1. General Introduction

SPECIFIC AIMS

The main aims of the present project were to search for natural products that exhibit activity against toxic effects and potentiate currently used anticancer drug, Doxorubicin.

- The first step in our studies was an *a priori* selection, based partly on literature surveys, of plants and plant products. Fermented Papaya Preparation (FPP) made from fermentation of *Carica Papaya* fruit was subjected to a systematic screening for its various healing properties.

- The purpose of the further work was to investigate the protective as well as healing property of FPP towards organ toxicity induced by Doxorubicin.

- An additional aim of our work was to authenticate our findings in *in vivo* studies with Rat model with that of *in vitro* studies in cell lines.

1. INTRODUCTION

1.1 CANCER

Cancer is one of the most prevailing ailments in the world. It is estimated that cancer is the cause for 13% of all deaths worldwide (Guire 2015) and in the next two decade 70% increase in the new cases are expected. More than 100 types of existing cancer include breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and lymphoma. Symptoms of cancer vary depending on the type and each one requires unique diagnosis and treatment (Martel 2012). In cancer, tumours are developed in the form of lumps or masses of tissue due to alteration in the normal cell division. Leukaemia is the exception where normal blood function is forbidden by abnormal cell division in the blood stream. Tumours can grow and interfere with the digestive, nervous, and circulatory system where they release hormones that alter body function. Invasion is a process in which healthy tissues are destroyed due to the movement and streaming of a cancerous cell throughout the body using the blood or lymphatic systems. The invading cancerous cell manages to divide and grow, making new blood vessels to feed itself in a process called angiogenesis (Ungefroren *et al.,* 2011).

1.1.1 CAUSES OF CANCER

Most of the cancers are caused due to environmental or genetic factors. Major environmental factors causing cancer are tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and pollutants (Anand *et al.,* 2008). Several cancers occur due to gene mutation. These mutations affect three main types of genes—proto-oncogenes,

tumour suppressor genes and DNA repair genes. Proto-oncogenes regulate normal cell division or inhibit normal cell death. They produce protein products that are involved in normal cell growth and division. When their DNA is mutated due to certain factors, they are converted to oncogenes. Onco-protein, produced by oncogenes, activate the signaling cascade continuously, resulting in an increased production of factors that stimulate growth and differentiation while refraining apoptosis. Tumour suppressor genes are involved in controlling cell growth and division, regulate cell division by correcting the DNA mutations and controlling the Programmed cell death. However, cells with certain mutations in tumour suppressor genes may divide in an uncontrolled manner. DNA repair genes are involved in fixing damaged DNA. Cells with mutations in these genes tend to develop additional mutations in other genes. These mutations may further lead the cells to become cancerous (Hanahan and Weinberg 2000).

The treatment regimens for cancer include surgery, radiotherapy, chemotherapy, immunotherapy, targeted therapy and hormone therapy. The therapeutic strategy is based on three factors, the histological nature of the lesion; the assessment of the extension of the tumour process and the evaluation of the general disease state. Nature of tumour is different depending on the different cell families and may not always show sensitivity towards chemotherapy or radiotherapy. As different parts of body are made up of different tissues, they may have multiple types of tissues and cells. Therefore, the cancer is classified based on the cells from which they originate. The treatment regime, therefore, follows only after proper evaluation of probable cause and evolution of tumour growth.

1.1.2 TYPES OF CANCERS

Carcinoma: All cancers developing in the breast, prostate, lung, pancreas and colon are called carcinoma. Many of the common cancers come under this group. This involves the disturbance in cellular growth of epithelial tissue (Delisle and De Vauchelle 1990).

Sarcoma: Sarcoma is a cancer that develops from the connective tissue, which include bone, cartilage, fat, nerves etc. These tissues originate in mesenchymal cells outside the bone marrow (Delisle and De Vauchelle 1990).

Lymphoma and leukemia: These two classes of cancer arise from hematopoietic cells. It is the disorder in white blood cell growth, originating from bone marrow. (Varricchio and Claudette 2004). It includes Hodgkin's lymphoma, non-Hodgkin's lymphomas, multiple myelomas and immunoproliferative diseases. About 90% of lymphomas are non-Hodgkin's lymphoma.

Leukemia is the most common type of cancer in children as well as adults. Both acute and chronic forms of lymphoblastic and myelogenous leukemia are found with acute form more commonly observed in children (Delisle and De Vauchelle 1990).

Germ cell tumour: Cancers derived from pluripotent cells, most often occuring in the testicle or the ovary (seminoma and dysgerminoma respectively).

Blastoma: Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in adults (Delisle and De Vauchelle 1990).

1.1.3 TREATMENTS FOR CANCER

Surgery is used at the stage when tumour formed can be removed. Radiation, chemotherapy, or both might be used to shrink the tumour before or after surgery.

In Radiation therapy high energy rays like x-rays are used to kill cancer cells and shrink tumours The radiation may be provided from outside the body (external radiation) or from radioactive materials put right into the tumour (internal or implant radiation). Though a painless process its side effects like tissue damage is distressing.

Hormone therapy is sometimes used to treat certain kinds of prostate and breast cancers where hormones required for growth of cells are blocked with drugs. For example, estrogen bindining sites are blocked by anti-estrogen drugs in breast cancer cells. Immunotherapy is treatment designed to boost the cancer patient's own immune system to help fight the cancer.

Targeted therapy is a treatment that targets exclusively the cancer cells and causes less damage to healthy cells (Bahls and Fogarty 2002).

1.2. CHEMOTHERAPY

Chemotherapy a modality to cure cancer includes introduction of drugs (chemicals) to destroy cancer cells in mitosis stage. It is widely used to cure cancers like testicular cancer and Hodgkin lymphoma. When used prior to surgery (breast cancer), the reduced tumour size makes its removal effortless. In neo adjuvant treatment, chemotherapy is given before other treatments but in adjuvant treatment it is used after the surgery or radiotherapy. The purpose of this treatment is to avoid the recurrence of cancer by killing

off any cancer cells that have broken away from the main tumour before surgery. Sometimes chemotherapy is used along with radiotherapy in the procedure called chemo-radiation. But it can increase side effects. Chemotherapy is a procedure that is predominantly used in the treatment of cancer disease with metastasis and propagation. Depending on the primary location and the extension of the disease, chemotherapy can be curative or can decrease the symptoms and slows down the cancer growth (often called as palliative cancer). In majority of cases, chemotherapy leads to the increase in survival span, while in others it results in the removal or suppression of the disease (Alecsandru and Cornel 2007).

The application of cytotoxic chemotherapy has shown undesired side effects that are not effective in tumour degeneration in some patients. The heterogeneity of the neoplasm is the reason for unexpected response to chemotherapy. Anticancer drugs have their own type of sensitive and resistant primary tumours, and the use of combined drugs leads to an extreme complexity of possible therapeutic schemes (Alecsandru and Cornel 2007).

The side effects of chemotherapy are numerous and may include fatigue, hair loss, easy bruising and bleeding, infection, anaemia, nausea and vomiting, appetite changes, constipation, diarrhoea; mouth, tongue, and throat problems such as sores and pain with swallowing; nerve and muscle problems such as numbness, tingling, and pain; skin and nail changes such as dry skin and colour change; urine and bladder changes and kidney problems, weight changes, chemo brain that affects concentration and focus, mood changes, changes in libido and sexual function and fertility problems.

There are several groups of chemo drugs that work on different phases of the cell cycle.

Alkylating agents: Their main representatives are Cyclophosphamide , Ifosfamide, Meclorethamine, Chlorambucil, Melphalan, Lomustine, Thiotepa and Dacarbacine (DTIC). The alkyating agents rupture the cross links of the DNA spirals, interfering with replication and transcription.

Antimetabolites: Vinca alkaloids, Cytosine-Arabinoside, 5-Fluorouracil, Methotrexate, Vincristine and Vinblastine. They interact with special enzymes, leading to the inhibition of that enzyme or the subsequent synthesis of an aberrant molecule that cannot function normally. These chemo drugs introduce false substances in DNA and cause inhibition of the division spindle.

Enzymes- L-Asparaginase. Asparagin cause enzymatic cleavage.

Unclassified chemotherapeutics: Platinum bonds (Cisplatin, Carboplatin), Hydroxyurea and Taxol. They produce cross linking of DNA chains (similarly to alkylating agents) and cause destruction of enzymes.

Antitumour antibiotics: Doxorubicin, Actinomycin D, Mitoxantrone and Bleomycin. These antibiotics inhibit topoisomerase II and cause Intercalation.

1.2.1. DOXORUBICIN

Doxorubicin (FIG.1.1) is a drug used in cancer chemotherapy. Doxorubicin is available as a powder for injection and in liquid solution, or as Liposomal formulations under the brand name Adriamycin. It comes under the class of drugs named anthracycline.

STRUCTURE OF DOXORUBICIN

(C_{27}-H_{29}-N-O_{11})

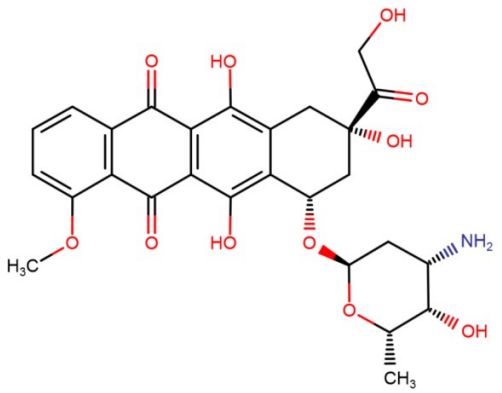

FIG.1.1

Anthracyclines (or anthracycline antibiotics) are derived from streptomyces bacterium *Streptomyces peucetius* var. *caesius* (Fujiwara A 1985) . The anthracyclines are among the most effective anticancer treatments ever developed and are effective against more types of cancer than any other class of chemotherapeutic agents (Weiss 1992; Minotti *et al.,* 2004; Peng X 2005).They inhibit the DNA-dependent RNA synthesis, as well as DNA duplication by rapidly penetrating the cells, getting fixed in the nuclear structures and intercalating with DNA.

Doxorubicin is a 14-hydroxylated version of daunorubicin which is the immediate precursor of doxorubicin in its biosynthetic pathway. Initially it was found that only one non-wild type species, *Streptomyces peucetius* subspecies *cesius* ATCC 27952, had the ability of producing doxorubicin (Lomovskaya *et al.,* 1999). Subsequently, under special

environmental conditions, or by the introduction of genetic modifications, other strains of Streptomyces could also produce doxorubicin (Grimm *et al.,* 1994). In 1996, Strohl's group discovered, isolated and characterized dox A, the gene encoding the enzyme that converts daunorubicin into doxorubicin (Dickens *et al.,* 1996). By 1999, they produced recombinant dox A, a cytochrome P450 oxidase, and found that it catalyzes multiple steps in doxorubicin biosynthesis, including steps leading to daunorubicin (Walczak *et al.,* 1999). These findings were significant as it became clear that all daunorubicin-producing strains have the necessary genes to produce doxorubicin. Doxorubicin is commonly used in the treatment of a wide range of cancers, including haematological malignancies and carcinomas.

Haematological malignancies (blood cancers, like leukaemia and lymphoma):

Doxorubicin is often used in combination chemotherapy as a component of various chemotherapy regimens. However, in spite of its high antitumour efficacy, use of this drug in chemotherapy has been largely limited due to its cardiac, renal, pulmonary, hepatic, testicular, and hematological toxicities (Singal *et al.,* 1987; Fadillioˇglu *et al.,* 2003).

Some of the common side effects of doxorubicin include hair loss, myelosuppression (a compromised ability of the body's bone marrow to produce new blood cells), nausea and vomiting, oral mucositis, oesophagitis, diarrhoea, skin reactions (including hand-foot syndrome) and localised swelling and redness along the vein in which the drug is delivered (Rossi 2013; Brayfield 2014). Like all anthracyclines, doxorubicin works by intercalating DNA, with serious adverse effects. It is believed to cause cardiomyopathy,

including oxidative stress, downregulation of genes for contractile proteins, and p53 mediated apoptosis (Kanu *et al.,* 2010). Doxorubicin affects the cancer cells by inhibiting DNA replication and RNA transcription by intercalation of ligand in DNA strand. Doxorubicin mechanism of cytotoxicity include its interference with DNA binding and arylation, DNA crosslinking, DNA unwinding, DNA strand separation, and helicase activity. It disrupts topoisomerase-mediated DNA repair and causes direct membrane damage due to oxidation of lipids. DNA topoisomerases change the topology of DNA by forming single- (type I topoisomerases) or double-strand (type II topoisomerases) breaks in the double helix. This relaxes the torsional stress that occurs when the DNA double helix unwinds when DNA and RNA polymerases access the DNA.Type II topoisomerases also untangle and separate the replicated DNA during cell division. Topoisomerase interactive agents inhibit relegation of DNA cleaved by topoisomerase I & II and induce protein-linked breaks in the DNA. These lesions in the ongoing DNA replication or RNA transcription lead to cytotoxic DNA damage, causing cell-arrest, apoptosis, or cell necrosis (DeVita *et al.,* 2005).

Formation of Doxorubicin metabolites by the liver may play an essential role in heart damage that are more cytotoxic than Doxorubicin (Václavíková *et al.,* 2008; Nabekura *et al.,* 2008; Zhang, *et al.,* 2009; Danz *et al.,* 2009; Panda *et al.,* 2009). Several reports suggest that Doxorubicin induced apoptosis plays an important role in its cytotoxicity that is linked to formation of reactive oxygen species (ROS) derived from redox activation of Doxorubicin (Kalyanaraman *et al.,* 1980; Sawyer *et al.,* 1999; Kotamraju *et al.,* 2000). Most studies hypothesized that the primary pathogenic mechanisms of doxorubicin induced cytotoxicity are mediated via its ability to generate reactive oxygen species

(ROS) including lipid peroxides, super oxide anions, hydroxyl radicals and hydrogen peroxides (Abd El-Aziz *et al.,* 2001; Kalender *et al.,* 2005; Yagmurca *et al.,* 2007).

In the last decade, many studies were focussed to reveal the mechanism behind the doxorubicin mediated cardiomyopathy caused by oxidative stress. It was noted that redox cycling transformation take place in cytoplasm, endoplasmic reticulum and mitochondria (Sardão *et al.,* 2009). It was clear that, oxidative changes in mitochondrial DNA are responsible for disturbances in mitochondrial protein synthesis, due to which, the product of the electron transfer chain was not only four electron reduced oxygen (H_2O) but also ROS-one, two, and three electron products, H_2O_2, and HO*, respectively (Boelsterli 2003). The symptoms during the latent period of doxorubicin induced cardiomyopathy were not clinically evident but studies showed that, in the due course of time the oxidation of mtDNA by ROS appears again (Berthiaume *et al.,* 2007; Zhao *et al.,* 2010). The insufficiency of mitochondrial function becomes apparent with passage of time after treatment is initiated, which is evident by enhanced participation of glycolytic pathway in ATP synthesis. Gradually, in the long run, cardiomyocytes lose their functioning (Zhang *et al.,* 2009; Shabanah *et al.,* 2012) and consequently lead to heart failure (Thompson *et al.,* 2010; Pointon *et al.,* 2010).

Doxorubicin is an intercalating agent, which does not exceed the hematoencephalic barrier as it is inactivated in the liver and eliminated by biliary route. But NADPH-dependent cellular reductases convert Doxorubicin to semiquinone free radicals (Domitrović *et al.,* 2009). Such reactive oxygen species increase intracellular Ca2+ by activating phospholipases via lipid peroxide, which further damages liver membranes that results ALT release and hepatocyte death (Ogawa *et al.,* 1987).

1.3. HERBAL TREATMENT

All over the world, thousands of indigenous plants are investigated, which have been used for treatment of ailments since prehistoric times. Herbs, which have medicinal properties, are prevalent in India since ages. Herb is a part of plant or whole plant used as perfume, flavouring agent, or therapeutic properties. The medicinal herbs are considered as one type of dietary supplement. They are sold in the market in the form of tablets, capsules, powders, teas, extracts, and fresh or dried plants and form the part of Ayurveda medicines. Many sages have studied and experimented with herbs to arrive at accurate conclusions about the efficacy of different plants and herbs that have medicinal value. Most of the Ayurvedic herbs, thus formulated, are free of side effects or reactions. In Ayurveda these herbs have been used as medicine as early as 1900 B.C. Many herbs and minerals used in Ayurveda have been described by ancient Indian herbalists such as Charaka during the 1st millennium BC. The Sushruta Samhita in the 6th century BC described 700 medicinal plants, 64 preparations from mineral sources, and 57 preparations based on animal sources. Thus ayurvedic medicine has quite complex formulas with many medicines having 30 or more ingredients (Aggarwal and Paridhar 2007).

The World Health Organization (WHO) estimates that presently 80 percent of the population of some Asian and African countries uses herbal medicine for some aspect of primary health care. According to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants (WHO 2012). Several chemical compounds present in plants as part of their normal metabolic activities. These chemicals are divided into primary metabolites and secondary metabolites.

Primary metabolites like carbohydrates, proteins and lipids are found in all plants whereas unique secondary metabolites and pigments are found only in a particular genus or species (Harborne *et al.,* 2001). At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants. Among 120 active compounds currently isolated from the higher plants and widely used in modern medicine, 80% show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant *et al.,* 2001).

Many of these phytochemicals are the compounds present in plants which have the capability to alter the activity of several cell signaling pathways. These can lead to modulation of inflammatory processes, regulation of cytoprotective mechanism and regulation of cell growth and differentiation (Surh 2003; Aggarwal and Shishodia 2006). Resveratrol, curcumin, genistein, capsaicin, epigallocatechin gallate (EGCG), quercetin, β-carotene, and lycopene are among the most widely studied phytochemicals (Benzie and Watchel 2011).

Carotenoids are also a type of phytochemicals which are widespread in the plant kingdom. Approximately 1000 naturally occurring variants of carotenoids have been identified. At least 60 carotenoids occur in the fruits and vegetables commonly consumed by humans. Besides the pro-vitamin A, carotenoids, α- and β-carotene, and β-cryptoxanthin, lycopene and the hydroxy carotenoids (xanthophylls) lutein and zeaxanthin are the main carotenoids present in the diet. Basic function of these carotenoids is to harvest light as auxillary components which help in quenching of excited molecules, such as singlet oxygen, that might be formed during photosynthesis. Phenolic compounds are also common in dietary plants. They are synthesized in large

varieties, and belong to several molecular families, such as benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins. Over 8000 plant phenols isolated are antioxidants by virtue of their hydrogen-donating properties of the phenolic hydroxyl groups (Lindsay and Astley 2002).

Most of the products categorized as herbal and traditional plant medicines are antioxidant-rich dietary plants or isolated phytochemicals. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants generally terminate these chain reactions. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids (Sies 1997; Vertuani *et al.,* 2004). In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Davies 1995; Sies 1997). However, ROS also have useful cellular functions, such as redox signaling. Thus, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level (Rhee 2006).

Healthy cells have low levels of reactive oxygen species. Risk of cancer is amplified, due to mutations in DNA that is caused by increase in ROS by inflammation or environmental factors. There are mechanisms in the cancerous cells which increase the defence against ROS, not allowing the cells to exceed the threshold for death. Therefore increase in ROS, below the threshold level, leads to activation of signalling pathways that favour cell growth, migration, and proliferation. Furthermore, many cancer therapies (e.g., radiation, chemotherapy) induce massive amounts of ROS that exceed the ROS

threshold and induce cancer cell death (Trachootham *et al.,* 2009). Due to the severe side effects of the chemicals administered in the cancer patients, the fear of the treatment is terrifying than the disease itself. The lethal side effects of chemotherapeutic agents include the devastating production of ROS. Therefore, several studies have been carried out in which herbs are used to lower the side effects of chemotherapy. An increasing amount of facts suggest that a simultaneous treatment of chemotherapy and chemo-preventive agents with antioxidant action may boost the efficacy of chemotherapeutics (Lee *et al.,* 2008; Aydin *et al.,* 2011).

Several reports suggest that doxorubicin induced apoptosis plays an important role in its cytotoxicity that is linked to formation of reactive oxygen species (ROS) derived from redox activation of doxorubicin (Kalyanaraman 1980; Sawyer *et al.,* 1999; Kotamraju S 2000). To reduce the toxic effects of doxorubicin, several pharmacologic agents such as antioxidants, hematopoietic cytokines and iron-chelating agents are being used (Li *et al.,* 2006; Yeh *et al.,* 2007). Several plants, with antioxidant properties have been investigated to prevent the ailments associated with free radicals. Studies have shown that various antioxidants have beneficial effects against doxorubicin induced organ toxicity in mice. Boghdady *et al.,* (2013) showed that grape seed proanthocyanidins and *ginkgo biloba* extract mediate their protective effect against doxorubicin induced cardiac injury through antioxidant, anti-inflammatory and antiapoptotic mechanisms. Studies have also revealed that *Terminalia paniculata* bark extract exerts equipotent cardioprotective and ionotropic activity in doxorubicin induced myocardial infarction in rats (Davey and Atlee 2011). Coumarin, hemidesmine, hemidine, hemidesine and rutin present in the root extract of *Hemidesmus indicus* is shown to have cardiprotective effect against

doxorubicin induced oxidative stress (Mahsa *et al.,* 2013). Further, *Vaccinium macrocarpon* that contains proanthocyanidins, flavonols and quercetin are protective against doxorubicin induced cardiotoxicity in rats (Ahmed *et al.,* 2010). Similarly, free radical scavengers such as melatonin and alpha-lipoic acid have been proved to ameliorate myocardial toxicity induced by doxorubicin (Liu *et al.,* 2002). Antioxidants such as vitamin E successfully provide protection from cardiac cell damage with a simultaneous decrease in lipid peroxidation (Speyer *et al.,* 1985). Modulatory effects of Rosemary leaves aqueous extract on Doxorubicin-Induced histological lesions, apoptosis and oxidative stress in mice were demonstrated by Ahmed R Rasha et al. (2010). In Chinese medicine also herbal products have been shown to protect against oxidative stress induced by adriamycin chemotherapy (Xu-Jun and Wie 2008). Jadhav (2013) has reported ameliorating effect of *Luffa acutangula* Roxb. on doxorubicin induced cardic and nephro toxicity. Natural plant components such as Rutin and Hesperidin have been reported to protect the liver against doxorubicin induced liver toxicity (Walaa *et al.,* 2014). Another plant product Berberine has also shown protective effects on doxorubicin-induced hepatotoxicity in mice (Zhao 2012). Studies have also revealed the efficiency of olive leaf extract treatment on doxorubicin-induced cardiac, hepatic and renal toxicity in rats (Alkın *et al.,* 2015). Thus experimental studies in animals have demonstrated that antioxidants from natural products provide protection against doxorubicin induced toxicity and also improve the therapeutic efficacy in cancer patients (Václavíková, et al. 2008).

1.3.1. FERMENTED PAPAYA PREPARATION

Many studies have been carried out on plant extracts as dietary food supplements. An extensive range of advantageous activity for the human health has been advocated for such dietary supplements, at least in part to their antioxidant activity (Rice-Evans *et al.,* 1996). Reports have suggested that the ability of antioxidant nutrients to influence cell response and gene expression, providing a novel and different mechanistic outlook underlying the biological activity of plant derived nutraceuticals (Prajda *et al.,* 1995; Csokay *et al.,* 1997; Virgili *et al.,* 1998). Fermented papaya preparation (FPP) is one such plant derived nutraceutical made by yeast fermentation of Carica papaya Linn., and is used as a health supplement in different parts of the world. FPP is rich in amino acids and carbohydrates that are polyphenolic in nature. Many reports on papaya are either based on the papaya fruit (Mahattanatawee 2006; Melo 2006; Simirgiotis *et al.,* 2009) or the leaf (Canini *et al.,* 2007), where reference to their phenolic, allosides and glucoside composition have been reported. Papaya skin, pulp and seeds contain a variety of phytochemicals, alkaloids and flavonoids including carotenoids and polyphenols, glucosides, and anthraquinone (Netsuwan 2013). FPP has a different composition due to the production process involving yeast fermentation. The composition identified in Table.1 has been authenticated by the Japanese Food Research Laboratories. The nature of the carbohydrates identified in FPP is the subject of ongoing research. Several findings imply that the extent of fermentation can influence its anticariogenic activity in vivo to exceed that of non fermented extracts. During the bio fermentation process, the formation of novel oligosaccharides enhances FPP's dietary composition and improves its remedial properties (Santiago *et al.,* 1992; Marotta *et al.,* 2007). FPP is one of the dietary

supplements to be extensively documented for its amazing free radical scavenging capability, modulation of endogenous phase II antioxidant enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase, therodoxin, xanthine oxidase) and simultaneous improvement of the overall natural defence mechanisms of the immune system. The functional activities of FPP have been found to remain unaffected despite subjection to harsh storage conditions highlighting FPP to be an actively stable and highly reliable dietary supplement (Santiago *et al.,* 1992).The molecular mechanisms of the bio efficacy of FPP are being defined. Earlier studies have indicated that FPP had antioxidant functions in vitro and in vivo. This property is achieved by the virtue of its ability to inhibit lipid peroxidation and protect supercoiled plasmid DNA against ferric nitrilotriacetate (Fe-NTA) plus H_2O_2 induced single and double strand breaks. This protects human T-lymphocytes challenged with Fe-NTA/H_2O_2 (Rimbach *et al.,* 2000). Property of FPP to act as a macrophage activator, as a result of its ability to augment nitric oxide synthesis and the secretion of TNF-α (a central regulatory cytokine in macrophage antimicrobial activity) has been suggested (Rimbach *et al.,* 2000). Tumour necrosis factor-alpha (TNF-α) is a cytokine that under normal conditions induces inflammation, tumour inhibition, and apoptotic cell death. However, when the former undergoes deregulation, it acts as a breast tumour promoter, enhancing the proliferation of chemically induced mammary tumours (Rivas *et al.,* 2008). Phenolic antioxidants can block the increase of TNF- α at the transcriptional level in the nucleus. This suggests the molecular mechanism of phenolic antioxidants through control of cytokine induction (Ma and Kinner 2002). Interestingly, Carica papaya is known to exhibit antibacterial activity that inhibits growth of gram-positive and gram-negative organisms, independent of the

stage of the fruits maturity.This is a property that is highly relevant for the treatment of chronic skin ulcers whereby it promotes wound healing (Dawkins *et al.,* 2003). Marotta et al. (2000) have demonstrated that FPP modulates atrophic and metaplastic changes of gastric mucosa in chronic atrophic gastritis patients. A study has also shown the protective effect of FPP on H_2O_2-induced cytotoxicity in PC12 cells.

Further, the clinical data supports the use of FPP as a dietary supplement in the management of type 2 diabetes mellitus because of its ability to effectively reduce fasting blood glucose levels, low-density lipoprotein/high density lipoprotein ratio, and inflammatory biomarkers such as C-reactive protein and uric acid (Danese 2006, Somanah 2012). Collard and Roy (2010) has reported a reduction in inflammation of the gums in FPP-supplemented rats with gingivitis. In this context, FPP may prove to be a valuable asset in reducing the risk of developing oral pathologies such as dental caries and gingivitis.

Despite postulations of the involvement of β-D-glucans (the major structural constituent of yeast cell walls) and complex amino acid and carbohydrates, the mechanisms behind the immunomodulatory role of FPP are still to be clarified (Islam *et al.,* 2008). Previous studies have suggested that FPP decreased ROS generation and effectively inhibited the oxidative stress induced GSH decrease in neuronal cells, thus protecting them from apoptosis through both antioxidant and bax/bcl-2 sensitive pathways (Imao *et al.,* 1998). As it has antioxidant properties, FPP even provides the protection against the iron-mediated oxidative DNA damage in the plasmid by significantly blocking oxidative damage to DNA and proteins, probably both due to hydroxyl radical scavenging as well as iron chelating properties (Rimbach 2000). Antioxidative potential of FPP is mediated

by scavenging ROS by upregulating expression of genes related to the oxidative status, such as glutathione peroxidase, superoxide dismutase and catalase (Marotta *et al.,* 2010), as well as by chelating excess iron (Aruoma *et al.,* 2010; Prus and Fibach 2012). Free iron species, such as the intracellular labile iron pool (LIP) participate in chemical reactions that generate ROS (Haber-Weiss and Fenton reactions) (Kruszewski 2003). The composition of FPP's principal components has been previously reported (Aruoma *et al.,* 2010), but the active ingredient(s) have not been defined. It is conceivable that the activity of FPP depends on synergy among different ingredients in addition to other components in its environment (Fibach and Ginsberg 2015).

Oxidative stress-induced damage is implicated in a variety of diseases such as cancers, hemolytic anemias, diabetes, arthritis, cardiovascular dysfunctions and neurodegenerative disorders (e.g., stroke, Alzheimer's disease and Parkinson's disease). These conditions could potentially benefit from treatment with FPP. For example, neuroprotective potential evaluated in an Alzheimer's disease cell model showed that the toxicity of the β-amyloid can be significantly modulated by FPP. Apoptotic pathways such as the c-jun N-terminal kinase and p38-mitogen activated protein kinase are preferentially activated by pro-inflammatory cytokines and oxidative stress resulting in cell differentiation and apoptosis. FPP has been shown to modulate the H_2O_2-induced ERK, Akt and p38 activation with the reduction of p38 phosphorylation as well as reduced the extent DNA damage (Aruoma *et al.,* 2010).

Table 1.1 - Composition of FPP. It is prepared using a biotechnological process adhering to the ISO9001 and 14001 using non genetically modified Papayas. The composition analysis was carried out and authenticated by the Japan Food Research Laboratories, Fukuoka, Japan.

Component	Level of component per 100g FPP
Moisture(vaccum oven method)	8.3g
Protein[a]	0.3 g
Fat	<0.1g
Ash	<0.1 g
Carbohydrates[b]	91.3 g
Energy[c]	366 kcal
Sodium	0.5 mg
AMINO ACIDS	
Arginine	16 mg
Lysine	6 mg
Histidine	6 mg
Phenylalanine	12 mg
Tyrosine	8 mg
Leucine	18 mg
Isoleusine	10 mg
Methionine	5 mg
Valine	14 mg
Alanine	13 mg
Glycine	11 mg
Proline	12 mg
Glutamic acid	40 mg
Serine	11 mg
Threonine	8 mg
Aspartic acid	23 mg
Tryptophan	2 mg
Cystine	Not detected

a The Nitrogern to protein conversion factors was 6.25.
b The formula used was 100- (moisture + protein + fat + ash)
c Energy conversion factors were in accordance with the notification no.176 (2003).Standards of nutrition labelling, Ministry of Health, Labour and Welfare, Japan.