NUTRIENT CONTENT, PHYTOCHEMICAL, ANTIOXIDANT, ANTIMICROBIAL AND ANTICANCEROUS ACTIVITY OF *LEUCAS ASPERA* (WILLD.) LINK. AND *PASSIFLORA EDULIS* SIMS

By

AKHILA.I

(14PFN001)

A THESIS SUBMITTED TO

THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND

HIGHER EDUCATION FOR WOMEN

COIMBATORE – 641 043

In partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION

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Certified as a Bonafide Research Work

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Signature of the Guide

29/4/16

Signature of the

Head of the Department

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I. INTRODUCTION

"Nature is the Best Physician"

-Hippocrates

"Nature is God's Amazing Pharmacy"

-Marks

Plants are one of five big groups (kingdoms) of living things. They are autotrophic eukaryotes, which mean they have complex cells, and make their own food. Usually they cannot move (not counting growth). Plants include familiar types such as trees, herbs, bushes, grasses, vines, ferns, mosses, and green algae. The scientific study of plants, known as Botany, has identified about 350,000 extant (living) species of plants. Fungi and non-green algae are not classified as plants.

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs, antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the rest source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However such plants should be investigated to better understand their properties, safety and efficiency (Rumaisa *et al*., 2013).

Undoubtedly medicinal plants are relevant in both developing and developed nations of the world as sources of drugs or herbal extracts for various chemotherapeutic purposes. Also the use of plant derived natural compounds as part of herbal preparations used as alternative sources of medicaments continues to play major roles in the general wellness of people all over the world. Higher plants, as sources of medicinal compounds continue to play a dominant role in maintenance of human health since antiquities. Over 50 per cent of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker *et al.,* 1995 and

Cordell, 1995). In developing countries, especially in rural contexts people usually turn to traditional healers when in diseased conditions and plants of ethno botanical origin are often presented for use.

The WHO estimated that 80 per cent of people worldwide rely on herbal medicines for some aspect of their primary health care. In the past 20 years in the United States, increasing public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in the use of herbal medicines. For most herbs, the specific ingredient that causes a therapeutic effect is not known. Whole herbs contain many ingredients, and it is likely that they work together to produce the desired medicinal effect. Many factors determine how effective an herb will be. For example, the type of environment (climate, bugs, soil quality) in which a plant grew will affect its components, as will how and when it was harvested and processed.

It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25 per cent of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80 per cent. Thus the economic importance of medicinal plants is much more to countries such as India than to rest of the world. These countries provide two third of the plants used in modern system of medicine and the health care system of rural population depend on indigenous system of medicine. Plants especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and reduced toxicity (Khan and Iqbal, 2011).

Medicinal plants are one of the important components of biodiversity. Status of biodiversity in India India's biodiversity constitutes five per cent of the World biodiversity on two per cent of the earth surface. Number of plant species in India is estimated to be over 45,000 representing about seven per cent of the World flora. This includes over 15,000 flowering plants of which 4900 species are endemic to the country. Out of eighteen 'hotspots' two hotspots are in India - the Eastern Himalayas and Western Ghats. India is one among the twelve mega diversity nations in the World (http://saffron.pharmabiz.com).

The history of medicinal plants in India can be traced to Vedic period. The Rig Veda, perhaps the oldest repository of human knowledge, written about 4500-1600 BC claims about 99 medicinal plants, the Yajur Veda, 82 plants and Sama Veda 100 plants. Atharvana Veda deals with 288 plants and almost all having medicinal ingredients used to cure deadly diseases (Ahmed, 2010).

Indian medicinal plants are claimed to induce para immunity, the non-specific immune modulation of essentially macrophages, granulocytes, NK cells and lymphocytes and complement functions (Ghule *et al*, 2006).

The medicinal properties of plants have been investigated due to their potent pharmacological activities, low toxicity and economic viability (Prashant *et al*., 2008). This revival of interest in plant derived drugs is mainly due to the current widespread belief that green medicine is safe and more dependable than the costly synthetic drug, many of which may have adverse side effects (Jigna and Sumitra, 2006). The development could lead to new drug discovery or advance use of indigenous herbal medicines.

The preservative effect of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues. It has been reported that there is an inverse relationship between antioxidative status and incidence of human diseases such as cancer, aging, neurodegenerative disease and atherosclerosis (Morales *et al*., 2008).

Leucas aspera (Willd.) Link. (Family: *Lamiaceae*) commonly known as *'Thumbai* is distributed throughout India from the Himalayas down to Ceylon. *Leucas aspera* is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long. Flowers are white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, and ciliate with long slender hairs. Fruit nutlets, 2.5 mm long, oblong, brown, smooth, inner face angular and outer face rounded (Srinivasan *et al*., 2011).

The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide and emmenagogue. Leaves are

considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites. *Leucas aspera* is used for treatment of respiratory tract disorders, edema, gastrointestinal disorders, pain, and as an antidote to poison. In Indian traditional medicine, the leaf juice of *Leucas aspera* is used to treat psoriasis, chronic skin eruptions, and chronic rheumatism (Kirtikar and Basu, 1991).

Different types of chemicals such as glucosides, tannins, saponins, sterols, oleic acid, linoleic acid, linolenic acid, palmitic acid, stearic acid, oleanolic acid, ursolic acid, nicotin etc. have already been isolated from the leaves, flower and seeds of this plant (Islam and Kato-Noguchi, 2012).

According to Augustine *et al*., (2014), the anti‑cancer activity of *Leucas aspera* exhibits anti‑cancer activity through stimulation of macrophage cells which is a key component of immune system. The present study was, therefore, undertaken to explore the preliminary phytochemical screening, acute toxicity studies and immunomodulatory activity of *Leucas aspera* aerial parts on cellular and humoral immune responses.

Passiflora edulis Sims (Passion fruit) belongs to the genus Passiflora, comprising about 500 species that are distributed in warm temperatures and tropical regions. The passion fruit, in general, prefer subtropical and frost free environments. *P. edulis* Sims (Family- Passifloraceae) is a vigorous climber. They cling to anything they can grab. The leaves are evergreen and alternate, three lobed leaves when mature. They grow quickly and 15 – 20 feet per year once established. They should have strong support. Their life cycle seems to be short in five to seven years, but new plants can be planted and fruit can happen the same year. Several species are grown in the tropics for edible fruits, the most widely grown being *P. edulis* (McGuire, 1999). The fruits are yellow when ripen with a maximum size of a lemon. The pulp inside the fruits is light yellowish orange in color with a pleasant odor. When the fruits are hanging down from the branches, it looks very ornamental (Ariharan *et al*., 2013).

The ethnobotanical literature has also indicated that the Passiflora plant contains a variety of compounds, including alkaloids, phenols, glycoside flavonoids, and cyanogenic constituents (Dhawan *et al*, 2004). The leaf extract of Passiflora species has been shown to possess anxiolytic and sedative activity, as well as treatment for diabetes and hypertension, and anti-inflammatory, cytotoxic, antioxidant, antibacterial, and antifungal properties (Ramaiya *et al*., 2014). In support of these claims, a study by Birner and Nicolls (1973) has reported the isolation of an antibacterial

and antifungal compound called Passicol from *P. edulis*. This plant has a continuing history of use in Ayurveda and homeopathic medicine as a treatment for a number of ailments (Ingale and Hivrale, 2010). The seeds possess an antifungal protein (Passiflin) (Lam and Ng, 2009) and an antifungal peptide (Pe-AFP1), which protects the plant from invasion by pathogenic fungi (Pelegrini, *et al*., 2006).

Antioxidants are type of molecules that neutralize harmful free radicals, produced through a chain of reactions that damage living cells, spoil foods; degrade material such as rubber, gasoline, lubricating oil. Antioxidants terminate these chain reactions through the removal of free radical intermediates and inhibition of other oxidation reactions. The use of antioxidants in pharmacology is intensively studied as oxidative stress might be an important part of many human diseases particularly stroke and neurodegenerative incidents. Antioxidants, therefore, are routinely added to meals, oils, food stuffs and other materials to prevent free radical damage. A lot of new plant species have been investigated in the search for novel antioxidants but there is still a demand to find more information on the oxidant potential of plant species (Emran *et al*., 2012).

The search for antibacterial agent with new mode of actions will always remain an important and challenging task. To a bacterium, the human body is a collection of environmental niches that provide the warmth, moisture and food necessary for organism to grow. The bacteria have acquired genetic traits that enable them to enter the environment, remain in a niche, gain access to food sources, and escape clearance by host immune and non-immune protective responses unfortunately many of the mechanisms that bacteria use to maintain their niche and the by-products of bacterial growth are incompatible with system of the human host (Kannan *et al*., 2011). Over the last three centuries, intensive efforts have been made to discover clinically useful antimicrobial drugs. It is believed that by using new compounds which are not based on the existing synthetic antimicrobial agents, antibiotic resistance can be considerably minimized. These has led to the increasing interest in traditional ethno medicine which is believed will lead to discovery of novel therapeutic agents (Akanbi *et al*., 2011).

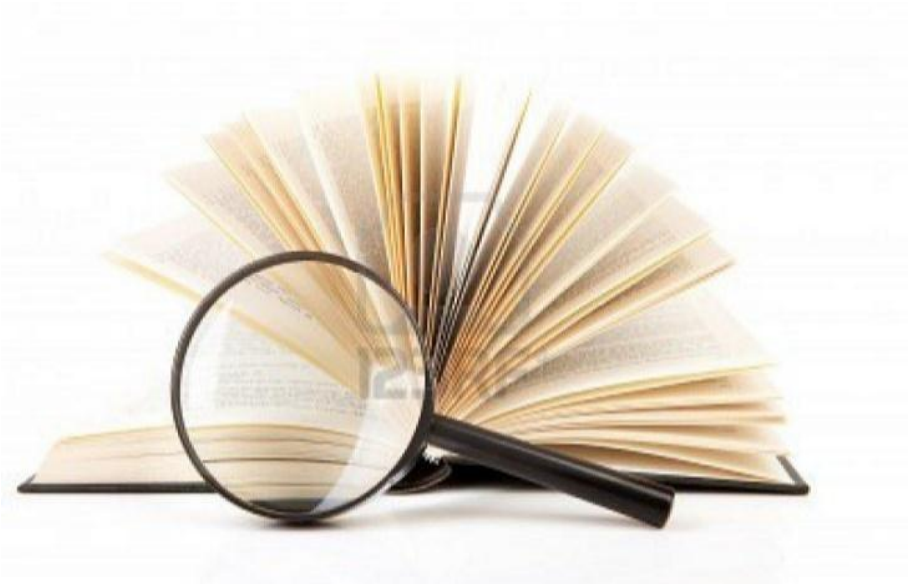
Cancer is one of the most life-threatening diseases and causes serious health problems in both developed and developing countries. It is a group of diseases characterized by the deregulated proliferation of abnormal cells that invade and disrupt surrounding tissues (Gennari *et al*., 2007). Tumour growth and systemic metastasis are highly dependent on angiogenesis. It is

a process involved in growth of new capillaries from existing ones, which is a regular process that rarely occurs under normal conditions. Many diseases are driven by persistent unregulated angiogenesis. Angiogenesis is a normal process in growth and development of blood vessels and is a process essential for tumour growth (Devi *et al*., 2013).

Therefore, investigations for finding new anticancer compounds are imperative and interesting. After taking into consideration the immense side effects of synthetic anticancer drugs, many researchers are making concerted efforts to find new and natural anticancer compounds. The screening of plant extracts has been of great interest to scientists in the search for new drugs for effective treatment of several diseases (Dimayuga and Garcia, 1991).

Even though studies have been done on phytochemical and antioxidant potentials of *Leucas aspera* and fruit of *Passiflora edulis*, there are less scientific studies to establish the medicinal properties of the leaves of these plants. Moreover research on anticancerous activity of the leaves of the two plant species is less though the two plants are common in occurrence. According to Khan and Iqbal (2011), King and Young (1999) and Pettinelli (2009) the nutritional and phytochemical components of plants vary according to the crop species and variety, growing conditions like soil moisture level, temperature, soil texture and structure, soil pH, disease and insect problems, weather conditions and cultural practices. Keeping these points in mind an attempt has made to explore the nutrient content, phytochemical, antioxidant, antimicrobial, anticancerous activities of these two plant leaves and the study "Nutrient Content, Phytochemical, Antioxidant, Antimicrobial and Anticancerous Activity of *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims." was planned with the following objectives:

- Assess the nutrient composition of plant leaves
- Determine the phytochemical 01components qualitatively
- Determine antioxidant activity
- Determine the antimicrobial activity
- Purify and identify the bioactive components using chromatographic methods
- Determine the anticancerous activity in the best answering plant leaves from the previous analyses.



Review of Literature

II. REVIEW OF LITERATURE

The review of literature pertaining to the study on **"Nutrient content, Phytochemical, Antioxidant, Antimicrobial and Anticancerous Activity of *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims."** is discussed under the following headings:

F. Importance of Medicinal Plants
G. Nutritional Value of Medicinal Plants
H. Phytochemical and Antioxidant Activity of Medicinal Plants
I. Antimicrobial and Anticancerous Activity of Medicinal Plants
J. Botanical Description and Health Benefits of *Leucas aspera* and *Passiflora edulis*

A. IMPORTANCE MEDICINAL PLANTS

Medicinal plants are defined as the plants have the ability to synthesize a wide variety of chemical compounds that are to perform important biological functions and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than ten per cent of the total. Chemical compounds in plants mediate their effect on the human body through process identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects (Tapsell *et al*., 2006 and Lai and Roy, 2004).

Each and every human civilization on earth has been rooted in the biodiversity of nature. Biodiversity provides humankind enormous direct benefits and indirect essential services through natural ecosystem function and stability. It comprises about five to more than fifty millions of species of which 270,000 are plant species. The World Health Organization (WHO) estimates that up to 80 per cent of the world's people rely on plants for their primary health care. Plants contain chemical constituents such as tannins, flavonoids, steroids, saponins, glycosides, phenolics, terpenes, alkaloids, waxes, essential oils, carbohydrates, amino acids, proteins etc.

The presence or absence of some of these constituents has been found useful in the placement of the plants in their taxonomic categories. Particular chemical constituents or drugs are found to be present in particular plants or plant parts that are used for many useful purposes (Johnson *et al*., 2008).

Since ancient times, the medicinal properties of plants have been investigated for scientific advancement throughout the world due to their potent radical scavenging activities. As antioxidants have been reported to prevent oxidative stress and damage caused by free radical, they can interfere with the oxidation process by reacting with free radicals, chelating agents, catalytic metals and also by acting as oxygen scavengers (Gill and Tuteja, 2010 and Hazra *et al*., 2010). As a result, a recent upsurge of interest has been made in the therapeutic potentials of plants as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as ascorbic acid, butylated hydroxyanisole and butylated hydroxytoluene, are commercially available, they are quit unsafe and their toxicity is a problem of concern (Vinay *et al*., 2010).

The investigation of medicinal properties of various plants attracted an increasing interest since last couple of decades because of their potent pharmacological activities, convenience to users, economic viability and low toxicity (Prashant *et al*., 2008). This regained interest to plant-derived medicines is basically due to the multidrug resistance of many antibiotics as well as current widespread perception that green medicine is safe and dependable than the expensive synthetic drugs most of which have adverse effects (Jigna and Sumitra, 2006). This belief and perception could lead to the exploration of new indigenous herbal medicines.

In the USA, 74 per cent of drugs are based on plants (Singh, 2002). A large portion of the world population, especially in developing countries like India depend directly on the traditional medicine system for a variety of diseases. Approximately 20 per cent of the plants found in the world have been submitted for pharmacological and biological screening (Suffrendi *et al*., 2004). Several thousands of plants (approximately 20000 by traditional practice) are used medicinally, mainly as herbal preparations in the indigenous system of medicine in India and the sources of

very potent and powerful drugs which have stood the test of time and modern chemistry has not been able to replace most of them (Shariff *et al*., 2008).

According to Das *et al*., (2011), the importance of the antioxidant constituents of plant materials in the maintenance of health and protection from ageing-related diseases has intrigued scientist for a long time. Therefore, the effectiveness of phytochemicals in the treatment of various diseases may lie in their antioxidant effects. Phenolic compounds such as flavonoids, alkaloids, phenolic acids, stilbenes, lignans, lignin and tannins, found in both edible and nonedible plants, are well known as scavengers of free radicals and have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic acid is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

The modern pharmaceutical industry is highly dependent on plant-based medicines, with more than 50 per cent of drug substances derived from natural resources. Plants are known to produce phytochemicals, which are potential sources of anticarcinogenic, anticancer, antimicrobial, and antioxidant activity; these compounds include flavonoids, phenolic acids, and tannins (Ramaiya *et al*., 2014).

B. NUTRITIONAL VALUE OF MEDICINAL PLANTS

India is known for its rich biodiversity reflecting the use of plant materials both for food and medicinal purposes. Many herbs are recognized as important sources of beneficial compounds including the ingredients for functional foods. These functional foods promote better health to prevent chronic illness. Some ingredients that make food functional are dietary fibres, antioxidants, oligosaccharides, essential fatty acids (omega-3), lactic acid bacteria cultures and lignins. Many of these are present in medicinal plants. Indian system of medicine believes that complex disease can be treated with complex combination of botanicals unlike in the west, with single drugs. Whole foods are hence used in India as functional foods rather than supplements, such as onion, garlic, mustard, red chilli, turmeric, cloves, cinnamon, saffron, curry leaf, fenugreek and ginger. These have been shown to have many beneficial effects such as lipid lowering, sugar lowering, anti hypertensive and anti tumour properties (Dixit *et al*., 2005 and

Kulkarni *et al*., 2006). During the last decade many studies have been reported on extraction and efficacy of biologically active compounds isolated from plant species used for natural therapies or herbal medicine exhibiting increasing interest in this area (Ryos and Recio, 2005).

Gopalan et al., (2010) opines that, life cannot be sustained with adequate nourishment and needs adequate food for growth, development and to lead an active life and healthy life. Plants can manufacture the foods they need from simple chemicals derived from the soil, water and carbondioxide of the air. Higher organisms on the other hand do not possess this capacity to manufacture food from simple chemicals and hence they depend on plants or other animals for obtaining the food they need. Procuring enough food for obtaining enough food for its survival is the main aim of the life's struggle in all the higher organisms.

Man needs a wide range of nutrients to perform various functions in the body and to lead a healthy life. The nutrients include proteins, fat, carbohydrates, vitamins and minerals. Protein, fat and carbohydrate are sometimes referred to as proximate principles. They are oxidized in the body to yield energy which the body needs. Although proteins provide energy, their primary function is to provide amino acids for building body proteins. Fat provides essential fatty acids which have a vitamin like function in the body. Together with water, which is also an essential element, the proximate principles form bulk of the diet. Vitamins and minerals do not supply energy but they play an important role in the regulation of the metabolic activity in the body and help in the utilization of the proximate principles. Minerals also used for the formation of body structure and skeleton (Gopalan *et al.*, 2010).

Plants have great importance due to their nutritive value and continue to be a major source of medicines as they have been found throughout human history 30 to 40 percent of today's conventional drugs used in the medicinal & curative properties of various plants are employed in herbal supplements botanicals, nutraceuticals and drug. Plant materials form major portion of the human diet hence their nutritive value is important (Nile and Khobragade, 2009).

Carbohydrates, termed to reflect their chemical nature as hydrates of carbon, occur widely in plants, animals and many microorganisms serve both structural and metabolic functions. Though animals can synthesize some carbohydrates from fat and protein, a major portion of their carbohydrate is obtained from plant source (Sivakumar, 2013).

As per Kalyanasundaram (2013), one sixth of the wet cell mass is contributed by proteins. Though proteins can also provide energy as carbohydrates, they are used for this purpose only when diet is inadequate in carbohydrates and fats. The major function of protein is building muscle mass and other tissues. Because twenty different amino acids can be arranged in many different combinations proteins exhibit very diversified functions.

Fats are group of diverse organic compounds sharing the common physical property of being insoluble in water and soluble in non-polar solvents. In general, functions of fats are classified as structural, metabolic, regulatory, nutritional and storage. Dietary fats are concentrated source of energy, enhance palatability and satiety, facilitate the absorption of fat soluble vitamins (A, D, E and K) and provide the essential fatty acids (Ghafoorunissa and Vajreswari, 2013).

Vitamins are organic substances present in small amounts in many foods. They are required for carrying out many vital functions of the body and many of them are involved in the utilization of the major nutrients like proteins, fat and carbohydrates. Although they are needed in small amounts, they are essential for health and well being of the body. The minerals and trace elements present in the body form a part of body structural component and some others act as catalytic agents in many body reactions. For example bones and skeleton are made up mainly of calcium, magnesium and phosphorus, and iron is a component of blood (Gopalan et al., 2010).

Hence, the evaluation of nutritional significant of medicinal plants helps to understand the medicinal use of that plant. All of these nutrients like carbohydrate, fats, protein, lipids etc are important for physiological function of human body. The quality and quantity of protein in the seed are important factors in the selection of plants for nutritive value and plant improvement programs (Shah *et al*., 2014).

C. PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS

According to Hasler and Blumberg (1999) phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics and about 150 phytochemicals have been studied in detail (Saxena *et al*., 2013).

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds (Costa *et al*., 1999). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (King and Young, 1999).

Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Hahn, 1998).

Study findings suggest that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity (Mathai, 2000). Phytochemicals may detoxify substances that cause cancer. They appear to neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens. For example, according to data summarized by Meagher and

Thomson (1999), genistein prevents the formation of new capillaries that are needed for tumor growth and metastasis.

Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic antitumor activity (Tapas *et al*., 2008).

In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours (Bruyne *et al*., 1999), and as antiinflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara, *et al*., 2005).

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

Among plant secondary metabolites terpenoids are a structurally most diverse group; they function as phytoalexins in plant direct defense, or as signals in indirect defense responses which involves herbivores and their natural enemies (McCaskill and Croteau, 1998). In addition,

terpenoids can have medicinal properties such as anticarcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), antiulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol (Langenheim, 1994 and Dudareva *et al*., 2004).

Many saponins are known to be antimicrobial, to inhibit mould, and to protect plants from insect attack. Saponins may be considered a part of plants' defense systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants (Lacaille-Dubois and Wagner, 2000).

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Phenolics acid possesses diverse biological activities, for instance, antiulcer, anti inflammatory, antioxidant (Sylva *et al*., 2007), cytotoxic and antitumor, antispasmodic, and antidepressant activities (Ghasemzadeh *et al*., 2010).

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress (Qusti *et al*., 2010). The phenolic compounds mainly show antioxidant capacity due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. These compounds also exhibit a wide spectrum of medicinal properties other than antioxidant properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects (Jacob, 1995).

According to Nigam and Sodhi (2014) an antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electron from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Free radicals are very unstable molecules with an unpaired electron and are important intermediates in natural processes involving control of vascular tone, cytotoxicity and neurotransmission. Free radicals cause many human diseases like cancer, Alzheimer's disease, cardiac reperfusion abnormalities, kidney disease and fibrosis etc. Antioxidants play many vital functions in a cell and have many beneficial effects when present in foods.

As per Sharma *et al*., (2013) oxidative free radicals are generated by metabolic reactions create a chain reaction leading to membrane and other lipid peroxidation, DNA damage, etc. This has been implicated in atherosclorosis (oxidated LDL is more atherogenic), cancers, neurodegenerative and inflammatory bowel diseases. Many endogenous and dietary compounds like superoxide dismutase, ferritin, transferrin, reruloplasmin, tocopherol, carotene and ascorbic acid have antioxidant and free radical scavenging properties. Small amounts of reactive oxygen species are continually formed in the body in the cell membrane and close to the cells organelles. They act where they are generated. Hence, they can damage most cell structures including membrane lipids, proteins, enzymes and nuclic acids.

Several herbs and herbal formulations are available for the scavenging activity. In addition to this there is a global trend to revive the traditional systems of medicines and renewed interest in the natural remedies for treating human ailments. Antioxidants have important preventive roles, not only on undesirable changes in the flavor and nutritional quality of food, but also on tissue damage in various human diseases. Almost all organisms are well protected against free radical damage by either enzymes or compounds, such as ascorbic acid, α-tocopherol and gluthione (Sharma *et al*., 2013).

D. ANTIMICROBIAL AND ANTICANCEROUS ACTIVITY OF MEDICINAL PLANTS

Plants, as the source of medicine, have been playing an important role in the health services around the globe (Thomson, 2010). Use of herbal medicines worldwide represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases.

Microorganisms have the potential to cause diseases. Human body is very prone to viral, bacterial and fungal infections. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. But due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases multiple drug resistance has been developed (Davis, 1994 and Ahamad *et al*., 1998).

In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions (Monroe and Polk, 2000). This creates a need of new effective and safe antimicrobial therapeutic agents. In this regard naturally occurring medicinal plants having active constituents which show the antimicrobial activity may provide the wide area of research. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body (Agrawal et al., 1996).

Antimicrobial activity of extracts of medicinal plants depends on some parameters like plant material used, technique employed, growth medium and most importantly microorganism tested. For better research better quality of plant material should be selected. The solvent and the extraction system may both modify the final results. Different extracts of a medicinal plant may show different results (Kapoor *et al*., 2015).

The International Agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within five years of diagnosis) in 2012 worldwide. By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year. Therefore there is a constant demand to develop new, effective, and affordable anticancer drugs. Approximately 60 per cent of drugs currently used for cancer treatment have been isolated from natural products and the plant kingdom has been the most significant source (Solowey *et al*., 2014).

A great deal of pharmaceutical research done in technologically advanced countries like USA, Germany, France, Japan and China has considerably improved quality of the herbal medicines used in the treatment of cancer. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Certain biological response modifiers derived from herbs are known to inhibit growth of cancer by modulating the activity of specific hormones and enzymes. Some herbs reduce toxic side effects of chemotherapy and radiotherapy. Scientists all over the world are concentrating on the herbal medicines to boost immune cells of the body against cancer. By understanding the complex synergistic interaction of various constituents of anticancer herbs, the herbal formulations can be designed to attack the cancerous cells without harming normal cells of the body. So far, pharmaceutical companies have screened more than 25,000 plants for anti-cancer drugs. This should tell us that looking for single ingredients to attack cancer might be missing the point. Just as cancers are a product of disturbances in the body, so herbs can correct the disturbances as well as control many cancers. Herbal system of medicine has been practiced for thousands of years. Phytoconstituents derived from the herbs *Vinca rosea, Taxus species, Achyranthes bidentata, Allium sativum, Aloe vera, Angelica sinensis, Astragals membranaceus, Glycine max, Glycyrrhiza glabra, Hordeum vulgare, Hydrocotyle asiatica, Medicago sativa, Morinda citrifolia, Panax pseudoginseng, Saussurea lappa, Taxus wallichiana, Tinospora cordifolia, Viscum album, Withania somnifera, Zingiber officinale* etc. have been used in various formulations to enhance activity of immune cells of the body that promotes production of cytokines including interleukin, interferon, tumor necrosis factor and colony stimulating factor. These formulations help the body to fight cancer more effectively and reduce toxic side effects of chemotherapy and radiotherapy stages of cancer (Sakarkar and Deshmugh, 2011).

E. BOTANICAL DESCRIPTION AND HEALTH BENEFITS OF *LEUCAS ASPERA* AND *PASSIFLORA EDULIS*

a) *Leucas aspera* (Willd.) Link.

Leucas aspera (Willd.) Link. (Family: *Lamiaceae*) commonly known as 'Thumbai' is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperient,

diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites (Srinivasan *et al*., 2011). The taxonomical classification is given below and a glimpse of the plant in its native habitat is presented in Plate 1a.

Taxonomical classification

Kingdom	: Plantae
Subkingdom	: Tracheobionta, Vascular plant
Super division	: Spermatophyta, Seed plant
Division	: Angiosperma
Class	: Dicotyledonae
Sub-class	: Gamopetalae
Series	: Bicarpellatae
Order	: Tubiflorae
Family	: Labiatae
Genus	: *Leucas*
Species	: *aspera*

Botanical description

Leucas aspera is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long. Flowers are white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle tipped, ciliate with long slender hairs. Calyx variable, tubular, 8-13 mm long; tube curved, contracted above the nutlets, the lower half usually glabrous and membranous, the upper half ribbed and hispid; mouth small, very oblique, not villous, the upper part produced forward; teeth small, triangular, bristle-tipped, ciliate, the upper tooth being the largest. Corolla 1 cm long; tube 5 mm long and pubescent above, annulate in the middle; upper lip 3 mm long, densely white-woolly; lower lip about twice as long, the middle lobe obviate, rounded, the lateral lobes small, subacute. Fruit nutlets, 2.5 mm long, oblong, brown, smooth, inner face angular and outer face rounded (Kirtikar and Basu, 1975 and Hooker, 1984).

Health Benefits

According to Rai *et al*., (2005), the juice of the leaves is used as local application for psoriasis, chronic skin eruptions and chronic rheumatism. As per Sadhu *et al*., (2003), *Leucas aspera* exhibits prostaglandin (PG) inhibitory and antioxidant activities. The experiment showed inhibition at 3-4 g/mL against PGE1- and PGE2- induced contractions in guinea pig ileum and a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect. Phytochemical investigation suggested the presence of nectandrin B, mesodihydroguaiaretic acid, macelignan, acacetin, apigenin 7-O-[6′-O-(p-coumaroyl)-3-D-glucoside], chrysoeriol, apigenin, erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3- methoxyphenyl)propan-1-ol, myristargenol B and machilin C, (-)-chicanine, (7R,8R)- and (75,85)- licarin A.

In vitro study of chloroform and ether extracts of *L. aspera* revealed its antifungal activity against *Trichophyton* and *Microsporum gypseum*. The minimum inhibitory concentration was found to be 5mg/mL and also this study proves that *Leucas aspera* had both fungistatic and fungicidal actions (Thakur *et al*., 1987).

The smoke of *L. aspera* is more toxic to the filarial vector mosquito, Culex quinquefasciatus than the synthetic mosquito mats, which contain 4% d-allethrin (Selvaraj *et al*., 1994). The methanol extract of *L. aspera* flowers, its fractions, the alkaloidal residue and the expressed flower juice showed good antibacterial activity for methanol extract and methanol fraction with maximum activity for the alkaloidal residue (Mangathayaru *et al*., 2005).

The essential oils from *L. aspera* possessed bacteriostatic activity against *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella typhi*, *Klebsiella aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas pyocyanea* and *Dys. Flexneri* (Rao and Narasimha, 1971).The ethanolic extract was subjected to acetic acid induced writhing inhibition, 1,1-diphenyl-2- picryl hydrazyl (DPPH) free radical scavenging assay and brine shrimp lethality bioassay for screening of antinociceptive, antioxidant and cytotoxic activity, respectively. The ethanolic extract of *L.aspera* root produced significant inhibition in acetic acid induced writhing in mice at the doses of 250 and 500 mg/kg. The extract showed a significant free radical scavenging activity with an IC50 of 8µg/ml. The extract showed significant lethality to brine shrimp (Rahman *et al*., 2007).

The particle size has been tested for freshly prepared *L.aspera* extract Silver Nanoparticles solution was observed with the stability solution 50 per cent and the size of 23 nm in a study by Ramya *et al*., (2014). The zeta potential value reveals that surface change stability of synthesized silver nanoparticles was about -28.90. This study shows that, the medicinal plant *L.aspera* (23 nm) has sustained smaller particles in the colloidal state. The extracts show inhibition zone of 6mm (after 24 hours) and 7 mm (after 48 hours) against E.coli (MTCC40) compared with antibiotic disc tetracycline of 5mm (after 24 hours) and 8mm (after 48 hours). The inhibitory zone of 4mm (after 24 hours) and 6mm (after 48 hours) compared with tetracycline antibiotic disc which was 3mm (after 24 hours) and 4mm (after 48 hours) against *Salmonella enterica* (MTTC3219).

Different parts of this plant (root, flower, leaf, stem) have been found to have antioxidant, antibacterial and cytotoxic effect (Chew, *et al*., 2012). The efficacy of whole plant extracts of L. aspera has been proven on larvicidal and pupicidal activities against the malarial vector Anopheles stephensi (Kovendan *et al*., 2012). Leaves of L. aspera are useful in chronic rheumatism, psoriasis, scabies, chronic skin eruptions and their juice is used as antibacterial agent. Its chloroform and ether extracts possess antifungal activity (Rahman *et al*., 2007). Traditionally, the whole plant is taken orally for analgesic, antipyretic, antirheumatic, anti inflammatory and antibacterial treatment and its paste is applied topically to inflamed areas (Gani, 2003). Apart from this, the entire plant is also used as an insecticide and indicated in traditional medicine for coughs, colds, painful swelling and chronic skin eruption (Chopra *et al*., 2002). The plant possesses wound healing property and is used in cobra venom poisoning (Mangathyaru *et al*., 2006).

b) *Passiflora edulis* Sims.

Passion fruit are grown mostly in tropical and sub-tropical part of the world. Passion fruit, a native of tropical America (Brazil), belonging to the family Passifloraceae is a high value and export oriented crop. Passion fruit stands out not only for its exotic and unique flavor and aroma but also for its amazing nutritional and medicinal properties (Phamiwon and John, 2016). The taxonomical classification is given below and a picture of plant is provided in Plate 1b.

Taxonomical classification

Kingdom	: Plantae
Phylum	: Spermatophyta
Subphylum	: Angiospermae
Class	: Dicotyledonae
Order	: Violales
Family	: Passifloraceae
Genus	: Passiflora
Species	: Passiflora edulis

Botanical description

Passion fruit is a highly nutrient responsive perennial crop, grows mostly as vine with a shallow root system (root density confined to top 20cm soil depth). The leaves are tri-lobed, long and deep green. These leaves are gloss on upper surface and dull on lower surface. The vine grows to a length of 15-20 feet. The fruit has circular or oval shape with membranous sac which is highly aromatic as seen inside when a fruit is cut. The sac is filled with aril (pulp) and juice. When the fruit is mature, the outer skin turn purple or yellow colour (Narayan, 2013).

As per Phamiwon and John (2016), of the estimated 500 species of Passiflora in the family Passifloraceae, only one P. edulis Sims, has the exclusive designation of passion fruit, preferably seen growing at an altitude of 800-1500 m above sea level. Within this species, there are two distinct forms, the standard yellow (Passiflora edulis f. flavicarpa Deg.) and the purple (Passiflora edulis f. edulis), differing in pH and starch content between the yellow (pH 2.8 and 0.06% starch) and the purple (pH 4.2and 0.74% starch), with comparatively higher amylose content in former (8.7%) than latter type (5.8%).In purple passion fruit, cool temperatures are favorable for flower initiation and fruit set(23-18o), while relatively high temperatures seem necessary for promoting juice production (23-33oC) and improvement in quality. In India, passion fruit cultivation is confined to Kerala, Tamil Nadu (Nilgiri hills and Kodai Kenal), Karnataka (Coorg) and northeastern states (Mizoram, Nagaland, Manipur and Sikkim).

Health Benefits

In Nagaland fresh leaves of *Passiflora edulis* is boiled in little amount of water and extract is drunk for the treatment of dysentery and hypertension. The flowering and fruiting portion are dried and preserved and used as a drug in preparation of certain proprietary products. The root extracts are also used in the treatment of ulcers and haemorrhoids (Jamir, 1999).

The root has been used as a sedative and vermifuge in West Indies, Mexico and Netherlands. In Italy the plant has been used as anti-spasmodic and sedative. In Mauritius the tincture and extract of plant has been used as a remedy for insomnia due to various nervous conditions but not due to pain. The root has been used as a diuretic and a decoction of leaves as an emetic. It has been used as a sedative, diuretic, anthelmintic, anti-diarrheal, stimulant and also treatment for hypertension, menopausal symptoms and colic of infants in South America. The fruit of *Passiflora edulis* is regarded as a digestive stimulant and used a remedy for gastric carcinoma. Fruits are eaten to get relief from constipation (Phamiwon and John, 2016).

An experimental study on albino rats of which 100,200,300,400 mg/kg body weight was administered indicating % reduction of blood glucose was 6.31, 7.14, 6.73 and 6.00 respectively for each dose. It was also found that 200mg/kg body weight was the most effective in reducing blood glucose levels with a maximum fall rate of 47.25% after 3 hours of glucose administration (De-Pari, *et al*., 2002). The presence of phenols and flavonoids may be responsible for the observed hypoglycemic activity of *Passiflora edulis* (Devaki *et al*., 2011).

On supplementation of 30g/day of *Passiflora edulis* flavicarpa fruit peel flour for 60 days among type II diabetic patient, blood glucose, glycated hemoglobin was significantly reduced. The presence of fibers, pectin which forms viscous mixture can change the gastric emptying time, increases satiety and delay the absorption of simple carbohydrates, also ability to form complexes with bile salts increases the cholesterol excretion (Sunitha and Devaki, 2009).

Supplementation of Passion fruit (*Passiflora edulis*) juice 250 ml twice a day before and after practice for three months (90 days) to traditional Thang ta athletes of Manipur enhanced their sports performance in terms of muscular endurance, strength and their general health and

nutritional status as revealed by increase in haemoglobin and reduction in cholesterol (Anel and Subapriya, 2011).

Passion fruit has a significant content of iron, potassium, zinc and manganese. The diet contain five per cent flour of passion fruit peel reduces blood glucose by 59 per cent in diabetic rats reaching the normal glycemic amount (112.6mg/dl). The mechanism is due to the presence of fiber, tannins and phenolic compounds (Queiroz *et al*., 2012) which reduce the digestion and absorption of carbohydrates, increased the sensitivity of muscle and adipose tissue to insulin (Jocelem *et al*., 2010).

The decoction of *passiflora edulis* dried leaves given to mice was found to possess sedative activity, increasing the total sleep induced by diazepam. The total sleep time increased from 31 ± 11 min in the control group to 77.6 ± 15 min and 78.3 ± 16 min in treatment group with extracts at the dose of 132.3 mg and 1325 mg/kg. CNS effect of the decoction in sedation could be interacting with benzodiazepine receptor and not GABA. Inhibitory effect on STR-induced seizure is probably on glycine and not GABA, while NMDA induced turning effect was reduced in dose dependent manner and was mediated through NMDA receptor and blocked by D-AP7. The decoction of *passiflora edulis* brought about these central actions by interacting with either inhibitory glycine or NMDA amino acid neurotransmitters. It also showed anti-convulsant activity protecting against seizure (Chandala *et al*., 2000).

The petroleum ether (*Passiflora edulis* leaf) crude extract at (500µg/disc) showed anti-bacterial activity of *B. megaterium* and *P. aeruginosa*. The chloroform crude leaf extracts (500µg/disc) showed moderate antibacterial activity (gram positive bacteria like B. *megaterium, B.Subtilis, S.Aureus and Sarcina Lutea*, gram negative bacteria like *E.Coli, P. aeruginosa, S.Paratyphi, S.Typhi, Shigella Boydii , Vibrio Mimicus*) with the average zone of inhibition of 7-10 mm by disc diffusion method (De-Pari *et al*., 2002). The aqueous extract showed zone of inhibition at a maximum concentration (200µg/disc) against *E. coli* (9.5mm), *K. pneumoniae* (7.2mm), *P. mirablis* (4.7mm), *P. aeruginosa* (6mm), *S. flexineri* (8.1mm), *S. typhi* (7.3mm) respectively (Ripa *et al*., 2009).

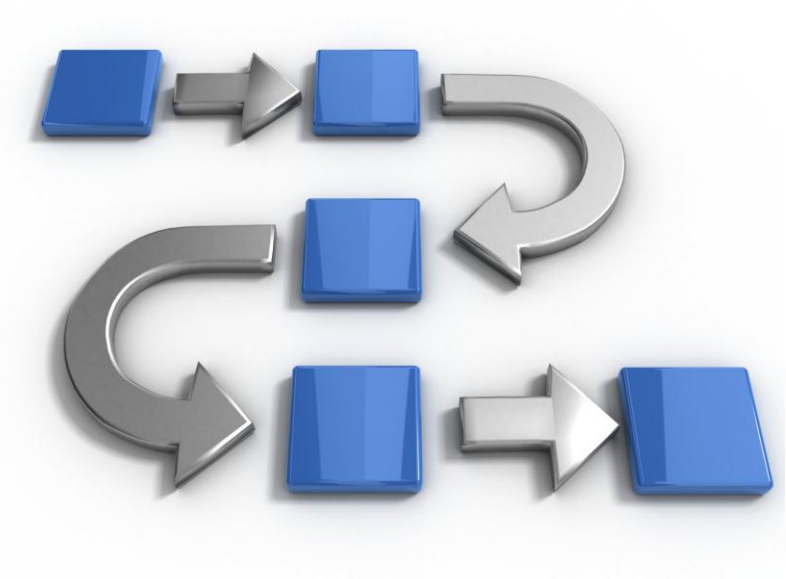
The petroleum ether and chloroform extracts of *Passiflora edulis* leaf on DPPH free radical scavenging assay showed antioxidant activity with IC50 of 58.88µg/ml and 56.85µg/ml respectively (Elisabeth *et al*., 2011). A concentration of 1100 µg mL−1 of *Passiflora edulis* leaf aqueous extract was able to scavenge 50% of DPPH radical (Razia *et al*., 2014).

The leaf extract of *P. edulis* exhibited potential antioxidant activity exhibiting an IC50 value of 875±87.83 µg/ml studied by 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) quenching assay at the concentration on 1000 (µg/ml) (Souza *et al*., 2012). When three different concentrations of each extract (0.1, 1 and 10 g/ml) were used in the TRAP assay to assess the antioxidant activity, there was a significant antioxidant capacity in vitro only at final concentrations of 1 and 10 µg/ml. The phenolic compounds present in the extract of *Passiflora edulis* might be the major contributors to the antioxidant activities (Sunitha and Devaki, 2009).

Patients with asthma were studied in a four week with oral administration of *Passiflora edulis* peel extract (150 mg/d) or placebo pills of which the prevalence of wheeze, cough, as well as shortness of breath was reduced significantly (Sharma *et al*., 2007). Passion fruit contains high amount of vitamin A and vitamin C both of which are strong anti-oxidants. They neutralize free radicals and protects from cancer. The flavonoids further enhance the potency of passion fruit in providing antioxidant to body and protecting from cancer. Recent studies have also shown that in cancer patients, passion fruit can kill the cancerous cells in vitro studies (Martina *et al*., 2007).

Plate 1a – *Leucas aspera* in its native habitat in Attappadi

Plate 1b – *Passiflora edulis* in its native habitat in Manjeri



Methodology

III. METHODOLOGY

The methodology followed in the present study titled **"Nutrient Content, Phytochemical, Antioxidant, Antimicrobial and Anticancerous Activity of *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims",** comprises the following steps:

A. Selection and Authentication of Plants
B. Preparation of Plant Leaf Extracts
C. Qualitative and Quantitative Analysis of the Leaf Extracts for
    1. Nutrient Content
    2. Phytochemical Components
    3. Antioxidant Activity
    4. Antimicrobial Activity
    5. Purification and Identification of Bioactive Components
    6. Anticancer Activity

A. SELECTION AND AUTHENTICATION OF PLANTS

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. Cancer is one of the most serious diseases that damage human health in the modern world and the second largest deadly disease just below heart disease (Heron *et al*., 2008). Recent studies show that oxidative stress and neoangiogenesis plays good role in the initiation and development of cancer (Bergers and Benjamin, 2003). It is also reported that activated macrophages have an important role in cancer therapy, since they can kill tumor cells (Ramesh *et al*., 2002).

Several studies are being carried out to establish the properties of many medicinal herbs. Traditional medicine makes tall claims on the medicinal properties of *Leucas aspera* and *Passiflora edulis*. Studies have been done on phytochemical and antioxidant potentials of *Leucas aspera* and fruit of *Passiflora edulis*. However, there are less scientific studies to establish the medicinal properties of the leaves of these plants. Moreover research on anticancerous activity of the leaves of the two plant species is less though the two plants are common in occurrence. Khan and Iqbal (2011), King and Young (1999) and Pettinelli (2009) claim that the nutritional and

phytochemical components of plants vary according to the crop species and variety, growing conditions like soil moisture level, temperature, soil texture and structure, soil pH, disease and insect problems, weather conditions and cultural practices. Hence, the two plants *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims. were selected for this study.

Leucas aspera (Willd.) Link. is a medicinal herb that belongs to the family Lamiaceae. It is popular as "Thumbai" throught out the Indian sub continent. It is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial and cytotoxic activities (Prajapathi *et al*., 2010). The juice of the leaves is used as remedies for psoriasis, chronic skin eruptions and chronic rheumatism (Kirtikar and Basu, 1991). The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L.aspera* leaves are also used as insecticides and mosquito repellent in rural area. The plant extract with honey is a good remedy for stomach pain and ingestion (Reddy *et al*., 1993). *Passiflora edulis* Sims. (Passion fruit) is a wild species belonging to the family Passifloraceae. The plant is a shallow rooted, perennial, tentril climbers. It is reported to possess cytotoxic, antioxidant activity (Ripa *et al*., 2009), antimicrobial and antifungal activities (Johnson, *et al*., 2008). Bum *et al*., (2008), Kamaldeep *et al*., (2004), Movafegh *et al*., (2008) and Santos *et al*., (2006) claims that different parts of this plant have been used in traditional medicine for the treatment of insomnia, sedation, epilepsy, hypnotic, hypertension, diuretic, reduction of cholesterol and triglycerides, bronchitis, asthma, colds, antispasmodic, stomach pain, tetanus, boils and intestinal tumors.

Studies claim that ingestion of natural antioxidants from *Leucas aspera* and *Passiflora edulis* reduces the risk of arthritis, cardiovascular diseases, cancer and other diseases associated with ageing (Augustine *et al*., 2013 and Uttara *et al*., 2009). Hence, it was hypothesised that these plant may contain a molecule with antioxidant, neoangiogenesis inhibition and macrophage stimulatory properties may improve the conventional anticancer treatment.

Based on the above hypothesis, adequate amounts of fresh leaves of two plants namely *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims were collected from the local area of Attappadi, **Palakkad District** and Manjeri, **Malappuram District** of Kerala respectively. The plants were authenticated by Dr.G.V.S. Murthy, Scientist 'F', Botanical Survey of India, Tamil

Nadu Agricultural University (Authentication Certificate of *Leucas aspera* (Willd.) Link.- Appendix I; Authentication Certificate of *Passiflora edulis* Sims.- Appendix II).

B. PREPARATION OF PLANT EXTRACT

The leaves were dried at room temperature at 25ºC under shade and away from sunlight for one week and were ground into powder with the help of mechanical grinder.

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures (Handa *et al*., 2008). The extracts prepared for this study included:

a) **AQUEOUS EXTRACTION**: 1g of samples of the two plants were immersed separately in 20ml of deionised water and kept in orbital shaker for 24 hours at 60-80 rpm for the extraction and filtered using Whatmann No.1 filter paper to get pure extract (Razia *et al*., 2014).

b) **METHANOL EXTRACTION**: Shade dried powder of 1g of sample of each plant leaf powder was taken in conical flask and 20ml of Methanol was added and was covered tightly with a polythene cover and kept on orbital shaker for 24 hours at 60-80 rpm and later it was filtered using Whatmann No.1 filter paper to get pure extract (Razia *et al*., 2014).

c) **ACETONE EXTRACTION**: 1g of each plant leaf powder was weighed taken in a conical flask and added 20ml of acetone. Then was covered tightly with a polythene cover and kept on orbital shaker for 24 hours at 60-80 rpm and later it was filtered using Whatmann No.1 filter paper to get pure extract (Razia *et al*., 2014).

Plate 2a and Plate 2b shows the aqueous, methanol and acetone extracts of *Leucas aspera* and *Passiflora edulis* respectively.

C. QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE LEAVES EXTRACTS

1. **NUTRIENT ANALYSIS:** The nutrients analysed were energy, carbohydrate, protein, fat, moisture, calcium, phosphorus, iron, vitamin C and β-carotene. The energy content was determined from the values of protein, fat and carbohydrates and multiplication of the content of these components with appropriate factors. One gram of carbohydrate or protein yield 4 kcal (16.8 KJ) and one gram of fat yield 9 kcal (37.8 KJ) (Gopalan *et al*., 2010).The carbohydrate content was estimated using Anthrone method (Snehal and Madhukar, 2012). The estimation of protein was done by Kjeldahl distillation that depends on the fact that organic nitrogen when digested with sulphuric acid in the presence of a catalyst is converted to ammonium sulphate, ammonia liberated by making the solution alkaline is distilled into a known volume of standard acid, which is then back titrated. The protein content is obtained by multiplying the nitrogen value with 6.25. Fat was estimated as crude ether extract of the dry material using Soxhlet apparatus. The moisture content was estimated by oven drying method. Other constituents were estimated as follows; iron by colorimetrically (Wong's method), calcium by titrimetric method. The ascorbic acid content was determined based on 2,6-dichlorophenol indophenol visual titration method. For estimation of β-carotene powdered samples were extracted in acetone and transferred into petroleum ether phase. The total β-carotene content was determined spectrophotometrically (Raghuramulu *et al*., 2003). Phosphorus analysis was carried out by measuring the blue colour, which is formed when the ash solution was treated with ammonium molybdate. The phosphomolybdate thus formed was reduced and read colorimetrically (Nagarajaiah and Prakash, 2011). The detailed experimental procedure for determining nutrient content in two plant leaves is enclosed in Appendix III.

2. **PHYTOCHEMICAL ANALYSIS:** The qualitative phytochemical tests for the identification of alkaloids, terpenoids, phenol and tannin, reducing sugar, saponins, flavonoids, quinines, protein and steroids were determined for all the extracts by the method described by Harborne (1998) and Sazada *et al* (2009). The detailed procedure for reagent preparation for phytochemical analysis are appended (Appendix IV).

Test for Alkaloids

To 1ml of extract added 1ml of Mayer reagent and a few drops of Iodine solution. The formation of yellow colour indicates the presence of alkaloids.

Test for Terpenoids

To 1ml of extract 1ml of concentrated Sulphuric acid (H_2SO_4) was added and heated for 2 minutes in a boiling water bath. The formation of grayish colour indicates the presence of terpenoids.

Test for Phenol and Tannins

To 1ml of extract 1ml of ferric chloride solution was added. A blue green/ black colour indicates the presence of tannins.

Test for Reducing sugar

To 1ml of extract 1ml of Fehling's A and Fehling's B solutions are added and heated in a boiling water bath. The formation of red colour indicates the presence of sugar.

Test for Saponins

To 1ml of extract 1 or 2ml of deionised water was added and shake well. The formation of 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids

To 1ml of extract a few fragments of magnesium ribbon and a few drops of concentrated hydrochloric acid was added. Pink scarlet colour appearance indicates the presence of flavonoids.

Test for Quinines

To 1ml of extract added 1ml of 1% sodium hydroxide. Blue green or red colour indicates the presence of quinines.

Test for Protein

To 1ml of extract a few drops of concentrated Nitric acid/ mercuric chloric acid was added. The formation of yellow colour indicates the presence of protein.

Test for Steroids

To 1ml of extract 1ml of Chloroform and concentrated Sulphuric acid was added along the sides of test tube. A red colour residue at the lower chloroform layer indicates the presence of steroids.

Finally from the results the best answering extract (methanol extract) was selected for both the plants for carrying out further analysis.

3. **ANTIOXIDANT ACTIVITY:** Quantitative determination of antioxidant content was determined by using DPPH Radical scavenging activity, FRAP, H_2O_2, Total phenol and Total Flavonids assays. The reagents required for antioxidant assay is appended (Appendix V).

    a) **1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical scavenging activity:** A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown above. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured (www.medallionlabs.com).

    b) The free radical scavenging activity of the extracts of two plant materials were determined according to the method of Williams *et al*., (1995) modified by Gunjan *et al*., (2011). To 100µl of plant extracts 100µl 0.1 Molar 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 400µl Tris. Hydrochloric acid solution was added in an eppendrof tube. This was incubated at room temperature for 30 minutes. The absorbance was measured in spectrophotometer at 517nm using distilled water as a blank. The percentage inhibition level of 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the selected two plant extracts was calculated according to the following equation:

Free Radical Scavenging (%) = $[(A_c - A)/ A_c] X 100$ where A_c is the absorbance of control and A is the absorbance of sample.

c) **Total Phenol:** A class of chemical compounds in organic chemistry which consist of a hydroxyl group (-OH) directly bonded to an aromatic hydrocarbon group is known as phenols or phenolics. Phenolic compounds are basically involving plant metabolic system and widely spread throughout the plant kingdom. Phenolic compounds have potential against oxidative damages diseases; therefore play a protective role through ingestion of fruits and vegetables. These compounds are very much essential for the growth of plant and involve in reproduction process of plants. These compounds produced during the response process against pathogens for defending injured plants. Antioxidant action of phenolic compounds is because of their high tendency to chelate metals with heavy metals like iron and copper. In human body as well as in plant system, free radicals are main component which can easily damage cell due to the presence of free electrons (www.chemistry.tutorvista.com).

The total phenol content of extracts was determined using Folin-Ciocalteu reagent using the method of Singelton *et al*., (1999). The 1ml of extract was mixed with 1ml of Folins phenol and 1ml of 20% sodium carbonate and incubated at 45ºC for 45 minutes. The absorbance was measured at 765nm in a spectrophotometer. The percentage of total phenolic content was calculated using the following formula:

Total Phenol Content (%) = $[(A_c - A)/ A_c] X 100$ where A_c is the absorbance of control and A is the absorbance of sample.

d) **Total Flavonoids:** The principle involved in Aluminium chloride (AlCl3) colorimetric method is that AlCl3 forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids (Bhaigyabati *et al*., 2014).

The total Flavonoid content was described by Kumaran and Karunakaran (2007). 1ml of Plant extract was treated with 0.1ml of 10% Aluminium chloride and 0.1ml of sodium tartarate or potassium acetate. Then 2.8ml of deionised water was added and incubated at room temperature for 30 minutes. The sample was measured at 415nm in a spectrophotometer. The Total flavonoid content (%) was determined using the formula given below.

Total Flavonoid content (%) = $[(A_c – A)/ A_c] X 100$ where A_c is the absorbance of control and A is the absorbance of sample.

e) **Ferric ion Reducing Ability of Plasma (FRAP - as a measure of antioxidant power) Assay:** Although a reductant is not necessarily an antioxidant, an antioxidant is commonly a reductant. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity44. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Priyanka, *et al*., 2013).

FRAP assay was performed according to the methods of Benzie and Strain (1999) with slightly modification by Rabeta and Faraniza (2013).To 1ml of sample added 1ml of phosphate buffer solution. After mixing 1ml of 0.1 % potassium ferric cyanide was added and incubated at 50ºC for 20 minutes. Added 1ml of 10 % Trichloro acetic acid and 1ml of deionised water. The measurements were taken at 700nm in a spectrophotometer. The ferric ion reducing antioxidant power (%) was determined using the formula given below.

Ferric ion reducing antioxidant power (%) = $[(A_c – A)/ A_c] X 100$ where A_c is the absorbance of control and A is the absorbance of sample.

f) **Hydrogen Peroxide Scavenging Activity:** Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential

thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu2+ ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate (Nagulendran *et al*., 2007).

The ability of *L.aspera* and *P.edulis* extracts to scavenge hydrogen peroxide was determined according to the method of Keser *et al*., (2012). *0*.5ml of sample was taken and added 2ml of 20mM hydrogen peroxide. Then 0.9ml of ethanol was added after mixing. Then it was incubated at room temperature for10-15 minutes. The measurements were taken at 230nm in a spectrophotometer. The percentage of hydrogen peroxide scavenging of both extracts and standard compounds were calculated:

Hydrogen Peroxide Scavenging Activity (%) = $[(A_c - A)/ A_c]$ X 100 where A_c is the absorbance of control and A is the absorbance of sample.

The reagents required for Antioxidant assay is appended (Appendix IV).

4. ANTIMICROBIAL ACTIVITY

Test organisms

The bacterial strains used in this study were clinical isolates obtained from the CBNR Laboratory, Coimbatore. The clinical isolates were *Bacillus subtilis* (gram positive), Klebsiella *pneumonia* (gram positive), *Pseudomonas aeruginosa* (gram negative) and *Escherichia coli* (gram negative).

Screening for Antibacterial activity of the plant extracts on Bacteria

The antibacterial activity of the plant extracts were tested by agar well diffusion method as per Sen and Batra (2012) and was slightly modified. The nutrient agar prepared aseptically by mixing 28g of nutrient agar in 1000ml distilled water and was sterilized along with four set of petri dishes in an autoclave at 121ºC for 15 minutes. Then the nutrient agar was poured into the petri plates. The test microorganisms of *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* were taken aseptically by sterile micropipette using microtips of

40µl. The organisms were spread with cotton swab and the plates were allowed to dry at 10-15 minutes. After drying, well were made with cork borer (5mm), and samples such as 40µl of extracts of two plants, methanol (positive control) and distilled water (negative control) were poured aseptically into the well separately and one Tobramycin (10 mcg) disc also was placed as a standard. The plates were allowed to stand until extracts have been completely absorbed by the medium. The plates were incubated at 37ºC for 24 hours. The effectiveness of these extracts was recorded by measuring the diameter of inhibition zone against each microorganism.

5. PURIFICATION AND IDENTIFICATION OF BIOACTIVE COMPONENTS

Chromatography is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a mobile phase through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated (Vergeese, 2010). In this study the methods used for purification and isolation of phytochemical components are column chromatography, paper chromatography, thin layer chromatography and gas chromatography-mass spectroscopy.

COLUMN CHROMATOGRAPHY

Column chromatography in chemistry is a method used to purify individual compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms upto kilograms. In this separation technique the stationary bed is within the tube. The particles of solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube or be concentrated on or along the inside tube well leaving an open, unrestricted path for the mobile phase in the middle part of the tube. Differences in the rates of movement through medium are calculated to different retention times of sample (Vergeese, 2010).

For this study Silica mess 200 column was used (15 X 3 cm). 1.5g of silica gel was used as stationary phase and was mixed with distilled water. The bottom of column was plugged with cotton to prevent the adsorbent pass out and then the silica gel suspension was poured into the column, set aside for 10 minutes and used. Then the methanol extract of *Leucas aspera* was poured for separation of the bioactive compounds. The fractions were collected in the time

interval of five minutes and totally nine fractions were collected. The same procedure was repeated for the separation of bioactive components in methanol extract of *Passiflora edulis*.

PAPER CHROMATOGRAPHY

According to Kamlesh (2009), paper chromatography has the widest field of application of all the chromatographic laboratory methods. It has gained its significance and popularity as an analytical method through the retention achieved by its aid in the fields of biochemistry and the organic chemistry of natural substances. Paper chromatography has these advantages: (a) simplicity and availability of materials and equipments; (b) high efficiency of separation; (c) the possibility of working with microgram quantities; (d) the characterization of substances in mixtures without prior separation of homologues; (f) its applicability to structural analysis; and (g) the study of reaction kinetics.

The paper chromatographic method was carried out using whatmann No.1 filter paper as per Singleton *et al*., (1999) and Markham, (1989). A volume of 1 µL of 1% methanolic solutions of standards and investigated extracts was spotted on the filter paper. One-dimensional paper chromatographic analysis was performed with Methanol: acetic acid: water in volume ratio 2: 2: 1. 15% acetic acid Spots were observed under UV light at 366 nm before and after spraying with the 2- aminoethyldiphenylborinate. The Rf value was calculated using the formula given below:

Rf value = Distance travelled by solute/ Distance travelled by solvent

Paper chromatography is supplemented by Thin Layer Chromatography which has an even higher resolving power but often uses more complicated equipment (Kamlesh, 2009).

THIN LAYER CHROMATOGRAPHY (TLC)

Thin Layer Chromatography is a chromatography technique used to separate mixtures. Thin Layer chromatography is performed on a sheet of glass, plastic or aluminum foil. Which is coated with thin layer of adsorbent material usually silica gel, aluminum oxide, or cellulose. This layer of adsorbent is known as stationary phase. After the sample has been applied on the plate a solvent or solvent mixture is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved (Vergeese, 2010).

The TLC was performed on the 20 × 20 cm plates precoated with microcrystalline cellulose. A volume of 1 µL of 1% methanolic solutions of standards and investigated extracts was spotted on the plates. One-dimensional TLC analysis was performed with Methanol: acetic acid: water in volume ratio 2: 2: 1. 15% acetic acid Spots were observed under UV light at 366 nm before and after spraying with the 2- aminoethyldiphenylborinate (Singleton *et al*., 1999 and Markham, 1989). The Rf value was calculated using the formula given below:

Rf value = Distance travelled by solute/ Distance travelled by solvent

Finally from the results the best answering plant extract was selected for carrying out GC-MS and MTT Assay.

GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GC-MS)

Gas Chromatography-Mass Spectroscopy is a method that combines the features of Gas-Liquid Chromatography and Mass Spectroscopy to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, and environmental analysis, explosive investigation and identification of unknown samples. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample (Vergeese, 2010).

GC - MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite -5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°c. The source temperature was maintained at 180°c. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification (Bilia, 2001).

6. ANTICANCER ACTIVITY (MTT ASSAY)

Media and reagents required for MTT Assay

The media required for MTT assay is DMEM (Dulbecoo's Modified Eagels medium, high glucose), FBS (Fetal Bovine Serum). MTT (3 - [4, 5- dimethylthiazol - 2 - yl] - 2, 5-diphenyltetrazolium bromide) is a yellow coloured water soluble tetrazolium dye. This is known as the MTT reagent. Mitochondrial enzyme lactate dehydrogenase, produced by metabolically active cells reduces MTT to water-insoluble formazan crystals. When dissolved in appropriate solvent, these formazan crystals exhibit purple colour.

Cytotoxicity Assay

The anticancer activities of samples on HeLa Cells was determined by MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5-diphyhyl tetrazolium Bromide] Assay was used to assess the cytotoxicity (Horiuchi *et al*., 1988). The cells were grown in DMEM medium (Hi Media , Mumbai) supplemented with 10% fetal bovine serum (FBS) (Hi Media, Mumbai), 100 U/ml penicillin and 100 µg/m*l* streptomycin (Hi Media, Mumbai) Cells were incubated in a humidified incubator contain 5% CO_2 at 37 ºC. After 24hrs the cells were seeded in to 96 well The cell culture suspension was washed with 1 X PBS (Phosphate Buffered Saline) and then added with 200 µl MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5-diphyhyl tetrazolium Bromide] solution to the culture flask. It was then incubated at 37°C for 3 hours, removed all MTT solution, washed with 1 X PBS and added with 300 µl DMSO (Dimethyl Sulfoxide) to each culture flask and incubated at room temperature for 30 minutes until all cells get lysed and homogenous color was obtained. The solution was then transferred to centrifuge tube and centrifuged at top speed for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD was measured at 540 nm using DMSO blank. Then the percentage viability and cell death was calculated as per Gunasekera *et al*., (1981) and Akinpelu and Onakoya (2006).

% Viability = [OD of Sample/ OD of Control] X 100

% Cell death = [(OD of Control - OD of Sample) / OD of Control] X 100

The research design of the study is given in Figure 1.

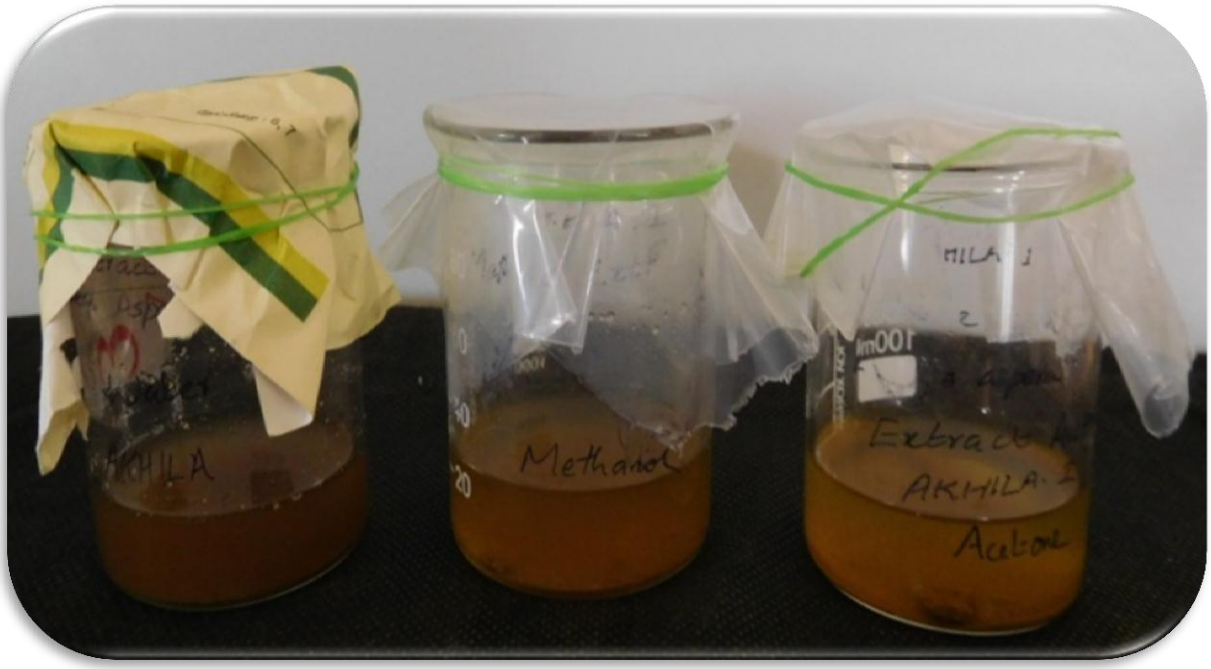


Plate 2a- Aqueous, methanol and acetone extract of *Leucas aspera*

Plate 2b- Aqueous, methanol and acetone extract of *Passiflora edulis*

SELECTION AND AUTHENTICATION OF PLANTS
Leucas aspera (Willd.) Link. and *Passiflora edulis* Sims.

Shade Drying (1week) and Powdering of Leaves

NUTRIENT ANALYSIS (QUANTITATIVE)

Energy, Carbohydrate, Protein, Fat, Moisture, Calcium, Iron, Phosphorus, Vitamin C, β-carotene

PREPARATION OF LEAF EXTRACT

- Aqueous extract
- Methanol extract
- Acetone extract

PHYTOCHEMICAL ASSAY (QUALITATIVE)
Alkaloids, Terpenoids, Phenol and Tannin, Reducing sugar, Saponins, Flavonoids, Quinines, Protein, Steroids

Selection of Best Answering Extract (Methanol Extract of *L.aspera* and *P.edulis*)

ANTIOXIDANT ASSAY : DPPH, FRAP, H_2O_2, Total phenol, Total Flavonoids

Antibacterial Activity

Column chromatography

Thin layer chromatography

Identification of Plant with Best Antioxidant and Antimicrobial Potentials (*Leucas*

GC-MS

ANTICANCER ACTIVITY (Cell death and Cell viability)

FIGURE 1 - RESEARCH DESIGN


RESULTS

IV. RESULT AND DISCUSSION

The results and discussion of the study entitled **"Nutrient Content, Phytochemical, Antioxidant, Antimicrobial, Anticancerous Activity of *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims",** is presented under the following headings:

A. Nutrient Content of the Leaf Powders
B. Phytochemical Components in the Leaf Extracts
C. Antioxidant Activity of the Leaf Extracts
D. Antimicrobial Activity of the Leaf Extracts
E. Bioactive Components in the Leaf Extracts
F. Anticancer Activity of *Leucas aspera* (Willd.) Link.

A. NUTRIENT CONTENT OF THE LEAF POWDERS

The nutrients present in the selected two plants namely *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims. are listed in Table I.

TABLE I

NUTRIENT CONTENT OF *LEUCAS ASPERA* AND *PASSIFLORA EDULIS*

Parameters	*Leucas aspera*	*Passiflora edulis*	*t value*
Energy (kcal)	353.61 ± 5.67	337.04 ± 5.50	16.79**
Carbohydrate (g)	77.8 ± 1.57	64.23 ± 1.98	8.90**
Protein (g)	9.60 ± 1.20	18.32 ± 1.32	7.24**
Fat (g)	0.41 ± 0.15	0.76 ± 0.22	3.67*
Moisture (g)	7.67 ± 0.67	11.81± 0.83	4.81**
Iron (mg)	6.80 ± 0.73	9.32 ± 0.80	4.74**
Calcium (mg)	28.67 ± 2.56	90.0 ± 3.50	34.56**
Phosphorus (mg)	368.0 ± 7.80	552.0 ± 9.73	68.17**
Vitamin C (mg)	62.0 ± 3.56	120.0 ± 5.24	96.29**
β- carotene (µg)	BDL	660.0	-

BDL- Below Detectable Level; ** - Significant at 1% level; * - Significant at 5% level

Passiflora edulis was found to be nutritionally superior to *Leucas aspera* with appreciably higher levels ($p<0.01$) of all nutrients estimated except for energy and carbohydrate.

The mean energy content of *Leucas aspera* and *Passiflora edulis* were found to be 353.61 kcal and 337.04 kcal respectively and the t value 16.79 indicates that it is significant at one per cent level. The mean carbohydrate content of *L.aspera* was 77.8 g, whereas in *P.edulis* it was 64.23 g and the t value 8.90 shows that it is significant at one percent level. The mean content of protein present in the leaves of *L.aspera* was 9.60 g and it was 18.32 g in *P.edulis*. The t value 7.24 indicates that it is significant at one percent level. The amount of fat content was found to be 0.41 g and 0.76 g in *L.aspera* and *P.edulis* respectively and the t value 3.67 indicates that it is significant at five percent level. The mean moisture content was 7.67 g and 11.81 g for *L.aspera* and *P.edulis* respectively and the t value 4.81 indicates that it is significant at one percent level.

The mean iron content was 6.80 mg for *L.aspera* and 9.32 mg for *P.edulis*. The t value 4.74 shows that it is significant at one percent level. The mean calcium content was more in *P.edulis* (90.0 mg) when compared to *L.aspera* (28.67 mg) and the t value 34.56 shows that it is significant at one percent level. The *P.edulis* contains 552.0 mg of phosphorus whereas *L.aspera* contains 368.0 mg of phosphorus. The t value 68.17 indicates that it is significant at one percent level. The mean vitamin C content in *L.aspera* was 62.0 mg and 120.0 mg in *P.edulis* and the t value 96.29 shows that it is significant at one percent level. The presence of β-carotene was below detectable level in *L.aspera* and it was 660.0µg in *P.edulis*.

Comparison of nutrient content in *Leucas aspera* (Willd.) Link. and *Passiflora edulis* with the nutrient value of some of the popular green leafy vegetables such as *Sebania grandiflora* (Agathi), *Brassica olerace* (Cabbage), *Trigonella foenum graecum* (Fenu greek leaves), *Solanum nigrum* (Manathakali leaves) and *Spinacia oleracea* (Spinach) (Gopalan *et al*., 2010) is presented in Table II and Figure 2a to 2j.

TABLE II

COMPARISON OF NUTRIENT CONTENT OF *LEUCAS ASPERA AND PASSIFLORA EDULIS* WITH OTHER GREEN LEAFY VEGETABLES

Parameters	***L.aspera***	***P.edulis***	***S.grandiflora***	***B.oleracea***	***T.foenum graecum***	***S.nigrum***	***S.oleracea***
Energy (kcal)	353.61	337.04	93.0	27.0	49.0	68.0	26.0
Carbohydrate (g)	77.8	64.23	11.8	4.6	6.0	8.9	2.9
Protein (g)	9.60	18.32	8.4	1.8	4.4	5.9	2.0
Fat (g)	0.41	0.76	1.4	0.1	0.9	1.0	0.7
Moisture (g)	7.67	11.81	73.1	91.9	86.1	82.1	92.1
Iron (mg)	6.80	9.32	3.9	0.8	1.93	20.5	1.14
Calcium (mg)	28.67	90.0	1130.0	39.0	395.0	410.0	73.0
Phosphorus (mg)	368.0	552.0	80.0	44.0	51.0	70.0	21.0
Vitamin C (mg)	62.0	120.0	169.0	124.0	52.0	11.0	28.0
β- carotene (µg)	BDL	660.0	5400.0	120.0	2340.0	-	5580.0

BDL- Below Detectable Level

Comparison of the nutrient content of *L.aspera* and *P.edulis* with that of nutrient rich green leafy vegetable such as *Sesbania grandiflora* (Agathi) showed that the energy, carbohydrate and protein content was higher in both the plants than Agathi (Energy- 93kcal; Carbohydrate- 11.8g; Protein- 8.4g). The fat and moisture content in *L.aspera* and *P.edulis* leaves was less than that of agathi leaves (Fat- 1.4g; Moisture- 73.1g). The iron and phosphorus content was higher in *L.aspera* and *P.edulis* than agathi leaves (Iron-3.9mg; Phosphorus- 80mg). The calcium content in agathi leaves (1130mg) was higher than in two plants *L.aspera* and

P.edulis. The vitamin C (169mg) and β- carotene (5400µg) content in agathi leaves was higher than that of *L.aspera* and *P.edulis*.

The energy, carbohydrate, protein, fat, iron and phosphorus content was higher in *L.aspera* and *P.edulis* than in *Brassica olerace* (Cabbage) (energy- 27 kcal; carbohydrate- 4.6 g; protein- 1.8 g; fat- 0.1 g; iron- 0.8 mg; phosphorus- 44.0 mg; vitamin C- 124.0 mg) and the moisture content (91.9 g) was found to be higher in cabbage. The calcium content was found to be higher than *L.aspera* and less than *P.edulis* than in cabbage (39.0 mg). The β- carotene content (120.0 µg) was less in cabbage than *P.edulis*.

Comparison with nutrient content of *L.aspera* and *P.edulis* with that of *Trigonella foenum graecum* (Fenugreek leaves) showed that the energy (49 kcal), carbohydrate (6.0 g), protein (4.4 g), iron (1.93 mg), phosphorus (51.0 mg), vitamin C (52.0 mg) was less in fenugreek leaves than *L.aspera* and *P.edulis*, whereas the fat (0.9 g) moisture (86.1 g) and calcium (395.0 mg) content was higher in fenugreek leaves. The β-carotene content (2340.0 µg) was high in fenugreek leaves than in *L.aspera* and *P.edulis*.

In *Solanum nigrum* (Manathakali leaves) the energy (68.0 kcal), carbohydrate (8.9 g), protein (5.9 g), phosphorus (70.0 mg) and vitamin C (11.0 mg) content was less than *L.aspera* and *P.edulis* and the fat (1.0 g), moisture (82.1 g), iron (20.5 mg) and calcium (410 mg) content was higher than *L.aspera* and *P.edulis*. The β-carotene content was absent in manathakkali.

The presence of energy (26 kcal), carbohydrate (2.9 g), protein (2.0 g), iron (1.14 mg), phosphorus (21.0 mg) and vitamin C (28 mg) was less in *Spinacia oleracea* (Spinach) than *L.aspera* and *P.edulis*, whereas the moisture (92.1 g) content was found to be higher in spinach. The fat (0.7 g) content in spinach was higher than *L.aspera* (0.41 g) and slightly less than *P.edulis* (0.76 g). The calcium content present in spinach was 73.0 mg which was higher than the calcium content in *L.aspera* (28.67 mg) and less than *P.edulis* (90.0 mg). The β-carotene content (5580.0 µg) was high in spinach than *L.aspera* and *P.edulis*.

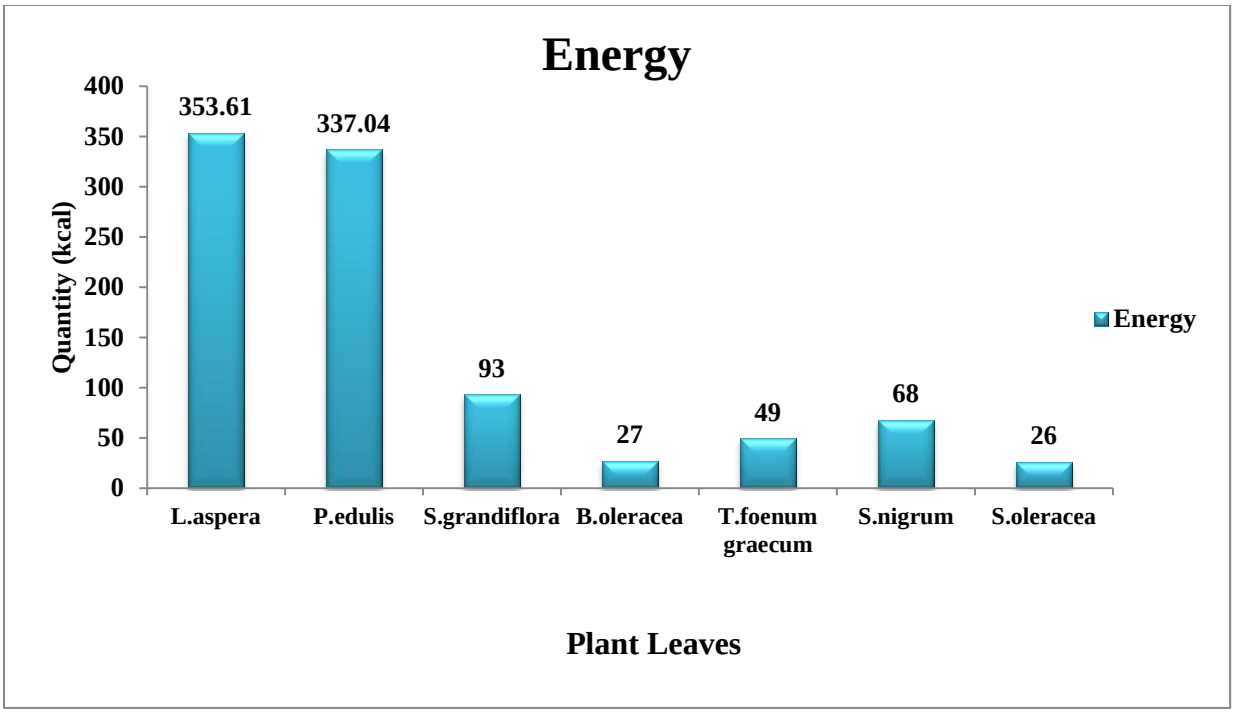


Figure 2a

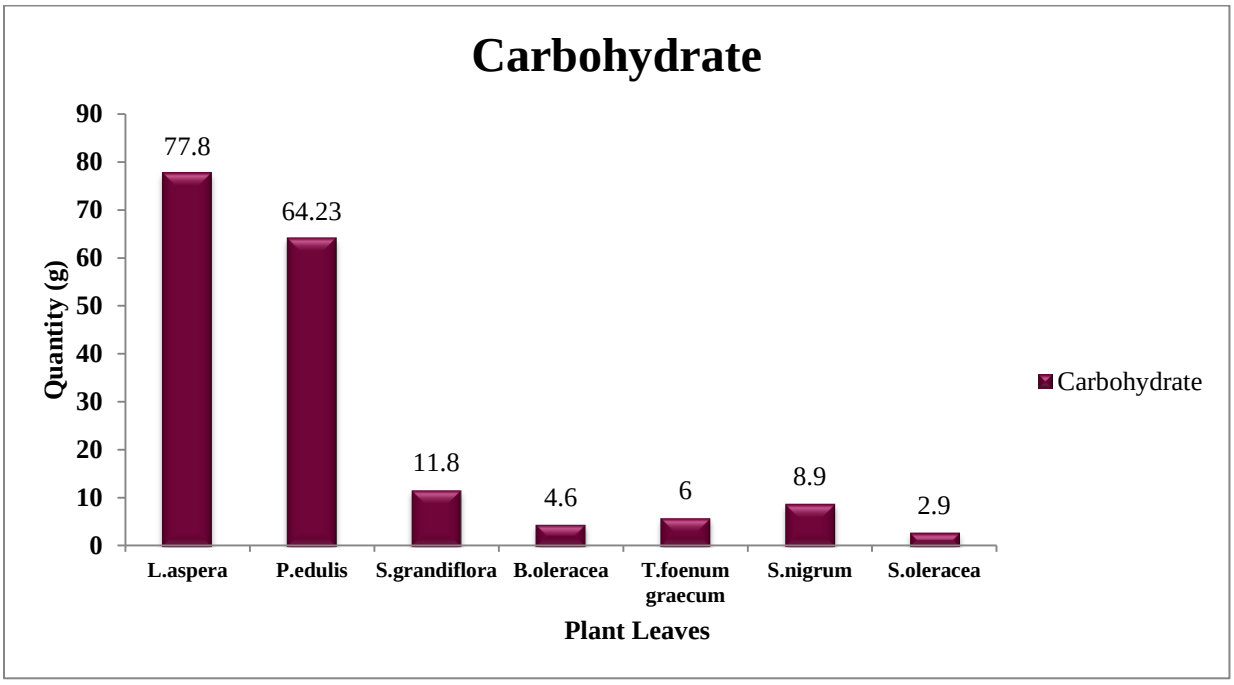


Figure 2b

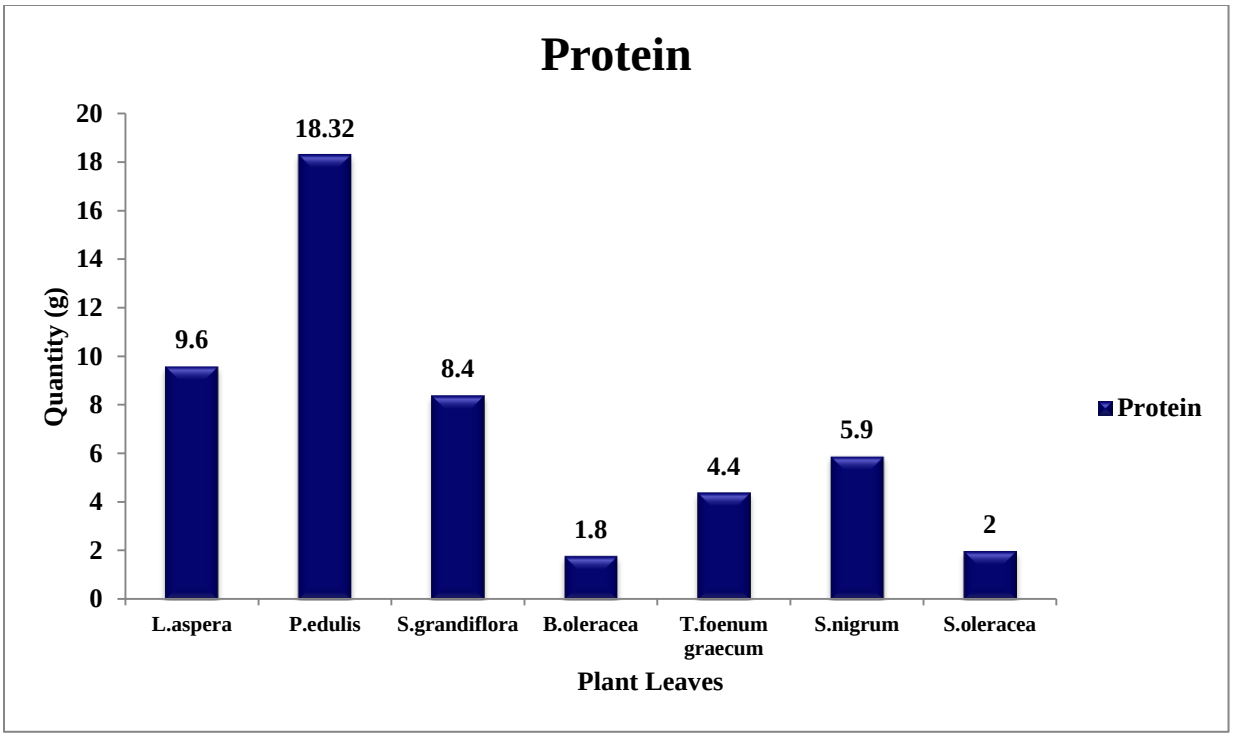


Figure 2c

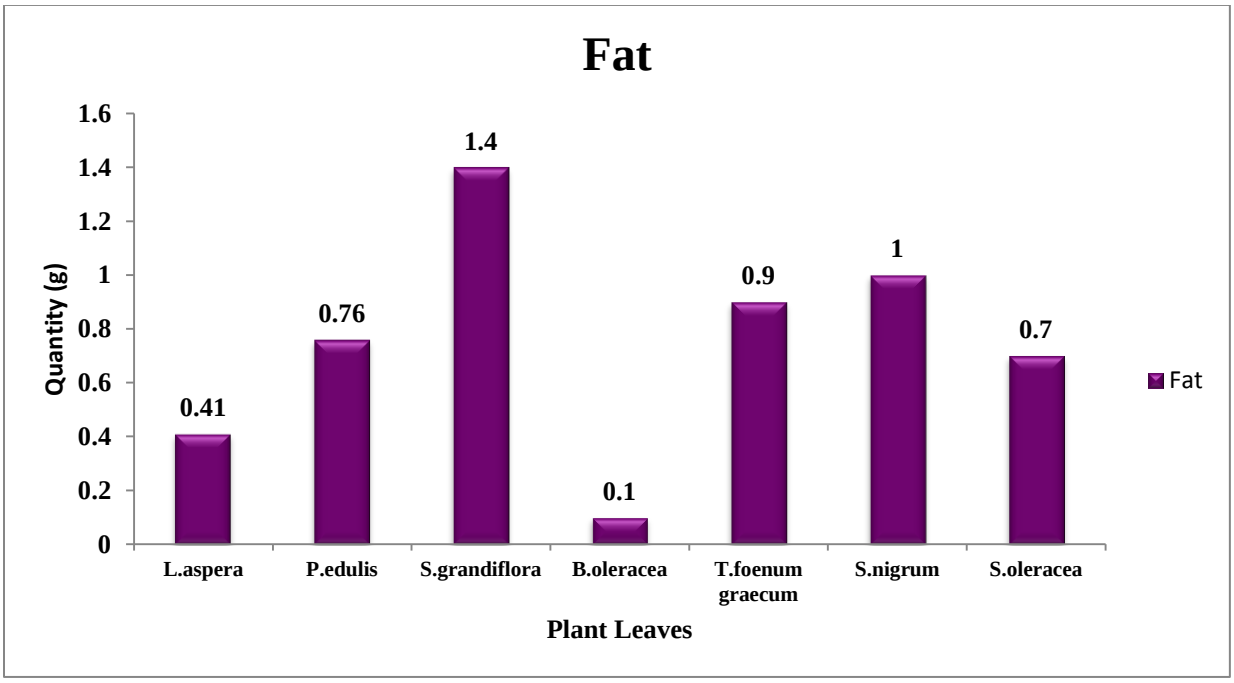


Figure 2d

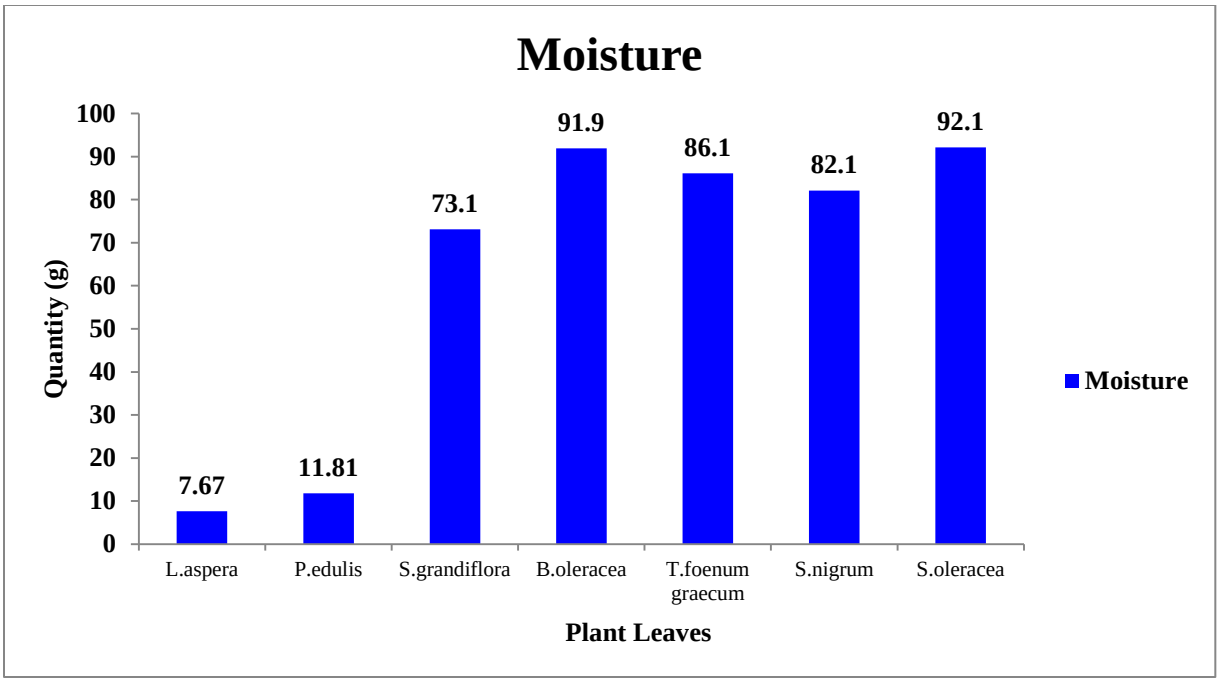


Figure 2e

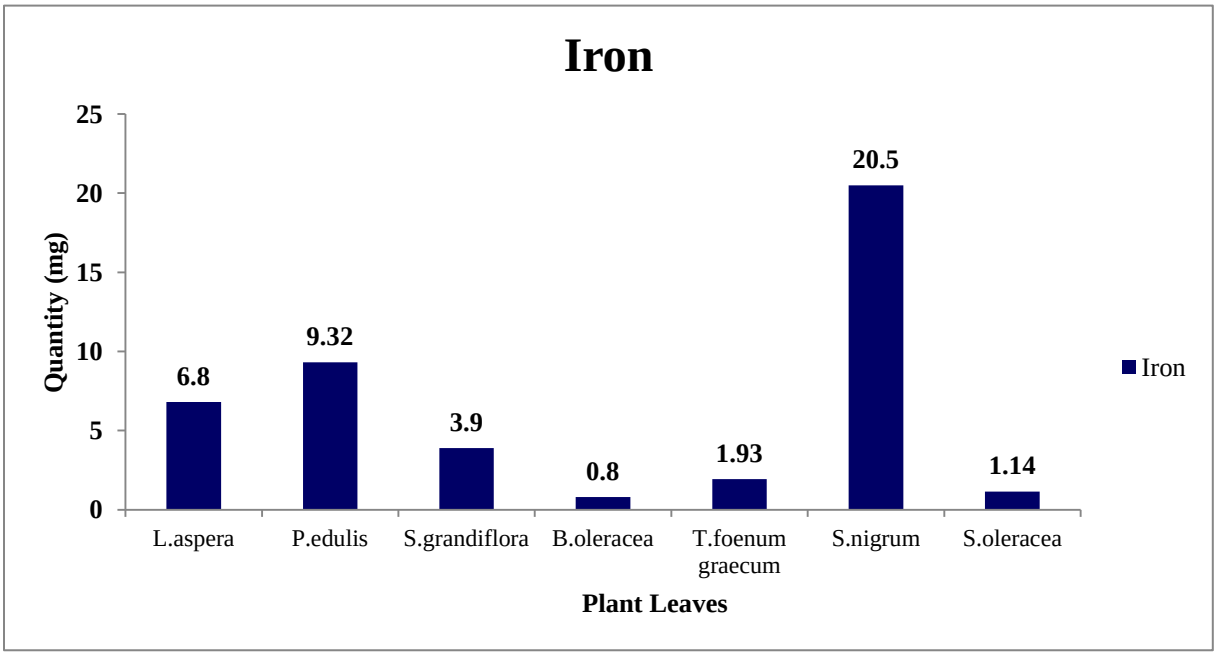


Figure 2f

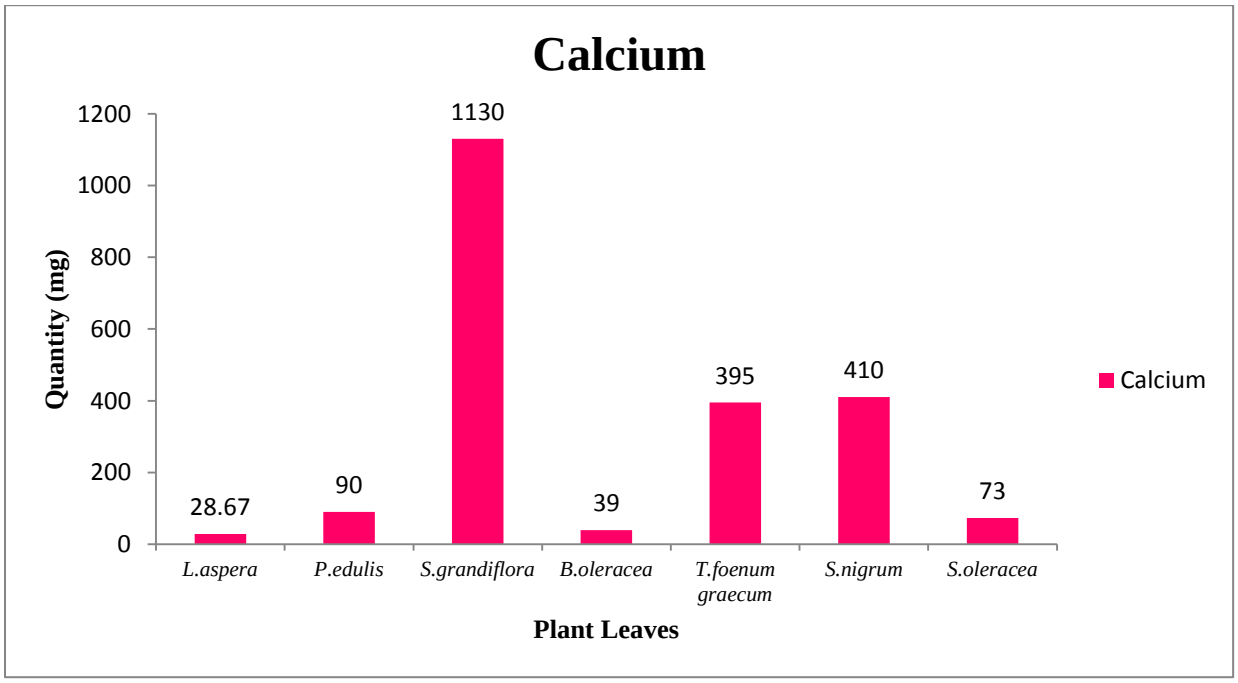


Figure 2g

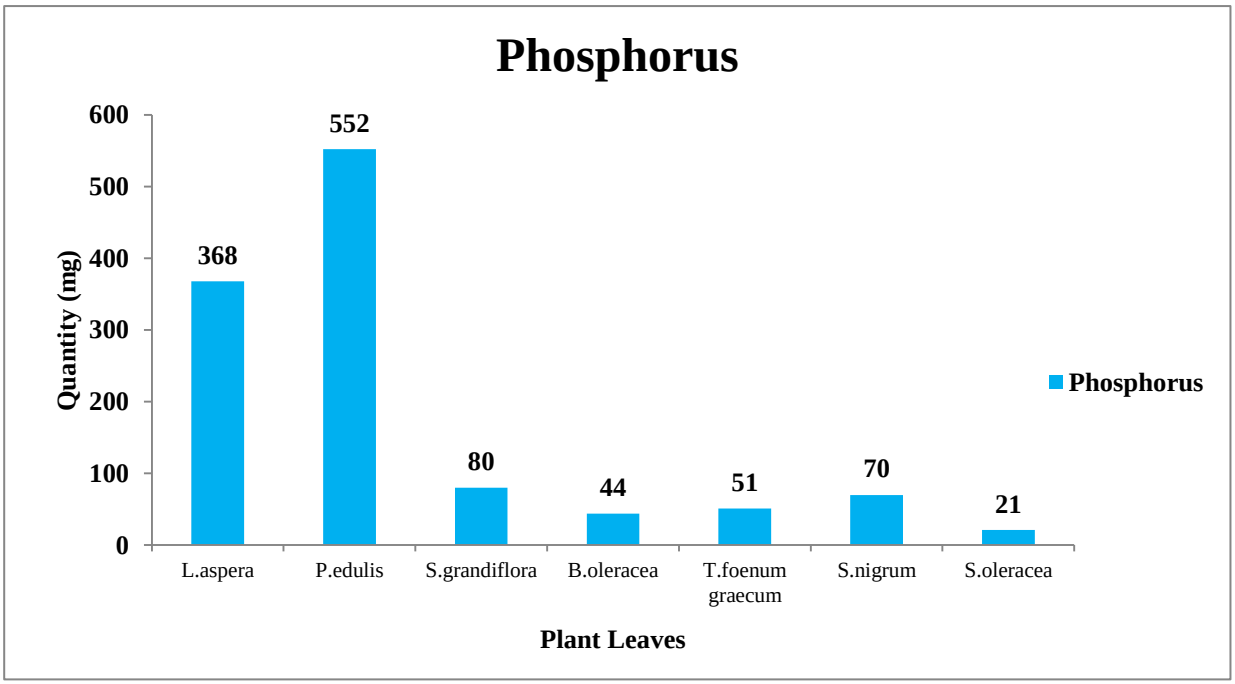


Figure 2h

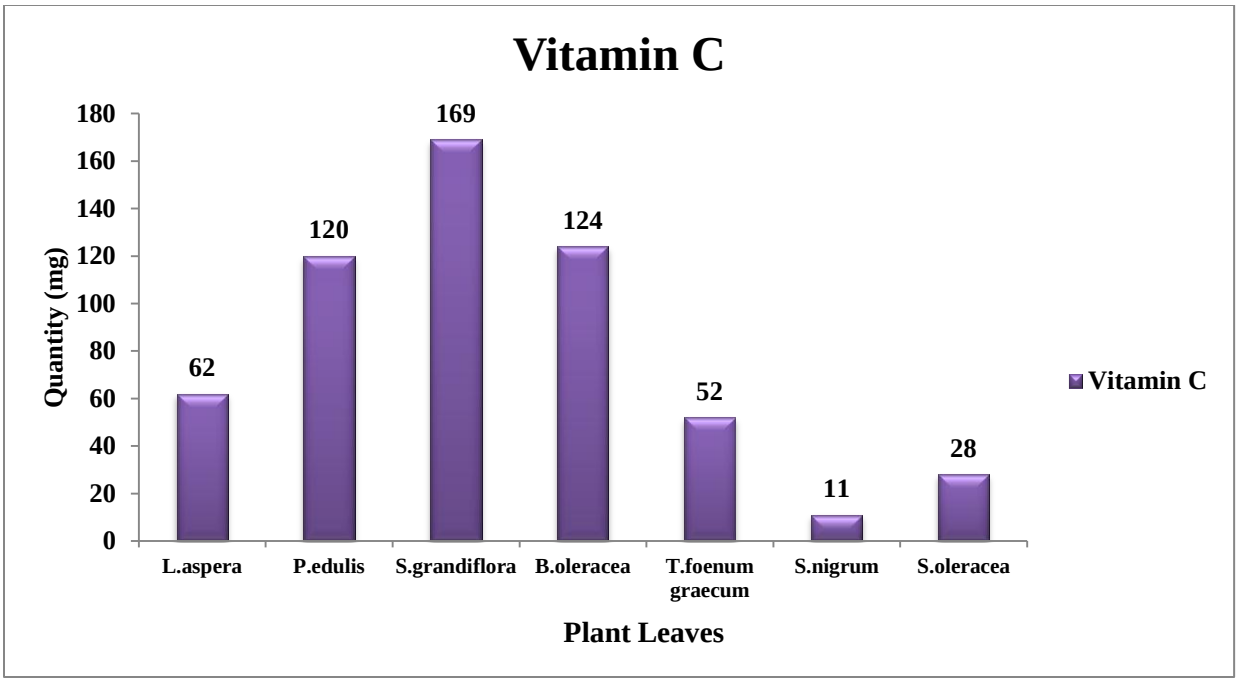


Figure 2i

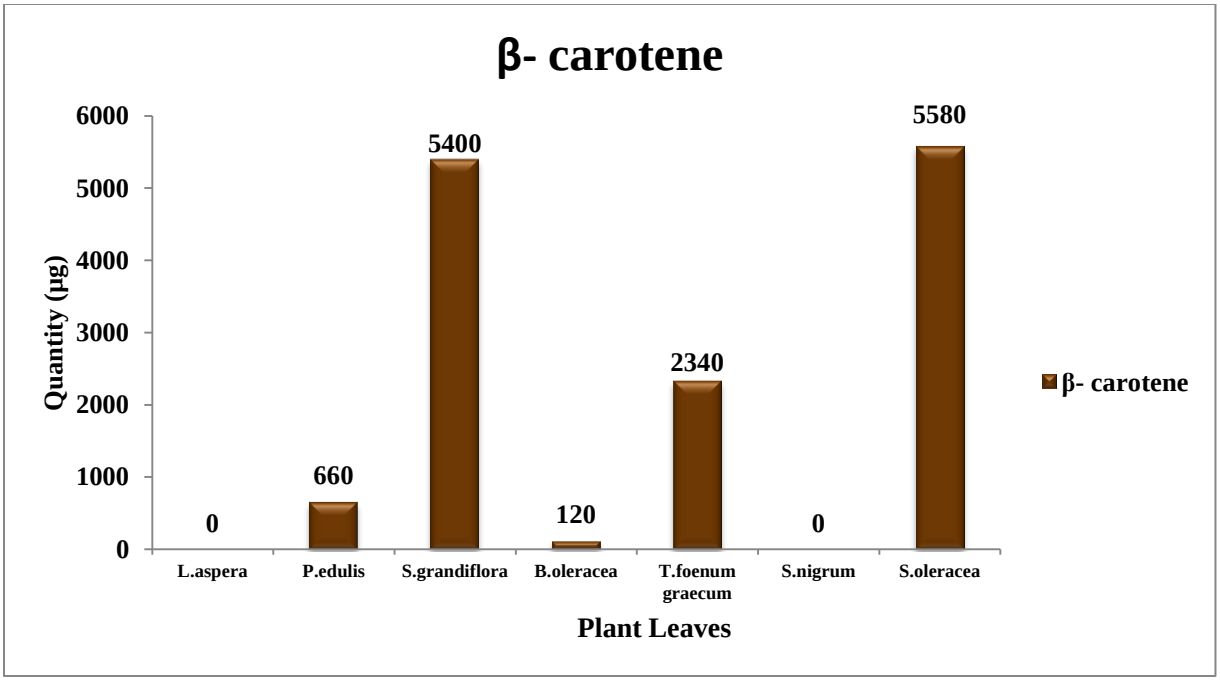


Figure 2j

B. PHYTOCHEMICAL COMPONENTS IN THE LEAF EXTRACTS

1. *Leucas aspera* (Willd.) Link.

The phytochemical components present in *Leucas aspera* (Willd.) Link. are presented in detail in Table III.

TABLE III

PHYTOCHEMICAL STATUS OF *LEUCAS ASPERA*

Phytochemicals	Inference	Extracts		
		Acetone	Methanol	Aqueous
Alkaloids	Formation of yellow colour	+	+	+
Terpenoids	Formation of grayish colour	+	+	+
Phenol and tannins	Formation of blue green/ black colour	+	+	+
Reducing sugar	Formation of red colour	-	-	-
Saponins	Foam formation	-	+	+
Flavonoids	Formation of pink scarlet colour	-	-	-
Quinines	Formation of blue green/ red colour	-	-	-
Protein	Formation of yellow colour	+	+	+
Steroids	Formation of red colour residue	+	+	+

Key: + Positive, - Negative

Phytochemicals are the individual chemicals from which the plants are made (Raaman, 2006). The phytochemical screening of *Leucas aspera* was carried out in acetone, methanol and aqueous extracts. The results revealed the presence of alkaloids, terpenoids, phenols, tannins, protein and steroids in acetone extract. The methanol and aqueous extract showed the presence of alkaloids, terpenoids, phenols, tannins, saponins, protein and steroids. Reducing sugars, saponins, flavonoids and quinines were absent in acetone extract whereas in methanol and aqueous extracts reducing sugars, flavonoids and quinines were found to be absent.

Among the three different extracts the more number of phytochemical compounds were present in methanol and aqueous extract than acetone extract. So from the results methanol extract was selected as the best extract for further analysis.

Plate 3a, 3b and 3c shows the identification of phytochemicals in acetone, methanol and aqueous extracts of *Leucas asprea* respectively.

The preliminary phytochemical screening of the extract of *L.aspera* revealed the presence of various bioactive components of which alkaloid, cardiac glycosides, terpenoids, and tannins were the most prominent in the study by Das *et al*., (2011).The results indicated the presence of phytochemicals in the order of ethanol> ethyl acetate> n-hexane extract. This data shows the higher yield of phytochemicals in ethanol extract.

Rumaisa *et al*., (2013) report the presence of tannins, proteins, steroids, glycosides, carbohydrates, saponins, flavonoids and alkaloids in chloroform and aqueous ethanol extracts of *L.aspera*. They opine that these chemical ingredients may be responsible for the various pharmacological actions such as antibacterial, antiulcer, anticancer, larvicidal and chemo protective activities although their specific roles remain to be investigated.

Similar findings have been reported by Rahman and Islam (2013) that the ethanol extract of *L.aspera* showed the presence of alkaloids, flavonoids, terpenoids, steroids, tannins, phlobatannins, saponins and glycosides.

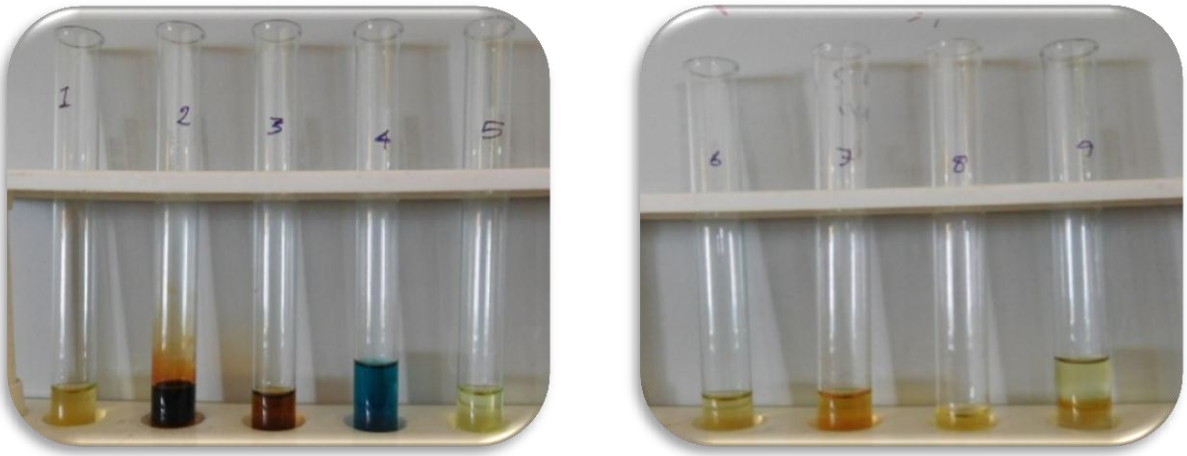


Plate 3a- Phytochemicals Identification in Acetone Extract of *Leucas aspera*

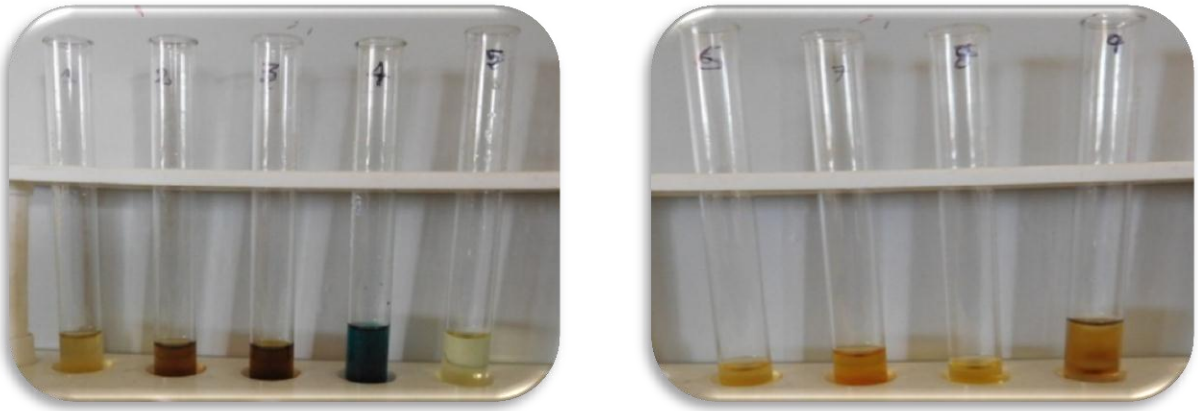


Plate 3b- Phytochemicals Identification in Methanol Extract of *Leucas*

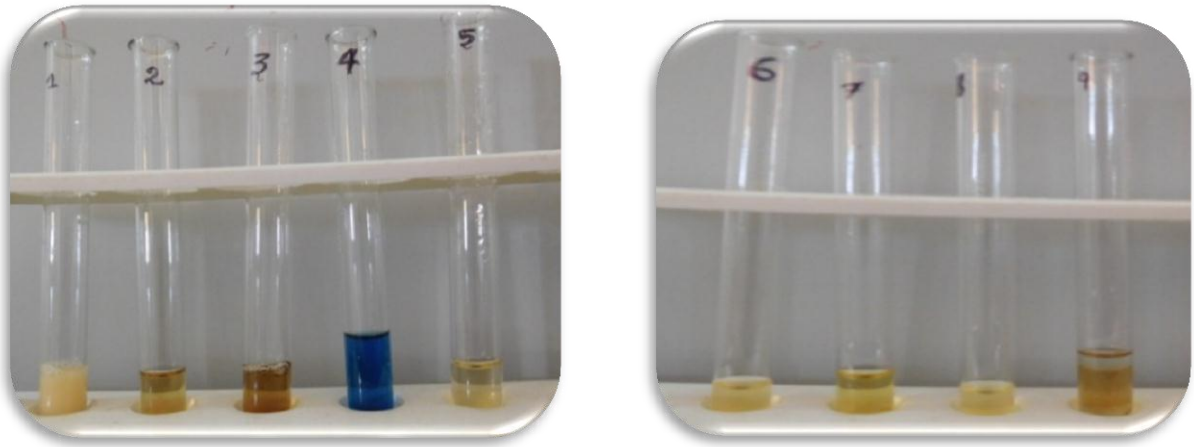


Plate 3c- Phytochemicals Identification in Aqueous Extract of *Leucas*

2. *Passiflora edulis* Sims.

Table IV presents the phytochemical status of the *Passiflora edulis* Sims.

TABLE IV

PHYTOCHEMICAL STATUS OF *PASSIFLORA EDULIS*

Phytochemicals	Inference	Extracts		
		Acetone	Methanol	Aqueous
Alkaloids	Formation of yellow colour	+	+	+
Terpenoids	Formation of grayish colour	+	+	+
Phenol and tannins	Formation of blue green/ black colour	+	+	-
Reducing sugar	Formation of red colour	-	-	-
Saponins	Foam formation	-	-	-
Flavonoids	Formation of pink scarlet colour	-	-	-
Quinines	Formation of blue green/ red colour	+	+	-
Protein	Formation of yellow colour	+	+	+
Steroids	Formation of red colour residue	+	+	+

Key: + Positive, - Negative

The phytochemical screening of *Passiflora edulis* in acetone, methanol and aqueous extracts, revealed the presence of alkaloids, terpenoids, phenols, tannins, quinines, protein and steroids in acetone extract as well as in methanol extract. The aqueous extract showed the presence of alkaloids, terpenoids, protein and steroids. Reducing sugars, saponins, flavonoids were absent in acetone as well as in methanol extract. In aqueous extracts phenols, tannins, reducing sugars, saponins, flavonoids and quinines were found to be absent.

Among the three different extracts the more number of phytochemical compounds were present in methanol and acetone extract than aqueous extract. So from the results methanol extract was selected as the best extract for further analysis.

Plate 4a, 4b and 4c shows the identification of phytochemicals in acetone, methanol and aqueous extracts of *Passiflora edulis* respectively.

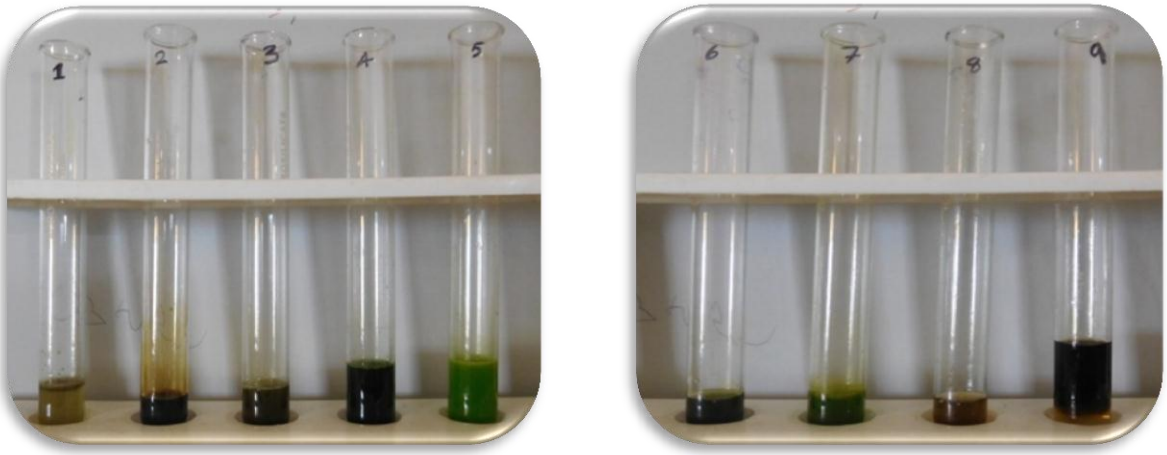


Plate 4a- Phytochemicals Identification in Acetone Extract of *Passiflora*

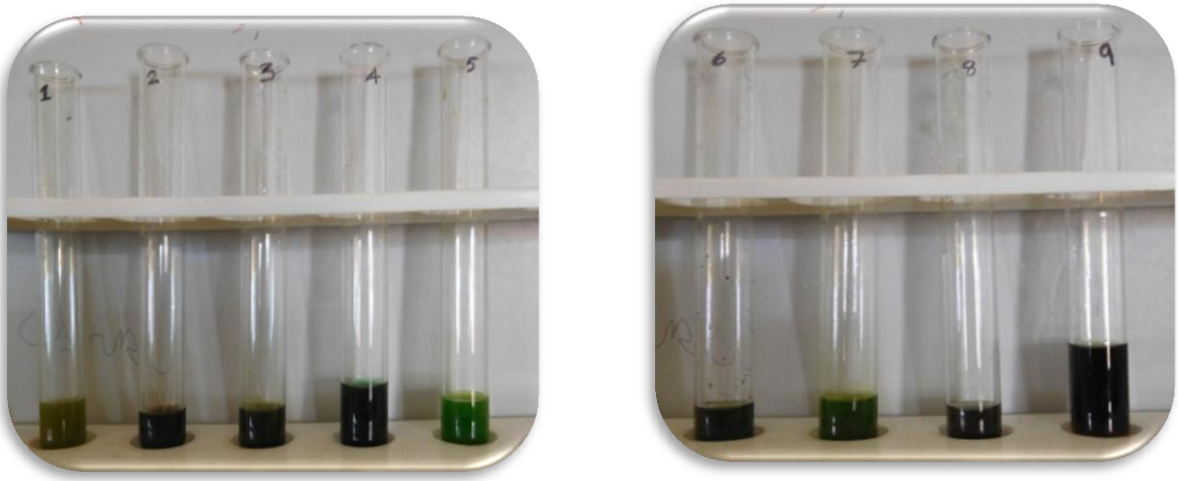


Plate 4b- Phytochemicals Identification in Methanol Extract of *Passiflora*

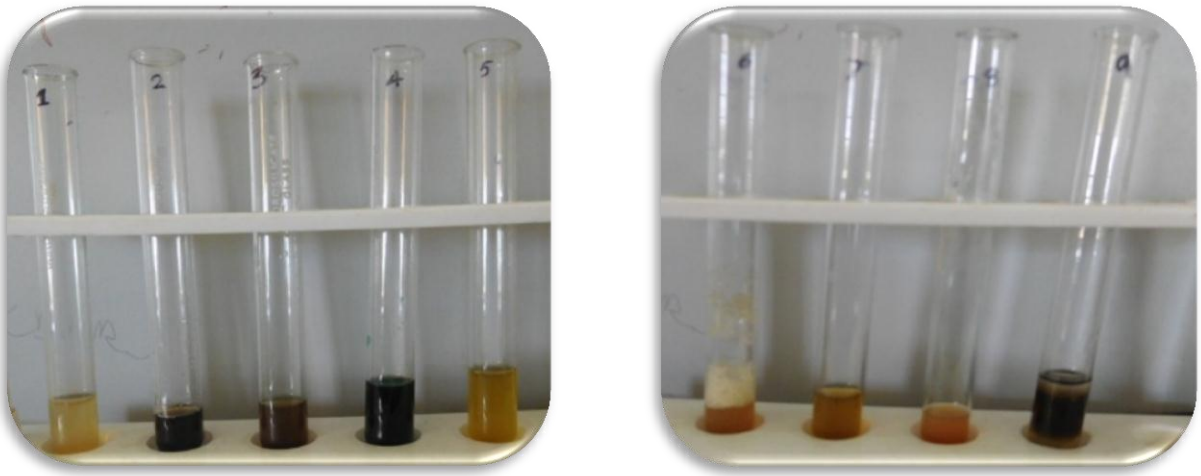


Plate 4c- Phytochemicals Identification in Aqueous Extract of *Passiflora*

Akanbi *et al*., (2011) revealed the presence of phytochemicals in the leaf, stem and fruit extracts (hexane, methanol, ethyl acetate and water) of *Passiflora edulis*. The results showed the presence of carbohydrates, glycosides, flavonoids, alkaloids, phenols and resin and balsams in leaf, stem and fruit extracts. Saponin and volatile oil are present only in leaf and fruit part, and tannin present only in the leaf and fruit parts of the plant. The test for terpenes gave negative result for all the plant parts examined.

C. ANTIOXIDANT ACTIVITY OF THE LEAF EXTRACTS

The antioxidant activity of *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims. are given in Table V and Figure 3.

TABLE V

ANTIOXIDANT ACTIVITY (%) OF *LEUCAS ASPERA* AND *PASSIFLORA EDULIS*

Antioxidant tests	*Leucas aspera* (Methanol extract)	*Passiflora edulis* (Methanol extract)	***t value***
DPPH	97.89 ± 2.46	74.12 ± 1.90	42.36**
Total phenols	33.05 ± 1.89	12.85 ± 0.76	51.19**
Total flavonoids	99.11 ± 4.56	96.93 ± 3.84	1.43^{NS}
FRAP	38.82 ± 1.69	13.78 ± 1.17	34.15**
H_2O_2	82.29 ± 2.40	11.48 ± 0.87	124.78**

** - Significant at 1% level; * - Significant at 5% level

1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

Plant leaves of *L.aspera* exhibit 97.89 per cent DPPH radical scavenging activity. Leaves of *P.edulis* have less DPPH radical scavenging activity (74.12 per cent). Thus *L.aspera* exhibits significantly higher DPPH radical scavenging activity ($p < 0.01$) than *P.edulis* and hence better antioxidant avtivity than *Passiflora edulis*.

According to Ali *et al*., (2013) the DPPH radical scavenging activity of *Leucas aspera* was found to increase with increasing concentration of the extract. This assay was based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) to decolorize in the presence of antioxidants.

The sample extract displayed significant reducing power which was found to increase with the increasing concentration.

The results obtained in the study by Chew *et al*., (2012) showed that root extract of *L.aspera* exhibited the greatest free radical scavenging activity among the plant extracts with a mean value of 32.36±1.19 per cent. On the other hand, the scavenging activity of flower, leaf and stem extracts was 26.39±0.07 per cent, 17.04±0.82 per cent and 13.42±0.56 per cent, respectively. The scavenging activity of these extracts was less compared with both antioxidants, BHT (65.67±0.58 %) and vitamin E (41.67±0.58 %).

In the study by Ripa *et al*., (2009), all the four extracts from the leaves of *P. edulis* exhibited potential antioxidant activity. The chloroform extract of stem scavenged 50 per cent DPPH free radical at the lowest inhibitory concentration (IC50: 51.28µg/ml). The petroleum ether extract of stem also revealed strong antioxidant activity (IC50: 54.01µg/ml). On the other hand, petroleum ether and chloroform extracts of leaf showed antioxidant activity with IC50 of 58.88µg/ml and 56.85µg/ml respectively. These results denote the presence of antioxidant principles in the extractives.

Total Phenol

Phenols are very important plant constituents. There is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups. The phenolic contents of the extract can also scavenge hydrogen peroxide by donating electrons and thereby neutralizing it to water. It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidants. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing super oxide anion (O2 -•), hydroxyl radical or peroxy radicals, quenching singlet and triplet oxygen or decomposing peroxides. Polyphenolic contents appear to function as good electron and hydrogen atom donors, and therefore, be able to terminate radical chain reaction involved in lipid peroxidation by converting free radicals and reactive oxygen species to more stable products. Thus, the antioxidant activity of *L.aspera*

extracts might be attributed to these modes of activity due to their alkaloids, phytosterols and flavonoid contents (Rumaisa *et al*., 2013).

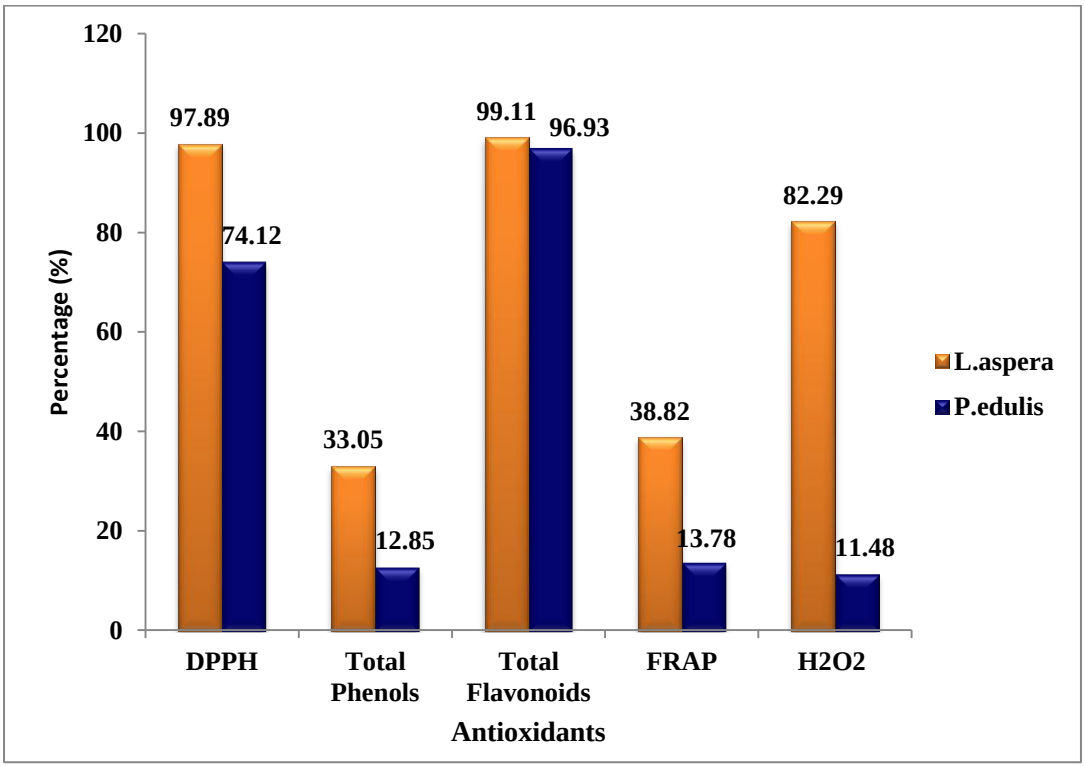


FIGURE 3

ANTIOXIDANT ACTIVITY (%) OF *LEUCAS ASPERA* AND *PASSIFLORA EDULIS*

Phenolic compounds are commonly found in both edible and inedible plants and plant parts. They have been reported to have multiple biological effects, including antioxidant activity. The content of phenolic compounds (mg/100g DW) in ethanolic, ethyl acetate and n-hexane extract was determined from regression equation of calibration curve (y = 0.0138x +0.1276, R2 = 0.9881) and expressed in gallic acid equivalents (GAE). Among the three extracts, the total phenolic contents of *L. aspera* ethanol extract was found to be promising with a value of 15.36 ± 0.512 GAE/g dry weight of extract (Das *et al*., 2011).

The total phenolic content in the ethanolic and aqueous extracts of *P.edulis* leaf showed the presence of highest concentration of phenolic compounds, 429 and 368 mg GAE/g extract respectively (Osma *et al*., 2013).

In the present study, the plant leaves of *L.aspera* had a total phenol content of 33.05 per cent, whereas leaves of *P.edulis* possessed 12.85 per cent total phenolic content. Hence it is clear that *L.aspera* had significantly higher ($p< 0.01$) phenolic content than *P.edulis*.

Total Flavonoids

As observed in the present study, the plant leaves of *L.aspera* contained 99.11 per cent total flavonoids and leaves of *P.edulis* had 96.93 per cent of total flavonoids. It is clear that both the plants have comparably high level of flavonoids content. Though flavonoids in *L.aspera* was slightly higher than in *P.edulis*, the difference was not statistically significant.

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties. Bhaigyabati *et al*., (2014), report total flavonoid content in the aqueous rhizome extract of *Hedychium rubrum, H. coronarium* and *H. spicatum* was found to be 3.22, 2.77 and 0.74 µg QE/100g of the extract respectively.

Ferric ion Reducing Ability of Plasma (FRAP - as a measure of antioxidant power) Assay

The plant leaves of *L.aspera* showed 38.82 per cent Ferric Ion Reducing Antioxidant Power. Leaves of *P.edulis* showed a significantly lower ($p< 0.01$) level of 13.78 per cent Ferric Ion Reducing Antioxidant Power.

Studies by Kaushik *et al*., (2012) show that the FRAP method depends upon the reduction of ferric tripyridyltriazine [Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II) - TPTZ) by a reductant at low pH. Fe (II)-TPTZ has an intensive blue color and can be monitored at 593 nm. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing range with antioxidant mixtures. The free radical scavenging power of different extracts of *Diplazium esculentum* leaves increased with an increasing amount of extract. Among all the extracts (petroleum ether, chloroform, acetone, methanol, and aqueous) aqueous extract was showing maximum antioxidant power (7.6 mM/dry wt. of extract) but methanol and acetone extracts were having lesser extent of free

radical scavenging power (4.16, 3.8 mM/dry wt. of extract, respectively). Petroleum ether and chloroform extracts were containing FRAP power in very less amount (0.47, 0.229 mM/dry wt. of extract, respectively). The range of antioxidant power lies between 0.229 to 7.6 mM/dry wt. of extract.

Hydrogen Peroxide Scavenging Capacity

According to Keser *et al*., (2012) extracts *Crataegus monogyna* were capable of scavenging hydrogen peroxide in an amount dependent manner. 100 µg of water and ethanol extracts of *C. monogyna* exhibited 15.44-30.13 per cent scavenging activity on hydrogen peroxide. On the other hand, using the same amounts, BHA and α- tocopherol exhibited 44.58 per cent and 39.26 per cent hydrogen peroxide scavenging activity. Based on their findings the authors opine that though hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells. Thus, the removing of H_2O_2 is very important for antioxidant defense in cell or food systems.

In the present study the plant leaves of *L.aspera* towered above ($p< 0.01$) those of *P.edulis* in terms of hydrogen peroxide scavenging activity (82.29 and 11.48 per cent respectively). This finding brings out the antioxidant potential of *L.aspera* in terms of H_2O_2 scavenging.

D. ANTIMICROBIAL ACTIVITY OF THE LEAF EXTRACTS

The antimicrobial activity (Zone of inhibition) of methanol extract of *L.aspera* and *P.edulis* were given in Table VI and Figure 4 and Plate 5a, 5b, 5c and 5d shows the zone of inhibition developed in *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* for the methanolic extracts of *Leucas aspera* and *Passiflora edulis*, methanol, distilled water and Tobramycin disc respectively.

TABLE VI

ANTIMICROBIAL ACTIVITY (ZONE OF INHIBITION in mm) OF *LEUCAS ASPERA* AND *PASSIFLORA EDULIS*

Bacteria	Distilled water	*L.aspera* (Methanol extract)	*P.edulis* (Methanol extract)	Methanol	Disc (Tobramycin)
Bacillus subtilis	Nil	3	2	1	9
Klebsiella pneumonia	Nil	12	Nil	9	9
Pseudomonas aeruginosa	Nil	4	3	Nil	8
Escherichia coli	Nil	10	7	10	8

In the present study, the zone of inhibition produced by the disc Tobramycin of 10mcg (standard) was found to be 9mm each for *Bacillus subtilis* and *Klebsiella pneumonia*. The zone of inhibition was 8mm each for *Pseudomonas aeruginosa* and *Escherichia coli*. In methanol the zone of inhibition developed was 1mm, 9mm, and 10mm against *Bacillus subtilis*, *Klebsiella pneumonia* and *Escherichia coli* respectively and against *Pseudomonas aeruginosa* methanol did not show zone of inhibition. The zone of inhibition produced by the methanolic extract of *L.aspera* was 3mm, 12mm, 4mm and 10mm against *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* respectively. In the methanolic extract of *P.edulis* the zone of inhibition developed was 2mm, 3mm and 7mm against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* respectively and against *Klebsiella pneumonia* the methanolic extract of *P.edulis* did not exhibit any zone of inhibition. Distilled water did not produce any zone of inhibition against any of the microorganisms.

The zone of inhibition produced by the methanolic extract of *L.aspera* (3mm) against *Bacillus subtilis* was found to be less than that of standard disc (9mm) and it was higher than the positive control methanol (1mm). For methanolic extract of *P.edulis* the zone of inhibition developed was 2mm against *Bacillus subtilis* which was found to be less than standard disc (9mm) and higher than that of positive control methanol (1mm).

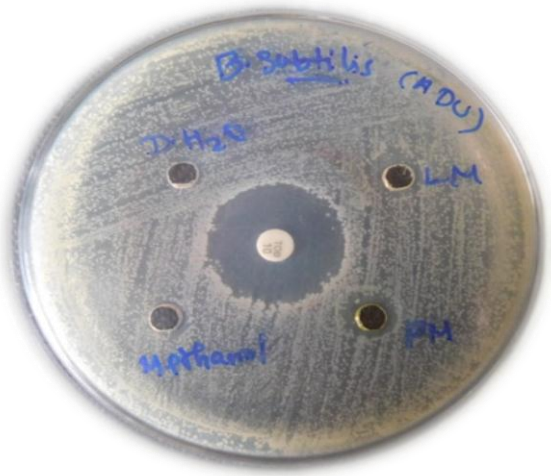


Plate 5a - Zone of inhibition developed against *Bacillus subtilis*.

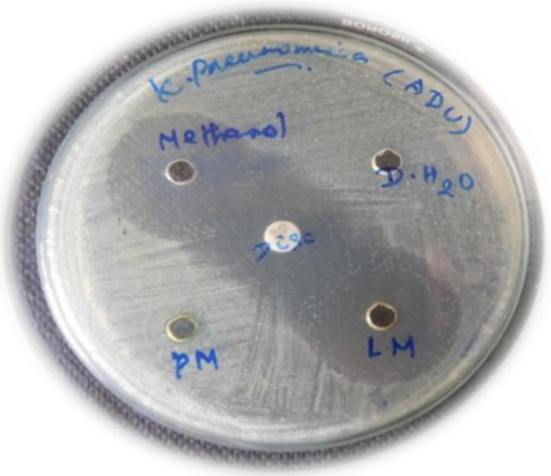


Plate 5b - Zone of inhibition developed against *Klebsiella pneumonia*.

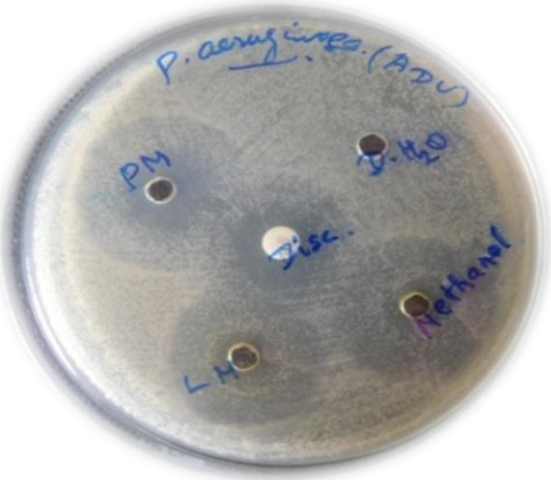


Plate 5c - Zone of inhibition developed against *Pseudomanas aeruginosa*.

Plate 5d - Zone of inhibition developed against *Escherichia coli*.

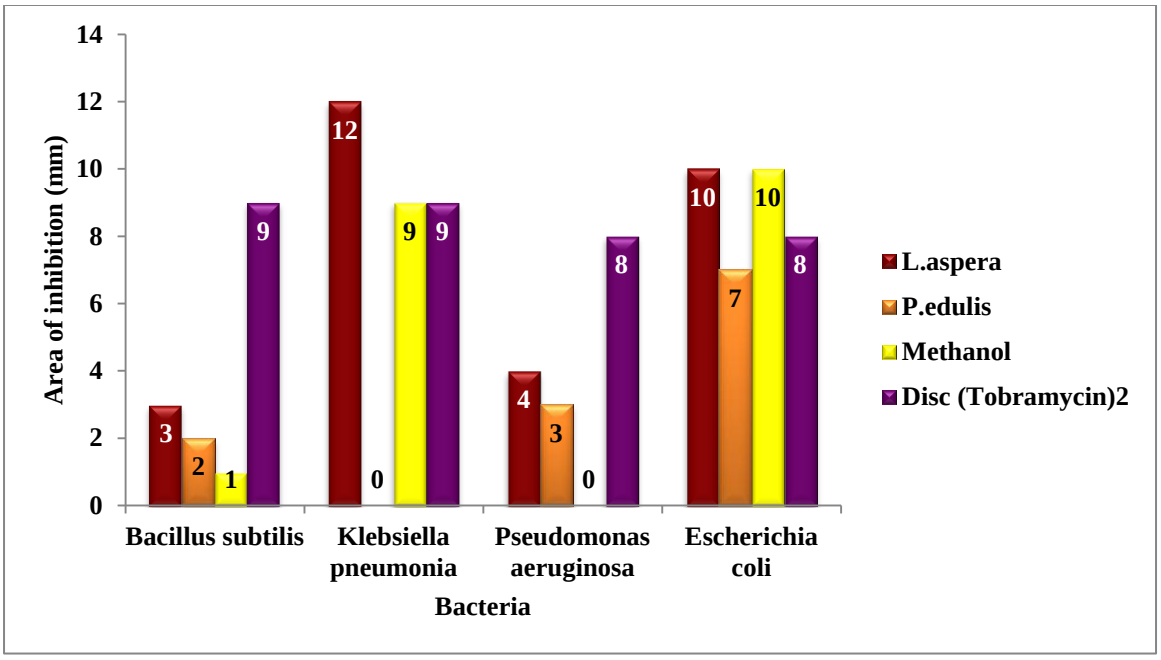


FIGURE 4

ANTIMICROBIAL ACTIVITY OF *LEUCAS ASPERA* AND *PASSIFLORA EDULIS*

The methanolic extract of *L.aspera* exhibited zone of inhibition (12mm) against *Klebsiella pneumonia* and it was higher than standard disc (9mm) and positive control methanol (9mm). The methanolic extract of *P.edulis* did not exhibit zone inhibition against this bacteria.

The zone of inhibition of methanolic extract of *L.aspera* was 4mm against *Pseudomonas aeruginosa* and it was less than standard disc (8mm) and higher than positive control methanol. The zone of inhibition was 3mm for the methanolic extract of *P.edulis*, which was less than standard disc (8mm) and greater than positive control methanol (Nil).

For *Escherichia coli* the zone of inhibition was 10mm for methanolic extract of *L.aspera* and it was greater than standard disc (8mm) and similar to that of positive control methanol (10mm). The methanolic of *P.edulis* exhibited 7mm zone of inhibition against the same bacteria and which was less than standard disc (8mm) and positive control methanol (10mm).

When examining the activity of both plant extracts against the four microorganisms the methanolic extract of *Leucas aspera* showed highest activity against *Klebsiella pneumonia* and

Escherichia coli, and the inhibition potential was higher than the standard. Antimicrobial activity of *Passiflora edulis* was found to be low except against *Escherichia coli*, in which the activity (7mm) was just 1mm below when comparing with the standard (8mm).

Similar results have been observed by Chew *et al*., (2012). The microorganisms used in the study of *L.aspera* were gram positive *Staphylococcus aureus* (*S.aureus*) and the Gram negative *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P.aeruginosa*), *Salmonella typhimurium* (*S.typhimurium*), *Salmonella choleraesuis* (*S. choleraesuis*) and *Shigella flexneri* (*S. flexneri*) were used for antimicrobial study. The mean zone of inhibition produced by the commercial antibiotic, chloramphenicol, was between 21.0 to 25.0 mm and was larger than those produced by all methanol extracts which was between 7.0 to 11.0 mm. Based on the results, the root extract showed the highest zone of inhibition compared with all the extracts against all the tested microorganisms, followed by the flower extract which was more active against Salmonella species compared with other tested microorganisms. Leaf extract was active against four microbes. On the other hand, methanol (negative control) did not exhibit any effect on all the tested microorganisms.

The study by Kannan *et al*., (2011) reveals that methanolic extract of *Passiflora edulis* were significantly effective against Gram positive bacteria *Bacillus subtilis* and Gram negative bacteria *E.coli*. Also methanolic extract was suggestively against *Staphylococcus aureus* and Gram negative bacteria *Salmonella typhi* when compared with standard ciprofloxacin.

E. BIOACTIVE COMPONENTS IN THE LEAF EXTRACTS

The methods used for purification and isolation of phytochemical components are column chromatography, paper chromatography, thin layer chromatography and gas chromatography-mass spectroscopy.

COLUMN CHROMATOGRAPHY

Plate 6a and 6c shows the process of separation of bioactive components from the methanolic extract of *Leucas aspera* and *Passiflora edulis* respectively. Similarly Plate 6b and 6d shows the collected fragments of methanolic extract of *Leucas aspera* and *Passiflora edulis* using column chromatography respectively.

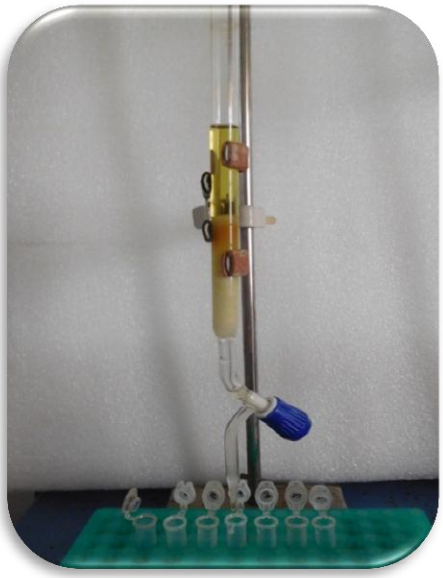


Plate 6a – Separation of Bioactive components in methanolic extract of *Leucas aspera* using Column Chromatography

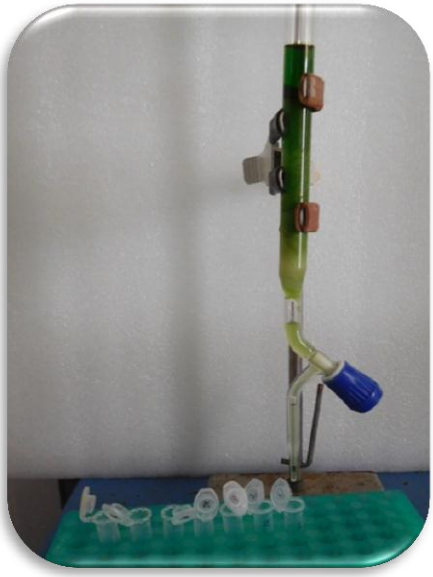


Plate 6c – Separation of Bioactive components in methanolic extract of *Passiflora edulis* using Column Chromatography

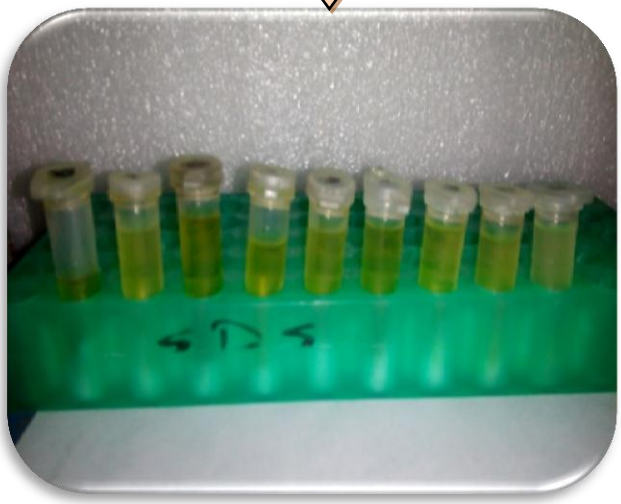


Plate 6b – Collected fragments of methanolic extract of *Leucas aspera* using Column Chromatography

Plate 6d – Collected fragments of methanolic extract of *Passiflora edulis* using Column Chromatography

PAPER CHROMATOGRAPHY

The Retention Factor (Rf) value obtained for two fractions are given in Table VII.

TABLE VII

Rf VALUE OF BIOACTIVE COMPONENTS USING PAPER CHROMATOGRAPHY

Leaf Extract	Rf value	
	1st fragment	2nd fragment
L.aspera (Methanol extract)	0.71	0.65
P.edulis (Methanol extract)	0.81	0.74

For *L.aspera* the Rf value obtained was 0.71 and 0.65 for first and second fragment respectively, while for *P.edulis* the Rf value was 0.81 and 0.74 for first and second fragment respectively. Hence the first fragment was selected to perform TLC for both the plants.

Plate 7a and 7b shows the distance travelled by the components present in methanolic extract of *Leucas aspera* and *Passiflora edulis* respectively.

THIN LAYER CHROMATOGRAPHY

The Rf value obtained for methanolic extracts of both the plants using TLC are shown in Table VIII and Plate 7c shows the distance travelled by the components present in methanolic extract of *Leucas aspera* and *Passiflora edulis* respectively.

TABLE VIII

Rf VALUE AND COLOUR OF BIOACTIVE COMPONENTS USING THIN LAYER CHROMATOGRAPHY

Leaf extracts	Rf value	Colour	Compound present
L.aspera (Methanol extract)	0.68	Light brown	Flavonoids
P.edulis (Methanol extract)	0.62	Green	Steroids

Plate 7a – Distance travelled by the components present in methanolic extract of *Leucas aspera* in paper chromatography

Plate 7b – Distance travelled by the components present in methanolic extract of *Passiflora edulis* in paper chromatography

Plate 7c – Distance travelled by the components present in methanolic extracts of *Leucas aspera* and *Passiflora edulis* in Thin Layer Chromatography.

Using TLC, the Rf value was 0.68 for methanolic extract of *L.aspera* and 0.62 for methanolic extract of *P.edulis*. For methanolic extract of *L.aspera* the colour obtained for the compound was light brown, indicating the presence of flavonoids. The colour obtained was green for methanolic extract of *P.edulis*, indicating the presence of steroids.

TLC profile of methanol extract was described in a study by Elumalai *et al*., (2015). Eight major bands were observed in long UV 372 nm. Rf was calculated as distance traveled by solute/distance traveled by solvent. Methanol extracts Rf values: Band-1: Rf 0.95 – Triterpenoids and steroid, Band-2: Rf 0.81 – Phenolic compound and catechin, Band-3: Rf 0.69 – Flavonoids-c-glycosides and mentione, Band-4: Rf 0.58 – Saponin, Band-5: Rf 0.46 – Terpene alcohols and quercertin, Band-6: Rf 0.38 – Sterols, Band-7: Rf 0.06 – Polyines and Band-4: Rf 0.04 – Unknown compound.

The TLC profile showed the presence of flavonoids and saponins in the study "TLC Fingerprint of Flavonoids and Saponins from Passiflora Species" by Birk *et al*., (2005).

In the present study, it was found that the *L.aspera* had better Rf value; it also had high antioxidant, antimicrobial activity. Hence it was selected as the plant with best potential, for GC-MS analysis and anticancer activity.

GC-MS STUDY

The bioactive components present in the leaves of *L.aspera* are shown in Table IX and Figure 5.

TABLE IX

THE BIOACTIVE COMPONENTS IN LEAF OF *LEUCAS ASPERA* (WILLD.) LINK.

Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
2Z,6E-Farnesol	C15H26O	222	0.83
d-Arabinose	C5H10O5	150	1.34
Xanthorrhizol	C15H22O	218	4.94
Stigmast-5-en-3-ol,(3á,24S)-	C29H50O	414	11.83
β-Sitosterol	C29H50O	414	11.83
Oxirane, trimethyl- (CAS)	C5H10O	86	1.84

The main bioactive compounds present in the leaf extract were 2Z,6E-Farnesol (0.83 %), d-Arabinose (1.34 %), Xanthorrhizol (4.94 %), Stigmast-5-en-3-ol,(3á,24S)- (11.83 %), ç-Sitosterol (11.83 %) and Oxirane, trimethyl- (CAS) (1.84).

Farnesol is a natural 15-carbon organic compound which is an acyclic sesquiterpene alcohol. Under standard conditions, it is a colorless liquid. It is hydrophobic, and thus insoluble in water, but miscible with oils. Farnesol is produced from 5-carbon isoprene compounds in both plants and animals. Phosphate activated derivatives of farnesol are the building blocks of most, and possibly all, acyclic sesquiterpenoids. These compounds are doubled to form 30-carbonsqualene, which in turn is the precursors for steroids in plants, animals, and fungi. As such, farnesol and its derivatives are important starting compounds for both natural and artificial organic synthesis. Farnesol has been suggested to function as a chemopreventative and anti-tumor agent (Joo and Jetten, 2009). Farnesol is used as adeodorant in cosmetic products because of its anti-bacterial activity (Kromidas, *et al*., 2006). Farnesol is used by the commensal, opportunistically pathogenic fungus *Candida albicans* as a quorum sensing molecule that inhibits filamentation (Hornby, 2015).

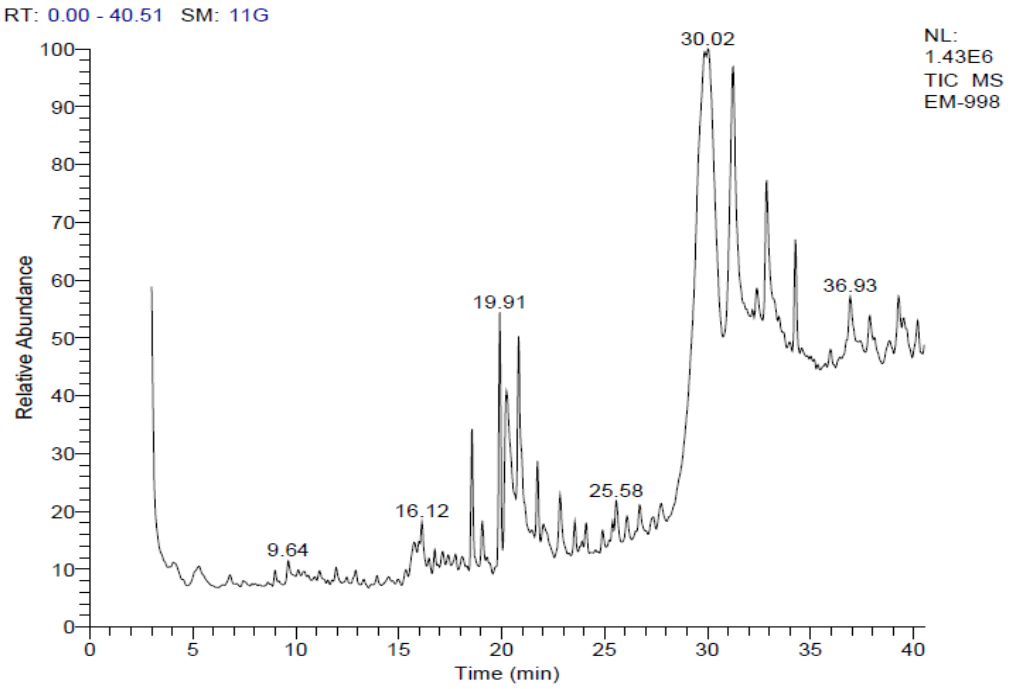


FIGURE 5

GC-MS OF LEAVES OF *LEUCAS ASPERA* (WILLD.) LINK.

Arabinose is an aldopentose – a monosaccharide containing five carbon atoms, and including an aldehyde (CHO) functional group. In synthetic biology, arabinose is often used as a one-way or reversible switch for protein expression under the P_{bad} promoter in *E. coli*. This on-switch can be negated by the presence of glucose or reversed off by the addition of glucose in the culture medium which is a form of catabolite repression. Some organic acid tests check for the presence of arabinose, which may indicate overgrowth of intestinal yeast such as *Candida albicans* or other yeast/fungus species (Guzman *et al*., 1995).

Xanthorrhizol (XNT) is the most active and abundant compound isolated from the essential oil of the rhizomes of *Curcuma xanthorrhizza*Roxb. It is categorized as a bisabolane-type sesquiterpenoid compound. It has antimicrobial, anti-inflammatory, antioxidant, antihyperglycemic, antihypertensive, antiplatelet, nephroprotective and hepatoprotective, estrogenic and antiestrogenic properties. Xanthorrhizol was first known to possess anticancer properties when it was tested on Sarcoma 180 ascites in mice. Sarcoma 180 ascites is a

transplantable tumor. Although the antitumor activity of XNT was found to be lower than α-curcumene, there is lack of mechanism studies on how XNT inhibited tumor growth of Sarcoma 180 ascites (Oon *et al*., 2015).

(3b)-stigmast-5-en-3-ol is a plant phytosterol found commonly in many plants. Other common names of (3b)-stigmast-5-en-3-ol includes Betasitosterol, (3b)-stigmast-5-en-3-ol, 22:23-dihydrostigmasterol, alpha-dihydrofucosterol, cinchol, cupreol, rhamnol, quebrachol and (3b)-stigmast-5-en-3-ol. The anti-proliferative effect of (3b)-stigmast-5-en-3-ol, has been investigated using in vitro models and also has been documented for its cholesterol lowering effect. Hwang and co-workers reported on the enhancement of glucose uptake by betasitosterol through LKB1-mediated AMPK activation in in vitro model. However its mechanistic role in triggering insulin signaling cascade for augmenting glucose transport needs exploration. Against this background, the present study aims at finding the mechanistic action of (3b)-stigmast-5-en-3-ol on the insulin signaling cascade using L6 myotubes, a well established skeletal muscle model for evaluating the anti-diabetic potential (Sujatha *et al*., 2010).

β-sitosterol is an ancient molecules in plant kingdom. It has the ability to inhibit the absorption of cholesterol, cancer-cell growth, angiogenesis, invasion and metastasis. β-sitosterol is well-known natural sterol in composition of known herbal drugs for treatment of benign prostatic hyperplasia and prostate cancer. Besides, the compound elevated enzymatic and nonenzymatic antioxidant in cells making it effective anti-diabetic, neuroprotective and chemoprotective agent as well. High potential of this compound and its analogues in treatment of various illnesses, classifies this compound as the noteworthy drug of the future, although its role in treatment of BPH is now approved via clinical trial confirmations (Saeidnia *et al*., 2014).

Oxirane is a colorless and flammable gas at room temperature and pressure. Ethylene oxide is a bactericidal, fungicidal, and sporicidal disinfectant. It is effective against most micro-organisms, including viruses. It is used as a fumigant for foodstuffs and textiles and as an agent for the gaseous sterilization of heat-labile pharmaceutical and surgical materials (https://pubchem.ncbi.nlm.nih.gov).

The GC-MS analysis of aerial part of *L.aspera* by Parimaladevi *et al*., (2014) showed the presence of 3-Allyl-6-methoxyphenol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Hydroquinone, Phenol, 2,6-dimethoxy-, 2-Methoxy-4-vinylphenol, Phenol, 2-methoxy- and Phenol.

G. ANTICANCER ACTIVITY OF *LEUCAS ASPERA* (WILLD.) LINK.

The percentage cell viability and cell death of HeLa cells after treating with the plant extract is given in Table X and Figure 6 and Plate 8a shows the t flask containing HeLa cells. Plate 8b and Plate 8c shows the 96 well plate before and after the formation of Formazan after treating with methanolic extract of *Leucas aspera* repectively. Plate 9a and 9b shows the microscopic view of HeLa cells before and after the formation of Formazan respectively.

TABLE X

ANTICANCER ACTIVITY OF *LEUCAS ASPERA*

Concentration (µl)	OD Sample (L.aspera)	OD Control	Cell Death		Viability	
			%	r value	%	r value
25	0.197	0.372	47.04	0.987**	52.96	0.992**
50	0.167		55.10		44.90	
75	0.134		63.98		36.02	

** - Significant at 0.01 level.

It was observed that cell death increased and cell viability decreased with increase in concentration of the methanolic extract of *L.aspera*. For 25µl concentration the cell death and cell viability was 47.04 per cent and 52.96 per cent respectively. For 50µl concentration the cell death and cell viability was 55.10 per cent and 44.90 per cent respectively. For 75µl concentration of leaf extract 63.98 percent cell death and 36.02 per cent cell viability was observed.

It is thus inferred that the anticancerous activity of *L.aspera* is due to the presence of 2Z, 6E - Farnesol, Xanthorrhizol, Stigmast-5-en-3-ol,(3á,24S)-, β-Sitosterol compounds in the leaf extract.

Studies by Parimaladevi *et al*., (2014) shows the aqueous extract of different parts of *Leucas aspera* possess good cytotoxic effect against *Ehrlich ascites carcinoma* cell lines. Augustine *et al*., (2014) report that, the ethyl acetate extract of *L.aspera* aerial parts are effective in inhibiting the Daltons lymphoma *in vitro* and *in vivo*. The biochemical and histological studies revealed that anti cancer effect of *L.aspera* mediated through macrophage stimulation, anti angiogenesis and free radicals scavenging.

High positive correlation (r = 0.987 at p< o.o1) was observed between counteraction of plant extract and percentage cell death. Similarly high negative correlation (0.992 at p< 0.01) was recorded between counteraction of plant extract and percentage cell viability.

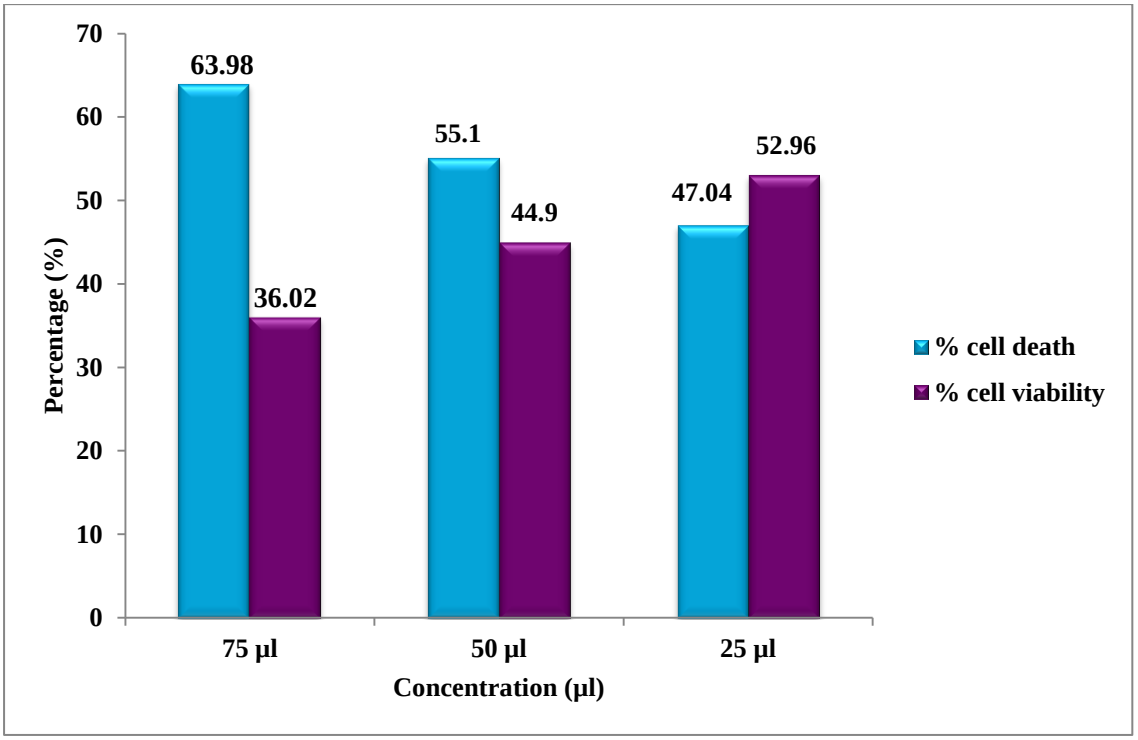


FIGURE 6

ANTICANCER ACTIVITY OF *LEUCAS ASPERA*

Plate 8a - t flask containing HeLa cells

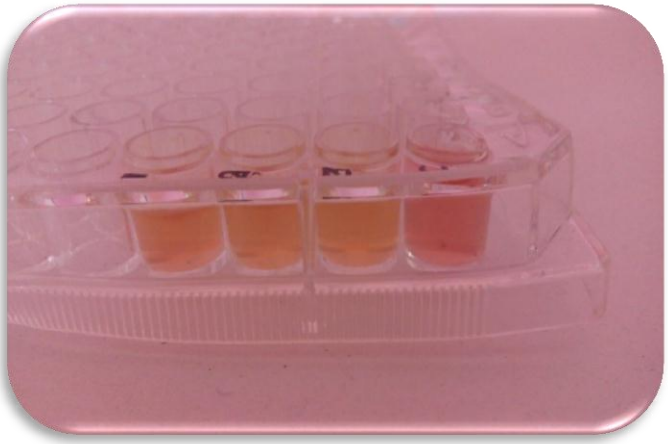


Plate 8b – 96 Well plate before Formazan formation (Leucas aspera- methanolic extract)

Plate 8c – 96 Well plate after Formazan formation (Leucas aspera- methanolic extract)

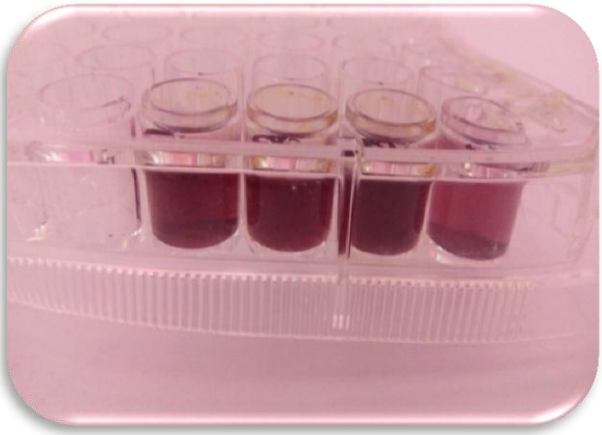


Plate 9a – Microscopic view of HeLa cells

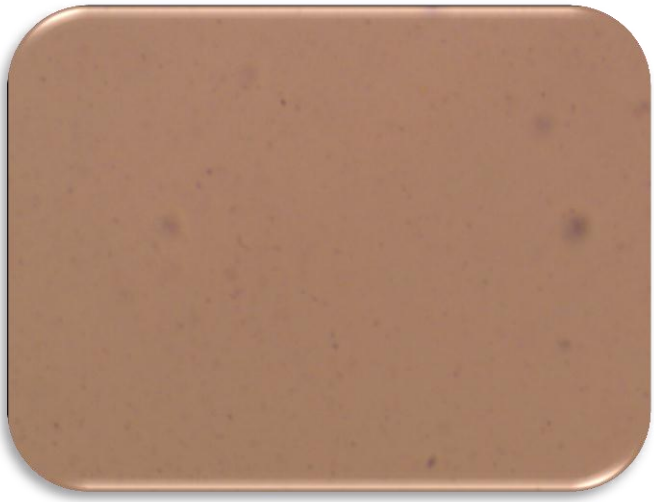


Plate 9b – Microscopic view of HeLa cells after Formazan formation

The present study thus shows that the leaves Thumbai (*Leucas aspera*) and Passion fruit (*Passiflora edulis*) have nutritive value almost on par with popular green leafy vegetable consumed in India. Both the plants contain phytochemicals such as alkaloids, terpenoids, phenols and tannins, protein and steroids in aqueous, methanolic and acetone extracts. Exception was the presence of saponins in *L.aspera* and quinines in *P.edulis*. The antioxidant activity was higher in *L.aspera* than *P.edulis* in all the antioxidant assays such as DPPH radical scavenging activity, Total phenol, Total flavonoids, Ferric ion reducing ability of plasma (as a measure of antioxidant power) and Hydrogen peroxide scavenging activity. The leaves of *L.aspera* exhibited best antimicrobial activity against *Bacillus subtilis* (gram positive), Klebsiella *pneumonia* (gram positive), *Pseudomonas aeruginosa* (gram negative) and *Escherichia coli* (gram negative). The TLC profile of the methanolic extract of plant leaves showed the presence of flavonoids and steroids in *L.aspera* and *P.edulis* respectively. The GC-MS study identified the bioactive components such as 2Z, 6E-Farnesol, Xanthorrhizol, Stigmast-5-en-3-ol (3á,24S)-, ç-Sitosterol and Oxirane, trimethyl- (CAS). All these favor anticancerous activity of plant extract. The cytotoxicity study showed that the cell death increased and cell viability decreased with increase in concentration of the methanolic extract *L.aspera*.

Summary and Conclusion

V. SUMMARY AND CONCLUSION

A medicinal plant is a plant that has similar properties as conventional pharmaceutical drugs. Human beings have used plants for the treatment of diverse ailments for thousands of years. *Leucas aspera* is a species within the *Leucas* genus and the Lamiaceae family. *Leucas aspera* is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities. *Leucas aspera* is used in the traditional medicine of the Philippines to treat scorpion bites. It is also an antipyretic, it is a herb that has the ability to help reduce fevers. Of the estimated 500 species of *Passiflora,* in the family Passifloraceae, only one, *Passiflora edulis* Sims, has the exclusive designation of passion fruit, without qualification. Some medical uses for the passion fruit include treating osteoarthritis, hypertension, anxiety, and its usefulness as a sedative. The leaves of passiflora edulis was found to contain antioxidant, antibacterial, antifungal and cytotoxic activities.

The nutrient composition, phytochemical components, antioxidant activity, antimicrobial activity and anticancerous activity of the leaves of *Leucas aspera* (Willd.) Link. and *Passiflora edulis* Sims were carried out using different analytical methods. The nutrients components like energy, carbohydrate, protein, fat, moisture, calcium, phosphorus, iron, vitamin C and β-carotene and the presence of phytochemicals such as alkaloids, terpenoids, phenol and tannin, reducing sugar, saponins, flavonoids, quinines, protein and steroids were determined for aqueous, methanol and acetone extracts. From the results the best answering extract (methanol extract) was selected for both the plants for carrying out further analysis.

The antioxidant activity of these plants were determined by DPPH radical scavenging activity, total phenol content, total flavonoid content, ferric ion reducing ability of plasma, and hydrogen peroxide radical scavenging activity. The antimicrobial activity of plant extracts were determined using agar well diffusion method against *Bacillus subtilis* (gram positive), Klebsiella *pneumonia* (gram positive), *Pseudomonas aeruginosa* (gram negative) and *Escherichia coli* (gram negative) bacteria. Then using chromatographic techniques such as column chromatography, paper chromatography, thin layer chromatography the bioactive components were separated. From the results obtained from phytochemical, antioxidant, antimicrobial and

thin layer chromatography *Leucas aspera* was selected as the plant with best potential for carrying out GC-MS and anticancerous activity. The HeLa cells were used to assess the anticancerous activity of the methanolic extract of *Leucas aspera*.

The results obtained from this study are summarized below:

- *Passiflora edulis* was found to be nutritionally superior to *Leucas aspera* with appreciably higher levels ($p<0.01$) of all nutrients estimated except for energy and carbohydrate.
- The mean energy content of *Leucas aspera* and *Passiflora edulis* were found to be 353.61 kcal and 337.04 kcal respectively and the t value 16.79 indicates that it is significant at one per cent level. The mean carbohydrate content of *L.aspera* was 77.8 g, whereas in *P.edulis* it was 64.23 g and the t value 8.90 shows that it is significant at one percent level. The mean content of protein present in the leaves of *L.aspera* was 9.60 g and it was 18.32 g in *P.edulis*. The t value 7.24 indicates that it is significant at one percent level. The amount of fat content was found to be 0.41 g and 0.76 g in *L.aspera* and *P.edulis* respectively and the t value 3.67 indicates that it is significant at five percent level. The mean moisture content was 7.67 g and 11.81 g for *L.aspera* and *P.edulis* respectively and the t value 4.81 indicates that it is significant at one percent level.
- The mean iron content was 6.80 mg for *L.aspera* and 9.32 mg for *P.edulis*. The t value 4.74 shows that it is significant at one percent level. The mean calcium content was more in *P.edulis* (90.0 mg) when compared to *L.aspera* (28.67 mg) and the t value 34.56 shows that it is significant at one percent level. The *P.edulis* contains 552.0 mg of phosphorus whereas *L.aspera* contains 368.0 mg of phosphorus. The t value 68.17 indicates that it is significant at one percent level. The mean vitamin C content in *L.aspera* was 62.0 mg and 120.0 mg in *P.edulis* and the t value 96.29 shows that it is significant at one percent level. The presence of β-carotene was below detectable level in *L.aspera* and it was 660.0µg in *P.edulis*.
- Comparison of the nutrient content of *L.aspera* and *P.edulis* with that of nutrient rich green leafy vegetable such as *Sesbania grandiflora* (Agathi) showed that the energy, carbohydrate and protein content was higher in both the plants than Agahi (Energy- 93kcal; Carbohydrate- 11.8g; Protein- 8.4g). The fat and moisture content in *L.aspera*

and *P.edulis* leaves was less than that of agathi leaves (Fat- 1.4g; Moisture- 73.1g). The iron and phosphorus content was higher in *L.aspera* and *P.edulis* than agathi leaves (Iron- 3.9mg; Phosphorus- 80mg). The calcium content in agathi leaves (1130mg) was higher than in two plants *L.aspera* and *P.edulis*. The vitamin C (169mg) and β- carotene (5400µg) content in agathi leaves was higher than that of *L.aspera* and *P.edulis*.

- The energy, carbohydrate, protein, fat, iron and phosphorus content was higher in *L.aspera* and *P.edulis* than in *Brassica olerace* (Cabbage) (energy- 27 kcal; carbohydrate- 4.6 g; protein- 1.8 g; fat- 0.1 g; iron- 0.8 mg; phosphorus- 44.0 mg; vitamin C- 124.0 mg) and the moisture content (91.9 g) was found to be higher in cabbage. The calcium content was found to be higher than *L.aspera* and less than *P.edulis* than in cabbage (39.0 mg). The β- carotene content (120.0 µg) was less in cabbage than *P.edulis*.
- Comparison with nutrient content of *L.aspera* and *P.edulis* with that of *Trigonella foenum graecum* (Fenugreek leaves) showed that the energy (49 kcal), carbohydrate (6.0 g), protein (4.4 g), iron (1.93 mg), phosphorus (51.0 mg), vitamin C (52.0 mg) was less in fenugreek leaves than *L.aspera* and *P.edulis*, whereas the fat (0.9 g) moisture (86.1 g) and calcium (395.0 mg) content was higher in fenugreek leaves. The β-carotene content (2340.0 µg) was high in fenugreek leaves than in *L.aspera* and *P.edulis*.
- In *Solanum nigrum* (Manathakali leaves) the energy (68.0 kcal), carbohydrate (8.9 g), protein (5.9 g), phosphorus (70.0 mg) and vitamin C (11.0 mg) content was less than *L.aspera* and *P.edulis* and the fat (1.0 g), moisture (82.1 g), iron (20.5 mg) and calcium (410 mg) content was higher than *L.aspera* and *P.edulis*. The β-carotene content was absent in manathakkali.
- The presence of energy (26 kcal), carbohydrate (2.9 g), protein (2.0 g), iron (1.14 mg), phosphorus (21.0 mg) and vitamin C (28 mg) was less in *Spinacia oleracea* (Spinach) than *L.aspera* and *P.edulis*, whereas the moisture (92.1 g) content was found to be higher in spinach. The fat (0.7 g) content in spinach was higher than *L.aspera* (0.41 g) and slightly less than *P.edulis* (0.76 g). The calcium content present in spinach was 73.0 mg which was higher than the calcium content in *L.aspera* (28.67 mg) and less than *P.edulis* (90.0 mg). The β-carotene content (5580.0 µg) was high in spinach than *L.aspera* and *P.edulis*.

- The phytochemical screening of *Leucas aspera* revealed the presence of alkaloids, terpenoids, phenols, tannins, protein and steroids in acetone extract. The methanol and aqueous extract showed the presence of alkaloids, terpenoids, phenols, tannins, saponins, protein and steroids. Reducing sugars, saponins, flavonoids and quinines were absent in acetone extract whereas in methanol and aqueous extracts reducing sugars, flavonoids and quinines were found to be absent. Among the three different extracts the more number of phytochemical compounds were present in methanol and aqueous extract than acetone extract. So from the results methanol extract was selected as the best extract for further analysis.
- The phytochemical screening of *Passiflora edulis* in acetone, methanol and aqueous extracts, revealed the presence of alkaloids, terpenoids, phenols, tannins, quinines, protein and steroids in acetone extract as well as in methanol extract. The aqueous extract showed the presence of alkaloids, terpenoids, protein and steroids. Reducing sugars, saponins, flavonoids were absent in acetone as well as in methanol extract. In aqueous extracts phenols, tannins, reducing sugars, saponins, flavonoids and quinines were found to be absent. Among the three different extracts the more number of phytochemical compounds were present in methanol and acetone extract than aqueous extract. So from the results methanol extract was selected as the best extract for further analysis.
- Plant leaves of *L.aspera* exhibit 97.89 per cent DPPH radical scavenging activity. Leaves of *P.edulis* have less DPPH radical scavenging activity (74.12 per cent). Thus *L.aspera* exhibits significantly higher DPPH radical scavenging activity ($p< 0.01$) than *P.edulis* and hence better antioxidant avtivity than *Passiflora edulis*.
- The plant leaves of *L.aspera* had a total phenol content of 33.05 per cent, whereas leaves of *P.edulis* possessed 12.85 per cent total phenolic content. Hence it is clear that *L.aspera* had significantly higher ($p< 0.01$) phenolic content than *P.edulis*.
- The plant leaves of *L.aspera* contained 99.11 per cent total flavonoids and leaves of *P.edulis* had 96.93 per cent of total flavonoids. It is clear that both the plants have comparably high level of flavonoids content. Though flavonoids in *L.aspera* was slightly higher than in *P.edulis,* the difference was not statistically significant.

- The plant leaves of *L.aspera* showed 38.82 per cent Ferric Ion Reducing Antioxidant Power. Leaves of *P.edulis* showed a significantly lower ($p< 0.01$) level of 13.78 per cent Ferric Ion Reducing Antioxidant Power.
- The plant leaves of *L.aspera* towered above ($p< 0.01$) those of *P.edulis* in terms of hydrogen peroxide scavenging activity (82.29 and 11.48 per cent respectively). This finding brings out the antioxidant potential of L.aspera interms of H_2O_2 scavenging.
- The zone of inhibition produced by the disc Tobramycin of 10mcg (standard) was found to be 9mm each for *Bacillus subtilis* and *Klebsiella pneumonia*. The zone of inhibition was 8mm each for *Pseudomonas aeruginosa* and *Escherichia coli*. In methanol the zone of inhibition developed was 1mm, 9mm, and 10mm against *Bacillus subtilis*, *Klebsiella pneumonia* and *Escherichia coli* respectively and against *Pseudomonas aeruginosa* methanol did not show zone of inhibition. The zone of inhibition produced by the methanolic extract of *L.aspera* was 3mm, 12mm, 4mm and 10mm against *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli* respectively. In the methanolic extract of *P.edulis* the zone of inhibition developed was 2mm, 3mm and 7mm against *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* respectively and against *Klebsiella pneumonia* the methanolic extract of *P.edulis* did not exhibit any zone of inhibition. Distilled water did not produce any zone of inhibition against any of the microorganisms.
- The zone of inhibition produced by the methanolic extract of *L.aspera* (3mm) against *Bacillus subtilis* was found to be less than that of standard disc (9mm) and it was higher than the positive control methanol (1mm). For methanolic extract of *P.edulis* the zone of inhibition developed was 2mm against *Bacillus subtilis* which was found to be less than standard disc (9mm) and higher than that of positive control methanol (1mm).
- The methanolic extract of *L.aspera* exhibited zone of inhibition (12mm) against *Klebsiella pneumonia* and it was higher than standard disc (9mm) and positive control methanol (9mm). The methanolic extract of *P.edulis* did not exhibit zone inhibition against this bacterium.

- The zone of inhibition of methanolic extract of *L.aspera* was 4mm against *Pseudomonas aeruginosa* and it was less than standard disc (8mm) and higher than positive control methanol. The zone of inhibition was 3mm for the methanolic extract of *P.edulis*, which was less than standard disc (8mm) and greater than positive control methanol (Nil).
- For *Escherichia coli* the zone of inhibition was 10mm for methanolic extract of *L.aspera* and it was greater than standard disc (8mm) and similar to that of positive control methanol (10mm). The methanolic of *P.edulis* exhibited 7mm zone of inhibition against the same bacteria and which was less than standard disc (8mm) and positive control methanol (10mm).
- When examining the activity of both plant extract against the four microorganisms the methanolic extract of *Leucas aspera* showed highest activity against *Klebsiella pneumonia* and *Escherichia coli*, and the inhibition potential was higher than the standard. Antimicrobial activity of *Passiflora edulis* was found to be low except against *Escherichia coli*, in which the activity (7mm) was just 1mm below when comparing with the standard (8mm).
- In paper chromatography for *L.aspera* the Rf value obtained was 0.71 and 0.65 for first and second fragment respectively, while for *P.edulis* the Rf value was 0.81 and 0.74 for first and second fragment respectively.
- Using TLC, the Rf value was 0.68 for methanolic extract of *L.aspera* and 0.62 for methanolic extract of *P.edulis*. For methanolic extract of *L.aspera* the colour obtained for the compound was light brown, indicating the presence of flavonoids. The colour obtained was green for methanolic extract of *P.edulis*, indicating the presence of steroids.
- The main bioactive compounds identified from the leaf extract were 2Z,6E-Farnesol (0.83 %), d-Arabinose (1.34 %), Xanthorrhizol (4.94 %), Stigmast-5-en-3-ol,(3á,24S)- (11.83 %), ç-Sitosterol (11.83 %) and Oxirane, trimethyl- (CAS) (1.84 %).
- It was observed that cell death increased and cell viability decreased with increase in concentration of the methanolic extract of *L.aspera*. For 25µl concentration the cell death and cell viability was 47.04 per cent and 52.96 per cent respectively. For 50µl concentration the cell death and cell viability was 55.10 per cent and 44.90 per cent respectively. For 75µl concentration of leaf extract 63.98 percent cell death and 36.02 per cent cell viability was observed.

- It is thus inferred that the anticancerous activity of *L.aspera* is due to the presence of 2Z, 6E - Farnesol, Xanthorrhizol, Stigmast-5-en-3-ol,(3á,24S)-, β-Sitosterol compounds in the leaf extract.
- High positive correlation ($r = 0.987$ at $p< 0.01$) was observed between counteraction of plant extract and percentage cell death. Similarly high negative correlation (0.992 at $p< 0.01$) was recorded between counteraction of plant extract and percentage cell viability.

The present study thus authenticates that the leaves Thumbai (*Leucas aspera*) and Passion fruit (*Passiflora edulis*) have nutritive value almost on par with popular green leafy vegetable consumed in India. However it was observed that the leaves of *P.edulis* was superior in terms of micronutrients and protein compared to those of *L.aspera*. Both the plant leaves contained almost similar amounts of phytochemicals. However *L.aspera* exhibited significantly higher levels of antioxidant ($p< 0.01$) and antimicrobial activity. TLC profile showed that *L.aspera* contained flavonoids and was better than *P.edulis* in terms of Rf value.

Moreover *L.aspera* exhibited highly impressive anticancer activity as revealed by the cell death and cell viability of HeLa cells treated with the plant extract. While cell death showed a positive relationship, cell viability decreased with increase in concentration of *L.aspera* leaf extract.

Recommendation

Based on the findings of the present study, the following recommendations can be made for future research to develop cancer preventing neutraceuticals and food based products using medicinal plants. Future research should focus on:

1. Development of medicinal drugs using *Leucas aspera*.
2. Microencapsulation of antioxidants present in the plant leaf extract after removing the anti nutritional factors if any and in vivo studies on rats and its clinical trials.
3. Safety, efiiciency and efficacy studies of the developed drugs.
4. Analyse the feasibility and availability of such medicinal drugs.
5. Supplementation studies

Bibliography

BIBLIOGRAPHY

Agrawal, P., Ravi, B. and Singh, R.B (1996). Randomized, placebo-controlled, single-blind trial of holy basil leaves in patients with non-insulin dependent diabetes mellitus. International Journal of Clinical Pharmacology and Therapeutics, 34. pp: 406-409.

Ahamad, I., Mehmood, Z. and Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimi-crobial properties, Journal of Ethno pharmacology, 62. pp: 183-193.

Ahmed, M. (2010). Medicinal Plants. MJP publishers, Chennai. ISBN: 978-81-8094-073-6. pp: 1-2.

Akanbi, B.O., Bodunrin, O.D. and Olayanju, S. (2011). Phytochemical Screening and Antibacterial Activity of *Passiflora edulis*. Researcher. ISSN: 1553-9865. Vol. 3(5). pp: 9-12.

Akinpelu, D.A. and Onakoya, T.M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in South-Western. African J Biotech, 5(11). pp: 1078-81.

Ali, M.S., Sayeed, M.A., Nabi, M.M. and Rahman, M.A.A. (2013). In vitro Antioxidant and Cytotoxic Activities of Methanol Extract of *Leucas aspera* Leaves. Journal of Pharmacognosy and Phytochemistry. ISSN 2278- 4136 IC. Journal No: 8192. Volume 2. Issue 1. pp: 8-13.

Anel, T.C. and Subapriya, M.S. (2012). Nutritional status of traditional and non- traditional female athletes in Manipur. International Journal of Sport Sciences and Nutrition, pp: 485-491.

Ariharan, V.N., Devi, V.N.M. and Prasad, P.N. (2013). Nutraceutical studies on *passiflora edulis* – passion fruit. International Journal of Pharma and Bio Sciences. ISSN: 0975-6299; 4(4). pp: 176 – 179.

Augustine, B.B., Dash, S., Lahkar, M., Amara, V.R., Samudrala, P.K. and Thomas, J.M. (2014). Evaluation of immunomodulatory activity of ethyl acetate extract of *Leucas aspera* in Swiss albino mice. International Journal of Green Pharmacy, IP: 223.30.225.254. pp: 84-85.

Augustine, B.B., Dash, S., Lahkar, M., Lihite, R.J., Kumar, P.S. and Sathish, P. (2013). Effect of *Mirabilis jalapa* linn flowers in experimentally induced arthritis and consecutive oxidative stress. Int J Pharm Sci, 5. pp: 190–3.

Augustine, B.B., Dash, S., Lahkar, M., Sarma, U., Samudrala, P.K. and Thomas, J.M. (2014). *Leucas aspera* inhibits the Dalton's ascitic lymphoma in Swiss albino mice: A preliminary study exploring possible mechanism of action. Pharmacogn Mag. 10(38). pp: 118–124.

Baker, J.T., Borris, R.P., Carte, B., Cordell, G.A., Soejarto, D.D., Cragg, G.M., Gupta, M.P., Iwu, M.M., Madulid, D.R. and Tyler, V.E. (1995). Natural product drug discovery and development: New perspective on international collaboration, Journal of Natural Product, 58. pp: 1325-1357.

Benzie, I.F.F. and Strain, J.J. (1999). Ferric reducing antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in enzymology, 299. pp: 15-27.

Bergers, G. and Benjamin, L.E. (2003). Tumorigenesis and the angiogenic switch. Nat Rev Cancer. 3. pp: 401–10.

Bhaigyabati, T.H., Devi, P.G. and Bag, G.C. (2014). Total Flavonoid Content and Antioxidant Activity of Aqueous Rhizome Extract of Three Hedychium Species of Manipur Valley. Research Journal of Pharmaceutical, Biological and Chemical Sciences. ISSN: 0975-8585. Vol. 5(5). pp: 970-976.

Bilia, A.R., Salvini, D., Mazzi, G. and Vincieri, F.F. (2001). Characterization of Calendula Flower, Milk-Thistle Fruit, and Passion Flower Tinctures by HPLC-DAD and HPLC-MS. Chromatographia, 53. pp: 210-215.

Birk, C.D., Provensi, G., Gosmann, G., Reginatto, F.H. and Schenkel, E.P. (2005). TLC Fingerprint of Flavonoids and Saponins from Passiflora Species. Journal of Liquid Chromatography & Related Technologies. ISSN: 1082-6076. 3(2). pp: 1-7.

Birner, J. and Nicolls, J.M. (1973). Passicol, an antibacterial and antifungal agent produced by Passiflora plant species: preparation and physicochemical characteristics. Antimicrobial Agents and Chemotherapy, vol. 3, no. 1, pp. 105–109.

Bruyne, T.D., Pieters, L., Deelstra, H. and Vlietinck, A. (1999). Condensed vegetables tannins: biodiversity in structure and biological activities. Biochemical System Ecology, 27. pp: 445–59.

Bum, E., Ngah, E., Ekoundi, C., Dong, C., Mbomo, R., Rakotonirina, S. (2004) Sedative and anticonvulsant properties of *Passiflora edulis:* dried leaves decoction in mice. Afr J Trad Compl Alternative Med.1. pp:63-71.

Chandala, M., Garg, A., Lutjohann, D., Klaus, V.B, Scott, M.G., Lind, J.B. (2000). Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. The New England journal of medicine, 342. pp: 1392 – 98.

Chew, A.L., Jessica, J.J.A. and Sasidharan, S. (2012). Antioxidant and antibacterial activity of different parts of Leucas aspera. Asian Pacific Journal of Tropical Biomedicine. Vol. 2(3). pp: 176-180.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. (2002). Glossary of Indian medicinal plant. New Delhi: Council of Scientific and Industrial Research (CSIR), pp: 153.

Cordell, G.A. (1995). Changing strategies in natural products chemistry. Phytochemistry, 40. pp: 1585-1612.

Costa, M.A., Zia, Z.Q., Davin, L.B. and Lewis, N.G. (1999) Chapter Four: Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In Recent Advances in Phytochemistry, vol. 33, Phytochemicals in Human Health Protection, Nutrition, and Plant Defense, ed. JT Romeo, New York, pp: 67-87.

Das, B.K., Das, B., Arpita, F.K., Morshed, M.A., Uddin, A., Bhattacherjee, R. and Hannan, J.M.A. (2011). Phytochemical screening and antioxidant activity of *leucas aspera*. International Journal of Pharmaceutical Sciences and Research, ISSN: 0975-8232, Vol. 2, Issue 7. pp: 1746-1752.

Davis, J. (1994). Inactivation of the antibiotics and the dissemination of resistance genes. Science, Vol.264. pp:375-382.

De-Pari, F., Raque, D., Petry., Flavio, H., Reginatto. and Gosmann, G. (2002). Pharmacochemical Study of Aqueous Extracts of *Passiflora alata* Dryander and *Passiflora edulis* Sims Acta farm. Bonaerense, 21. pp: 5-6.

Devaki, K., Beulah, U., Akila, G., Sunitha, M. and Gopalakrishnan, V.K. (2011). Hypoglycemic activity of *passiflora edulis* sim leaves extract on wister albino rats. International research journal of pharmacy, 2. pp: 170 – 172.

Devi, S.M., Vinothini, K., Shalini, V., Masilamani, K., Sivakumar, D. and Pandian, A. (2013). Angiosuppresive activity of *Leucas aspera* (Willd) Linn. using Chicken Chorioallantoic Membrane (CAM) assay. International Journal of Innovative Research in Science, Engineering and Technology, Vol. 2, Issue 11, ISSN: 2319-8753. pp: 6327.

Dhawan, K., Dhawan, S. and Sharma, A. (2004). Passiflora: a review update. Journal of Ethnopharmacology, vol. 94, no. 1. pp: 1–23.

Dimayuga, R.E. and Garcia, S.K. (1991). Antimicrobial screening of medicinal plants from Baja California sur, Mexico. J Ethnopharmacol, Vol. 31. pp: 181-187.

Dixit, P., Devasagayam, T.P.A. and Ghaskadbi, S.S. (2005). Antioxidant effect of germinated fenugreek. Phytotherapy Res. Vol.19. pp: 977-983.

Dolara, P., Luceri, C., Filippo, C. D., Femia, A.P., Giovannelli, L., Carderni, G., Cecchini, C., Silvi, S., Orpianesi, C. and Cresci, A. (2005). Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. Mutation Research, 591. pp: 237–46.

Dudareva, N., Pichersky, E. and Gershenzon, J. (2004). Biochemistry of plant volatiles. Plant Physiology, 135. pp: 1893–1902.

Elisabeth, N.B., Charlotte, E., Dongc, C., Rigobert, E.M. and Vincent, S. (2011). Sedative and anticonvulsant properties of passiflora edulis dried leaves decoction in mice. Afr.J.Trad cam, 1. pp: 63 – 71.

Elumalai, D., Hemalatha, P. and Kaleena, P.K., (2015). Larvicidal activity and GC–MS analysis of Leucas aspera against Aedes aegypti Anopheles stephensi and Culex quinquefasciatus. Journal of the Saudi Society of Agricultural Sciences. King Saud University. Available at www.ksu.edu.sa, www.sciencedirect.com.

Emran, T.B., Rahman, M.A., Hosen, S.M.Z., Saha, D. and Dey, T.K. (2012). Antioxidant property of ethanolic extract of *Leucas aspera* Linn. Bulletin of Pharmaceutical Research, ISSN: 2249-6041. Vol. 2(1). pp: 46-9.

Gani A. (2003). Medicinal plant of Bangladesh. Chemical constituents and uses. Dhaka: Asiatic Soc of Bangladesh; 27. pp: 277.

Gennari, C. D., Castoldi. and Sharon, O. (2007). Natural products with taxol-like anti-tumor activity: Synthetic approaches to eleutherobin and dictyostatin. Pure Medica and Appl.Chem, 79(2). pp: 173.

Ghafoorunissa and Vajreswari A, 2013. Carbohydrates. Textbook of Human Nutrition. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi. ISBN: 978-81-204-1742-7. Pp: 33-46.

Ghasemzadeh, A., Jaafar, H.Z.E. and Rahmat, A. (2010). Antioxidant activities, total Phenolics and flavonoids content in two varieties of Malaysia Young Ginger (Zingiber officinale Roscoe). Molecules, 15. pp: 4324-4333.

Ghule, B.V., Murugananthan, G., Nakkat, P.D. and Yeole, P.G. (2006). Immunostimulant Effects of Capparis Zeylanica Linn.Leaves.J.Ethanppharmacol, 108. pp: 311-315.

Gill, S.S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem, 48(12). pp: 909-930.

Gopalan, C., Sastri, B.V.R. and Balasubramanian. (2010). Nutritive value of Indian foods. National Institute of Nutrition, ICMR, Jamsai-Osmania, Hyderabad, India. pp: 1-2, 9, 47-50, 60-61.

Gunasekera, S.P., Kinghorn, A.D., Cordell, G.A. and Farnsworth, N.R. (1981). Plant Anticancer Agents. XIX. Constituents of Aquilariamalaccensis. J Nat Prod, Vol. 44(5): 569-72.

Gunjan, G., Rajkumar, R., Ashok, K. and Lanzar, M. (2011). Therapeutic potential of polar and non polar extracts of Cyanthilium cinereum in Vitro. Evid Based Compl Alternative Med, 10. pp: 1-11

Guzman, L.M., Belin, D., Carson, M.J. and Beckwith, J. (1995). Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. J Bacteriol. Vol. 177(14). pp: 4121–30.

Hahn, N.I. (1998). Is Phytoestrogens Nature's Cure for What Ails Us? A Look at the Research. Journal of the American Dietetic Association, 98. pp: 974- 976.

Handa, S.S., Khania, S.P.S., Longo, G. and Rakesh, D.D. (2008). Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology. Vol: 4(5). pp: 590-609.

Harborne, J.B. (1998). Phytochemical Methods: Aguide to modern techniques of Plant analysis. 3rd ed. Chapmen and Hall, London ISBN: 0-412-57270-2, pp: 302.

Hasler, C.M. and Blumberg, J.B. (1999). Symposium on Phytochemicals: Biochemistry and Physiology. Journal of Nutrition; 129. pp: 756S-757S.

Hazra, B., Sarkar, R., Biswas, S. and Mandal, N. (2010). Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis*. BMC Complement Altern Med, 10. pp: 20.

Heron, M.P., Hoyert, D.L., Xu, J., Scott, C. and Tejada-Vera, B. (2008). Deaths: Preliminary data for 2006. Natl Vital Stat Rep, 56. pp: 1–52.

Hooker, J.D. (1984). The Flora of British India. London, the Muston Company. pp: 690.

Horiuchi, N., Nakagava, K., Sasaki, Y., Minato, K., Fujiwara, Y. and Nezu, K. (1988).In vitro antitumor activity of mitomycin C derivative (RM- 49) and a new anticancer antibiotic (FK973) against lung cancer cell lines determined by tetrazolium dye (MTT) assay. Cancer Chemother Pharmacol, 22. pp: 246-50.

Hornby. J.M. (2015). Quorum Sensing in the Dimorphic FungusCandida albicans Is Mediated by Farnesol.asm.org.

Ingale, G. and Hivrale, A. U. (2010). Pharmacological studies of Passiflora sp. and their bioactive compounds. African Journal of Plant Science, Vol. 4, No. 10. pp: 417–426.

Islam A.K.M. and Kato-Noguchi H. (2012). Allelopathic Potentiality of Medicinal Plant *Leucas aspera.*, International Journal of Sustainable Agriculture. ISSN 2079-2107. Vol: 4 (1). pp: 01-07.

Jacob, R.A. (1995). The integrated antioxidant system, Nutrition Research, 15. pp: 755-766.

Jamir, T.T., Sharma, H.K. and Dolui, A.K. (1999). Folklore medicinal plants of Nagaland, India, Fitoterapia, 70. pp: 395-401.

Jigna, P and Sumitra C. (2006). In-vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr J Biomed Res, 9. pp :89-93.

Jocelem, M.S., Talita, B., Debora, N.M., Sonia, M.D. and Maria, L. (2010). Effects of different concentration of passion fruit peel (*passiflora edulis*) on the glycemic control in diabetic rat. CCienc technol Aliment campinas, 30. pp: 784 – 786.

Johnson, M., Maridass, M. and Irudayaraj, V. (2008). Preliminary Phytochemical and Anti-Bacterial Studies on *Passiflora edulis*. Ethnobotanical Leaflets 12. pp: 425-432.

Joo, J.H. and Jetten, A.M. (2009). Molecular mechanisms involved in farnesol-induced apoptosis. Cancer Lett. 287(2). pp: 123-35. doi: 10.1016/j.canlet.

Kalyanasundaram, S. (2013). Carbohydrates. Textbook of Human Nutrition. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi. ISBN: 978-81-204-1742-7. Pp: 18-32.

Kamaldeep, D., Sanju, D. and Anupam, S. (2004). *Passiflora:* a review update. J Ethnopharmacol, 94. pp:1-23.

Kamlesh B. (2009). Chromatography, Campus Book International, New Delhi, ISBN: 81-87815-22-1. Pp: 24.

Kannan, S., Devi, B.P. and Jayakar B. (2011). Antibacterial evaluation of the methanolic extract of Passiflora edulis. Hygeia journal for drugs and medicines, all rights reserved. Vol. 3(1). Pp: 46-49.

Kapoor, A., Kaur, G. and Kaur, R. (2015). Antimicrobial activity of different herbal plants extracts: a review. World journal of pharmacy and pharmaceutical sciences. ISSN 2278 – 4357. Volume 4, Issue 07. pp: 422-459.

Kaushik, A., Jijta, C., Kaushik, J.J., Zeray, R., Ambesajir, A. and Beyene, L. (2012). FRAP (Ferric reducing ability of plasma) assay and effect of Diplazium esculentum (Retz) Sw. (a green vegetable of North India) on central nervous system. Indian Journal of Natural Products and Resources. Vol. 3(2). pp. 228-231.

Keser, S., Celi.k, S, Turkoglu, S., Yilmaz, O. and Turkoglu, I. (2012). Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn. Chemistry Journal, ISSN 2049-954X, Vol. 02, Issue 01. pp: 9-12.

Khan, N.A and Iqbal, S.A. (2011). Importance of medicinal plants. Discovery of publishing house Pvt. Ltd. New Delhi, ISBN: 978-81-8356, pp: 2, 257-259.

King, A. and Young, G. (1999). Characteristics and Occurrence of Phenolic Phytochemicals. Journal of the American Dietetic Association, 24. pp: 213-218.

Kirtikar, K.R. and Basu, B.D. (1991). In: Indian Medicinal Plants, Vol. III, Deharadun: Lalit Mohan Basu, pp: 2019-2020.

Kovendan, K., Murugan, K., Vincent, S. and Barnard, D.R. (2012). Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd. (Lamiaceae) and bacterial insecticide, *Bacillus sphaericus*, against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). Parasitol Res. Vol: 110(1). pp: 195-203.

Kromidas, L., Perrier, E., Flanagan, J., Rivero, R. and Bonnet, I. (2006). Release of antimicrobial actives from microcapsules by the action of axillary bacteria. Int J Cosmet Sci, Vol: 28 (2). pp: 103–108.

Kulkarni, S.D., Tilak, J.C., Acharya, R., Rajurkar, N.S. and Devasagayam, T.P.A. (2006). Evaluation of the antioxidant activity of wheatgrass (*Triticum aestivum*) as a function of growth and under different conditions. Phytotherapy Res, 20. pp: 218-227.

Kumaran, A. and Karunakaran, A.J. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India LWT, 40. pp: 344-52

Lacaille-Dubois, M.A. and Wagner, H. (2000). Bioactive saponins from plants: An update. In Studies in Natural Products Chemistry; Atta-Ur-Rahman, ed. Elsevier Science. Amsterdam, 21. pp: 633-687.

Lai, P.K. and Roy, J. (2004). Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem. Vol: 11 (11). pp: 1451-60.

Lam, S.K. and Ng, T.B. (2009). Passiflin, a novel dimeric antifungal protein from seeds of the passion fruit. Phytomedicine, Vol. 16, No. 2. pp. 172–180.

Langenheim, J.H. (1994). Higher plant terpenoids: A phytocentric overview of their ecological roles. Journal of Chemical Ecology, 20. pp: 1223- 1280.

Mangathayaru, K., Lakshmikant, J., Sundar, N.S., Swapna, R., Grace, X.F. and Vasantha, J. (2005). Antimicrobial activity of leucas aspera flowers. Fitoterapia. Indian J Pharm Sci, 52. pp: 81-85.

Mangathayaru, K., Thirumuragan, D., Patel, P.S., Pratap, D.V.V., David. D.J. and Karthikeyan, J. (2006). Isolation and identification of nicotine from *Leucas aspera* (wild) link. Indian J Pharm Sci, 68. pp: 88-90.

Markham, K.R. (1989). Methods in Plant Biochemistry. Academic Press, London, Pp: 197-237.

Marks, D. (2014). God's Amazing Pharmacy. Create space publishing. Available at www.barnesandnoble.com.

Martina, R., Marcos, R.O., Pereir, T., Flavio, H.R., Dal, F. and Jose, F.M. (2007). Antioxidant and antiglycation properties of *Passiflora alatam* and *Passiflora edulis* extracts. Food Chemistry, 100. pp: 719–724.

Mathai, K. (2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump, 271. pp: 274-275.

McGuire, C.M. (1999). *P. incarnata* (Passifloraceae): a new fruit crop. Economic Botany, 53. pp. 161–176.

Meagher, E. and Thomson, C. (1999). Vitamin and Mineral Therapy. In Medical Nutrition and Disease, 2nd ed., G Morrison and L Hark, Malden, Massachusetts: Blackwell Science Inc. pp: 33-58.

Molyneux, R.J., Nash, R.J. and Asano, N. (1996). Alkaloids: Chemical and Biological Perspectives, Vol. 11, Pelletier SW, ed. Pergamon, Oxford. pp: 303.

Monroe, S. and Polk, R. (2000), Antimicrobial use and bacterial resistance. Current Opin-ion Microbiology, 3. pp: 496-501.

Morales, G., Paredes, A., Sierra, P. and Loyola, L.A. (2008). Antioxidant activity of 50% aqueous-ethanol extract from *Acantholippia deserticola*. Biol Res, 41(2). pp: 151-155.

Movafegh, A., Alizadeh, R., Hajimohamadi, F., Esfehani, F. and Nejatfar, M. (2008). Preoperative oral *Passiflora r*educes anxiety in ambulatory surgery patients: A double-blind, placebo-controlled study. Int Anesth Res Soc.,106. pp: 1728-32.

Nagarajaiah, S.B. and Prakash, J. (2011). Chemical composition and antioxidant potential of peels from three varieties of banana. Asian Journal of Food and Agro Industry, ISSN: 1906-3040, Vol: 4(01). pp: 31-46.

Nagulendran, K., Velavan, S., Mahesh, R. and Begum, V.H. (2007). In Vitro Antioxidant Activity and Total Polyphenolic Content of *Cyperus Rotundus* Rhizomes, Vol: 4(3). pp: 440-449.

Narayan, D. (2013). Health benefits of Passion Fruit, Biotech articles; Vol. 10, No. 35, pp: 23.

Nigam, J. S. and Sodhi, J.S. (2014). Some medicinal plants with antioxidant activity- a review. International Journal of Pharmacy and Biological Sciences. ISSN: 2230-7605. Volume 4, Issue 1. pp: 173-178.

Nile, S.H. and Khobragade, C.N.N. (2009). Determination of Nutritive Value and Mineral Elements of some Important Medicinal Plants from Western Part of India. Journal of Medicinal Plants. Volume 8, Supplement No. 5. pp: 164-169

Oon, S.F., Nallappan, M., Tee, T.T., Shohaimi, S., Kassim, N.K., Saariwijaya, M.S.F. and Cheah, Y.H. (2015). Xanthorrhizol: a review of its pharmacological activities and anticancer properties. Cancer Cell International, DOI: 10.1186/s12935-015-0255-4.

Osma, J.A., Maldonado, M.E., Chamorro, N.L., Varela, S.S.A., Landázuri, P., (2013). Antioxidant and antiproliferative activity of ethanolic and aqueous extracts from leaves and fruits juice *Passiflora edulis.* Perspectivas en nutrición humana. ISSN: 0124-4108. Vol. 15 (1). pp: 162-169.

Parimaladevi, S.T., Raj, C.D., Sudha, P. and Brindha, P. (2014). GC-MS Analysis and in vitro Cytotoxicity and Antioxidant Studies on *Leucas aspera*. Asian Journal of Chemistry; Vol. 26, No. 12. pp: 3675-3678.

Pelegrini, P.B., Noronha, E.F. and Muniz, M.A.R. (2006). An antifungal peptide from passion fruit (*Passiflora edulis*) seeds with similarities to 2S albumin proteins," Biochimica et Biophysica Acta, vol. 1764, no. 6. pp. 1141–1146.

Pettinelli, D. (2009). Plant Tissue Analysis Interpretation Sheet. Soil Nutrient Analysis Laboratory; 6 Sherman Place, Unit 5102, Storrs, CT 06269-5102. Union Cottage, Depot Campus, Mansfield. pp:1-4.

Phamiwon, Z.A.S. and John, S. (2016). Diabetes and Medicinal Benefits of *Passiflora edulis.* World Journal of Pharmaceutical Research. ISSN: 2277– 7105. Volume 5, Issue 3. pp: 453-465.

Prajapathi, M.S., Patel, J.B., Modi, K. and Shah, M.B. (2010). *Leucas aspera*: A review. Phocg Rev, 4. pp: 85-87.

Prashant, K.R., Dolly, J., Singh, K.R., Gupta, K.R. and Watal, G. (2008). Glycemic properties of Trichosanthes dioica leaves. Pharm Biol, Vol: 46(12). pp: 894-899.

Priyanka, B., Anitha, K., Shirisha, K., Janipasha, S.K., Dipankar, B. and Rajesh, K. (2013). Evaluation of anti oxidant activity of ethanolic root extract of *Albizia lebbeck* Benth. International Research Journal of Pharmaceutical and Applied Sciences. ISSN: 2277-4149. Vol: 3(2). pp: 93-101.

Queiroz, M.S.R., Janebro, D.I., Cunha, M.A.L., Medeiros, J.S. and Sabaa-Srur, A.U. (2012). Effect of the yellow passion fruit peel flour (Passiflora edulis f. flavicarpa deg.) in insulin sensitivity in type 2 diabetes mellitus patients. Nutrition Journal, 11. pp: 1-7.

Qusti, S.Y., Abo-khatwa, A.N., Lahwa, M.A.B. and Qusti, A. (2010). Antioxidant food items cited in Holly Quran. European Journal of Biological Sciences, Vol: 2(1). pp: 164-169.

Raaman, N. (2006). Phytochemical Techniques. New India Publishing Agency, New Delhi. ISBN: 81-89422-30-8. Vol: 2(1). pp: 197.

Rabeta, M. S. and Faraniza, N.R. (2013). Total Phenolic content and ferric reducing antioxidant power of the leaves of and fruits of *Gareinia atrovirdis* and *Cynometra cauliflora*. International Food Research Journal, Vol: 20(4). pp: 1691- 1696.

Raghuramulu, N., Nair, M.K. and Kalyansundaram, S. (2003). A Manual of laboratory techniques. National Institute of Nutrition, ICMR, Jamsai-Osmania, Hyderabad, India. pp: 167.

Rahman, M.A. and Islam, M.S. (2013). Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract. Asian Pacific Journal of Tropical Biomedicine, Vol: 3(4). pp: 273-279.

Rahman, M.S., Sadhu, S.K. and Hasan, C.M. (2007). Preliminary antinociceptive, antioxidant and cytotoxic activities of *Leucas aspera* root. Fitoterapia, 78. pp: 552-555.

Rai, V., Agarwal, M., Agnihotri, A.K., Khatoon, S., Rawat, A.K. and Mehrotra, S. (2005). Pharmacological evaluation of Leucas aspera. Nat Prod sci, Vol: 5(4). pp: 169- 179.

Ramaiya, S.D., Bujang, J.S. and Zakaria, M.H. (2014). Assessment of Total Phenolic, Antioxidant, and Antibacterial Activities of Passiflora Species. The Scientific World Journal, Article ID: 167309. Vol: 20(14). Pp: 2.

Ramesh, H.P., Yamaki, K. and Tsushida, T. (2002). Effect of fenugreek (*Trigonella foenumgraecum*) galactomannan fractions on phagocytosis in rat macrophages and on proliferation and IgM secretion in HB4C5 cells. Carbohydr Polym, 50. pp: 79–83.

Ramya, M., Subapriya, M.S. and Nalini, B. (2014). Synthesis of Eco-Friendly Plant Mediated Nanoparticles from *Leucas aspera* (Thumbai) and its Antimicrobial properties in Food Packaging during Disaster Risk Management. Proceedings of National Conference on packaging techniques to hasten Emergency food Relief, Elsevier publications. pp: 31-39.

Rao, B. and Narasimha, G.V. (1971). Antimicrobial actions of some essential oils. IV. Effect of organic compounds. Riechstoffe, Aromen, Koerperpflegemittel. Nat Prod sci, Vol: 5(4). pp: 169- 179.

Razia, M., Beulah, M. and Sivaramakrishnan, S. (2014). Phytochemical, GC-MS, FT-IR Analysis and antibacterial Activity of *Passiflora edulis* of Kodaikanalregoin of Tamilnadu. ISSN: 2278-4357, Volume 3, Issue 9. pp: 435-441.

Reddy, K.M., Viswanathan, S., Thirugnanasabmantham, D., Santa, R. and Lalitha, K. (1993). Analgesic activity of *L. Aspera*. Fitoterapia, 64. pp:151-154.

Ripa, F.A., Mahmuda, H., Laizuman, N. and Monirul. (2009). Antibacterial, cytotoxic and antioxidant activity of *passiflora edulis* sims. European Journal of Scientific Research, 31. pp: 592-598.

Rumaisa, Y., Latha, B., Soumya, C.K., Shahul, S. and Sadhiya, N. (2013). Phytochemical studies on *Leucas aspera*. Journal of Chemical and Pharmaceutical Research. ISSN: 0975-7384. Vol. 5(4). pp: 222-228.

Ryos, J. and Recio, M. (2005). Medicinal plants and antimicrobial activity. J. Ethnopharmacology, 100. pp: 80-84.

Sadhu, S.K, Okuyama, E., Fujimoto, H. and Ishibashi, M. (2003). Separation of *Leucas aspera*, a medicinal plant of Bangladesh guided by prostaglandin inhibitory and antioxidant activities .Chem pharm bull(Tokyo). pp. 176–182.

Saeidnia, S., Manayi, A., Gohari, A.R. and Abdollahi, M. (2014). The Story of Beta-sitosterol- A Review. European Journal of Medicinal Plants, Vol: 4(5). pp: 590-609

Sakarkar, D.M. and Deshmukh, V.N. (2011). Ethnopharmacological Review of Traditional Medicinal Plants for Anticancer Activity. International Journal of PharmTech Research CODEN (USA): IJPRIF ISSN: 0974-4304. Vol. 3, No.1. pp: 298-308.

Santos, K.C., Tessaro, S.M., Davet, S., Weber, M., Monteiro, R.M. and Moraes, C. (2006). Sedative and anxiolytic effects of methanolic extract from the leaves of *Passiflora actinia*. Braz Arch Biol Technol., 49. pp: 565-73.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013). Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry. IC Journal No: 8192. ISSN: 2278- 4136. Vol: 1(6). pp: 156-159

Sazada, S., Verma, A., Rather, A.A., Jabeen, F. and Maghvansi, M.K. (2009). Preliminary Phytochemical analysis of some important medicinal and aromatic plants. Adv.in Biol. Res., 3. pp: 188-195.

Selvaraj, R., Revathy, C., Charles, A. and Manoharan. S. (1994). Toxicity evaluation of herbal smoke and synthetic mosquito mat on culex quinquefasciatus. Geobias. J. Ethnopharmacology, 59. pp: 80-84.

Sen, A. and Batra, A. (2012). Evaluation of Antimicrobial activity of different solvent extract of medicinal plant media Azedarach.L, International journal of Current Pharmaceutical Research. ISSN: 0975-7066, Vol.4, Issue 2. pp: 274-281

Shah, S., Siraj-ud-Din., Muhammad, Z. and Rehmanullah. (2014). Nutritional analysis and nutrients composition of various parts of Sageretia thea (Osbeck). International Journal of Biosciences. ISSN: 2220-6655. Vol. 4, No. 1. pp: 1-7.

Shariff, N., Sudharshana, M.S., Umesha, S. and Hariprasad, P. (2006). Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. African Journal of Biotechnology, 5. pp: 946 – 950.

Sharma, S.K., Singh, L. and Singh, S. (2013). A review on medicinal plants having antioxidant potential. Indian Journal of Research in Pharmacy and Biotechnology. ISSN: 2321-5674. Vol: 1(3). pp: 404-409.

Sharma, Z.F., Moriguchi, R., Satoru, L.U., Foo, Y. and Pooya, T. (2007). Oral administration of purple passion fruit peel extract attenuates blood pressure in female spontaneously hypertensive rats and humans. Nutrition research, 27: 408-16.

Silva, E.M., Souza, J.N.S., Rogez, H., Rees, J.F., Larondelle, Y. (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chemistry, 101. pp: 1012-18.

Singh, J.S. (2002). The biodiversity crisis, A multifaceted review. Curr. Sci, 82. pp: 638-647.

Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Methods Enzymol, 299. pp: 152-178.

Sivakumar, B. (2013). Carbohydrates. Textbook of Human Nutrition. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi. ISBN: 978-81-204-1742-7. Vol: 4(2). pp: 1-17.

Snehal, P. and Madhukar, K. (2012). Quantitative estimation of biochemical content of various extracts of *stevia rebaudiana* leaves. Asian Journal of Pharmaceutical and Clinical Research, ISSN: 0974-2441.Vol 5, Issue 1. pp: 115-117.

Solowey, E., Lichtenstein, M., Sallon, S., Paavilainen, H., Solowey, E. and Galski, H.L. (2014). Evaluating Medicinal Plants for Anticancer Activity. Hindawi Publishing Corporation. Scientific World Journal. Vol: 20(14), Article ID 721402, 12 pages.

Souza, M., Barbalho, S.M., Damasceno, D.C., Rudge, M.V., Campos, K. and Madi, A.C. (2012). Effects of *Passiflora edulis* (yellow passion) on serum lipids and oxidative stress status of Wistar rats. Journal of Medicinal Food, 15. pp: 78–82.

Srinivasan, R., Raval, B., Suvarchala, A., Honey, A., Tejaswini, A. and Neeraja, P. (2011). *Leucas aspera* - Medicial plant: A Review. Int J Pharma Biosci, Vol: 2(1). pp: 153-159.

Srinivasan, R., Ravali, B., Suvarchala, P., Honey, A., Tejaswini, A. and Neeraja, P. (2011). *Leucas aspera* - Medicinal Plant: A Review. International Journal of Pharma and Bio Sciences. ISSN: 0975-6299. Vol 2, Issue 1. Pp-153-159.

Stuffness, M. and Douros, J. (1982). Current status of the NCI plant and animal product program. Journal of Natural product, 45. pp: 1-14.

Suffrendi, J.B., Sader, H.S., Goncalves, A.G., Reis, A.O., Gales, A.C., Varella, A.D. and Younes, R.N. (2004). Screening of antimicrobial extracts from plants native to Brazillian Amazon rain forest and Atlantic forest. Brazil. J. Med. Biol. Res, 37. pp: 379 – 384.

Sujatha, S., Anand, S., Sangeetha, K.N., Shilpa, K., Lakshmi, J., Balakrishnan, A. and Lakshmi, B.S. (2010). Biological evaluation of (3b)-STIGMAST-5-EN-3-OL as potent anti-diabetic agent in regulating glucose transport using in vitro model. International Journal of Diabetes Mellitus, 2. Pp: 101–109.

Sunitha, M. and Devaki, K.C. (2009). Antimicrobial activity of *Passiflora edulis* sims leaves. Adv pharmacol toxicol., 10. pp: 171 – 174.

Tapas, A.R, Sakarkar, D.M. and Kakde, R.B. (2008). Flavonoids as Nutraceuticals: A Review.Tropical Journal of Pharmaceutical Research, 7. pp: 1089-1099.

Tapsell, L.C., Hemphill, I. and Cobiac, L. (2006). Health benefits of herbs and spices: the past, the present, the future. Med. J. Aust. 185(4 Suppl). pp: S4-24.

Thakur, D.K, Misra, S.K and Choudhari, P.C. (1987). In vitro trails of plant extracts and chemicals for their antifungal activity. Indian. J. Animal health, 40. pp: 964-972.

Thomson, G.E. (2010). Further consideration of Asian Medicinal plants in treating common chronic disease in West. Journal of Medicinal Plants Research, Vol: 4(2). pp: 125.

Uttara, B., Singh, A.V., Zamboni, P. and Mahajan, R.T. (2009). Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol, 7. pp: 65–74.

Vergeese, J. (2010). Analytical Chromatography. Pacific Books International, New Delhi. ISBN: 978-93-80472-05-8, Pp: 1-2, 38, and 235.

Vinay, R.P., Prakash, R.P. and Sushil, S.K. (2010) Antioxidant activity of some selected medicinal plants in western region of India. Adv. Biol. Res. Vol: 4(1). pp: 23-26.

Williams, W.B., Cuvelier, M. E. and Berset, C. (1995). Use of a Free radical method to evaluate antioxidant activity. Food Science and technology, 28. pp: 25-30.

Wink, M., Schmeller, T. and Latz-Briining, B. (1998). Modes of action of allelochemical alkaloids: Intraction with neuroreceptors, DNA and other molecular targets. Journal of chemical Ecology, 24: 1888- 1937.

WEBLIOGRAPHY

- http://chemistry.tutorvista.com/organic-chemistry/phenolic-compounds.html
- http://saffron.pharmabiz.com
- http://www.medallionlabs.com/downloads/antiox_acti_.pdf
- https://pubchem.ncbi.nlm.nih.gov/compound/Oxirane#section=Top
- http://blog.gaiam.com/quotes/authors/hippocrates

Appendices

APPENDIX I

AUTHENTICATION CERTIFICATE OF *LEUCAS ASPERA*

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी.एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No. BSI/SRC/5/23/2016/Tech. 210

दिनांक/Date: 29th February 2016

सेवा में / To

Ms. Akhila. I
II M. Sc. Food Science & Nutrition
Department of Food Science & Nutrition
Avinashilingam University for Women
Coimbatore – 641 043

महोदया/Madam,

The plant specimen brought by you for identification is identified as *Leucas aspera* (Willd.) Link. - LAMIACEAE. The identified specimen is returned herewith for preservation in their college/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

GVS Murthy 29/2/16

(डॉ. जी.वी.एस.मूर्ति/Dr. G.V.S. Murthy)
वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष/
Scientist 'F' & Head of Office

वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष
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Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयंबत्तूर / Coimbatore - 641 003

APPENDIX II

AUTHENTICATION CERTIFICATE OF *PASSIFLORA EDULIS*

भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FORESTS & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
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टी.एन.ए.यू कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

संसं. भा.व.स./द.क्षे.के./No. BSI/SRC/5/23/2016/Tech. / 211

दिनांक/Date: 29th February 2016

सेवा में / To

Ms. Akhila. I
II M. Sc. Food Science & Nutrition
Department of Food Science & Nutrition
Avinashilingam University for Women
Coimbatore – 641 043

महोदया/Madam,

The plant specimen brought by you for identification is identified as *Passiflora edulis* Sims - PASSIFLORACEAE. The identified specimen is returned herewith for preservation in their college/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

GVS Murthy 29/2/16

(डॉ. जी.वी.एस.मूर्ति/Dr. G.V.S. Murthy)
वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष/
Scientist 'F' & Head of Office

वैज्ञानिक 'एफ' एवं कार्यालय अध्यक्ष
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Southern Regional Centre
कोयंबत्तूर / Coimbatore - 641 003

APPENDIX III

PROCEDURES FOR NUTRIENT ANALYSIS

1. ESTIMATION OF CARBOHYDRATE

Principle

Carbohydrates are hydrolysed into simple sugar using dilute Hydrochloric acid (HCl). In hot acidic medium glucose is dehydrated to hydroxyl methyl furfural. This compounds forms with anthrone, a green colour product within absorption maximum at 630 nm.

Reagents

a. 2.5N HCl
b. Anthrone reagent: Dissolved 200 mg anthrone in 100 ml of ice cold H_2SO_4 prepared best before use.
c. Stock standard: Dissolved 100 mg of glucose in 100 ml of water.
d. Working standard: 10 ml of stock standard solution is diluted in 100 ml of distilled water.

Procedure

Weighed 100 mg of sample in a boiling tube. Hydrolyse it by keeping it in boiling water bath and boiled for 3 hours with 2.5N HCl and cooled to room temperature. Then it was neutralysed with solid sodium carbonate until effervescence ceases. Then make up the volume to 100 ml and centrifuge and collected the supernatant and 0.5 ml was taken for analysis. Similarly standard also prepared and made up to 1 ml in all the test tubes by adding distilled water. Then added 4 ml of anthrone reagent and heated for 8 min and cooled rapidly and read at 630 nm in colorimeter. The values obtained were plotted in graph and calculated the amount of carbohydrate present in the sample tube.

Calculation

0.5 ml of mineral solution contains X γ of carbohydrate

Therefore 100 ml of mineral solution contains Y γ of carbohydrate

100 ml of mineral solution was made up from Z g of the food stuff.

Therefore Z g of sample contains Y γ of carbohydrate

Hence, 100 g of sample will contain = (Y x 100)/ (Z x 1000) = P mg of carbohydrate

2. ESTIMATION OF PROTEIN

Micro – Kjeldhal method

Principle

The nitrogenous compounds of plasma or serum are converted into ammonium sulphate by boiling with concentrated sulphuric acid. It is subsequently decomposed by addition of excess of alkali and the liberated ammonia absorbed into a boric acid solution containing bromocresol green indicator by steam distillation. Ammonia forms a loose compound, ammonium borate with boric acid, which is titrated directly against standard sulphuric acid.

Reagents

a. 40% NaOH in distilled water
b. 2% Boric acid: Dissolve 20g of reagent grade boric acid in about 500ml of hot distilled water and add 2ml of 0.1% bromocresol green in alcohol (or aqueous solution of the sodium salt) and finally make up the volume to1 L with distilled water.
c. 1N H_2SO_4 (standardised)

Procedure

Transfer plasma (0.2 ml) or any other biological material into a 50ml long necked micro Kjeldal flask and wash the sides of the flask with distilled water. Add 2 ml of concentrated sulphuric acid and digest on a heated sand bath or on the micro digestion unit. When charring begins and white fumes appear in the flask, stop digestion for a while to allow the flask to cool and add a few drops of hydrogen peroxide. Continue the digestion till the solution becomes clear. If necessary, repeat the addition of hydrogen peroxide and digestion, to hasten the process. Add distilled water and boil for a few minutes and allow the flask to cool.

Take 10 ml of the boric acid solution in a 100ml conical flask and place in such a way that the tip of the condenser outlet of the steam distillation apparatus dips below the surface of the boric acid solution. If the colour of the boric acid solution has faded, 2 to 3 drops of bromocresol green indicator may be added. Transfer the digested sample completely by means of repeated rinsing to the chamber of the steam distillation apparatus. The chamber should be previously cleared of any contaminating ammonia by repeated washings. Add about 8 ml of 40% NaOH (or a quantity enough to make the mixture alkaline) to the digest in the chamber. Start the steam generation and make the distillation set airtight. Steam distill till about 30 ml distillate is collected into receiving flask with a little distilled water.

The solution in the receiving flask is coloured blue at this stage. Titrate the contents against 0.01N H_2SO_4 till the original green colour is obtained. Run the blank preparation through all the steps by taking distilled water in place of sample.

Calculations

1 ml of 0.01N H_2SO_4 = 0.00014g nitrogen. Since average nitrogen content of most proteins is 16%, 1 g nitrogen = 100/16 g protein = 6.25. If the titre value for sample minus the titre value of blank is t,

Protein content of the sample in 100 ml = t x 0.00014 x (100/16) x (100/0.2) = t x 0.4375 g.

3. ESTIMATION OF FAT

Fat is estimated as crude ether extract of the dry material. The dry sample (5-10 g) is weighed accurately into a thimble and plugged with cotton. The thimble is then placed in a soxhelt apparatus and extracted with anhydrous ether for about 16 h. The ether extract is filtered into a weighed conical flask. The flask containing the ether extract is washed 4 to 5 times with small quantities of ether and the washings are also transferred. The ether is then removed by evaporation and the flask with the residue dried in an oven at 80-100ºC, cooled in a desiccator and weighed.

Calculation

Fat content (g/100 g sample) = (Weight of ether extract x 100)/ Weight of the sample (equivalent to fresh sample taken)

4. ESTIMATION OF MOISTURE

About 10g of the material is weighed into a weighed moisture box and dried in an oven at 100 to 105ºC and cooled in a desiccators. The process of heating and cooling is repeated till a constant weight is achieved.

Calculation

Moisture % = [(Initial weight – Final weight) X 100]/weight of the sample

5. ESTIMATION OF IRON

Wong's method

Principle

Iron (Fe) is determined colorimetrically with ferric iron which gives a blood red colour with potassium thiocyanate.

Reagents

a. 30% H_2SO_4
b. 7% Potassium persulphate solution
c. 30% potassium thiocyanate solution: 40 g KCNS is dissolved in 90 ml glass distilled water, 4 ml of acetone is added and the volume made up to 100 ml.
d. Standard iron solution: 702.2 mg ferrous ammonium sulphate is dissolved in 100 ml glass distilled water and after addition of 5 ml of 1:1 HCl, the solution is made up to 1 L and mixed thoroughly (0.1 mg Fe/ml). The standard solution is prepared fresh once in six months.
e. Working standard solution (10 µg Fe/ml) is prepared by diluting the above solution 10 fold.

Procedure

To an aliquot (6.5 ml or less) of the mineral solution, enough water is added (if necessary) to make up to a volume of 6.5 ml followed by 1.0 ml potassium persulphate solution and 1.5 ml 40% potassium thiocyanate solution. The red colour that develops is measurd within 20 min at 540 nm. The red colour that develops is measurd within 20 min at 540 nm. Similarly the standard iron solution is taken in series (1 – 5 ml) in a test tube and added the reagents mentioned above in same order and a standard graph is prepared.

Calculation

-----ml of mineral solution contains X γ of iron

Therefore 100 ml of mineral solution contains Y γ of iron

100 ml of mineral solution was made up from Z g of the food stuff.

Therefore Z g of sample contains Y γ of iron

Hence, 100 g of sample will contain = (Y x 100)/ (Z x 1000) = P mg of iron

6. ESTIMATION OF CALCIUM

Titrimetric method

Principle

Calcium is precipitated as oxalate and is titrated with standard potassium permanganate.

Reagents

a. 4% ammonium oxalate solution
b. Dilute ammonia solution: 2 ml of liquor ammonia with 98 ml water.
c. 1N H_2SO_4
d. 0.01 N Potassium permanganate solution
e. 0.01 N oxalic acid: Sodium oxalate is dried in an oven at 100-105ºC for 12 h. Exactly 0.67 g is dissolved in redistilled water. Five ml concentrated H_2SO_4 is added and solution made up to 1 L after it has cooled down.

Standardisation of potassium permanganate solution: 25 ml of 0.01 N oxalic acid is transferred to an Erlenmeyer flask. One ml of concentrated H_2SO_4 is added, warmed to about 70ºC and titrated against $KMnO_4$ solution, till the pale pink colour remains.

The normality of $KMnO_4$ solution = (25 x 0.01)/ Titre value (ml)

Procedure

Two ml of sample is taken into a 15 ml centrifuge tube. Add 2 ml of distilled water and 1 ml of ammonium oxalate solution and mix thoroughly and leave overnight. Again the contents are mixed and centrifuged for 5 min at 1500 rpm. The supernatant liquid is poured off and the centrifuge tube drained by inverting the tube for 5 min on a rack (care should be taken not to disturb the precipitate). The mouth of the centrifuge tube is wiped with a piece of filter paper. The precipitate is stirred and the sides of the tubes are washed with 3 ml of dilute ammonia. It is centrifuged again and drained as before. The precipitate is washed once more with dilute ammonia to ensure the complete removal of ammonium oxalate. The precipitate is dissolved in 2 ml of 1N H_2SO_4. The tube is heated by placing it in a boiling water bath for 1 min and titrated against 0.01 N $KMnO_4$ solution to a define pink colour persisting for at least 1 min.

Calculation

1 ml of 0.01 N $KMnO_4$ is equivalent to 0.2004 mg of calcium.

mg of calcium/ 100 ml serum = [(X-b) x 0.2004 x 100]/ 2

where, X = Volume in ml of 0.01 N $KMnO_4$ required to titrate the sample, b = Volume in ml of 0.01 N $KMnO_4$ required to titrate 2 ml of H_2SO_4 (blank). If the normality of $KMnO_4$ is 'a', the value obtained in the above formula should be multiplied by the factor, a/ 0.01.

7. ESTIMATION OF PHOSPHORUS

Fiske and Subba Row method

Principle

The phosphate containing solutions are treated with molybdic acid to produce phosphomolybdic acid which is reduced by the addition of 1, 2, 4-aminonaphtholsulphonic acid to giving a blue colour.

Reagents

a. Ammonium molybdate solution – I: Dissolved 25 g of reagent grade ammonium molybdate in about 200 ml of water and transferred in to a one litre volumetric flask containing 500 ml of 10 N H_2SO_4 and then made up to the mark with water and mixed. This solution is stable indefinitely.

b. Ammonium molybdate solution – II: I: Dissolved 25 g of reagent grade ammonium molybdate in about 200 ml of water and transferred in to a one litre volumetric flask containing 300 ml of 10 N H_2SO_4 and then made up to the mark with water and mixed. This solution is stable indefinitely.

c. Aminonaphtholsulphonic acid reagent: 195 ml of 15% sodium bisulphate solution is taken in a glass stoppered cylinder and 0.5 g of 1, 2, 4-aminonaphtholsulphonic acid is added to it followed by 5 ml of 20 % sodium sulphite. It is stopperd and shaken until the powder is dissolved. If the solution is not complete, more of sodium sulphite, 1 ml at a time is added with shaking. The solution is then transferred to a brown glass bottle and stored in the cold. This solution is usable for four week.

d. Standard phosphorus solution: 35.1 mg of pure potassium dihydrogen phosphate was weighed and dissolved in water. Added 10 ml of 10 N H_2SO_4 and made up to 100 ml with water.

e. Working standard: This was prepared by diluting 10 ml of stock standard to 100 ml with water. One ml of this solution contains 8 µg of phosphorus.

Procedure

0.1 ml of the mineral solution was taken in two test tubes. 1ml of ammonium molybdate-II and 0.4 ml of 1, 2, 4-aminonaphtholsulphonic acid was added and the volume was made up to 10 ml with distilled water. For standard 1-5 ml was taken and added 1ml of ammonium molybdate-I and 0.4 ml of 1, 2, 4-aminonaphtholsulphonic acid and made up to 10 ml. All the tubes were mixed well and allowed to stand for 15 min. Simultaneously a blank was prepared by mixing 8.6 ml of water, 1 ml of ammonium molybdate-I and 0.4 ml of 1, 2, 4-aminonaphtholsulphonic acid. The colour developed was read in a colorimeter using a red filter of wavelength 660 mµ.

Calculation

0.1 ml of mineral solution contains X γ of phosphorus

Therefore 100 ml of mineral solution contains Y γ of iron

100 ml of mineral solution was made up from Z g of the food stuff.

Therefore Z g of sample contains Y γ of phosphorus

Hence, 100 g of sample will contain = (Y x 100)/ (Z x 1000) = P mg of phosphorus

8. ESTIMATION OF VITAMIN C

Dye method

Principle

The blue colour produced by the reduction of 2, 6 - dichlorophenol indophenols by ascorbic acid is estimated colorimetrically.

Reagents

a. Acetate buffer, pH 4.0: 300 g of anhydrous sodium acetate, 700 ml of water and 1L of glacial acetic acid are mixed.
b. Dye solution: 25 mg of the sodium salt of 2, 6 - dichlorophenol indophenols is dissolved in distilled water and made up to 200 ml.
c. 6% Metaphosphoric acid (HPO_3)
d. Ascorbic acid standard (1 mg/ml): 100 g of pure ascorbic acid dissolved in 100 ml of 6% HPO_3.

Procedure

A weighed quantity of the material is blended with a convenient volume of 6% HPO_3 and slurry diluted to obtain a final mixture containing approximately 20 µg of ascorbic acid per ml (for example, in case of leaves, 5 g of the sample can be blended with 6% HPO_3 to make 50 ml and 5 ml of the slurry further diluted to 50 ml).

The mixture is then filtered and 2 – 5 ml of the filtrate placed in a 50 ml separating funnel (A). The same amount of the extractant (6% HPO_3) is taken in two more separating funnels, B and C. Funnel B serves as the dye blank, and to funnel C, which serves as standard, is added 0.1 ml of the ascorbic acid standard solution. An equal volume of acetate buffer is added to all the three funnels, followed by 2 ml of the dye solution. Xylene, 10 ml is then added quickly and the contents shaken for 6-10 s. After the layers separate, the lower water layer is removed and the colour in the xylene extract measured in a spectrophotometer at 500 nm.

Calculation

If the ODs of A, B and C are a, b, and c,

then the ascorbic acid contained in the extract = [0.1 x (b-a)]/ (b-c) mg.

The ascorbic acid content of the material can then be calculated by applying the necessary dilution factors.

9. ESTIMATION OF β-CAROTENE

Principle

The individual carotenoids are separated on a column of calcium hydroxide or alumina and determined spectrophotometrically. The values for their respective vitamin A potency are used to arrive at the total vitamin A value of the foodstuff.

Procedure

Preparation of the sample: 25g of freshly ground sample is allowed to stand overnight in a mixture of 100 ml of petroleum ether: acetone (1:1). The extract is then filtered and the residue on the filter, washed twice with successive 500 ml portions of the mixture till all yellow colour is extracted. The pooled filtrate is shaken with 50 ml

portions of water and water washings discarded. This is repeated twice. The solvent layer is then dried over anhydrous sodium sulphate and concentrated under reduced pressure (flash evaporation) to a final volume of 4 ml.

Separation of pigments using calcium hydroxide column: A column of 30 x 1 cm is packed with 6 g of calcium hydroxide under gentle suction. About 1- 2 g of anhydrous sodium sulphate is placed on top of the packed column. The column is initially wet by passing petroleum ether.

Two ml of he concentrated extract containing about 40-100 µg of the total carotene is loaded on the column. Light suction is employed and a 1 % acetone in petroleum ether is used as developing solvent.

The separated bands are individually eluted and their spectra studied in a spectrophotometer to identify the different carotene fractions.

For quantitative estimation of different carotene fractions, standard graphs are obtained with graded concentrations of pure β-carotene dissolved in petroleum ether and this curve is used for determining the concentrations of the solutions of pigments whose main absorption is around 460 nm.

Separation using alumina column: Two ml of the concentrated carotene extract is loaded on to a column of alumina (10 x 1 cm) containing 3% anhydrous sodium sulphate. The β-carotene is eluted with petroleum ether containing 3% acetone. The volume of the eluate is made up to 5 ml and OD is measured at 450 nm.

Calculation

1 OD is equivalent to 4 µg /ml of β-carotene when measured in a cell of 1 cm light path.

The relative potencies of different carotenoids reported in the literature are used to calculate the vitamin A value in the case of each carotenoid fraction. The sum of the values of all the fractions present in the chromatogram of a particular food stuff gives its true vitamin A value.

APPENDIX IV

REAGENT PREPARATION FOR PHYTOCHEMICAL AND ANTIOXIDANT ANALYSIS

PHYTOCHEMICAL ANALYSIS

1. Mayer reagent: 1.35g of mercuric chloride was added to 60ml of distilled water. Prepare a 5g of mercuric chloride solution in 10ml of water. Makeup the volume to 100ml of distilled water.
2. Fehling's A Solution: Dissolve 1.75g of copper sulphate ($CuSO_4$) in 25ml of distilled water.
3. Fehling's B Solution: Dissolve 8.75g of PST (Potassium Sodium Tartarate) and 2.5g of Sodium Hydroxide (NaOH) in 25ml of distilled water.
4. 2% NaOH: Dissolve 2 g sodium hydroxide in 100 ml of distilled water.
5. 2% Ferric Chloride: Dissolve 2g of ferric chloride in 100 ml of distilled water.

ANTIOXIDANT ANALYSIS

a) DPPH Assay

Tris.HCl preparation: Dissolve 0.7875 g of Tris HCl in 100 ml of Deionised water.

b) Total Phenol

20% Sodium Carbonate: Dissolve 20g of Na_2CO_3 in 100ml Deionised water.

Folins Ciocalte Reagent: 1:1 dilution (Folins:Phenol).

c) Total Flavonoids

10% $AlCl_3$ Preparation: Dissolve 10g of $AlCl_3$ in 100ml Deionised water.

1M Potassium acetate: Dissolve 9.814 g of Potassium acetate in 100 ml Deionised water.

[Potassium acetate = (Mol.Wt x Req.Vol x Req.conc.)/1000]

d) FRAP

Preparation of 10% TCA

(10% x Req.Volume)/100 = x g + 5ml D. H_2O

1% Ferric Chloride

(0.1% x Req. Volume)/100 = x g + 5ml D. H_2O