher antioxidant efficiency and reactivity than that of both extracts of flaxseed.

- Total Phenol Content:** Total Phenol Content is calculated for aqueous extracts of FRTE Mix as well as ingredients separately for comparison of their properties is shown in Table VII and Figure 4.

TABLE VII

TOTAL PHENOL CONTENT (mg/g)

Ingredients	Total Phenols mg/g
Flaxseeds	122
<i>Annona squamosa</i> leaf powder	142
Garlic	124
FRTE Mix	125

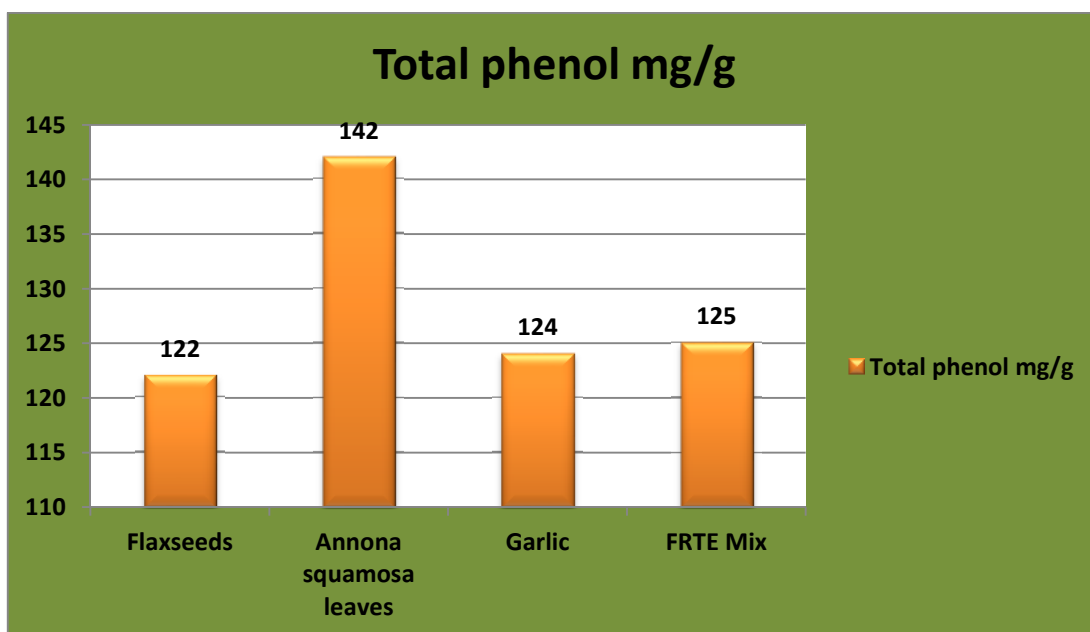


Figure 4- Total Phenol Content

The data on Total Phenol for aqueous extract of Flaxseeds, *Annona squamosa* leaf powder, garlic and FRTE mix was estimated and among them *Annona squamosa* leaf powder extract has 142mg/g of total phenols, garlic aqueous extract has 124mg/g of total phenols, flaxseeds aqueous extract has 122mg/g of total phenols. When *Annona*

squamosa leaf powder is mixed with flaxseed and garlic to develop FRTE Mix, the total phenols has decreased to 125mg/g.

This finding is on par with the results of Roberto *et al.*, (July 2015) in which they conducted study on ethanolic extract of flaxseed and reported that ethanolic extract of flaxseed has highest total phenol content of 130mg/g at maximum concentrations. This shows that ethanolic concentration of flaxseed has more total phenol content than aqueous extract.

C. ANTICANCER POTENTIAL OF FRTE MIX

Anticancer potential of FRTE Mix on He-La cell lines is given in the Table VII and Figure 5.

TABLE VIII

Anticancer Activity of FRTE Mix

Concentration(μ l)	Cell Death (%)	Cell Viability (%)
10	20.2	79.8
20	32	68
30	58.4	41.6

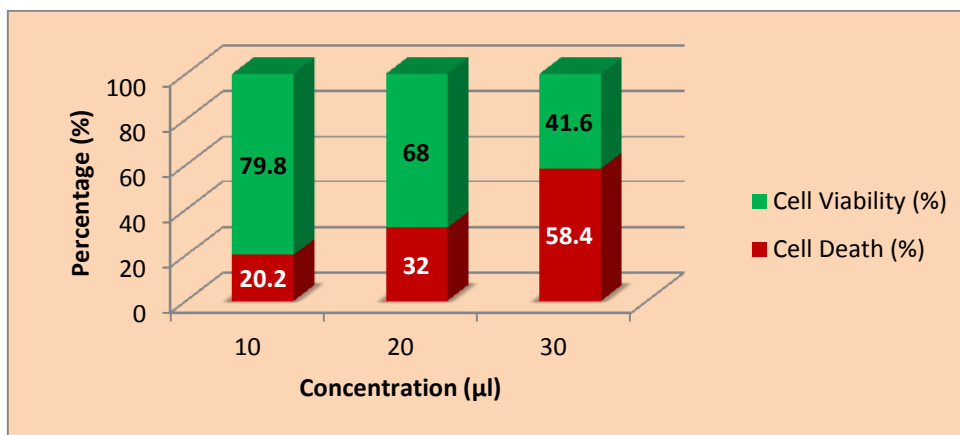


Figure 5- Anticancer activity

The Aqueous extract of FRTE Mix was analysed for cytotoxic activity against HeLa cancer cell lines in 96- well plate in 10 μ l, 20 μ l and 30 μ l of FRTE Mix respectively. HeLa cells with 10 μ l shows 20.2percent of cell death and 79.8 percent cell viability of cancer cells, 20 μ l concentration shows 32 percent cell death and 68 percent cell viability of cancer cells and HeLa cells with 30 μ l concentration shows maximum of 58.4 percent cell death and 41.6 percent cell viability.

This finding is on par with the results of Yunyun *et al.*, (2017) on breast cancer cell lines, SKBR3 and MDA-MB-231. Their experimental data suggests that flaxseed lignans significantly enhanced the ability of chemotherapeutic agents to cause cytotoxicity in SKBR3 and MDA-MB-231 breast cancer cells. They observed that high concentrations of Flaxseed methanolic extract inhibited cell proliferation and induced apoptosis of breast cancer cells which is in accordance with the present study. Consumption of the FRTE Mix would finitely show positive effect in cancer prevention.



Plate – 2a. Addition of MTT dye in 96-well plate.

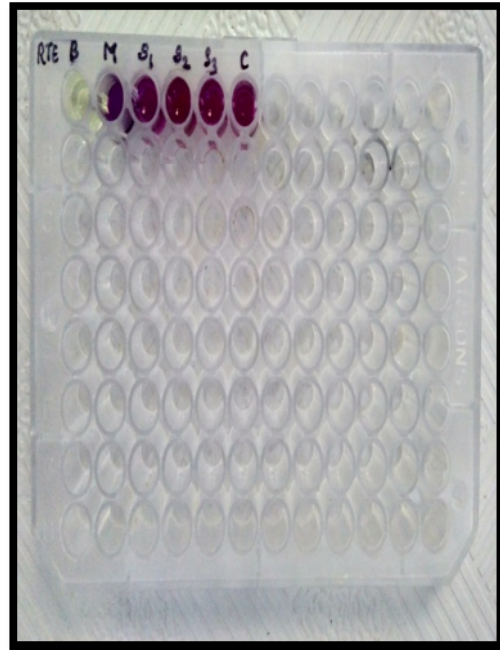
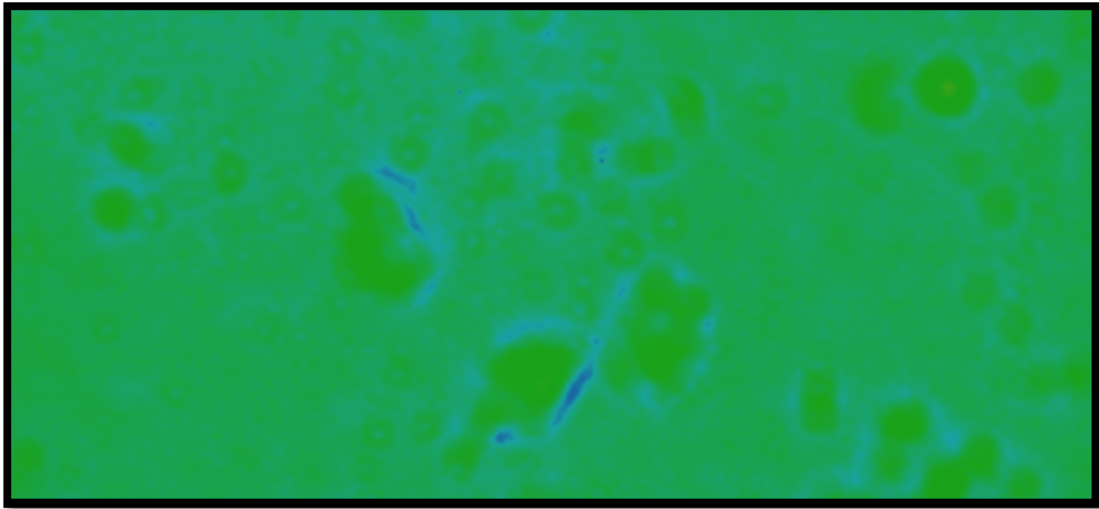


Plate – 2b. 96-well plate after addition of MTT dye

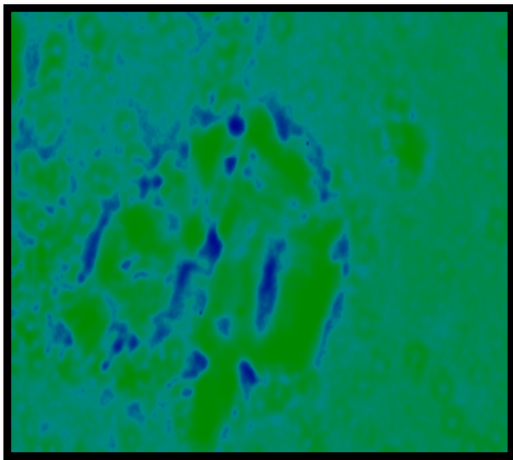


Plate 2c- Incubation of 96-well plate for 24 hours

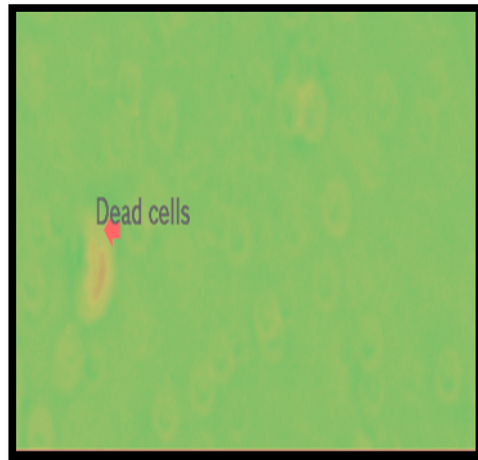
Plate 2- Anticancer



**Plate 3a. Control under Inverted
Fluorescent Microscope**



**Plate 3b. HeLa Cells treated
with Lowest Concentration
(10µl) of aqueous extract of
FRTE Mix**



**Plate 3c. HeLa Cells treated
with Highest Concentration
(30µl) of aqueous extract of
FRTE Mix**

**Plate 3. HeLa Cells in Inverted
Fluorescent Microscope**

D. SHELF LIFE OF FUNCTIONAL READY TO EAT MIX

Shelf life of FRTE Mix is shown in Table IX.

TABLE IX
SHELF LIFE OF FRTE MIX

Day	Sample (g)	Nutrient broth (ml)	Microbial growth
0	0.5	5	-
7	0.5	5	-
14	0.5	5	-
21	0.5	5	-
28	0.5	5	-
35	0.5	5	-

Shelf life analysis of FRTE Mix stored in glass container was done on day of preparation (Day 0) has no microbial content and OD reading was not taken, the microbial content was checked on seventh day showed the same result i.e. no microbial growth and no changes in sensory qualities of FRTE Mix, from then analysis of shelf life was done on every seven days showed no microbial growth and no changes in sensory attributes. On 28th day there was no microbial growth but, there was decrease in salt content and all other sensory attributes remain the same. On 35th day there was no microbial growth but showed minor changes in other sensory qualities. Similar results were obtained in a study conducted by Jyothirmay.*et.al* (2016). Where color of the flaxseed powder is retained well for six months and sensory qualities have minor changes.

The present study thus demonstrated that the aqueous extract of FRTE Mix developed in present study shows promising potentials as a functionally active food mix with appreciable potentials in antioxidant and anticancer activity and is also microbiologically safe for more than a month at ambient temperature.

V. SUMMARY AND CONCLUSION

Functional ready To Eat (FRTE) Mix ingredients have promising health benefits which are selected based on the previous articles of each and are used to formulate a health mix which can be taken along with rice, Idli, dosa etc. Traditional Ready To Eat (TRTE) Mix was taken as basis to formulate FRTE Mix by adding *Annona squamosa* leaf powder in different proportions to TRTE Mix and acceptability trials were carried out before further study. Three variations with 15g, 30g and 50g of *Annona squamosa* shade dried leaf powder and then compared with TRTE Mix. the three variations are prepared and sensory evaluation was done as compared to that of the TRTE Mix.

In sensory evaluation, Functional Ready To Eat (FRTE) Mix variations with Traditional Ready To Eat (TRTE) Mix. V1, V2, V3 were scored for appearance, color, flavour, texture, taste and overall acceptability. Among the three variations, V2 was found to be significantly ($P < 0.05$) superior to the other two variations (V1 and V2) in appearance, color, flavour texture and taste. Moreover it was found to be on par with the Traditional (TRTE) Mix in terms of appearance and overall acceptability. Among the three variations V2 was most acceptable and hence it was chosen for analysis study as the Functional Ready To eat (FRTE) Mix.

Antioxidant activity of FRTE Mix was analysed by three methods namely DPPH Radical scavenging Activity is calculated for aqueous extracts of Functional RTE Mix as well as ingredients separately for comparison of their antioxidant properties in which aqueous extract of Flaxseeds exhibited 58 percent of DPPH Scavenging while *Annona squamosa* leaf powder extract had 71percent, Garlic extract had 55 percent and the overall DPPH Scavenging power of the FRTE mix was 85 percent of radical scavenging action. Among the three ingredients, *Annona squamosa* leaf powder exhibited highest inhibition rate of 71percent.

Total Antioxidant is also calculated for aqueous extracts of Functional RTE Mix as well as ingredients separately for comparison of their properties in which aqueous extract of *Annona squamosa* leaf powder had 70 percent, Flaxseeds had 58 percent, garlic had 55 percent of antioxidants. When these ingredients were mixed with *Annona*

squamosa leaf powder, developed FRTE mix showed 75 percent of Total Antioxidants. Total Phenol Content is calculated for aqueous extracts of FRTE Mix as well as ingredients separately for comparison of their properties in which aqueous extract of Total Phenol for aqueous extract of Flaxseeds, *Annona squamosa* leaf powder, garlic and FRTE mix were estimated and among them *Annona squamosa* leaf powder extract has 142mg/g of total phenols, garlic aqueous extract has 124mg/g of total phenols, flaxseeds aqueous extract has 122mg/g of total phenols. When *Annona squamosa* leaf powder is mixed with flaxseed and garlic to develop FRTE Mix, the total phenols has decreased to 125mg/g. From the Antioxidant study it can be known that DPPH radical scavenging activity and Total Antioxidant percentage has increased when the flaxseed, garlic and *Annona squamosa* leaves are mixed. The total phenol content is decreased.

Anticancer activity of FRTE Mix is done on HeLa cell lines. The Aqueous extract of FRTE Mix was analysed for cytotoxic activity against HeLa cancer cell lines in 96-well plate in 10 μ l, 20 μ l and 30 μ l of FRTE Mix respectively. HeLa cells with 10 μ l shows 20.2 percent of cell death and 79.8 percent cell viability of cancer cells, 20 μ l concentration shows 32 percent cell death and 68 percent cell viability of cancer cells and HeLa cells with 30 μ l concentration shows maximum of 58.4 percent cell death and 41.6 percent cell viability. From the anticancer analysis it is came to know that all the three ingredients which are used to formulate has positive effect on cancer cell death and helps in preventing oxidative stress and cancers.

Shelf life analysis of FRTE Mix stored in glass container was done on day of preparation (Day 0) has no microbial content and OD reading was not taken, the microbial content was checked on seventh day showed the same result i.e. no microbial growth and no changes in sensory qualities of FRTE Mix, from then analysis of shelf life was done on every seven days showed no microbial growth and no changes in sensory attributes. On 28th day there was no microbial growth but, there was decrease in salt content and all other sensory attributes remain the same. On 35th day there was no microbial growth but showed minor changes in other sensory qualities.

Thus the Functional Ready To Eat (FRTE) Mix developed in the present study shows promising potentials as a functionally active food mix with appreciable antioxidant and anticancer activity. In addition it has the additional advantage of being microbiologically safe for 35 days at ambient temperature.

Recommendations for future study

- The FRTE Mix could be used as a day to day dietary as well as a therapeutic food.
- The efficacy of the FRTE could be tested on animal and human models.
- Other food mixes could be developed from inexpensive indigenous ingredients as well.

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**ANTIOXIDANT, ANTICANCER POTENTIALS AND SHELF LIFE OF A
FUNCTIONAL READY TO EAT MIX**

SENSORY EVALUATION FORM

Name:

Age:

Designation:

STANDARDIZATION OF READY TO EAT MIX

CRITERIA	SCORES FOR PRODUCT			
	Standard	Variation 1	Variation 2	Variation 3
Appearance				
Colour				
Flavour				
Texture				
Taste				
Overall Acceptability				

9- Point Hedonic Scale	
9	Like Extremely
8	Like Very Much
7	Like Moderately
6	Like Slightly
5	Neither Like nor Dislike
4	Dislike Slightly
3	Dislike Moderately
2	Dislike Very Much
1	Dislike Extremely

Potter,(2013)

INSTITUTIONAL HUMAN ETHICS COMMITTEE



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Dr. Anitha Subash

19th March 2018

To
Ms. Nallala Amrutha Varshini
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Nallala Amrutha Varshini,

Ref: Your proposal No. IHEC/17-18/FSN/08 entitled
“Antioxidants Anticancer Potential and Shelf Life of a
Functional Ready to Eat (RTE) Mix” submitted for approval
of the IHEC on 14th December.

The Institutional Human Ethics Committee of our University hereby
grants approval to your research proposal No. IHEC/17-18/FSN/08
entitled “Antioxidants Anticancer Potential and Shelf Life of a
Functional Ready to Eat (RTE) Mix” submitted by you. The
Approval number for the same is AUW/IHEC/ FSN -17-18/XMT/08.

We wish you all the best in your research endeavours.

Regards,

S. Uma Mageshwari
Dr. S. Uma Mageshwari
Member Secretary

