IDENTIFICATION AND ISOLATION OF BIOACTIVE COMPOUNDS IN CRANBERRY-HERBAL INFUSION

By

N.MAALAVIKA

(20PFN012)

A THESIS SUBMITTED TO

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE

AND HIGHER EDUCATION FOR WOMEN

COIMBATORE – 641043

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

THE DEGREE OF

MASTER OF SCIENCE

IN

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R. Balaccisork 27/5/2022
Signature of the Supervisor

M. Sylvia 27/5/22
Signature of the Head of the Department

CERTIFICATE

This is to certify that the thesis entitled, **"IDENTIFICATION AND ISOLATION OF BIOACTIVE COMPOUNDS IN CRANBERRY-HERBAL INFUSION"** submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore in partial fulfilment of the requirements for the award of the degree of Master of Science in **Food Science and Nutrition**, is a record of original research work done by **N.MAALAVIKA** with Register Number **20PFN012** during the period of this study under the Supervision and Guidance of **Dr.(Mrs.).R.BALASASIREKHA**, Assistant Professor (SS), Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641043, Tamil Nadu, India.

27/05/2022

Signature of the Supervisor

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DECLARATION

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27/05/2022

Signature of the Supervisor

Signature of the Candidate

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I. INTRODUCTION

Herbal infusions have become more popular in recent years Herbal infusions are hot beverages that can be consumed as a daily beverage or for medical purposes. Herbal infusions or tisanes are extracts made from plant or herbal ingredients. Steeping an element in a liquid produces an infusion. The flavour and a roma of the soaked substance are subsequently absorbed by the liquid. Herbs and fruits are steeped in boiling water to make an infusion. They can be steeped for a minute to an hour, depending on the strength of the flavour (Monbaliu.S *et al*.,2010).

Herbal infusions have more health advantages than other forms of tea. Herbal infusions are a great way to include mindfulness to the daily routine. Fruits and herbs are rich in vitamins, minerals and antioxidants. There are different methods to preserve the fruits and herbs with its at most nutritional value, one of which is herbal infusion in the form of powder. This infusion can be consumed along with snacks and tea and caffeinated drinks can be replaced with infusion. Hygienically prepared infusions are manufactured mechanically with creative packaging and infusion is favourable for adolescents, adults, middle age adults and senior adults due to its color, flavour, taste, nutritional value and it's shelf life.

Herbs have a long history of being used as preservatives, colourants, flavour enhancers, and even medicinal substances. Increased usage of herbs as culinary flavourings is also in practice for many years. Ethnic cuisines exist in different parts of the world, and because of this, consumption of herbs vary significantly. Assessment of dietary intake of herbs is difficult due to their wide range of applications and the fact that they are ingested in combination with other foods and in trace levels. Herbal concentration in foods typically range from 0.5-1 percent. Phytochemicals are responsible for the beneficial characteristics of herbs. Compounds such as terpenes and terpene derivatives are among the key phytochemical types associated with herbs. Glycosides, alkaloids such as piperine and chavicene, and capsaicinoids are among the other chemicals. Herbs have antioxidative, chemopreventive,antimutagenic, anti-inflammatory, and immunological modulatory properties that help people stay healthy. Herbs are mentioned in dietary guidelines as good sources of antioxidant chemicals and as a salt alternative (Kurian.A *et al*.,2012).

Herbal plants are an essential food ingredient. They not only improve the flavour of food (taste and scent), but also have a positive impact on human health. Thecontent influences their properties. Essential oils, phenolic compounds, tannins, bitter compounds, organic acids, and mucilage are examples of physiologically active substances. Enzymes, vitamins, phytoncides, and other active compounds are also found in herbs. For the quality of herbal material, however, the concentrations of basic nutrients and mineral components are just as significant as the quantity of bioactive chemicals. Phenolics and essential oil components are found in herbs. Phenolic chemicals have anti-inflammatory, anti-allergic, anti-microbial, and anti-cancer properties in addition to their antioxidant action. Herbs, like other plants, have a wide range of phenolic content. The phennoic diterpenes, carnosic acid and its derivative, camosol, and the phenolic acid, rosmarinic acid, are herb-specific phenolics. Lutein, apigenin, hispidulin, quercetin, and kaempferol are examples of flavonoids found in plants (Hedges, L. J *et al*.,2007).

Vitamin C an antioxidant is a necessary vitamin for humans, and its capacity to transfer electrons has pleiotropic effects. It is a cofactor for a number of biosynthetic and gene-controlling enzymes. Vitamin C aids immune protection by supporting both the innate and adaptive immune systems' cellular functioning. It protects the epithelial barrier function against infections and enhances the skin's oxidant scavenging capacity, potentially defending against oxidative stress from the environment. Vitamin C accumulates in phagocytic cells like neutrophils and can boost chemotaxis, phagocytosis, reactive oxygen species production, and eventually microbial death. It's also required for apoptosis and macrophage clearance of wasted neutrophils from infection sites, reducing necrosis/NETosis and potential tissue injury.

Vitamin C's involvement in lymphocytes is less apparent, however it has been demonstrated to improve B- and T-cell differentiation and proliferation, most likely due to its gene-regulating properties. Vitamin C insufficiency lowers immunity and makes more susceptible to diseases. As a result of the increased inflammation and metabolic demands, infections have a major impact on vitamin C levels. Furthermore, vitamin C supplementation appears to be effective in both preventing and treating respiratory and systemic infections.

Dietary vitamin C intakes that give adequate, if not saturating plasma levels (i.e., 100–200 mg/day), optimise cell and tissue levels, are required for prophylactic infection prevention. Treatment of established infections, on the other hand, necessitates much greater (grams) vitamin doses to compensate for the increased inflammatory response and metabolic requirement.(Anitra C Carr *et al*.,2017).

Vitamin C is required for human survival and must be consumed in right quantities for better immunity and good health.Vitamin C has the property of being an electron donor. All of its known functions are accounted for vitamin C is a powerful water-soluble antioxidant in humans because it is an electron donor. Human disorders including atherosclerosis and cancer may be caused in part by oxidant damage to tissues such as lipid, protein, and DNA oxidation. These oxidative biomarkers have been detected in humans. Vitamin C's antioxidant properties cause atherosclerosis and type I diabetes mellitus are the two common human diseases that may be caused or exacerbated by oxidant damage. It may also play a role in the pathophysiology of type 1 diabetes, diabetic complications, chronic renal failure, end-stage renal disease, and hemodialysis, rheumatoid arthritis, neurodegenerative illnesses, and other diseases.

During acute conditions like myocardial infarction, oxidants are thought to cause additional harm to organ systems. Acute pancreatitis, sepsis, and inflammatory illnesses are all linked to cigarette smoking's long-term effects. Vitamin C's effects on vascular endothelium protects endothelial Nitric Oxide from oxidation while also enhancing its synthesis. In healthy people, vitamin C and the other antioxidant vitamin, vitamin E, appear to improve vascular endothelial function. Patients with cardiovascular illness and patients with coronary artery disease (patients) have low levels of vitamin C in the blood have been linked to hypertension and poor endothelial function.

Vitamin C, which can be found in fruits and vegetables, has been shown to protect NO from oxidation and improve endothelial dysfunction. Vitamin C's effects on vascular responsiveness, intestinal iron absorption, and oxidant reduction in the stomach. These effects are assumed to be mediated by vitamin C's antioxidant properties. It may help with vascular disease, hypertension, iron absorption, and stomach cancer prevention (Padayatty, S. J *et al*.,2003).

Vitamin C intake from fruits and vegetables has been linked to malignancies of the oral cavity, larynx-pharynx, oesophagus, lung, stomach, and colon-rectum. Only breast cancer was found to be inversely related to vitamin C intake among hormone-dependent malignancies. There is also an inverse connection between vitamin C intake and cancer risk in populations consuming up to 110 mg per day, but not in populations consuming higher doses. A level of vitamin C intake beyond which tissues may be saturated and no more help in cancer prevention can be obtained.

Ascorbic acid's antioxidant properties prevent the generation of carcinogenic N-nitroso compounds, which are linked to gastric and lung cancer. Vitamin C is actively secreted in the gastric juice, and ascorbate levels in the gastric juice are lower in individuals with gastritis and *Helicobacter pylori* infection. Vitamin C administration enhances gastric ascorbate, lowers cancer biomarkers and oxidative damage in the gastric mucosa, and may lower the incidence of gastric cancer in these patients. (R. A. Jacob *et al*., 2002).

The link between nutrition and health has been well established, and many people in many countries think that food provides more than just energy. Herbs and natural compounds made from plants are among the world's oldest remedies. Plants have long been used to treat a wide range of ailments, including cancer, the second leading cause of death after heart disease. Over 30,000 plants were analysed for anticancer characteristics by the National Cancer Institute, and numerous studies were conducted to prove the anticancer potential of plants or their natural components.Plants and dietary agents, on the other hand, are utilised in cancer prevention and therapy rather infrequently in the medical field. Herbal infusions are popular in the Mediterranean and throughout the world. According to a study of 1260 cancer patients in Palestine, 60.9 percent of them used herbs, mostly in the form of decoctions. These drinks are typically made with aromatic plants from the *Lauracae, Umbelliferae, Lamiaceae, Myrtaceae*, and *Compositae* families. Several studies on the plants used to make herbal infusions have discovered that several of these plants have potent antioxidant and anticancer properties. On the other hand, excessive use of these herbal infusions may have unintended and negative repercussions. (Talib *et al*., 2020).

Common name of cranberry is karonda.The *vaccinium subg. Oxycoccus* (cranberry) is an American native fruit. Its native range stretches from the East Coast

to the Central United States and Canada, and from Southern Canada in the north to the Appalachians in the south, in moderate temperature zones.The plant has a perennial habit and grows as a low-growing, trailing, woody vine. Anthocyanins can be found in a variety of fruits, but especially in red, purple, and blue berries. The typical anthocyanin amount of a ripe cranberry at harvest is 95 mg/100 g, with reports of anthocyanin levels as high as 124 mg/100 g fresh fruit weight. Sources of flavonols and flavonoid content of chosen foods range from 30 mg/100 g fresh fruit weight to 48 mg/100 g.3-0-galactoside, delphinidin 3-0-galactoside, pelagonidin 3- O-galactoside, and malvidin 3-0-O-arabinoside make up around 75% of the flavonols in cranberries.The flavan-3-ol level of a ripe cranberry at harvest averages 7 mg/100 g, with content as high as 11 mg/100 g fresh fruit weight.The proanthocyanidin content of raw cranberries can range from 410 mg/100 g fresh fruit weight to 505 mg/100 g, while higher levels have been documented. The proanthocyanidin content of cranberry berries from the early black cultivar appears to be much higher. The detection of hydroxycinnamate esters in the whole fruit in levels averaging 15-20 mg/100 g fresh fruit was achieved using liquid chromatography combined with mass spectrometer analysis of various cranberry cultivars (Cote.J *et al.*,2010).

A number of factors influence cardio vascular disease (CVD), including oxidative stress and inflammation. Antioxidants and anti-inflammatory chemicals such as poly aluminum chloride, anthocyanins, and flavonol glycosides are abundant in cranberries, with quantities varying depending on cultivar. LDL cholesterol oxidation has been linked to the development of cardio vascular disease (CVD). By inhibiting LDL cholesterol oxidation, inducing LDL receptor expression, and enhancing cholesterol absorption, cranberry consumption may help to prevent CVD (A.B.Howell *et al*.,2009) .

The Vaccinium genus contains a lot of antioxidants. Berries from all of the major Vaccinium species (*V. macrocarpon, V. oxycoccos L, V. vitis-idaea L*.) have been shown to have a considerable antioxidant potential. Cranberry fruits protect the vascular endothelium from oxidative damage, such as the oxidation of low-density lipoproteins and oxidative and inflammatory damage. Cranberry antioxidant activity is positively associated with vitamin C concentration (Tunde Jurikova *et al.*,2018)

Cranberry's Culinary Applications in Indian Cuisine Cranberries are made into juices, sauces, syrups, and dried cranberries that have been sweetened. They can be eaten as a jelly or a compote, or cooked with a slurry to make cranberry sauce. Muffins, scones, and cakes with cranberries are delicious. Cranberry juice, which is incorporated into many mocktails and drinks, is also made with them. Cranberry wine is also made with it.

Multiple *in vitro* studies are being conducted to determine the cancer-inhibitory potential of cranberries and cranberry-derived compounds. Cranberries are thought to affect cell viability, proliferation, cell death, adhesion, inflammation, oxidative stress, and signal transduction pathways, according to preclinical research. The efficiency of cranberry-derived elements in terms of cell density and viability is the subject of many *in vitro* research at first. Assays serve as reasonable beginning points for assessing whether more mechanistic analysis is required. In India, breast cancer is the most often diagnosed invasive cancer among women. Because of the various types of tumours, breast cancer is extremely tough to cure. Cranberries have been proven to have anti-carcinogenic properties, such as inhibiting the growth of various cancer celllines in vitro and inhibiting the activity of Ornithine Decarboxylase (ODC) (Jie Sun *et al*.,2002).

Common name of thyme is ajwain. (thyme) *Thymus vulgaris L*. is a fragrant, medicinal plant of the Labiate family *(Lamiaceae)*, subfamily *Nepetoideae*, tribe *Mentheae*. The genus' distribution is Eurasian, with the Mediterranean region, particularly the Iberian Peninsula and North-West Africa, as its centre .Southern Europe, from Spain to Italy, is home to Vulgaris. It is widely grown there, as well as in most mild-temperate and subtropical regions, such as those found in Southern and Central Europe. *Thymus vulgaris L.* is a 10-30 cm tall perennial evergreen subshrub. The little grey-green leaves are opposite, oblong-lanceolate to linear, and have an oblong-lanceolate to linear shape. Thyme was one of eight dried herbs out of more than 60 tested that had high total antioxidant contents of more than 75 mmol/100 g. T. vulgaris L. is used as a food and for medical purposes, and its monoterpene derivatives, such as p-cymene, thymol, and carvacrol, have a high commercial value.

Thymus genus are valuable medicinal plants with a wide range of therapeutic characteristics in their essential oils. Because of their pharmacological and biological

qualities, thymus species are considered therapeutic plants. Its major components, thymol and carvacrol, are responsible for its characteristics. Among all herbs, fresh thyme has the highest antioxidant content. *Thymus vulgaris L*. is commonly used in folk medicine to treat a wide range of ailments, including gastroenteritis and bronchopulmonary problems.Thyme's antioxidative properties are useful in both medicinal and non-medicinal conditions. Because oxidative stress plays a role in the aetiology of many human diseases, antioxidative supplementation is critical for chronic disease prevention. The antioxidant property serves as a preservative in non-medicinal applications, particularly in preventing lipid oxidation in food.Thyme herb or processed products can be used as a standalone flavouring or in the formulation of compounded seasonings, spices, essential oils, oleoresins, or other product mixes in culinary and/or food processing.

Thyme is used in practically every type of food, including beverages, cheese, fish, meat, salad dressings, sauces, vegetables, eggs, game and poultry, soups, and honey, usually, because of its sensory properties.The following features of thyme components describe the major functions of thyme in culinary and food processing: i) scent and taste, (ii) antioxidant, and (iii) antibacterial activity. Fresh green thyme leaves can also be utilised as a decorative green herb in culinary art. Food flavouringis clearly the primary application for thyme, while its antibacterial and antioxidant capabilities can be considered extra benefits of thyme products that have been addedto foods. The ability to successfully exploit all three thyme component benefits, namelyflavour and microbiological and oxidative spoiling prevention, is dependent on product requirements, processing factors, and food producer experience.(Stahl-Biskup and venskutonis,2012).

Common name of fennel is saunf. The fennel plant, *Foeniculum vulgare*, is also known as fennel. It belongs to the Umbelliferae (Apiaceae) family of medicinal plants. Fennel is a seasonal herb with a long history.The fennel plant originated in thesouthern Mediterranean region, and it now grows wild throughout the Northern, Eastern, and Western hemispheres, notably in Asia, North America, and Europe, thanks to naturalisation and cultivation.*Foeniculum vulgare*, is a perennial herb with soft, feathery, almost hair-like leaf that grows up to 6.6 feet (2 metres) tall. Fennel hasa reputation for promoting a healthy appetite and digestion. Fennel can aid in the expulsion of wind from the alimentary canal, so relieving the respiratory system and

providing relief from coughs and bronchitis. (S.K. Malhotra et al.,2012) *Foeniculum vulgare*, is a common spice and beverage in Egyptian cuisine, as well as in traditional medicine for its estrogenic, lactagogue, diuretic, antioxidant, immunological booster, and dyspepsia-relieving properties. Thymol, carvacrol, terpinenes, P-thymene and thymol methyl ether, and phenolic glycosides are among the phytochemicals found in it (Naglaa M Ebeed *et al.,*2010).

Common name of dill leaves is savaa .Dill (*Anethum graveolens Linn.*) is a seed spice crop and an aromatic herb used to flavour tea, pickles, and sweets. Minerals, proteins, and fibre abound in dill leaves. Spice, culinary, and medicinal purposes are the most common uses of the seeds. Dill is thought to have originated in South West Asia or South East Europe, and has been grown since antiquity . The dill crop is thought to have originated in Europe, Africa, and Asia. It is grown in nations such as India, Germany, Hungary, the Netherlands, Pakistan, and the United States. India and Pakistan are the primary producers of dill.Dill is a tall, hardy annual plant in the Apiaceae family. The leaves are fern-like, slender, and wispy. The glossy stem is normally single, erect, dull-green cylindrical, subdichotomously branched, and can grow to a height of 0.9-1.5 m. Small yellow blooms and yellowish brown, ridged umbels adorn the big umbels. Fruits that are flattened and ovoid in shape. The little yellow dill blossoms, unlike the flowers of most other umbel liferous crops, have a strong odour but little nectar.(R. Gupta *et al*.,2012). Eugenol, a bioactive component found in dill leaves, has significant anti-diabetic actions that help to lower blood sugar levels in the body. Dill leaves are known for their digestive properties. Fresh dill sprigs' anti-flatulent activity decreases gas generation in the alimentary canal, reducing bloating, flatulence, and abdominal distension. Bones are an important aspect of the human body because they offer muscles and organs shape, structure, and support. Dill leaves assist normal bone cell formation, regeneration, and maintenance by providing calcium, iron, vitamin D, thiamine, riboflavin, and fibre. This herb's antioxidants and vitamin C have been utilised to fight germs and protect the body against numerous diseases since ancient times. (Soumita Basu,2020).

Dill leaves are used for culinary purpose.The young leaves and stems of dill are often used for seasoning sauces and salads, for flavoring vinegar, and in pickles. The seeds are sometimes used in pastries, soups, and stews, but about two feet high and has much most often in dill pickles (Arthur E Hutchins *et al .,*1958).

Cranberry- Herbal infusion, is prepared from natural ingredients of different morphological plant parts, namely fruit,herbs and herb seed. Cranberry Herbal infusion are rich sources of natural bioactive compounds and Vitamin C. researches show that these bioactive compounds render a myriad of biological effects, such as antioxidant, anti-viral , anti –inflammatory, anti- allergic, anti-cancer,anti –thrombotic and vasodilatory action, as well as antimutagenicity, anticarcinogenicity and antiaging effects,among others. The present study is an attempt to Identify and Isolate Bioactive compounds in Cranberry-Herbal Infusion.

Hence the present study entitled "**Identification and Isolation of Bioactive Compounds in Cranberry- Herbal Infusion"** with the following objectives:

- develop and standardize cranberry-herbal infusion using selected herbs
- evaluate the develop cranberry-herbal infusion organoleptically
- identify the antioxidant vitamins and phytochemicals present in the cranberry herbal Infusion
- isolate and analyse the bioactive components present in cranberry – herbal infusion using GCMS and
- determine the cytotoxicity effect of cranberry-herbal infusion by MTT assay.

II. REVIEW OF LITERATURE

The Review of Literature pertaining to the study entitled **"Identification and Isolation of Bio Compounds in Cranberry -Herbal Infusion"** is presented under following headings.

A. **Taxonomy, morphology and distribution and nutrient content of cranberry and selected herbs**
B. **Bioactive compounds of cranberry and selected herbs**
C. **Traditional medicine and ethno medicinal uses of cranberry and selected herbs**
D. **Therapeutic properties of cranberry and selected herbs**
E. **Anticancer effect of cranberry and selected herbs**
F. **Role of herbs in human nutrition of cranberry and selected herbs**

A.Taxonomy,morphology,and distribution and nutrient content of cranberry and selected herbs

Vaccinium macrocarpon (Cranberry)

The botanical name for cranberry is *Vaccinium* macrocarpon.It belongs to the Ericaceae family , *Vaccinium macrocarpon* is a eudicots belonging to a varied group of Angiosperms. It also belongs to the asterid clade, which includes the Cornales and Ericales orders. Ericales is an extremely varied order with 22 families. At least two species, *Vaccinium macrocarpon* Ait. (big American cranberry) and V. oxycoccus L are included in this category (Polashock J *et al*., 2014).

Due to the great demand for production in the Latvian and European markets, the American cranberry fields in Latvia grow every year. *Vaccinium_macrocarpon* (American cranberry) was introduced from North America and is now widely cultivated. In Latvia, growing cranberries has been popular since 1990, and are rated as one of the most successful fruit crops right now. (Vilka *et al.*, 2015).

Thymus vulgaris (Thyme)

Thymus is included in many European pharmacopoeias, recommending its use in medicines, balms and active dietary supplements. The raw materials of Wild *Thymus serpyllum L.* and the cultivated *Thymus vulgaris L*., implanted in the State

Pharmacopoeia of Ukraine, are mainly used for the production of medicines in Ukraine. There are 15 to 40 species of the genus. Thyme is a compound of fragments of productive shoots and vegetables (leafy stems), leaves, parts of inflorescences and flowers (or parts of flowers, such as calyx and corolla) (Minarchenko V *et al*., 2019).

Gland-dotted and 5-10 mm length and 0.8-2.5 mm broad. The edges of their margins are recurved. The flowers are light violet, two-lipped, 5 mm long, with a hairy glandular calyx, and are borne in loose whorls on the branchlets or in terminal oval or spherical heads with leaf-like bracts in axillary clusters.Thymol (2-isopropyl-5 methylphenol), its isomer carvacrol, p-cymene, and -terpineol are the main volatile elements of thyme.Thymol and carvacrol are found in thyme leaves with 8.6 and 0.7 mg/g, respectively (Stahl-Biskup and Venskutonis,2012).

Biometric estimates of plants are carried out during the growing season which includes the number of days from two, four, six and eight , the height of the plant, the location of the crown of large size, small bed, the crown area of the bed, the stem number per plant, germination percentage, sprout speed, number of days first flowering and the number of days up to 50% of flowering. The largest and smallest total plant lengths were observed in subtraction 8s and 10s, respectively. Accessions 4 and 12 appear to have a smaller and smaller size of the canopy crown area, respectively. The largest and smallest values in the canopy crown area were found at entrances 4 and 6, respectively. The largest and smallest number of stem numbers in each plant were seen at intervals of 7 and 1, respectively (Marzieh Dalir *et al*., 2017).

Camphor Thyme (*Thymus camphoratus)* is described as a multi-branched tree, with a small diameter up to 40 cm, with small oval leaves and flowering heads, glittering pink or violet flowers, giving broad, red bracts. Stage Types Thymus is characterized by brightly colored veins, twisted genes (downward) and lower tomentose (dense with short hairs). The calyx is almost campanulate (metallic). *Thymus camphoratus* is separated from *T. carnosus* by closely relating to the frontal hairs of the upper leaves, and from *T._capitellatus* by the width of the inflorescence, the length of the calyces, and the fact that the upper calyx teeth of *Camphor Thyme* are usually ciliate (with hair extending from the edges). Thyme *Thymus camphoratus* is best identified in Quinta da Rocha, separated from closely related species *T.Vulgaris*(Simonson *et al*.,2014).

Thymus vulgaris L. (Lamiaceae) is a evergreen plant found in southern Europe and the Mediterranean. At least 20 different species of *T.vulgaris* were indetified based on the composition of essential oils. Moreover, this is the first analysis to showthe enantiomer distribution of *Thymus vulgaris* monoterpenoids. Enantiomers are best known for evoking odors in insects and in humans a specific chemo type act as a herbal remedy and medicine. *Thymus vulgaris* due to its enantiomeric distribution have a significant impact on its bioactivity, taste, and aroma profile. (Prabodh Satyal *et al*., 2016).

***Foneiculum vulgare* (Fennel)**

Iran is one of the most important producers of fennel seeds in the world (FAO, 2012) and unlike commercial fennel cultivation, Iranian fennel landraces have never been studied in full or in the seeds and production of essential oils or components of essential oils. Fennel med quality is associated with seed yield, essential oil content and concentration of active components. Fennel figures in Iran are scattered from the city of Chabahar (25 26◦N and 60 61◦E) off the coast of Oman to the city of Mako (40 39◦N and 44 45◦E) near Caucasia, from arid areas like the city of Yazd I 100 mm to humid areas such as Talesh town with 2000 mm annual rainfall, from m 21m ali Bandar's town ukuya to 2600 m high Fareydon shahr town, from Zarine obato town with an average annual temperature to edolobheni Ahvaz is 27 ◦C, so Iranian fennels live in very different environments and because of their familiarity with local geography since time immemorial, it is assumed that each region has its own fennel landrace and this title has been proven morphologically, genetically and cytogenetically. (Kaivan Bahman *et al.*, 2015)

Foneiculum vulgare is a perennial plant with long, soft, feathery, hairy leaves that grow up to 6.6 ft. (2 m) height. It is often grown in vegetable gardens because of its fragrant leaves and anise seeds, both of which are often harvested for use in cooking. Straight and cylinder, bright green, and smooth as it appears to be polished, it has many leafy branches that are cut into finer parts. The leaves grow up to 40 cm long; well-dispersed, with filiform ends (like thread), about 0.5 mm wide. The bright golden flowers, produced by large, flat umbels, with thirteen to twenty rays, bloom in July and August. Leaves,striate stem, leaving 3-4 pinnate, filiform segments, up to 1.6 in. (4 cm) height and leaf base sheathing are its characteristic features.It has a green,

clear, and smooth stem with strong straight branches and different leaves according to successive stages. Radiation is 5–30 numbers 0.39–2.4 inches long (1–6 cm) .The flowers are small, yellow, and are found in large flat umbrellas. Fruits are ovoid 0.12–0.2 inches (3–5 mm) long and 1.5–2.0 mm wide (Shamkant B. Badgujar *et al*., 2014).

The *vulgare* originates in the Old World- perhaps the Mediterranean region considering its use by the ancient Egyptians, Greeks and Romans- but it is widely introduced and practiced naturally all over the world and today is reported to be native to North, Central, and South America and the Caribbean Islands. sweet fennel and bitter fennel,Sweet fennel is not found in the wild; is grown in Bulgaria, France, Italy and Macedonia. Bitter fennel is grown in India, Argentina, Czech Republic, France, Germany, Hungary, Italy, Japan, Romania and South Russia. Indian fennel has a sweet anise flavor while Persian fennel has a strong anise flavor and flavor. (Datiles M J *et al*., 2015).

***Anethum graveolens* (Dill leaves)**

Green leafy vegetables are good sources of minerals as well as vitamins. *Dill (Anethum graveolens L*.) is a leafy, widespread leafy plant that belongs to the family Apiaceae (Umbelliferae) with an attractive taste. Dill originated in central Asia although wild dill and weed species abounded in the Mediterranean and West Asia. To test genetic diversity in different areas of dill (*Anethum graveolens L.*) based on morphological features and molecular markers, seeds of 37 parts collected in different parts of Iran in addition to the European ones, obtained by IARS (Iran- Isfahan Agriculture Research Station) were planted in the research field of the Faculty of Agriculture and Natural Resources in Shirvan, Ferdowsi University, Iran (37 ° 26″ north and 57 ° 45″ east, 1067 m above sea level) in 2009. done by random design of triple blocks. Seeds are sown in three rows with a spacing of 50 cm and a distance of 15 cm between plants. The sites were watered weekly. To quantify genetic diversity, 14 plants in the middle row of each section were harvested. (M. Solouki *et al*., 2012).

Dill (*Anethum graveolens L.)* is a native of the Mediterranean and Russia. It is cultivated in many European countries, USA and Canada, India, Pakistan, and Japan.(Sanda Andrei *et al.,*2018).

B.Bioactive compounds of cranberry and selected herbs

Vaccinium macrocarpon (Cranberry)

European cranberry, the *Vaccinium oxycoccos*, is an important representative and is group, is an important source of antioxidants and other active organisms, such as the well-known and studied American cranberry (*Vaccinium macrocarpon)*. European cranberry fruit is particularly rich in polyphenolic compounds anthocyanins (12.4–207.3 mg / 100 g fw), proanthocyanins (1.5–5.3 mg / 100 g fw), and flavonols, especially quercetin (0.52–15.4 mg). / 100 g fw), which in particular contributes to the antioxidant activity of the fruit. Small cranberry is also important because of its biological effects such as protection of urinary tract (proanthocyanidins), antibacterial and antifungal properties (quercetin, proanthocyanidins, anthocyanins), cardio protective (proanthocyanidins) and anticancer activities (proanthocyanidins) . (Tunde Jurikova *et al.*, 2018).

Berries are a rich source of a variety of unhealthy, nutritious, and bioactive compounds such as flavonoids, phenolics, anthocyanins, phenolic acids, stilbenes, and tannins, as well as nutritional compounds such as sugar, essential oils, and carotenoids, vitamins, and minerals. Bioactive compounds derived from berries have a strong antioxidant and anticancer effect. (Shivraj Hariram Nile *et al*., 2014).

Large varieties of bioactive compounds are found in cranberries - flavonols (quercetin 3-galactoside), flavan 3-ols (catechin and epicatechin), anthocyanins (cyanidin) 3-galactoside, cyanidin 3-arabinoside, peonidin 3-galactoside. Also peonidin 3-arabinoside), tannins (ellagitannins and proanthocyanidin), and extracts of phenolic acid (fer ulic acid, coumaric acid, coumaroylglucose, feruloylglu cose, caffeoylglucose, chlorogenic acid, ellagic acid, and p hydroxybenzoic acid). These compounds represent many promising health features (J.Cote *et al.*, 2010).

The cranberry fruit is a unique and rich source of bioactive polyphenol compounds. Among the different classes of polyphenols, the flavonoid family has the largest variety of compounds with pharmacological activity. The three major flavonoids found in whole cranberries are the flavonols, anthocyanins, and the flavanols that include monomeric flavan-3-ols and the polymeric flavan-3-ols called proanthocyanidins. Very minute quantities of other polyphenols may also be found in cranberries including flavanones (e.g., prunin), non-flavonoids such as phenolics acids

(e.g., benzoic acid), and anti-oxidant stilbenes (e.g., resveratrol). The role that specific cranberry flavonoids play in disrupting cariogenic virulence.(Nebu Philip *et al*., 2019).

American cranberry has a complex and rich phytochemical structure, especially flavan-3-ols, A-type procyanidins (PACs), anthocyanins, benzoic acid, and ursolic acid. Cranberries per 100 g new weight (FW) provides 419 ± 75 mg total flavan -3-ols, including 70 ± 13 mg oligomers with 4–6 DP, 63 ig 15 mg oligomers with DPs of 7–10, as well as 234 ± 49 mg polymers, while monomers, dimers, and trimers are available in low doses (7.3 ± 1.5, 26 ± 6, and 19 ± 3 mg, respectively) .The values of anthocyanins are significantly higher in cranberry.The main anthocyanins are 3-O-galactosides and 3-O-arabinosides of cyanidin and peonidin; a total of 13 anthocyanins, mainly 3-O-monoglycosides. Cranberry anthocyanin content increases maturity.Canberry also contains phenolic acids, including hydroxybenzoic and hydroxycinnamic acid.The main hydroxycinnamic acids in cranberry are p-coumaric, sinapic, caffeic, and ferulic acid, with a content range of 8.8 to 25 mg / 100. (Jeffrey B. Blumberg *et al*., 2013).

Cranberry juice has been used in the medical field for the past decade because of its many health benefits. Because of its properties, cranberries and their cellular components have received the attention of researchers. The main bioactive component of cranberry is proanthocyanidins. Its improved health benefits are now available in the treatment of UTI and many systemic diseases (Khuntia Prach *et al*., 2020)

Thymus vulgaris (Thyme)

Herbs rich in bioactive phytochemicals have been recognized to have biological functions and have many health-promoting effects. In this work, cold pressed thyme (*Thymus vulgaris L.*) oil (TO) was studied in lipid classes, fatty acid profile, tocols and phenolics content. Antioxidant activity and ability to release TO energy against free radicals (DPPH · and galvinoxyl) are limited. Neutral lipids account for the highest lipid content in the TO, followed by glycolipids and phospholipids. The major fatty acids in TO were linoleic, oleic, stearic, and palmitic. Γ-Tocopherol (60.2% of total tocols) followed by α-tocotrienol (26.9%) and α-tocopherol (9.01% of total tocols) were the main tocols. TO contained high concentrations of phenolic compounds (7.3 mg / g as GAE). TO had a strong antiradical action where 65% of DPPH · radicals and 55%

galvinoxyl radical were extinguished after 60 minutes of incubation. Rancimat tests showed that the import time (IT) for: Sunflower oil mixture (1: 9, w / w) was 6.5 h, while TO: sunflower oil mixture (2: 8, w / w) recorded higher IT (9h). flavus (32 mm) with low lethal compounds (MLC) ranging from between 80 to 320 µg / mL. In addition, TO has shown extensive spectra activity against food-borne pathogens including *S. aureus* (30 mm), *E. coli* (25 mm) and L. Monocytogenes (20 mm) with MLC ranging from between 160 to 320 µg / mL. (U-Adel MA Assiri *et al*., 2016).

bioactive compounds of thyme-rich corn oil and determine the antioxidant activity of its methanolic extraction. The formation of flexible compounds was investigated by Gas Chromatography-Mass Spectrometry (GC-MS), while analysis of phenolic compounds was performed by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). Antioxidant activities of methanolic flavored oil extract were tested using DPPH radical scavenging, β-carotene-linoleic acid bleaching, energy reduction and complete antioxidant testing. The most volatile oils were p-cymene (26.47%), α-thujene (24.06%) and γ-terpinene (14.30%). Its methanolic extract had higher Total Phenol Contents (TPCs) (53.99 mg 100 g-1) than crude oil (23.63 mg 100 g-1). Thymol was the main source of phenolic compounds in spicy oils (55.84%). Flavored methanolic oil extract showed higher antioxidant activity (185.22 mg Gallic Acid Equivalent (GAE) 100 g-1) than crude (100.66 mg GAE 100 g-1). In β-carotene-linoleic acid bleaching and DPPH radical scavenging assays, flavored oil methanolic showed higher activity than crude oil based on IC50 values. (Jabri Karoui *et al.*, 2016).

The effect of different concentrations on phenolic, volatile, antioxidant, and color properties of thyme (Thymus vulgaris L.) teas has been investigated in a recent study. Thyme teas are brewed using freshly boiled water at intervals of 0, 2, and 5 min. The effect of the time given to him on phenolics, volatiles, and antioxidant properties was clearly evident. Among the phenolic compounds identified in the samples were LC-MS-MS, luteolin-diglucuronide-glucuronide and protocatechuic acid-hexoside were identified for the first time in thyme tea, and the most prominent phenolic compound in all subsequent samples luteolin-diglucuronide. - glucuronide. An important correlation between phenolic compounds and antioxidant properties suggests that phenolic compounds affect the antioxidant capacity of infusions of thymetea. A total of 27 flexible computers were identified and calculated from samples . The

main chemical group of variables in thyme tea was terpenes and represented about 90% of the total composition of thyme tea. Among terpenes, thymol (61.74–78.82%), ip-cymene (8.92–17.90%) and carvacrol (4.41–5.62%) are the most abundant storms in all teas (Ahmet Salih Sonmezdag *et al*., 2018).

Foneiculum vulgare (Fennel)

Fennel seeds are a source of many nutrients, namely, sugars, minerals, essential oils, essential vitamins, proteins and fiber. They are also rich in essential oils and many flavonoids. Fennel seeds with a pleasant taste are popular all over the world and are widely used in cooking and medicine. Bread reinforced with fennel seed powder is therefore more likely to be accepted by consumers than white bread or other solid bread. In this study, bread samples were prepared by adding white flour with fennel seed powder at 3.0, 5.0, 7.0, 10.0 and 15.0% levels and their physical, sensory, antioxidant and staling properties. The crumb humidity increased and reached a high level of 7.0% degree of increase with a gradual increase in the strength of the crumb. An effort has been made to understand the role of fennel seed powder in bread samples during storage. Antioxidant activity (i.e. total phenolic content, ferric antioxidant capacity and 2, 2-diphenyl-l-picrylhydrazyl radical scavenging) showed an increase of 7.0% supplementation rate. Bread with a fennel seed content of between 5.0 and 7.0% showed high acceptance among samples of fortified bread. (Lipi Das *et al*., 2013)

Study of cytotoxic experiments, antimicrobial and confocal analysis of zinc oxide nanoparticles (ZnO NPs) derived from the novel plant product fennel (Foeniculum vulgare Mill.) Seed extract (FSE) .ZnO NPs analyzed using UV-Vis spectroscopy, XRD, FTIR, TEM techniques and EDX. The MTT cell cytotoxicity assaymeasured the proliferation and survival of MCF-7 cells treated in various FSE-derivedZnO NPs. Antimicrobial activity against pathogenic bacteria and yeast species has been investigated.The UV-Vis spectra showed two peaks at 438 nm and 446 nm, confirming the formation of the nanoparticle. SEM morphology results showed porousfrom 23–51 nm. The amount of antitumor activity (IC 50) was 50 µg / mL and 100 µg / mL. In addition, the morphological mutations of MCF-7, cells treated at different FSE sites of ZnO NPs are observed in cell culture transplanted with a temporary vector expressing pCMV6-XL4-GFP containing a base of C-terminal proteins GFP-tagged,

which led to an aptotic effect. Antimicrobial IZ increased No Inhibition to 18.00 ± 0.4. The IZ presented at the highest level was E. faecium VRE and yeast Cryptococcus sp. (18.00 ± 0.4. mm), followed by S. aureus (17.00 ± 0.2 mm) and P. aeruginosa andyeast C. parapsilosis (16 ± 0.4). mm). IZ was similar to that caused by nystatin in Cryptococcus sp., Which was significantly higher than amp treatment for S. aureus, P. aeruginosa, C. albicans, and C. parapsilosis. The MIC value of ZnO NP taken from the FSE was tested against E. faecium and C. albicans was 6.00 µg / mL (E. faeciumand C. albicans). It was 32.00 µg / mL (S. aureus, S. typhimurium and Cryptococcus sp.), 64.00 µg / mL (P. aeruginosa), and 128 µg / mL (C. parapsilosis). (Mohamad S AlSalhi *et al*., 2020)

Anethum graveolens (Dill leaves)

Research has been done to evaluate the role of Anethum graveolens L. (dill) leaf extract in controlling corticosteroid-induced type 2 diabetes in female mice. In animals treated with dexamethasone (1 mg / kg for 22 days) an increase in insulin and glucose concentration in blood serum and hepatic lipid peroxidation was observed. However, there has been a decrease in serum secretion of thyroid hormones and endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and decreased glutathione (GSH) in the liver. In animals treated with the same amount of dexamethasone at the same time (22 days) when they received leaf discharge (100 mg / kg b.wt / d.) 15 days ago your decrease in serum glucose and insulin levels was noted, indicating the ability of the plant to release corticosteroid-induced diabetes. Changes caused by Dexamethasone to thyroid hormone levels and hepatic LPO, SOD, CAT and GSH were also altered by plant extraction. (Sunanda Panda *et al.*, 2008).

Traditional medicine, especially Ayurveda, has established the use and expertise of many natural remedies and their medicinal properties. However, the Asian continent or specifics, India, can be considered the "Land of Spices". The story of herbal remedies has been passed down from generation to generation with the motto “Make Food Like Medicine”. Such an unusual and widely used herb Anethum Graveolens (Dill) has the potential to create a variety of bioactivities. The whole plant, used as a vrushya (natural aphrodisiac), vataghna (vata balance) eliminates free

radicals, anti-vrana (non-healing wounds), shoola (abdominal colic pain), to treat disorders and ulcers in the eyes, it plays an important role. in the enema during panchakarma (bastikarma), acts as a galactagogue, prevents uterine fibroids, increases milk production during lactation and above all improves the taste of food.

Through this domain, the large lacuna has an understanding of function and mechanism of action at the cellular level. Therefore, this highlights the therapeutic potential of Dill seeds and their potential targets with modern knowledge and effects using the ayurpharmacomic method (understanding the structure of an ancient remedy and exploring its aspects of medical science). In addition, visual experiments were performed to assess the activity-structured (SAR) relationship between lead phytobioactive and biomarker / pathological targets. These studies will better understand the potential mechanisms in the new target development of drugs for the development and development of drugs in the future, which will facilitate primary phytobioactive activity (Kounaina Khan *et al*., 2010).

C. Traditional medicine and ethno medicinal uses cranberry and selected herbs

Vaccinium macrocarpon (Cranberry)

Cranberry as a potent drug that can cure various ailments such as infection and treatment with Native Americans has a long history of use. Uropathogens cause urinary tract infections that are more common in women. Cranberries are used as a valuable medicine in the treatment of urinary tract infections. Due to its high content of flavonoids and phenolic acids cranberries are among the fruits in both quality and quantity of antioxidants. It helps to improve normal life and provides protection from disease. Cranberry extract contains a large amount of polyphenols and organic acids and because of these nutrients is a powerful anti-oxidant. Therapeutically it has low toxicity and high biocompatibility and can be used to treat and prevent infectious diseases.Urinary tract infections are more common in women and are caused by Uropathogens, cranberries are traditionally used and may be used to treat urinary tract infections. Due to its anti-oxidant effects cranberry extract has been used as a potentially protective agent in humans.(*Ghazala Shaheen et al.,2016).*

cranberry (Vaccinium macrocarpon) is a unique source of polyphenols such as flavonoids and phenolic acids that have been described to show beneficial effects

against urinary tract infections (UTIs), the second most common type of disease in the world. UTI can lead to significant illness, especially in healthy women due to high levels of recurrence and resistance to antibiotics. Alternative therapies and alternative therapies for prophylaxis and anti-UTIs have always been sought. Unlike cranberry, which has been widely recommended in traditional medicine for UTIs prophylaxis, probiotics have emerged as a new form of antibiotic use against these diseases and are the subject of new research in this area.

In addition to uropathogenic Escherichia coli (UPEC), common viruses cause mild UTIs, other etiological agents, such as Klebsiellapneumoniae or Gram-positive bacteria of Enterococcus and Staphylococcus genera. UTI protective effects of cranberry, probiotics and their new compound formulations. All of these current topics in understanding the protective effects of cranberry against UTIs.(Dolores González de Llano *et al*., 2020).

Thymus vulgaris (Thyme)

Thymol is a natural effect of phenol monoterpene based on cymene and isomer carvacrol. Potential use of this ingredient in the treatment of disorders affecting the respiratory, nervous, and cardiovascular systems. In addition, this compound also exhibits antimicrobial, antioxidant, anticarcinogenesis, anti-inflammatory, antispasmodic, and potency properties as a growth enhancer and immunomodulator. (Bahare Salehi *et al.,* 2018).

A major component of Thyme essential oil, thymol, is effective against Salmonella and Staphylococcus bacteria.The antiseptic and tonic properties of Thyme make it a useful tonic for the immune system in chronic, especially fungal, diseases as and an effective treatment for chest infections such as bronchitis, Cough, and pleurisy. Thyme and Thyme oil have it was used as an antiseptic, disinfectant, and mouthpiece you wash. Giving him a sweet taste can be taken with a small throat and chest infections, and new leaves may be chewed to relieve it sore throat. Thyme is prescribed for other asthma remedies, hay fever, and is often used to treat worms in children. Thyme has a long history of human use medicine to treat various diseases for example treatment respiratory diseases (cough, nasal congestion, asthma), in the form of tea, ointment, tincture, syrup or inhalation of smoke. It is also used to prevent vascular constipation, treatment toothache, urinary tract infections and dyspepsia. And

removes fungus from the stomach and intestines and is strong increase appetite due to its essential component of thymol, capable of killing germs and parasites**.** (Eqbal M. A. Dauqan *et al*., 2017).

Foneiculum vulgare **(Fennel)**

Ferula communis L., subsp. *communis*, namely giant fennel, has extensively been used in traditional medicine for a wide range of ailments. harmacological properties including antidiabetic, antimicrobial, antiproliferative, and cytotoxic activities. This plant has two different chemotypes, the poisonous and non-poisonous chemotypes. Each chemotype is endowed with various constituents and different activities. The poisonous chemotype exhibits anticoagulant and cytotoxic activities with sesquiterpene coumarins as major constituents, while the non-poisonous one exhibits estrogenic and cytotoxic effects with daucane sesquiterpene esters as the main compounds.

In addition, although various pharmacological properties have been reported for *F. communis*, anti-microbial activities of the plant have been investigated in most studies. Studies revealed that *F. communis* exhibits different biological activities, and contains various bioactive compounds. Although, antibacterial and cytotoxic activities are the two main pharmacological effects of this plant, further studies should focus on the mechanisms underlying these actions, as well as on those biological activities that have been reported traditionally.(Maryam Akaberi *et al*.,2015).

Anethum graveolens **(Dill leaves)**

Anethum is used as an ingredient in gripe water, given to relieve colic pain in babies and flatulence in young children. The seed is aromatic, carminative, mildly diuretic, galactagogue, stimulant, and stomachic. The essential oil in the seed relieves intestinal spasms and griping, helping to settle colic. The carminative volatile oil improves appetite, relieves gas, and aids digestion. Chewing the seeds improves bad breath. *Anethum* stimulates milk flow in lactating mothers and is often given to cattle for this reason. It also cures urinary complaints, piles, and mental disorders.(S Jana *et al*., 2010)**.**

D. Therapeutic Properties of cranberry and selected herbs

Vaccinium macrocarpon **(Cranberry)**

Cranberry extracts may provide beneficial health effects in treating various ailments, including cancer. the effect of proanthocyanidin (PAC) enriched cranberry (PAC-1) as a dual-acting therapeutic agent to regulate both cervical cancer function and angiogenesis in vitro. PAC-1 treatment of chemotherapy-resistant SKOV-3 cells inhibited cell continuity in the G2 / M phase, increased production of active oxygen (ROS), and induced apoptosis by opening up internal and external pathways. CACotoxicity of PAC-1 was partially based on ROS generation and could not be prevented in combination therapy with antioxidant glutathione. PAC-1 reduced the cellular activity of both SKOV-3 ovarian cancer cells and HUVEC endothelial cells in a dose-dependent manner and inhibited the activation of the pro-survival factor AKT. In addition, PAC-1 inhibits vascular endothelial growth factor (VEGF) - reactivates receptor phosphorylation in endothelial cells, which is associated with inhibition of endothelial tube formation in vitro. Our findings suggest that PAC-1 has strong anti-cancer and angiogenic properties and that a highly purified PAC from cranberry can be further enhanced to treat cervical cancer with combined or single-agent treatment (Kyu Kwang Kim *et al*., 2011).

Because of the high antioxidant activity of European cranberry fruit, the extract could be used as a meat additive to prevent lipid oxidation by inhibiting negative storage changes. Cranberry juice was added to thermally processed pig burgers that were held for seven days at 3 to 7 °C, and juice was also put to a raw beef stuffing in the quantity of 5% of the meat weight. The value of TBARS (thiobarbituric acid reactive chemicals), which are generated as a by result of lipid peroxidation of burgers, was reduced by twice or three times when cranberry juice was added at a concentration of 5%. Cranberry juice helps the raw beef filling keep its colour (Tunde Jurikova *et* al.,2018).

Bioactive polyphenol compounds of cranberry (Vaccinium macrocarpon) are known to have risk reduction effects against several areas of cariogenic virulence that are associated with dental caries pathogenesis. Dental caries is a polymicrobial multifactorial disease that occurs when acidogenic and aciduric members of the oral plant regularly receive selective environmental benefits over other commensal microorganisms, disrupt the homeostatic balance of plaque biofilm, and initiate the disease process. Although the disease is naturally polymicrobial, mutant streptococci (MS) act as key pathogens, as they are primarily the primary compound

exopolysaccharide (EPS) of the glucan-rich scaffold matrix. The ability to survive and thrive in low pH areas is an important factor in MS. Proton translocating F1F0-ATPases play a key role in protecting bacterial glycolytic enzymes and the sugar-transport system from environmental stress caused by biofilm acidification. Inhibition of the F1F0-ATPase proton pump can cause cytoplasmic acidification and thus affect the functioning of intracellular acid-sensitive glycolytic enzymes. . (Nebu Philip *et al*., 2019).

A phytochemical solution of aqueous cranberry-based oregano, rosemary, and *Rhodiola Rosea* was tested to determine its total phenolic content, antioxidant-related activity, and α-glucosidase-related diabetes prevention, pancreatic α-amylase inhibition, and hypertension-related to ACE - I inhibitory. jobs. Oregano water extracts contained 114.9 mg / g DW of phenolics which was the highest in all tested quotes, while 75% cranberries with 25% oregano compounds had the highest phenolic content (38.9 mg / g DW) among all the compounds tested. Oregano water extracts had the highest DPPH inhibitory activity (73.6%), while between compounds cranberry 75% and oregano 25% had the highest DPPH inhibitory activity (50.8%). These results showed a correlation (relationship between the total phenolic content and antioxidant activity.

Pure *Rhodiola Rosea* water extracts have the highest α-glucosidase inhibition, while the cranberry compound is 75% and 25 %. *Rhodiola Rosea* has a high inhibition between compounds. The case of α-amylase dehydration of *Rhodiola Rosea* has a very high inhibition, while 75% cranberry with 25% *Rhodiola Rosea* compound has a high inhibition between compounds. , among the extracts, 100% cranberry had a very high ACE-I activity, and a mixture of 75% cranberry and 25% rosemary had high ACE-inhibitory activity. -α-glucosidase, α-amylase, and ACE-I inhibitor activities suggest that inhibition depends on the phenolic profile of each extract unique and by combining synergistic combina in cranberry, health-enhancing performance has been enhanced. This improved function in relation to high α-glucosidase and α-amylase inhibitory activity demonstrates the ability to control diabetes, and high activity of ACE - inhibitor demonstrates high blood pressure control. (Apostolidis, Emmanouil *et al*., 2006)

cranberry extracts have been attracting the ever-growing attention of dentists. Potential benefits of cranberry extracts in reducing oral diseases, including dental

caries and periodontitis. The digestible cranberry extract combined with the weight of high polyphenols has very promising properties with respect to cariogenic and periodontopathogenic bacteria, as well as adhesive inflammatory responses and enzymes that degrade the outer matrix of the cell. Cranberry extracts can be anti-caries agents as they inhibit acid production, attachment, and biofilm formation by Streptococcus mutants. Proteins bind to Glucan, extracellular enzymes, carbohydrate production, and bacterial hydrophobicity, all are affected by cranberry components (C. Bodet *et al*., 2008)

***Thymus vulgaris* (Thyme)**

The purpose of this study was to study the therapeutic effect of trans fats and alcohol aqueous extracts of thyme leaves on blood glucose and the alloxan-induced lipid profile in diabetic rats. Flexible oils extracted by hydro distillation and alcohol aqueous extracts obtained using methanol. In experimental animals, fifty mice were divided into ten groups (n = 5). Mice Were injected (i.p) with alloxan-monohydrate (100mg / kg BW). Animals showing high blood glucose higher than 300 mg/dl were selected and used as diabetic rats. flexible oils and alcohol aqueous extracts of basil and thyme were administered at dose levels of 200 and 300 mg/kg body weight by mouth. The reduction effect on DPPH radicals was determined. Hypoglycemic and hypolipidemic effects of extracts were assessed by lowering blood glucose, plasma insulin, total cholesterol, triglyceride, LDL-cholesterol, ALT, AST, creatinine, uric acid, and bilirubin. Also, certain antioxidant parameters in the blood were tested with the determination of MDA, SOD, and GSH. The results showed a high concentration of activity with a concentration of 1000 ug/mg when the basal extracts of basil leaves were the highest (96.97 ± 0.58), followed by BHT (95.9 ± 3.18) and then alcohol-based extracts of thyme leaves (94.2 ± 58) . The results revealed that a group of fatty acids and alcohol aqueous extracts of thyme showed higher insulin levels compared with diabetes control (4.8Uu / ml). (See Shelbaya *et al*., 2013).

Thymol is a natural effect of phenol monoterpene based on cymene and the isomer carvacrol. Thymol (10–64%) is one of the main constituents of thyme essential oil (Thymus vulgaris L., Lamiaceae), a medicinal plant with several medicinal properties. Interest in the formulation of nutraceuticals, and based on thymol, is due to several studies that have explored the potential therapeutic use of this therapeutic

component of respiratory, neurological, and cardiovascular systems. In addition, this combination also exhibits antimicrobial, antioxidant, anticarcinogenesis, anti-inflammatory, and antispasmodic properties, as well as potency as a growth booster and immunomodulator. (Bahare Salehi *et al*., 2018)

***Foneiculum vulgare* (Fennel)**

Fennel seeds are a source of many nutrients, namely, sugars, minerals, essential oils, essential vitamins, proteins, and fiber. They are also rich in essential oils and many flavonoids. Fennel seeds with a pleasant taste are popular all over the world and are widely used in cooking and medicine. Bread reinforced with fennel seedpowder is, therefore, more likely to be accepted by consumers than white bread or other solid bread. In this study, bread samples were prepared by adding white flour with fennel seed powder at 3.0, 5.0, 7.0, 10.0, and 15.0% levels and their physical, sensory, antioxidant, and staling properties. The crumb humidity increased and reached a high level of 7.0% degree of increase with a gradual increase in the strengthof the crumb. An effort has been made to understand the role of fennel seed powder in bread samples during storage. Antioxidant activity (i.e. total phenolic content, ferric antioxidant capacity, and 2, 2-diphenyl-l-picrylhydrazyl radical scavenging) showed anincrease of 7.0% supplementation rate. Bread with a fennel seed content of between
5.0 and 7.0% showed high acceptance among samples of fortified bread. (Lipi Das *et al*., 2013)

Study of cytotoxic experiments, antimicrobial and confocal analysis of zinc oxide nanoparticles (ZnO NPs) derived from the novel plant product fennel (Foeniculum vulgare Mill.) Seed extract (FSE) . ZnO NPs analyzed using UV-Vis spectroscopy, XRD, FTIR, TEM techniques, and EDX. The MTT cell cytotoxicity assay measured the proliferation and survival of MCF-7 cells treated in various FSE-derived ZnO NPs. Antimicrobial activity against pathogenic bacteria and yeast species have been investigated. The UV-Vis spectra showed two peaks at 438 nm and 446 nm, confirming the formation of the nanoparticle. SEM morphology results showed porous from 23-51 nm. The amount of antitumor activity (IC 50) was 50 µg / mL and 100 µg / mL. In addition, the morphological mutations of MCF-7, cells treated at different FSE sites of ZnO NPs are observed in cell culture transplanted with a temporary vector expressing pCMV6-XL4-GFP containing a base of C-terminal proteins GFP-tagged,

which led to an aptotic effect. Antimicrobial IZ increased No Inhibition to 18.00 ± 0.4. The IZ presented at the highest level was E. faecium VRE and yeast Cryptococcus sp. (18.00 ± 0.4. mm), followed by S. aureus (17.00 ± 0.2 mm) and P. aeruginosa andyeast C. parapsilosis (16 ± 0.4). mm). IZ was similar to that caused by nystatin in Cryptococcus sp., Which was significantly higher than amp treatment for S. aureus, P. aeruginosa, C. Albicans, and C. parapsilosis. The MIC value of ZnO NP taken from the FSE was tested against E. faecium and C. Albicans was 6.00 µg / mL (E. faeciumand C. Albicans). It was 32.00 µg / mL (S. aureus, S. Typhimurium and Cryptococcussp.), 64.00 µg / mL (P. aeruginosa), and 128 µg / mL (C. parapsilosis). (Mohamad S AlSalhi *et al*., 2020)

***Anethum graveolens* (Dill leaves)**

The study was to determine the antioxidant potential of dried medicinal plants and fragrances by testing the quality and quantity of polyphenols by HPLC analysis. The results of a polyphenolic compound test obtained by RP-HPLC-DAD analysis showed that the flower of Anethum graveolens contains 13 times greater polyphenols than its stem. Feast flowers were characterized by a very high content of gallic acid (224.0 µg / g), epicatechin (199.5 µg / g), trans-caffeic acid (38.6 µg / g), trans-p-coumaric acid (76.1 µg / g), kaempferol (10.9 µg / g), and apigenin (49.4 µg / g) compared with other refined plants from the edible plant family. It was noted that compared with the root (Apium graveolens var. Rapaceum) and stem (Apium graveolens var. Dulce), the leaves of Apium graveolens var. rapacious have a high content of polyphenols (∑ 2,755.0 µg / g). (Dominika Kuling *et al*., 2021).

Research has been done to evaluate the role of Anethum graveolens L. (dill) leaf extract in controlling corticosteroid-induced type 2 diabetes in female mice. In animals treated with dexamethasone (1 mg/kg for 22 days) and increase in insulin and glucose concentration in blood serum and hepatic lipid peroxidation (LPO) was observed. However, there has been a decrease in serum secretion of thyroid hormones and endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and decreased glutathione (GSH) in the liver. In animals treated with the same amount of dexamethasone at the same time (22 days) when they received leaf discharge (100 mg/kg b.wt / d.) 15 days ago a decrease in serum

glucose and insulin levels was noted, indicating the ability of the plant to release corticosteroid-induced diabetes. Changes caused by Dexamethasone to thyroid hormone levels and hepatic LPO, SOD, CAT and GSH were also altered by plant extraction. (Sunanda Panda *et al*., 2008).

Traditional medicine, especially Ayurveda, has established the use and expertise of many natural remedies and their medicinal properties. However, the Asian continent, or specifics, India, can be considered the "Land of Spices". The story of herbal remedies has been passed down from generation to generation with the motto “Make Food Like Medicine”. Such an unusual and widely used herb Anethum Graveolens (Dill) has the potential to create a variety of bioactivities. The whole plant, used as a vrushya (a natural aphrodisiac), vataghna (Vata balance) eliminates free radicals, anti-Vrana (non-healing wounds), shoola (abdominal colic pain), to treat disorders and ulcers in the eyes, it plays an important role. in the enema during panchakarma (Basti karma), acts as a galactagogue, prevents uterine fibroids, increases milk production during lactation, and above all improves the taste of food. Through this domain, the large lacuna has an understanding of function and mechanism of action at the cellular level. Therefore, this highlights the therapeutic potential of Dill seeds and their potential targets with modern knowledge and effects using the ayurpharmacomic method (understanding the structure of an ancient remedy and exploring its aspects of medical science). In addition, visual experiments were performed to assess the activity-structured (SAR) relationship between lead phytoactive and biomarker / pathological targets. These studies will better understand the potential mechanisms in the new target development of drugs for the development and development of drugs in the future, which will facilitate primary phytoactive activity (Kounaina Khan *et al.*, 2010).

The purpose of this study was to study the therapeutic effect of trans fats and alcohol aqueous extracts of thyme leaves on blood glucose and the alloxan-induced lipid profile in diabetic rats. flexible oils extracted by hydrodistillation and alcohol aqueous extracts obtained using methanol. In experimental animals, fifty mice were divided into ten groups (n = 5). Mice injected (i.p) with alloxan-monohydrate (100mg / kg BW). Animals showing high blood glucose higher than 300 mg/dl were selected and used as diabetic rats. flexible oils and alcohol aqueous extracts of basil and thymewere administered at dose levels of 200 and 300 mg/kg body weight by mouth. The

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E. Anticancer effect of cranberry and selected herbs

Vaccinium macrocarpon (Cranberry)

Cranberry and its extracts may have anti-cancer properties. Cranberry and or its components may act as chemopreventive agents, reduce the risk of cancer by inhibiting cellular oxidation and inflammatory processes, while may have chemotherapeutic effects by inhibiting cell proliferation and angiogenesis, inducing cell apoptosis and reducing the ability of tumor cells to invade and metastasis. Limited in vivo studies have also documented the potential work of anti-cancer. Cranberry can be considered a combination of potentially effective anti-cancer compounds. (Mantzorou *et al.*, 2019)

At dosages of 5 to 30 mg/ml, cranberry phytochemical extracts effectively suppressed human breast cancer MCF-7 cell proliferation (P0,05). In comparison to the control groups, cranberry phytochemical extracts at a concentration of 50 mg/ml

resulted in a 25% greater ratio of apoptotic cells to total cells (P0.05). Phytochemical extracts of cranberries in concentrations ranging from 10 to 50 mg/ml. MCF-7 cells were considerably stopped at the GO/G1 phase (P0.05). The GIS index in the cranberry extract treatment group was consistently growing, whereas the GIS ratio in the control group was decreasing. MCF-7 cells had a GI/S index that was about 6 times greater than the control group (P0.05). The capacity of cranberry phytochemical extracts to decrease the proliferation of human breast cancer MCF-7 cells is at least partly attributed to the initiation of apoptosis as well as the GI phase arrest (Jie Sun *et al* .,2002).

Recent research reports have also suggested that cranberry fruit could be a potential natural source of cancer protection. Daily use of cranberry fruit powder (1500 mg daily for 30 days) lowers prostate specific antigen (PSA) in patients with prostate cancer. Some studies have shown that a frozen cranberry feed may have the potential to inhibit intestinal tumor development in APCmin / + mice of cranberry. metabolic components are accelerated and eliminated, indicating that they are less sensitive to bioavailability. (Prasain *et al*., 2020)

Phytochemicals of American cranberry (Vaccinium macrocarpon) fruit may be expected to contribute to the inhibition of colon cancer. Tissue-culture models were used to evaluate the effects of cranberry components on cell proliferation, apoptosis, and tumor cell formation. Several phytochemicals and fractions divided with whole cranberry fruit were previously reported to inhibit the growth and proliferation of breast, colon, prostate, and other lines of tumor cells. In HT-29 and HCT116 colon tumor cell lines, cranberry proanthocyanidins (PACs) and ursolic acid inhibit the formation of tumor colonies. Thus, cranberry phytochemicals have the potential to reduce carcinogenesis. (AM Liberty *et al.* , 2004)

***Thymus vulgaris* (Thyme)**

The commonly used plant that appears to have a high therapeutic value is Thymus vulgaris L. However, the effects of this plant on the misconduct of human CRC cells are still under investigation. The decrease was associated with apoptotic cell death as evidenced by an increase in caspase3/7 activity. TVE inhibits the malignant phenotype of colon cancer cells. Therefore, T. Vulgaris can have an anti-cancer effect

and some of its bioactive compounds may be effective treatments for human CRC. (Afnan Al-Menhali *et al*., 2015)

Thymus vulgaris (family: Lamiaceae) is an important treatment known for its health benefits. The anti-cancer activity was determined using a DNA ladder assay, achieved by blocking α-glucosidase. per 100 µg / mL essential oil of thyme produces DNA fragmentation and promotes apoptosis. Current research provides a scientific basis for possible use in controlling diabetes and breast cancer. Research continues to increase knowledge of the chemical composition of T. Vulgaris also provides evidence of the medicinal use of the plant by examining anti-cancer activities. (Abuzer Ali *et al*., 2021)

Thymus vulgaris has antitumor potential, according to multiple preclinical investigations. In the human BC T47D cell line, for example, the herb has showed considerable free radical scavenging activity as well as proapototic effects. Thymus vulgaris extract inhibited proliferation in a colorectal HCT116 cancer cell model in a concentration- and time-dependent way. Increased caspase-3/7 activity has been linked to lower proliferation rates and higher apoptosis. Thymus vulgaris also inhibits the migratory and invasive abilities of HCT116 cells .Dried *T. vulgaris* (as a haulm) was continuously used in both 0.1% and 1% concentrations in diet on the mammary- type mammary carcinomas model and syngeneic mouse model 4T1. After the autopsy, histopathological and molecular analysis of rodent mammary carcinomas was performed. In addition, in vitro testing using MCF-7 and MDA-MB-231 cells were performed. In mice, T. Vulgaris in both doses reduced the volume of 4T1 tissue by 85% (0.1%) and 84% (1%) compared to controls, respectively. In addition, treated plants showed a significant decrease in local necrosis/tumor localization and an indication of mitotic activity. In the mouse model, T. Vulgaris (1%) reduced tumor frequency by 53% compared with control. Vulgaris L. has shown significant chemopreventive and therapeutic activities against breast cancer screening. (Peter Kubatka *et al.*, 2019).

***Foneiculum vulgare* (Fennel)**

FSME can have amazing anti-cancer properties against breast cancer line (MCF7) and liver cancer line (Hepg-2). It also showed strong radical-scavenging activity (100%). Therefore, FSME may reduce oxidative stress and protect rat cells

from damage caused by active oxygen species. In addition, it can be used as a safe, effective, and easily accessible source of natural antioxidants to enhance the oxidative stability of fatty foods in the end. FSME also showed antitumor effect by altering lipid peroxidation and increasing the antioxidant protection system in EAC-infected mice with or without exposure to radiation (Ragaa Hosny Mohamad *et al.*, 2011).

The main components of both essential oils were anethole (55.33%) and estragole (11.57%) of fennel essential oil. The results revealed IC50 of 220 ± 5.7 and 60 ± 2.1µg / ml of fennel and geranium oil, respectively. The anti-cancer properties of the machines were investigated in a total oil mixture of 70, 50, and 25µg / ml. Specific apoptotic morphology and cytometric cell cycle flow analysis over apoptosis-related builder levels such as p53, caspase-3, mir-21, mir-92a, Bcl-2, and -ki-67 confirmed that fennel and geranium oil are compounds designed for cell cycle binding and apoptosis in MCF-7 cells. Furthermore, the oil mixture did not contain significant toxicity ($P < 0.01$) in normal in vitro human blood lymphocytes (El-Garawani *et al*., 2019).

Breast cancer is defined as a serious illness and is one of the most important causes of cancer-related death. When considering drug resistance, special attention is paid to natural compounds. This study aimed to test the anti-metastatic activity of fennel in a mouse model of breast cancer.The tumor size decreased significantly after nine days of fennel treatment. Fennel treatment has resulted in an increase in the production of E-cadherin to Ki-67 and E-cadherin in plant tissues. On the other hand, exposure to Ki-67 and HER2 decreases in the ovary. (Alireza Mehralikhani *et al* ., 2020).

***Anethum graveolens* (Dill leaves)**

Dill extracts have antiproliferative activity when tested on MK-1, HeLa, and B16F10 cell structures.Dill essential oil acts as an anti-cancer agent for hepatocellular carcinoma as it exhibits cytotoxic/antiproliferative activity and promotes cell death rate on HepG2 cells in 24h treatment. Further phytochemical studies have shown that dill containing flavonoids have been reported to have anticancer and antimutageniceffects according to their anti-oxidant and anti-inflammatory properties (Gomaa *et al.*,2020).

Different parts of Anethum graveolens L. (dill) (Umbelliferae) are used to treat liver disorders traditionally. It is one of the ingredients in various traditional medicines used to treat liver disorders and other diseases. The purpose of the present study was to evaluate the hepatoprotective effect of dill against liver damage in experiments. Dill essential oil is tested for hepatoprotective activity against Aspergillus parasiticus aflatoxin which causes hepatotoxicity in mice. Variousl. (Omid Hajikandi *et al*., 2015).

F. Role of herbs in human nutrition

Human nutrition is about nutrients. The human body is primarily made up of nutrients. Carbohydrates are a combination of organic and inorganic compounds which are essential for maintaining a healthy body. These nutrients such as proteins, carbohydrates, fats,vitamins, and minerals found in various plant and animal sources. Herbs are part of a plant leaf used for cooking – these can be used fresh or dried. Herbs are a great way to add flavor and color to any meal or beverage, whether sweet or without adding fat, salt, or sugar. In addition to taste and color, each has its own set of health benefits. (Sahib H.B *et al.*, 2010).

Herbs have been the most respected medical source in all of human history. They are widely used today to show that herbs are a growing part of modern, high-tech medicine. About 25-30 percent of modern prescription drugs contain chemical components derived from plants. The Indian medical system namely Ayurveda and ancient texts such as the Bashajya Ratnavali have an ancient tradition that offers a different understanding of the broader approach to dealing with asthma.(Ranjeeta.P *et al*., 2009).

The information about plants, herbs, and spices and their various roles and collections in improving human health is modest. Flavonoids in tea and anthocyanins in tart cherries are presented as examples of how to progress in understanding the active ingredients. Dietary compounds, their role in maintaining human health, and their interactions with established nutrients were determined to be priorities in the interim study (Balentine D. A *et al*.)

III. METHODOLOGY

The Methodology pertaining to the study titled "**Identification and Isolation of bioactive compounds in Cranberry-Herbal Infusion"** is discussed under the following sub-headings:

A. **Selection and identification of herbs and fruit**
B. **Collection of fresh herbs and fruit**
C. **Formulation and standardization of the infusion with selected herbs and fruit**
D. **Sensory evaluation of infusion**
E. **Analysis of infusion-antioxidant vitamins, phytochemicals, colour and TSS**
F. **Analysis of toxins in the infusion**
G. **Identification of bioactive compounds and MTT assay of freeze-dried infusion**
H. **Statistical analysis, interpretation of results and Ethical clearance**

A. Selection and identification of herbs and fruit

Herbs and fruits are rich in vitamins, minerals and antioxidants. The health benefits of herbs and fruit include controlling blood pressure, reducing heart disease and strokes, prevents cancer, reduces blood clots and has anti-inflammatory and anti-tumor properties. Fruit and herbs rich in vitamin C and having anticancer property was selected for the study. After a thorough study of literature, herbs and fruit rich in vitamin C and having anticancer property was selected. The fruit and herbs selected for the current study was cranberry and the herbs included thyme, fennel and dill leaves. The common and botanical names of the selected herbs and fruit is given in Table I.

Table I

SCIENTIFIC AND COMMON NAME OF FRUIT AND HERBS

Fruit/Herbs	Scientific Name	Common Name
Cranberry	*Vaccinium subg. Oxycoccus*	Karonda,Chirukili and Karvand.
Thyme leaves	*Thymus vulgaris*	Banajwain,Masho and Hasha
Fennel seeds	*Foeniculum vulgare*	Saunf,Varyali and Sonp
Dill leaves	*Anethum graveolens L*	Shopla,Soa-Kura and Savaa

Vaccinium subg. Oxycoccus (cranberry) are a group of evergreen dwarf shrubs. Cranberry fruits can be red, dark red, or dark purple, while those that are not completely matured are pale pink or white color (Boris V. Nemzer *et al*.,2022)**.**The cranberries which was selected and identified for the study was dark red in colour and sour in taste.

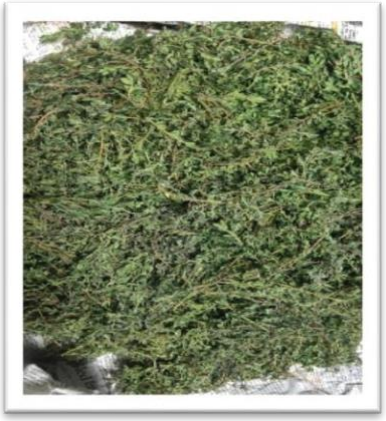
Thymus vulgaris (Thyme) is a aromatic and medicinal herb. *Thymus vulgaris* is indeed the most important species and is widely used as a flavouring agent, a culinary herb and a herbal medicine. (Stahl-Biskup, E *et al*.,2012).The thyme leaves which was selected and identified was green in color in thyme is due to the chlorophyll pigments present in it.Also it had sharp and minty flavour.

Foeniculum vulgare (fennel) is a biennial medicinal and aromatic plant. The fennel which was selected and identified for the current study was pale green in color and the pigment responsible for the pale green color is chlorophyll. The taste of fennel seeds were slightly sweet.

Anethum graveolens L (Dill leaves) a biennial or annual herb of the parsley family. The dill leaves which were selected and identified for the study was dark green in color and the pigments responsible for this dark green color is chlorophyll. The dill leaves were grassy and slightly tangy in taste.

The fruits and herbs and used in the study is presented in Plate I.

Cranberry
Vacciniumsubg. Oxycoccus



Thyme
Thymusvulgaris

Fennel
Foeniculumvulgare

Dill leaves
Anethum graveolens L

PLATE I

SELECTION OF HERBS AND FRUITS

B. Collection of fresh herbs and fruit

The fruits and herbs used for the formulation were namely cranberry (*Vaccinium subg. Oxycoccus)* and was collected from online as it was not available in the local markets. Fresh thyme leaves (*Thymus vulgaris)* was collected from the local areas of Ooty , Tamil Nadu; fennel seeds (*Foeniculum vulgare*) was collected from local areas of Saibaba Colony, Coimbatore, Tamil Nadu, fresh dill leaves was collected from the local areas of Edayarpalayam, Coimbatore, Tamil Nadu**.**

Cleaning and storage

The cranberry fruit was washed by soaking in water for 30 minutes. This helped to remove the dirt from the fruit. The fruits which are intact and fleshly, fully ripen are selected. The cleaned cranberry was then dried by spreading in a dry cotton cloth to remove the excess moisture. The herbs selected for the study namely, thyme leaves, fennel seeds and dill leaves purchased are in fresh form.

The leaves are taken, cleaned and washed in running tap water to remove the mud, dirt etc. Fully intact leaves, green in colour, not infected with insects' eggs was carefully taken and used for the study. The herbs that are washed are dried by spreading on a dry cotton cloth and dried at room temperature to remove the excess moisture content. To restore the quality and aroma of the fruit and herbs which was collected and cleaned was stored in a refrigerator

C. Formulation and standardization of the infusion with selected herbs and fruit

The ingredients used for the formulation of infusion are *Vaccinium subg. Oxycoccus* (cranberry), *Thymus vulgaris (*thyme)*, Foeniculum vulgare (*fennel), and *Anethum graveolens L* (dill leaves). Three of variations was formulated and standardized namely variation I, II and III is presented in Table II. Cranberries, thyme leaves, fennel and dill leaves were used in the preparation of infusion.

(i) Preparation of extract

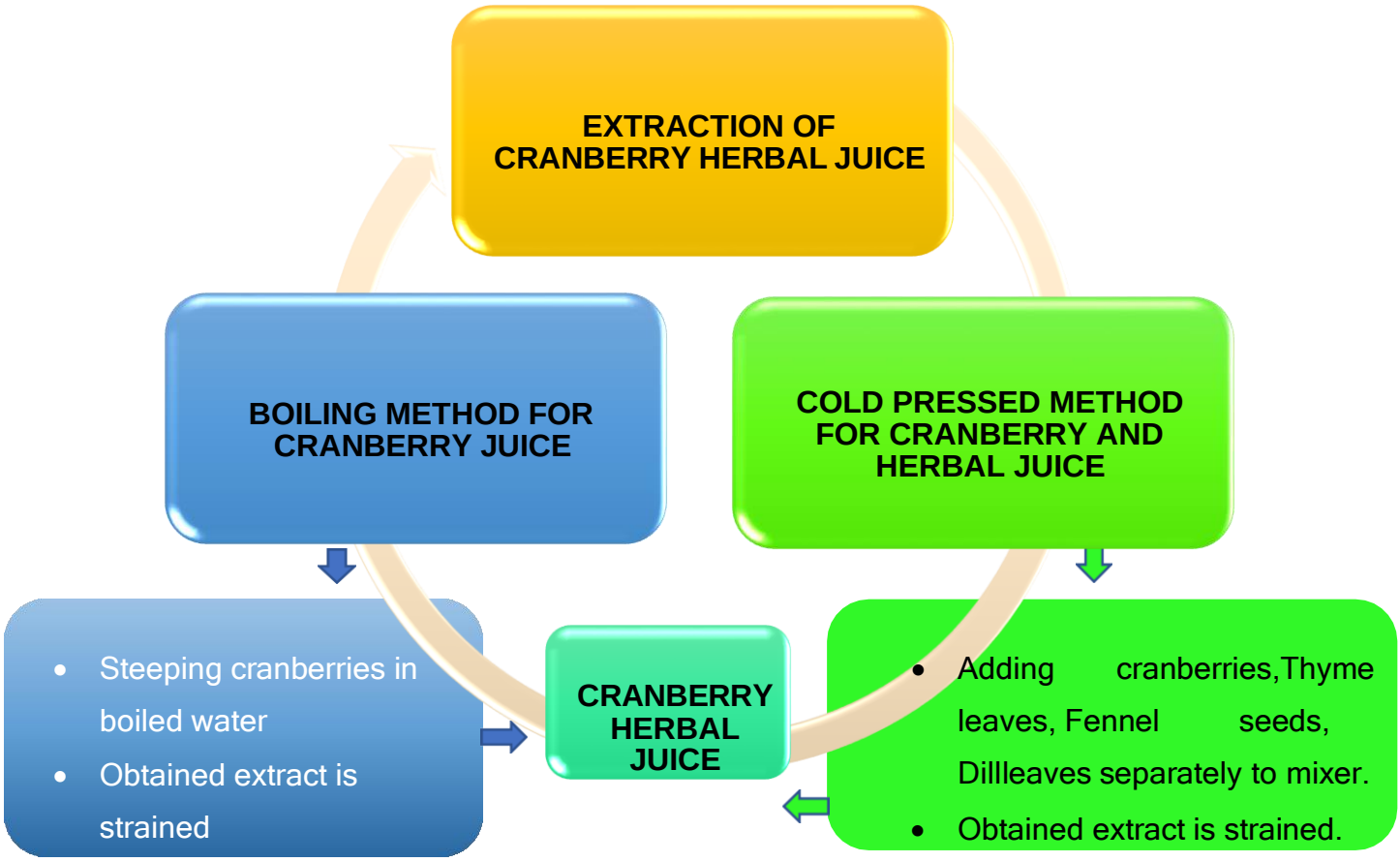


Figure 1

EXTRACTION METHOD OF CRANBERRY HERBAL JUICE

- **Boiling method 1 (for extraction of cranberry juice)**

Weighed quantity 250g of fresh cranberries are put in a pot and 250ml of water was added to it. Allowed it to boil for 20 minutes until the cranberries became soft and popped. The soft cranberries are then strained using the strainer to extract the juice. The total amount of juice extract obtained from 250g of cranberries was 100ml. After straining, the remaining residue was again added to the pot and added 200 ml of water and allowed to boil for 10 minutes. Again, the juice was strained and the total amount of juice obtained was recorded and is 75ml. The residue was again put into the pot for third time and added 200ml of water and was allowed to boil for 10

minutes. It was strained and 75ml of extract was obtained. The remaining residue was added to the pot and added 100ml of water. Allowed to boil for 10 minutes.100ml of the extract was obtained. The total amount of fruit residue obtained from the first set of 250g cranberries was 49.5g and total juice content was 350ml.

- **Boiling method 2 (for extraction of cranberry juice)**

The above method 1 was repeated except in the fourth extraction where only 50 ml of water was used for boiling. The boiling method 2 with 250g of cranberriesgave a total of 350 ml with the residue of 49.9g. The boiling method is depicted in PlateII.

TABLE II

METHOD OF PREPARATION OF CRANBERRY JUICE

Boiling Method	Water (ml)		Time (min)		Extract (ml)		Residue (g)	
	Method I	Method II	Method I	Method II	Method I	Method II	Method I	Method II
1st extraction	250	250	20	15	100	100		
2nd extraction	200	200	10	10	75	75		
3rd extraction	200	200	10	10	75	75		
4th extraction	100	50	10	10	100	100	49.5	49.9
TOTAL	**750**	**700**	**50**	**45**	**350**	**350**	**99.4**	

PLATE II

EXTRACTION OF CRANBERRY JUICE-BOILING METHOD

- **Cold pressed 1 for extraction of herbal juice and cranberry juice**

500g of cranberries was put into the mixer, then added 750ml of water and blended using high-speed blender. The extract was filtered using a strainer to remove the lumps if any. The total amount of extract collected was 1250ml, with 146.9g of leftover residue. The cold method of extraction of cranberry juice is depicted in Plate III.

PLATE III

EXTRACTION CRANBERRY JUICE-COLD PRESSED METHOD

- **Extraction of *Thymus vulgaris* (thyme) juice**

500g of fresh thyme leaves was put into the blender, along with 700ml of water, and the mixture was blended at high speed. The extract was filtered, yielding 2250ml of juice. The total weight of the leaf residue was 296 grams. The extraction of Thyme juice is depicted in Plate IV.

PLATE IV

EXTRACTION OF THYME LEAVES JUICE-COLD PRESSED METHOD

- **Extraction of *Foeniculum vulgare* (fennel) juice**

Washed 250g of fresh fennel seeds was soaked in 250 ml of water for 45 minutes. The fennel seeds were put into the blender, along with 250ml of water along with the soaked water, and the mixture was blended at high speed. The extract was filtered, yielding 1250ml of juice. The total weight of the fennel residue was 142grams.The extraction of Fennel juice is depicted in Plate V.

PLATE V

EXTRACTION OF FENNEL SEEDS JUICE-COLD PRESSED METHOD

- **Extraction of *Anethum graveolens L.* (Dill leaves) juice**

300g of fresh dill leaves was put into the blender, along with 200ml of water, and the mixture was blended at high speed. The extract was filtered, yielding 700ml of juice. The total weight of the leaf residue was 136 grams. The extraction of Dill juice is depicted in Plate VI.

PLATE VI

EXTRACTION OF DILL LEAVES JUICE-COLD PRESSED METHOD

From the extracted juice of Cranberry and herbs, three variation of infusions was prepared and compared with the standard. Table III gives the composition of ingredients used in the development of three variations. The extracted juice of cranberry, thyme, fennel and dill is depicted in Plate VII.

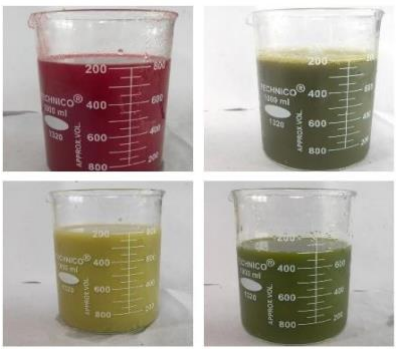


PLATE VII

EXTRACTION OF CRANBERRY AND HERBAL JUICE

(ii) Preparation of Standard

The infusion was prepared by weighing 5g of tea dust available in the market. The weighed 5g of tea dust was soaked in 10 ml of water for 10 minutes, added 100 ml of water and then boiled filtered and used as standard.

PLATE VIII

PREPARATION OF STANDARD

Preparation of variation I

Variation I was prepared by mixing the juice extract of cranberry and herbs as mentioned.100 ml of infusion was prepared by taking cranberry, thyme juice, fennel seed juice and dill leaves juice in the ratio 4:2:2:2 respectively and mixed thoroughly.

PLATE IX A

PREPARATION OF VARIATION 1

Preparation of variation II

Variation II was prepared by mixing the juice extract of cranberry and herbs as mentioned.100 ml of infusion was prepared by taking cranberry, thyme leaves juice, fennel seeds juice and dill leaves juice in ratio 7:1:1:1 respectively and mixed thoroughly.

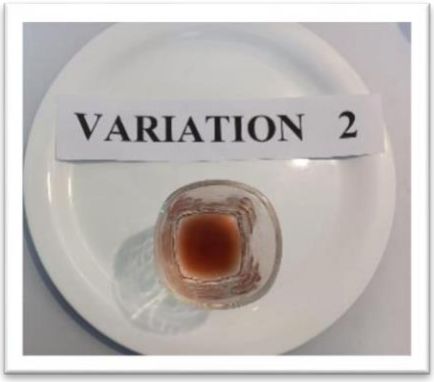


PLATE X A

PREPARATION OF VARIATION 2

Preparation of variation III

Variation III was prepared by mixing the juice extract of cranberry and herbs as mentioned.100 ml of infusion was prepared by taking cranberry thyme leaves juice, fennel seeds juice and dill leaves juice in ratio 6:12:14:14 respectively and mixed thoroughly.

PLATE XI A

PREPARATION OF VARIATION 3

TABLE III

COMPOSITION OF INFUSION IN VARIATIONS

Ingredients	Variation I (ml)	Variation II (ml)	Variation III (ml)
Cranberry juice	40	70	60
Thyme leaves juice	20	10	12
Fennel juice	20	10	14
Dill leaves juice	20	10	14

D.Sensory evaluation of infusion

Quality is a very important parameter for judging the edible nature of any food. Sensory evaluation is a scientific discipline that uses the human senses, smell, sight, taste, touch and hearing which evokes, measures, analyses and interprets responses to products which influence the quality of the product. Judging food quality is a matter of panelist's reaction to the sensory qualities of foods and is not a property of food itself. Psychological and social factors play an important role in acceptance. Appearance, colour, flavour and mouth feel decide the acceptance of the food. Hence, sensory evaluation of the standardized infusion along with the standard was carried out.

A score card is a tool that aids in evaluation by providing direction and degree of judgement through the use of appropriate defined scores. Scoring is a method of evaluating prepared foods on a numerical scale. The aspects such as look, colour, flavour, texture, taste, and overall acceptability of the recipes was scored on a 9-point hedonic scale, ranging from like exceedingly to detest severely. Scores 9 represents Like extremely and 1 represents Dislike extremely.

Panel members are the group testers chosen to participate in sensory test who were requested to assess food quality of samples presented for evaluation. Thirty semi-trained panel members was selected based on their health, co-operation,

willingness and knowledge of quality character and sensory analysis doing II M.Sc. and Ph.D. in the Department of Food Science and Nutrition.

The cranberry-herbal infusion prepared was presented to the semi-trained panel members for sensory evaluation at different points of time and was evaluated immediately after preparation, in order to prevent any changes in their quality, temperature changes. The sensory evaluation of the developed infusion was done in the mid-morning between 11am and 12pm. The portion sizes for all the products were kept consistent and uniform.

During the sensory evaluation, each panel member was permitted to take their time for evaluating the product that was shown to them. The finished item was neatly organized into Standard, Variation I, Variation II and Variation III and delivered to the semi-trained panel members. A pen and an evaluation form were given to the semi-trained panel members. Between tastings, the evaluator was given a glass of water to rinse, mouth. The panelist evaluated the infusion in terms of appearance, colour, flavour, taste and overall acceptability. The overall acceptability and the mean scores for each product was analyzed and the product that obtained the highest total and mean score was considered to be the best acceptable infusion.

PLATE XII

SENSORY EVALUATION OF CRANBERRY-HERBAL INFUSION

Preparation Freeze dried infusion

The infusion that was best accepted by the panel members as scored by sensory evaluation was identified and freeze dried.

Freeze drying or lyophilisation protects products because the material is dried by sublimation. This involves the transition directly from the solid to the gaseous phase, without passing through the liquid phase. The frozen product is dried under vacuum, without malting. The process finds a wide range of applications in preserving the characteristics of the initial substances.

The fundamental principle in freeze-drying is sublimation, the shift from a solid directly into a gas. Just like evaporation, sublimation occurs when a molecule gains enough energy to break free from the molecules around it. Water will sublime from a solid (ice) to a gas (vapor) when the molecules have enough energy to break free but the conditions aren't right for a liquid to form. It becomes a gas (Shukla,2011).

Power on the instrument and allowed the instrument to be in freezing mode for 10 minutes. Switched to "Warm Vacuums pump" and allowed to stand for 20 minutes. The sample was freeze dried to (20˚-80˚) and the pre-freezed sample was placed either on the Shelf or attached to the rubber valve. Changed the mode to main drying (vacuum setting will depend on the sample eutectic point). The instrument mode is run until the sample lyophilised. The sample is taken, removed acrylic chamber and base plate. Cleaned the condensing unit with warm water around 40˚C.Drained out the water through drain valve. Ensured that there is no moisture in the chamber (If the moisture is in the chamber that will affect the vacuum sensor) and closed it.

PLATE XIII

PREPARATION OF FREEZE DRIED CRANBERRY-HERBAL INFUSION

E. Analysis of infusion-Antioxidant Vitamins, minerals, Phytochemicals, Colour and TSS

(i) Analysis of antioxidant vitamins of the cranberry-herbal infusion

The nutrients present in freeze dried cranberry-herbal infusion was analysed in the Nutrition Laboratory of the Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, following the standard procedures. They include vitamin C, vitamin A, Vitamin E, calcium, and manganese. All the tests are done in triplicates to get concordant values.

Ash

The inorganic residue left over after the total combustion of organic matter is referred to as "ash." The ash content of food products is an index of product quality and nutritional value. When a high ash figure indicates the presence of an inorganic adulterant, the acid insoluble ash should be determined. By continuous heating, the substance gets charred which can be used for the determination of minerals present (AOAC, 2000).(Plate XIV)

PLATE XIV

ASHING

Vitamin C

The interaction between ascorbic acid and 2, 6-dichloroindophenol will be utilised to determine the amount of vitamin C in a sample using redox titration (DCIP). Because DCIP should only oxidize vitamin C and not other compounds that may be present, it is employed as a titrant and self-indicator throughout the titration. The material must be one color in the presence of excess ascorbic acid and another colour when the analyte has completely reacted to be a self-indicator. DCIP is red in acidic solutions, but it is converted to a colourless material when ascorbic acid is present. The solution will remain colourless as more DCIP is added until all of the vitamin C has reacted. The solution will turn light crimson due to the excess DCIP as soon as the next drop of DCIP solution is added, and the titration will be complete (Kramer, 2011).

PLATE XV

ESTIMATION OF VITAMIN C

Vitamin A

The vitamin A is rehydrated with light petroleum and saponified using an ethanolic potassium hydroxide solution. The light petroleum is removed by evaporation, and the remainder is dissolved in 2-propanol. Reverse-phase liquid chromatography is used to quantify the amount of vitamin A in 2-propanol juice under conditions that yield a single peak for all retinol isomers (AOAC, 2000).

Vitamin E

Standards and test samples are saponified in basic ethanol water solution, neutralized, and diluted, converting tocopherol esters to tocopherol, respectively. Alpha-tocopherol are quantitated on separate LC systems, using fluorescent detection (excitation 290 am, emission 330 mm) for alpha-tocopherol, Vitamin concentrations are calculated by comparison of peak heights or peak areas of vitamins in test samples with those of standards (AOAC,2002).

Calcium

Calcium is measured by precipitating it as calcium oxalate and comparing it to a standard potassium permanganate solution in dilute sulphuric acid (AOAC, 2000).

PLATE XVI

ESTIMATION OF CALCIUM

Manganese

The glassware and polyethylene containers used for analysis was washed with tap water, then soaked overnight in 6N HNO_3 solution and rinsed several times with ultra-pure water to eliminate absorbance due to detergent. The standard procedure described in AOAC was followed for the preparation of samples for analysis of heavy metals. Accurately weighed (0.5 g) sample was transferred into a silica crucible and kept in a muffle furnace for ashing at 450” C for 3 hours and then 5 ml of 6M HCI was added to the crucible. Further, the crucible containing acid solution was kept on a hot plate and digested to obtain a clean solution. The final residue was dissolved in 0.1 M

HNO_3 solution and made up to 50 mL. Standard solutions were prepared according to the Shimazdu Perkin Elmer Pure Atomic Spectroscopy Standards guidelines. Working standard solutions was prepared by diluting the stock solution with 0.1 M nitric acid for checking the linearity. (Srividhya.B *et* al.,2011).

(ii) Analysis of Phytochemicals present in the cranberry-herbal infusion

Preparation of aqueous extract

5g of the sample was taken and added 10 ml of water. The solution was centrifuged and the supernatant clear liquid was collected for further analysis. (Plate XVII)

Tannins-About 0.5g of the dried sample was boiled in 20ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black coloration (AOAC, 2005).

Terpenoids-To 0.5 ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids (AOAC, 2005).

Phenols-To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green indicates presence of phenols (AOAC, 2005).

Flavonoids-To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Development of yellow colour indicates the presence of flavonoids (AOAC, 2005).

Saponins (Foam test)-5ml sample extract was dissolved in 2.5 ml of dilute water and shaken vigorously till a stable persistent froth was obtained. The froth was mixed with 3 drops of olive oil and shaken vigorously and then emulsion was observed (AOAC, 2005).

Quinones-To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones (AOAC, 2005).

Glycosides-To 2ml of the extract, 3 ml of chloroform and 10% ammonia solution was added. Formation of pink colour indicates the presence of glycosides (AOAC, 2005).

Coumarins-To 1ml of the extract, 1ml of 10% sodium hydroxide was added. Formation of yellow colour indicates the presence of coumarins (AOAC, 2005).

PREPARATION OF THE

AQUEOUS EXTRACT OF CRANBERRY-HERBAL INFUSION

PLATE XVII

(iii) Analysis of Total soluble solids (TSS) and colour

Physiochemical properties of food samples are used to evaluate the acceptability of food samples and help in determining the quality deterioration (AACC, 2000). The physiochemical properties like total soluble solids and color are examined accordingly with the respective equipment.

Analysis of Total soluble solids (TSS)–Refractometer

Refractometer is an instrument used to measure the index of refraction of transparent materials, both solid and liquid. Refractive indices are often taken as a guide to the purity of raw materials, such as drying oils. The Abbé design is convenient and is used worldwide (Jan W. gooch.et *al*.,2007).

The total soluble solid content of a solution is determined by its refractive index. The degree of brix is a measurement taken with a refractometer. Brix is the name for the percentage by mass of total soluble solids in a pure sucrose solution calculated using a refractometer with a scale based on the relationship between refractive indices

at 20°C and the percentage by mass of total soluble solids in a pure aqueous sucrose solution. In a sucrose-containing fluid, this influences the solids concentration. It is carried out in order to calculate the sugar concentration in the sample. Brix is a unit of sugar concentration measurement that is normally proportional to the percentage of sucrose in a solution at 20°C.

Hence TSS is measured at that temperature to get an exact number. The light source was initially switched on. Water is used to calibrate the instrument at first. To get a distinct interface between the lit and dark portions, the refractometer scale knob was switched on. Total soluble solids was determined using the telescope scale (Abbe's Refractometer, 2020). (Plate XVIII)

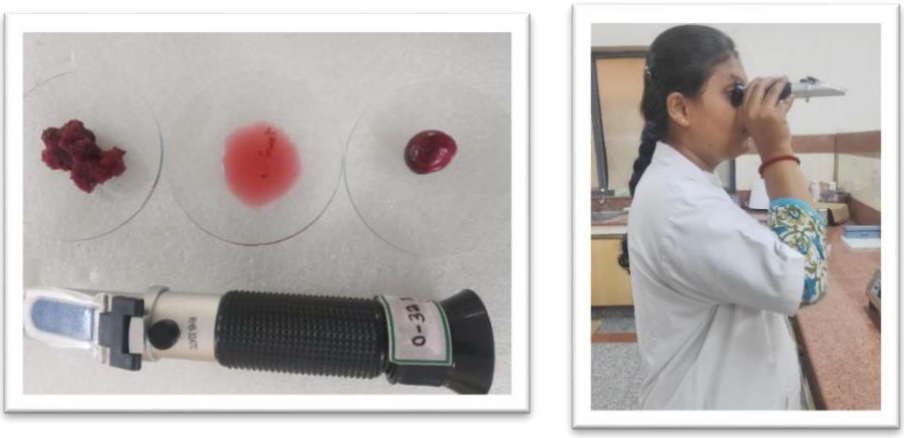


PLATE XVIII

ANALYSIS TOTAL SOLUBLE SOLIDS (TSS)

Analysis of Colour

A colour reader is a device that measures the colour and whiteness of paste, solid, or powdered foods or raw materials. It comes with a big LCD panel that shows measurement data from one of eight colour spaces/colorimetric indices, including L*a*b*, L*C*h, and Yxy.This instrument can be used to manage the colour of food products. It can also calculate the effect of storage on a product's whiteness throughout shipping (Konica Milota, 2022). The main page was displayed after the colour reader was turned on. By tapping the up and down buttons on the main page, "card search" was selected. The established colour library was selected from the colour chart's dropdown menu. The test button was pressed once the instrument was placed on the sample. The sample's test results will appear on the screen after hearing

the “beep” sound. It displays the color number that is the most similar to the sample. The test was repeated until same concordant values are obtained. (Plate XIX)

PLATE XIX

ANALYSIS OF COLOUR

F. Analysis of toxins in the infusion

The toxins present in the freeze-dried infusion was determined by brine shrimp lethality assay. The evolution of the toxic action of plant extracts is indispensable to consider a treatment safe; it enables the definition of the intrinsic toxicity of the plant, and the effects of acute overdose, a cheap and general bioassay that appears capable of detecting a spectrum of bioactivity present in crude extract is the brine shrimp lethality test. The lethality of the test sample in a simple zoological organism like the brine shrimp (*Artemia salina*) has been utilized by many researchers and has proven to be a useful tool in screening various chemical compounds found in various bioactivities. After 24 h the number of survivals of nauplii was counted and percentage of mortality was determined using the equation:

$$\% \text{ mortality} = \frac{\text{No. of dead nauplii}}{\text{initial no. of live nauplii}} \times 100$$

Preparation of samples

The given sample (Cranberry -herbal infusion) was weighed and dissolved in water to get 1mg/ml stock solution. The samples (Cranberry-herbal infusion) of

different volume 100, 250, 500, 1000, 1500µl is added to each beaker containing saline solution respectively.

Procedure:

30 shrimps were Introduced into the sample solution of various concentration. The movement of shrimp is monitored at intervals of 1, 2, 4, 6, 24 hours. Blank solution: 30 shrimps in Brine solution

Positive control: Potassium dichromate (1mg/ml).

The mortality of shrimp is calculated after 24 hours. For each of the sample, 30 shrimps were added to 25ml of the solution. The mortality of the shrimps was monitored as that of blank and positive control.

G. Identification of bioactive compounds and MTT assay of freeze-dried infusion

Gas Chromatography Mass Spectrometry GC-MS

A gas chromatography equipped with a MS detector (GC-MS-QP2010 Ultra) and a TR-5MS Thermo (30m x 0. 25µm) column was used. Electron Impact (EI) mass spectra were obtained at 70 eV. The MS system was programmed in the Selected Ion Monitoring (SIM) mode for confirmation. The working parameters were injector temperature, 230 °C; interface temperature, 280 °C; carrier gas He at 1 PATED ml. min. The oven conditions were 120 °C initial (5 min), increased to 190 °C at 10 °C min (1 min hold), increased to 250 °C at 5 C min (5 min hold). Increased to 280 °C at 5 °C min (5 min hold) and finally increased to 320 °C at 20 °C min' and hold during 2 min) (45 min total run). The injection mode was split, the split ratio 12:1 and the injection volume were 1.0 pl. For data processing and acquisition, Lab Solutions GC-MS solution Version 4.11 SU2 from Shimadzu was used. Quantification was accomplished by relative areas versus TPP used as Internal Standard (IS) in order to minimize deviations in responses and by compensating the variations of the system. TPP was chosen on the basis of its use in most QUECHERS procedures, as it gives sharp peaks and intense signal. The MS detection method was optimized firstly with the injection of a 1 mg L mix solution. Containing all the pesticides in full scan mode in order to obtain the times and to select the optimal precursor ions.

MTT assay of the infusion

The Cytotoxicity activity of freeze-dried infusion was determined by MTT assay. The cytotoxic assay was carried out using MCF-7 human breast carcinoma cells maintained as monolayer cultures in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% Fetal Calf Serum, Penicillin G (100 U/ml), *Streptomycin* (100 g/ml) and Gentamycin (50 ug/ml). Briefly L * 10 degrees * cells ml¹ seeded in each well of a 24 well plate and incubated overnight at 37°C in a humidified 5% CO_2/95% air atmosphere. The extracts were diluted in culture medium at different concentrations. When the cells in the 24 well plate were 80% confluent, medium was removed from each concentration at 50% growth (ICso) was determined (Jose *et al*.,2014) .

Aim: To determine the cytotoxicity effect of test compound on MCF-7 cell line by MTT assay.

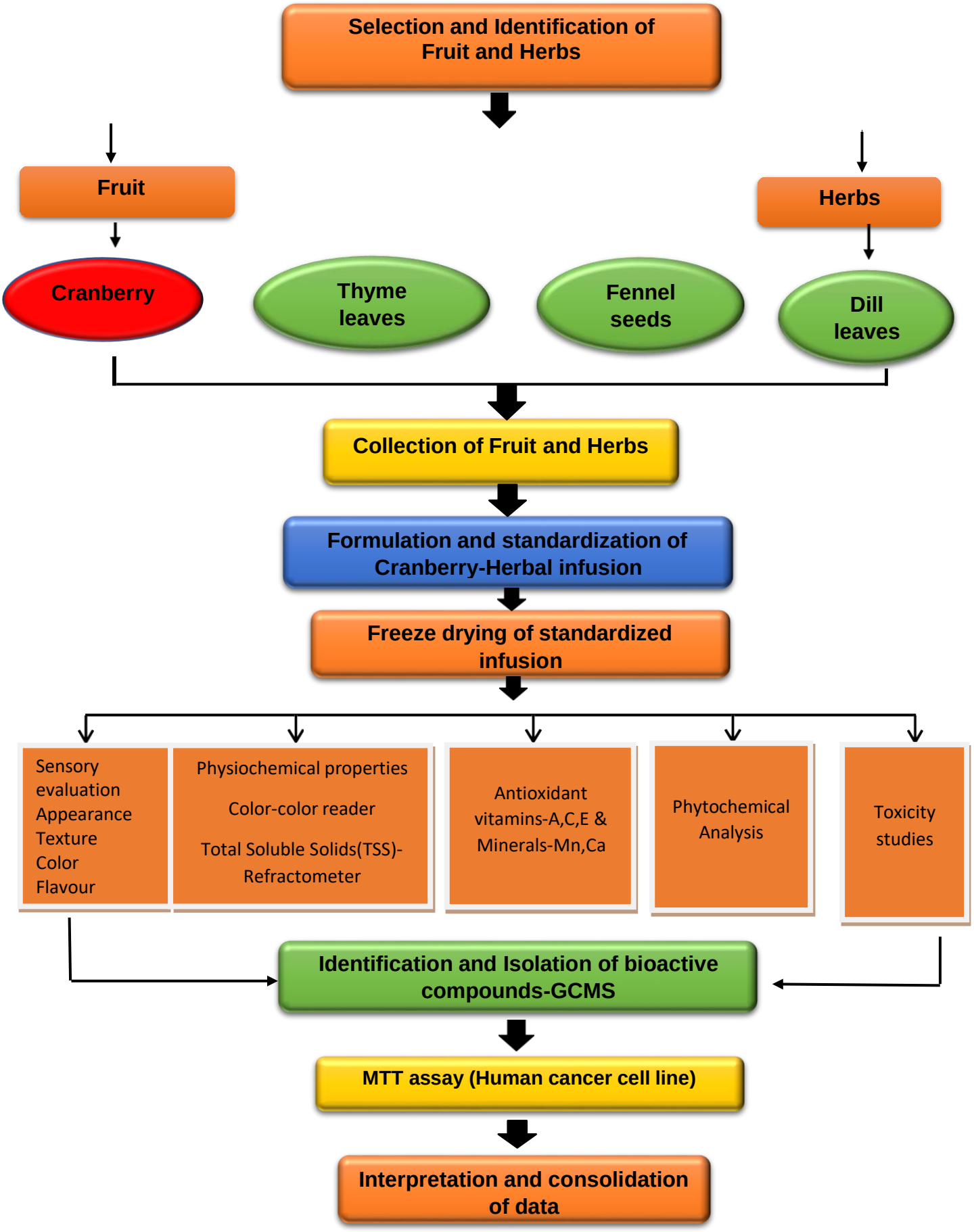
Seeded 200µl cell suspension in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Allowed the cells to grow for about overnight. Added appropriate concentrations of the test agent. Incubated the plate for 48hrs at 37°C in a 5% CO 2 atmosphere. After the incubation period, took out the plates from incubator, and removed spent media and added MTT reagent to a final concentration of 0.5mg/mL of total volume. Wrapped thewell and extracts at different concentrations. Were added and kept for overnight incubation. Culture medium was replaced with fresh medium prior to the addition of 20µl of MTT solution (0.5mg ml). Treatment of living cells with MTT produced a dark blue formazan product, whereas no staining is observed in dead cells. Wrap the plate with aluminum foil to avoid exposure to light. Returned the plates to the incubator and incubated for 3 hours. (Note: Incubation time varies for Different cell lines. Within one experiment, incubation time should be kept constant while making comparisons.). Removed the MTT reagent and then add 100µl of solubilization solution (DMSO). Gentle stirring in a gyratory shaker enhanced dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense Cultures. Read the absorbance on a Spectrophotometer or an ELISA reader at 570nm and 630nm uses a reference wavelength.

H. Statistical analysis, interpretation of the data and ethical clearance

The data was consolidated, tabulated and analyzed statistically to evaluate the

correlation and significance of the results obtained.

The study was presented in the Institutional Human Ethics Committee, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore and the approval was obtained. The human ethical clearance approval number is AUW/ IHEC/ FSN -20-21/XPD-12 and is presented in Appendix I.



RESEARCH DESIGN

Figure 2

IV. RESULTS AND DISSCUSSION

The Results and Discussion pertaining to the study "**Identification and Isolation of Bioactive Compounds in Cranberry-herbal Infusions"** is presented under following sub-headings:

A. **Standardized Cranberry- Herbal Infusion**
B. **Sensory characteristics of Cranberry- Herbal Infusion**
C. **Antioxidants Vitamins, Minerals, Phytochemicals, Colour and TSS of the freeze-dried Cranberry Herbal-Infusion**
D. **Toxins in the freeze-dried Cranberry -Herbal Infusion**
E. **Bioactive compounds and MTT assay of freeze-dried Cranberry- Herbal Infusion**

A. Standardized Cranberry- Herbal Infusion

The total amount of cranberry juice extracted from 500g of Cranberries by boiling method was 1450 ml in which the obtained residue after straining the juice from the cranberry was 99.4g.The total amount cranberry juice extracted from 500g of cranberries by cold pressed method was 750ml in which the obtained residue after straining the juice from the cranberry was 146.9g.The total amount of thyme leaves juice extracted from 500g of thyme leaves by cold pressed method was 700ml in which the obtained residue after straining the juice from thyme leaves was 296g.The total amount of fennel juice extracted from 250g of fennel by cold pressed method was 1250ml in which the obtained residue after straining the juice from fennel was 142g.The total amount of dill leaves juice extracted from 300g of dill leaves by cold pressed method was 200ml in which the obtained residue after straining the juice from dill leaves was 136g.

It was observed that boiling method for obtaining cranberry juice and cold-pressed method for obtaining herbal juice came out well and identified as the best method for the extraction and preparation of cranberry-herbal infusion.

Cranberry-Herbal Infusion was prepared by mixing the extracted juice of cranberry (70ml) and herbs in the ratio Thyme (10ml), Fennel (10ml) and Dill leaves (10ml) which was a total of 100ml and was standardized.

B. Sensory evaluation of Cranberry- Herbal Infusion

Sensory evaluation of Cranberry-Herbal Infusion was done using thirty semi-trained panel members. The evaluation report as given by the panel members is given in IV and Figure 3.

TABLE IV

SENENSORY EVALUATION OF CRANBERRY-HERBAL INFUSION

Criteria	Standard	Variation I	Variation II	Variation III
Appearance	7.60 ± 0.8	7.83 ± 0.89	**8.66 ± 0.82**	7.73 ± 0.62
Colour	7.53 ± 0.92	7.76 ± 0.84	**8.66 ± 0.74**	7.70± 0.69
Flavour	7.43 ± 0.71	7.7 ± 0.73	**8.63 ± 0.75**	7.56 ± 0.66
Consistency	7.43 ± 0.71	7.93 ± 0.89	**8.56 ± 0.76**	7.63 ± 0.65
Taste	7.2 ± 0.65	7.66 ± 0.78	**8.53 ± 0.76**	7.63 ± 0.65
Overall acceptability	7.34 ± 0.65	7.76 ± 0.68	**8.58 ± 0.69**	7.65 ± 0.48
T value Standard *vs* variation		7.01^{NS}	32.629^{NS}	4.718^{NS}

NS- Not Significant

Sensory evaluation of Cranberry-Herbal Infusion revealed that the appearance for standard was 7.60, variation I was 7.83, variation II was 8.66 and variation III was 7.73. The mean values obtained for colour was 7.73 for standard, 7.76 for variation I, 8.66 for variation II and 7.73 for variation III. The mean scores obtained for the flavour in Cranberry-Herbal Infusion ranged from 7.43 to 8.63, the score obtained for standard with a mean value of 7.43 followed by variation I with 7.7, variation II with 8.63 and variation III with 7.56 With regard to consistency, variation II showed the best result with a mean value of 8.56 followed by variation I with 7.93, variation III with 7.63 and standard with 7.43. The taste of the variation II scored maximum mean value of 8.58 followed by 7.2, 7.65 and 7.63 for standard, variation I and variation III respectively. The overall acceptability of Cranberry-Herbal Infusion revealed that variation II had a maximum mean score of 8.58 and was comparable with the standard of 7.44. This was followed by variation I and III with mean scores 7.76 and 7.66 respectively.

The overall acceptability was analyzed statistically using-test comparing standards with Variation I, II and III. It was observed that overall acceptability was not significant when standard and variations were compared.

It was observed from sensory evaluation that variation II was best accepted by the panel members. Hence further analysis was carried out for the best accepted variation.

The best accepted variation i.e. variation II was freeze-dried. A net weight of freeze-dried cranberry herbal infusion powder weighing 20g was obtained from 600ml of cranberry herbal juice. The freeze dried powder of cranberry-herbal infusion was further subjected to analysis.

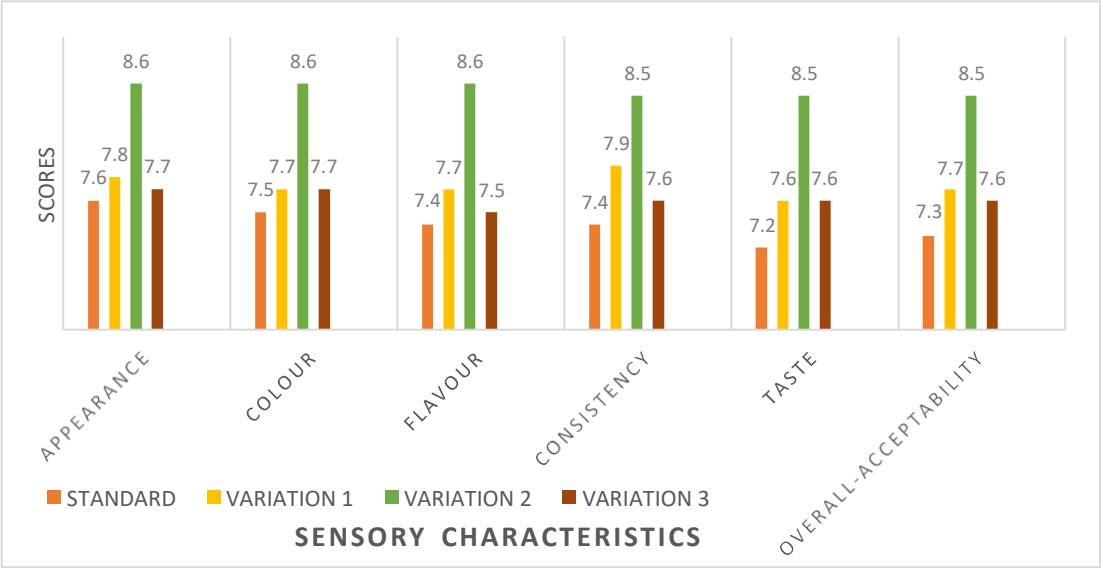


Figure 3

Sensory Evaluation of The Cranberry -Herbal Infusion

C. Antioxidants Vitamins, Minerals, Phytochemicals, Colour and TSS of the freeze-dried Cranberry Herbal-Infusion

Antioxidant vitamins in freeze dried cranberry-herbal infusion

The antioxidant vitamins present in Freeze-dried Cranberry-Herbal Infusion is depicted in Table V and Figure 4.The vitamins analyzed in cranberry-herbal infusion are vitamin A, vitamin C and vitamin E which are anti-oxidants in nature.

TABLE V

QUALITATIVE ANALYSIS OF ANTIOXIDANT VITAMINS IN CRANBERRY-HERBAL INFUSION

Vitamins	Values
Vitamin A	263 IU/100g
Vitamin C	10.11 mg/g
Vitamin E	6.1 mg/kg

The vitamin A content present in freeze dried cranberry-herbal infusion powder was **263 IU/100g.** Vitamin A helps in lowering the risk of cancer, supports bone health, promotes healthy growth and reproduction. Vitamin A protects eyes from night blindness and age-related decline.

Retinoic has the ability to control cell proliferation, usually by delaying the cell cycle, as well as produce immature and altered cells. Retinoids are one of the most powerful families of drugs for inducing cell differentiation, and as a result, they're a hot topic in cancer prevention and treatment. Despite extensive study, however, the side effects associated with most substances have limited their application in cancer chemoprevention (CathrineRoss *et al*., 2010).

The vitamin C present in freeze dried cranberry-herbal infusion powder was **10.11 mg/g.** Vitamin C is necessary for the growth, development and repair of all body tissues. It helps in functioning of the immune system.

Vitamin C is a necessary vitamin for humans, and its ability to transfer electrons has pleiotropic effects. It's a powerful antioxidant as well as a cofactor for several biosynthetic and gene regulatory enzymes. Vitamin C aids immune protection by supporting both the innate and adaptive immune systems' cellular functioning. Vitamin C protects the epithelial barrier function against infections and enhances the skin's oxidant scavenging capacity, potentially defending against oxidative stress from the environment. Vitamin C accumulates in phagocytic cells like neutrophils, where it can boost chemotaxis, phagocytosis, reactive oxygen species production, and eventually microbial death. Itis also required for apoptosis and macrophage clearance of wasted

neutrophils from infection sites, reducing necrosis and potential tissue damage (Anitra C *et al.*,2017).

The vitamin E content present in freeze dried cranberry-herbal infusion powder was **6.1 mg/kg.** Vitamin especially those that claim to have anti-aging benefits. Vitamin E supplements may prevent coronary heart disease, support immune function, prevent inflammation, promote eye health, and lower the risk of cancer.

There are four tocopherols and four tocotrienols in natural vitamin E. Although vitamin E is the body's primary lipid-soluble antioxidant, not all of its effects can be attributed to it. Vitamin E works as an antioxidant in cell membranes, preventing the spread of free radical reactions, while it has also been found to have pro-oxidant activity. The reaction between the tocopherol radical and other free radicals, which are conjugated to glucuronic acid and expelled through the bile or urine, produces non-radical oxidation products. Lipoproteins in the plasma transport vitamin E. Vitamin E is packed into chylomicrons after intestinal absorption and released into the systemic circulation *via* the lymphatic system. Part of the tocopherols delivered in chylomicrons are taken up by extrahepatic tissues due to the activity of Lipoprotein Lipase (LPL), and the remaining tocopherols are transferred to the liver by the remnant chylomicrons. A large amount of tocopherol is integrated into nascent Very Low- Density Lipoproteins (VLDL) by the action of the "-tocopherol transfer protein," while the excess -tocopherol and other forms of vitamin E are eliminated in bile (Herrera *et al*.,2001).

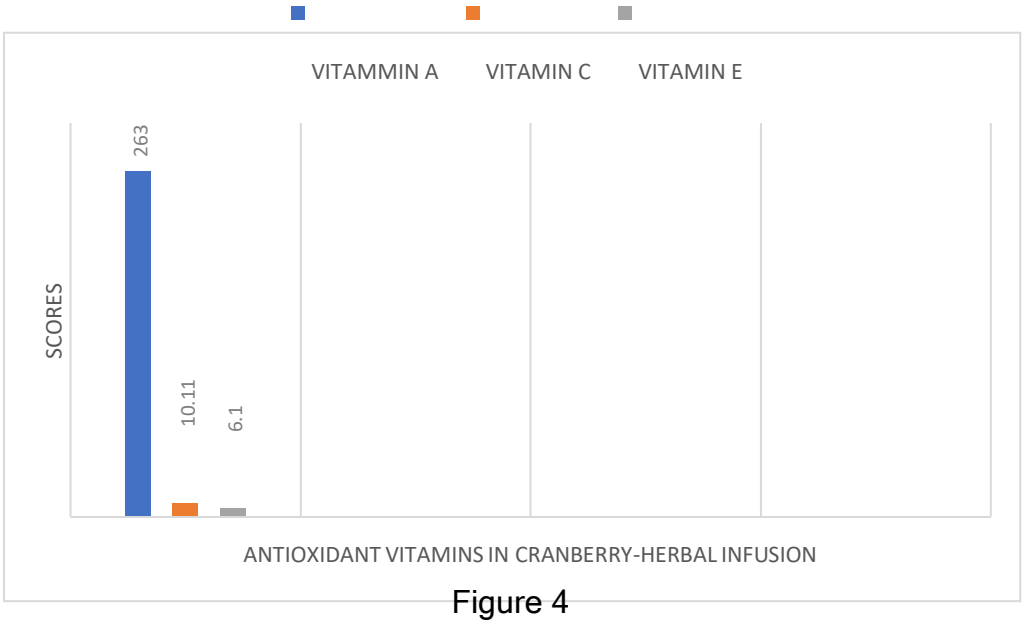


Figure 4

Antioxidant Vitamins in Freeze Dried Herbal Infusion

Minerals in freeze dried cranberry-herbal infusion

The minerals present in freeze- dried cranberry-herbal infusion powder is depicted in Table VI and Figure 5. Minerals present in cranberry-herbal infusion are calcium and manganese.

TABLE VI

MINERALS IN FREEZE- DRIED CRANBERRY-HERBAL INFUSION POWDER

Minerals	Values
Calcium	623 mg/100g
Manganese	2.6 mg/100g

The calcium content present in cranberry-herbal infusion was **623 mg/100g.**Calcium helps to build and maintain strong bones. Calcium helps in protecting against cancer,Diabetes and high blood pressure.

Calcium intake disparities between rich and poor populations are substantial. Appropriate calcium intake has been linked to a variety of health benefits, including reduced hypertensive disorders of pregnancy, lower blood pressure, especially in young people, prevention of osteoporosis and colorectal adenomas, lower cholesterol levels, and lower blood pressure in the offspring of mothers who consumed enough calcium during pregnancy. Some calcium supplements negative effects, such as iron deficiency, renal stone production, and myocardial infarction in the elderly, have been debunked by research. After discontinuing calcium supplementation, postpartum women should be monitored for bone resorption. The relationship between low calcium consumption and high blood pressure is mediated by parathyroid hormone, which causes vasoconstriction by increasing intracellular calcium in vascular smooth muscle cells (Cormick*et al*.,2019).

The manganese content present in freeze dried cranberry-herbal infusion was **2.6 mg/100g.**Manganese helps the body form connective tissue, bones, blood clotting

factors and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption and blood sugar regulation. Manganese is also necessary for normal brain and nerve function.

Manganese, an important trace metal, enters the brain through the blood-brain and blood-cerebrospinal fluid barriers. This process has some processes, and manganese transport into the brain may be aided by transferrin. Manganese metalloproteins, particularly glutamine synthetase in astrocytes, bind a considerable portion of manganese. Manganese is likely present in synaptic vesicles in glutamatergic neurons, and the manganese is dynamically linked to the neurons' electrical activity. Synaptic neurotransmission may be influenced by manganese delivered into the synaptic cleft. Dietary manganese shortage appears to impair manganese homeostasis in the brain, which may increase sensitivity to epileptic activities. This is likely followed by changes in neural activity (Atsushi Takeda *et al*.,2003).

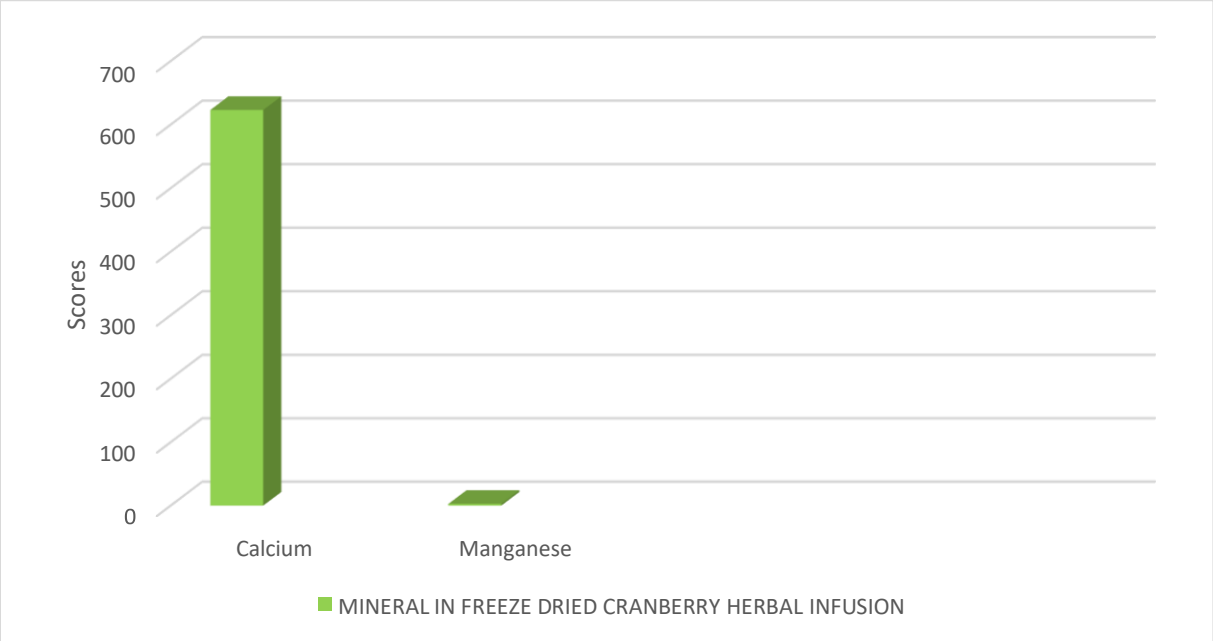


Figure 5

Minerals in Freeze- Dried Cranberry-Herbal Infusion Powder

Colour intensity of freeze dried cranberry-herbal infusion

Colour intensity was analyzed for the freeze dried cranberry-herbal infusion using a colour reader. A colour reader is a device that measures the colour and whiteness of paste, solid, or powdered foods or raw materials. It comes with a big LCD

panel that shows measurement data from one of eight colour spaces/colorimetric indices, including L*a*b*, L*C*h, and Yxy.

The colour intensity of the freeze dried cranberry-herbal infusion is depicted in Table VII and Figure 6.

TABLE VII

COLOUR INTENSITY OF FREEZE- DRIED CRANBERRY-HERBAL INFUSION

Extract	L*A*B
Cranberry juice	59.26*10.36*2.34
Thyme leaves juice	59.15*-1.51*22.91
Fennel seeds juice	52.52*-3.34*16.50
Dill leaves juice	63.90*-6.23*0.60
Freeze dried cranberry-herbal infusion	42.40*2.79*-0.09

The colour intensity of freeze-dried cranberry-herbal infusion powder wasbased on the L*A*B* values. The colour intensity of cranberry juice was 59.26*10.36*2.34* which represents white, red and yellow respectively. The colour intensity of thyme leaves juice was 59.15*-1.51*22.91* which represents white, green and yellow respectively. The colour intensity of fennel juice was 52.52*-3.34*16.50 which represents white, green and yellow. The colour intensity of dill leaves juice was63.90*-6.23*0.60 which represents white, green and yellow respectively. The colour intensity of freeze-dried cranberry-herbal infusion was 42.40*2.79*-0.90 which represents white, red and blue respectively. (Plate XX).

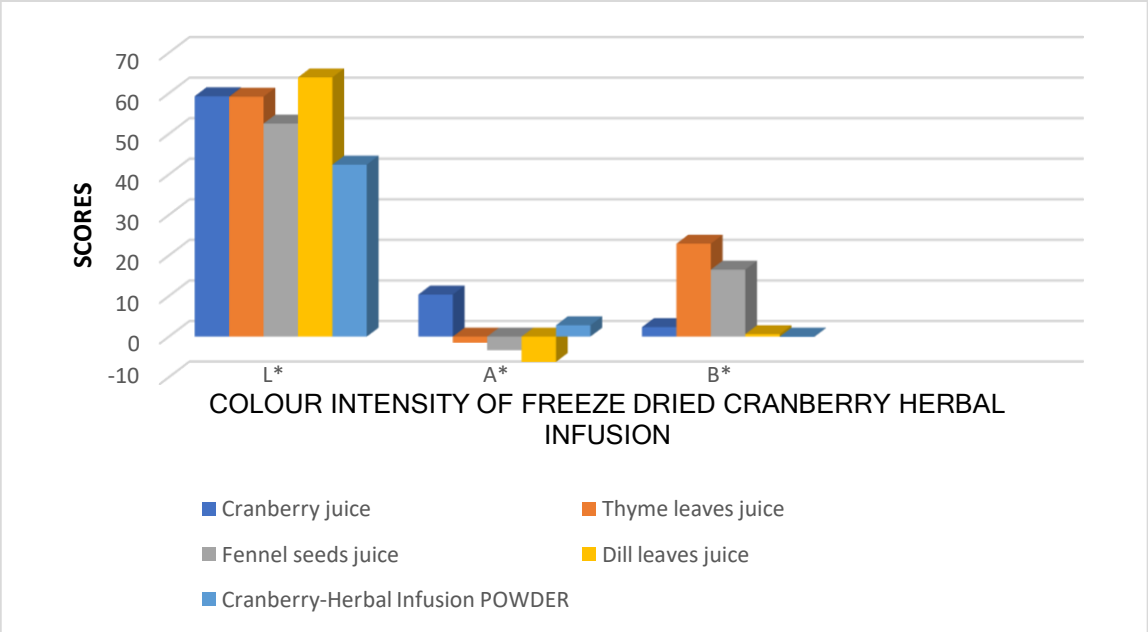


Figure 6

Colour Intensity of Freeze Dried Cranberry-Herbal Infusion

PLATE XX

COLOUR INTENSITY OF CRANBERRY-HERBAL INFUSION

Total Soluble Solids (TSS)

The Total Soluble Solids (TSS) was analysed using Brix Refractometer. The unit of measuring the Brix was ˚Bx. The Total Soluble Solids of the freeze dried cranberry-herbal infusion is depicted in Table VIII and Figure 7.

TABLE VIII

TOTAL SOLUBLE SOLIDS OF CRANBERRY-HERBAL INFUSION

INGREDIENTS	TSS (˚Bx)
Fresh cranberry fruit	5
Cranberry juice	0
Freeze Dried Cranberry-Herbal Infusion powder	0

The Total Soluble Solids (TSS) was analyzed for the cranberry fruit and freeze dried cranberry-herbal infusion. The sugar concentration of cranberry was analyzed using Brix Refractometer. TSS measures the sugar content in the cranberry and it was 5 Bx° for the fresh cranberry fruit. The TSS of cranberry juice was 0 Bx°.The TSS for freeze dried cranberry-herbal infusion was also 0 Bx° as it had no sugar content in it (Plate XXI).

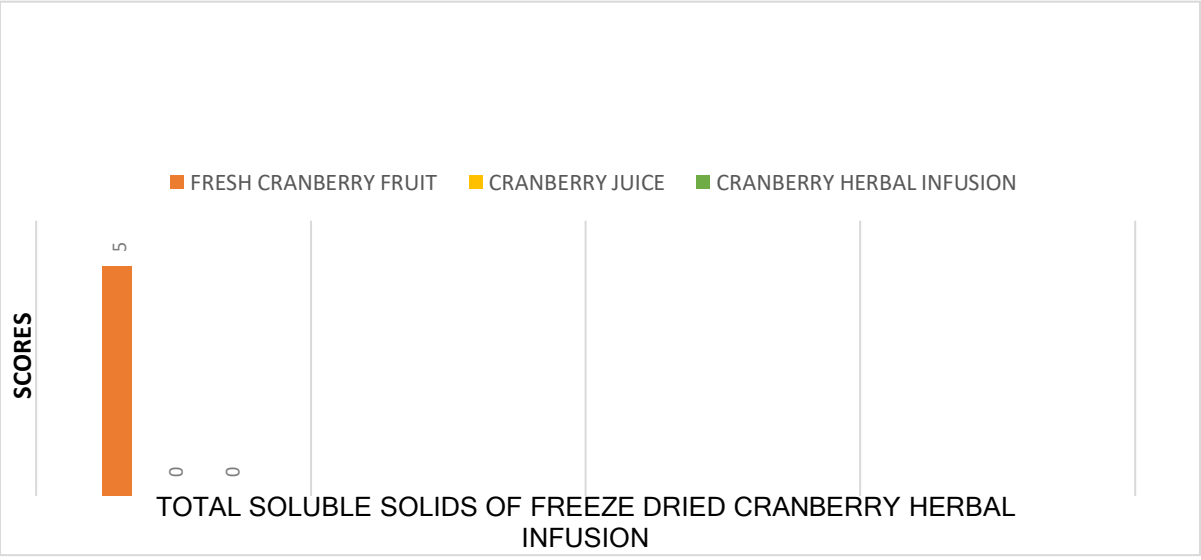


Figure 7

Total Soluble Solids of Freeze Dried Cranberry-Herbal Infusion

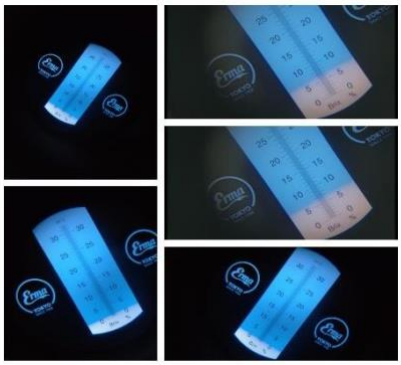


PLATE XXI

Total Soluble Solids OF CRANBERRY-HERBAL INFUSION

Phytochemicals in Freeze Dried Cranberry-Herbal Infusion

The qualitative analysis of phytochemicals in Freeze-dried Cranberry-Herbal Infusion is depicted in the Table IX.

TABLE IX

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN FREEZE DRIED CRANBERRY-HERBAL INFUSION

Phytochemicals	Aqueous extract
Tannins	+++
Terpenoids	+++
Phenols	++
Saponins	+++
Quinones	-
Glycosides	+++
Coumarins	+
Sterols	++
Flavonoids	++
Alkaloids	+

+++ Strongly present,++ Moderately present,+Weekly present,-Absent

Phytochemicals are important in the prevention and treatment of modern diseases like cancer, diabetes, Alzheimer's, and cardiovascular disease (Andrae-Marobela *et al.,* 2013). Qualitative analysis of phytochemicals was done in aqueous extract of cranberry-herbal infusion. The extracts was screened for phytochemicals like tannins, terpenoids, phenols, saponins, quinones, glycosides, coumarins, sterols, flavonoids and alkaloids.

The aqueous extract of freeze dried cranberry-herbal infusion showed a strong presence of phytochemical alkaloids, tannins, terpenoids, saponins and glycosides. Phenols, sterols and flavonoid were moderately present in aqueous extract; while alkaloids and coumarins were weakly present and quinones were absent in aqueous extract.

Toxins in the freeze dried cranberry-herbal infusion

The Brine Shrimp Lethality Assay (BSLA) is a simple and inexpensive bioassay used for testing the efficacy of phytochemical present in the plant extracts. The toxins present in Cranberry-Herbal Infusion is depicted in Table X, Figure 8 and Plate XXII.

TABLE X

TOXINS IN THE FREEZE DRIED CRANBERRY-HERBAL INFUSION

Sample	Concentration (µg/ml)	Mortality of brine shrimp (No of shrimps dead) (h)					
		1	2	4	6	24	% Molarity (at 24 h)
Cranberry-herbal infusion	100	0	0	0	0	2	7
	250	0	0	0	0	3	10
	500	0	0	0	0	3	10
	1000	0	0	0	0	6	20
	1500	0	0	1	1	11	37
$K_2Cr_2O_7$	**(1mg/ml)**	**30**	**-**	**-**	**-**	**-**	**100**
Blank	**Saline water**	0	0	0	0	0	0

The sample Cranberry-Herbal Infusion is comparatively less toxic than $K_2Cr_2O_7$ which shows maximum lethality of shrimps at higher concentration. The shrimps in the sample Cranberry-Herbal Infusion is found to be very less toxic in lower as well as in higher concentration. Even after 24h, only 2- 10 shrimps are found to be mortal at lowest and highest concentration.

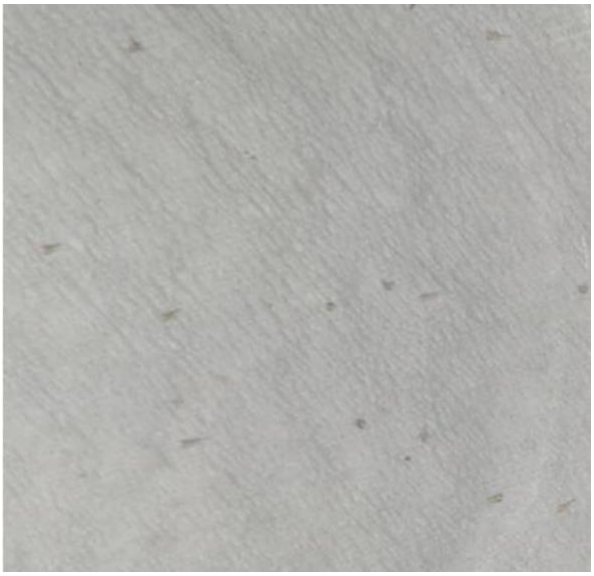


PLATE XXII

TOXINS IN THE CRANBERRY-HERBAL INFUSION

F. Identification of bioactive compounds and MTT assay of freezed dried infusion

Identification of bioactive compounds in Freeze dried Cranberry-herbal infusion

The bioactive compounds present in freeze dried cranberry-herbal infusion is depicted in Table XI and Figure 8.

TABLE X

BIOACTIVE COMPOUNDS IN FREEZE DRIED CRANBERRY-HERBAL INFUSION

Peak #	R.Time	Area	Area%	Height	Height %	A/H	Name
1	0.037	1536687	2.69	575042	5.91	2.67	**Propanoicacid,3,3'-dithiobis-(CAS) Dithiodi**
2	0.117	911147	1.60	146507	1.51	6.22	**1,1'-bibicyclo(2.2.2)octyl-4-carboxylicacid**
3	0.424	477261	0.84	513650	5.28	0.93	**TRANS-.BETA.-IONON-5,6-Epoxide**
4	**0.497**	**11426151**	**20.01**	**365857**	**37.61**	**3.12**	**Ethanol (CAS) Ethyl alcohol**
5	0.603	1948805	3.41	809772	8.32	2.41	**1,1,4,4-Tetradeuteriotertamethyl**
6	**0.665**	**29891090**	**52.35**	**245001**	**25.19**	**12.20**	**Trans-.Beta.-Ionon-5,6-Epoxide**
7	1.100	4988305	8.74	473214	4.86	10.54	**2-Butanone,3-hydroxy-(CAS) Acetoin**
8	**1.250**	**1995339**	**3.49**	**235127**	**2.42**	**8.49**	**1,2-Bis(trimethylsilyl)benzene**
9	1.752	1088284	1.91	132537	1.36	8.21	**Propanoicacid,2-hydroxy-,ethylester(CAS)**
10	1.908	293451	0.51	54458	0.56	5.39	**5-METHOXY-1-AZA-6-Oxabicyclo(3.1.)**
11	1.967	68929	0.12	27218	0.28	2.53	**1,2-Propadiene(CAS)Allene**
12	2.355	81368	0.14	39660	0.41	2.05	**1-Dimethyl(isopropyl)silyloxypropane**
13	2.450	49232	0.09	11484	0.12	4.29	**2-Hexynoicacid**
14	2.763	90644	0.16	47662	0.49	1.90	**Cyclohexene,4-ethenyl-1,4-dimethyl-**
15	3.477	165891	0.29	77244	0.79	2.15	**Bicyclo[2.2.1]heptan-2-one,1,3,3-trimethyl-**
16	3.608	66926	0.12	17328	0.18	3.86	**1-Propyne(CAS)Propyne**
17	4.545	177280	0.31	31545	0.32	5.62	**3-Butyn-1-ol(CAS)3-Butynol**
18	4.769	233521	0.41	38820	0.40	6.02	**Estragole**
19	5.982	309175	0.54	79326	0.82	3.90	**Phenol,2-methyl-5-(1-methylethyl)-(CAS)Ca**
20	6.150	130622	0.23	23557	0.24	5.54	**Ethyl1-Hexyl-4-Hydroxy-2(1H)-OX**
21	9.958	446022	0.78	121145	1.25	3.68	**Diethyl Phthalate**
22	13.913	155127	0.27	58293	0.60	2.66	**Hexadecanoicacid(CAS)Palmiticacid**
23	15.327	95161	0.17	36691	0.38	2.59	**TRIMETHYLSILYLDERIVATIVEOF2-MO**
24	17.925	73013	0.13	21127	0.22	3.46	**pnz-sar**
25	20.854	404522	0.71	47989	0.49	8.43	**Pyrazine,tetrakis(1-methylethyl)-(CAS)TET**
		57103953	**100.00**	**972793 8**	**100.00**		

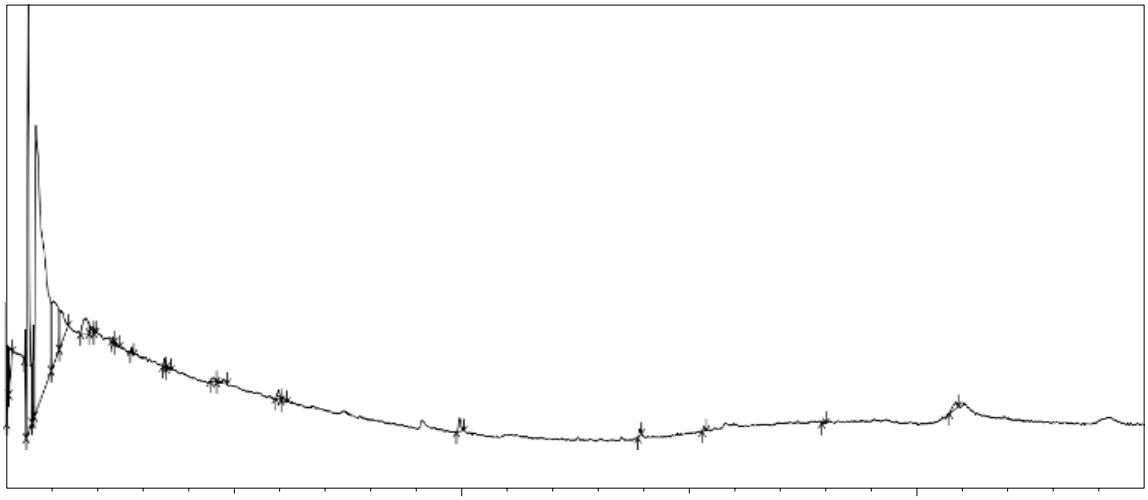


Figure 8

Bioactive Compounds in Freeze Dried Cranberry-Herbal Infusion

The bioactive compounds present in freeze dried cranberry-herbal infusion revealed that the highest peak obtained is for the component Ethanol (CAS) Ethyl alcohol with reaction time 0.497. The next highest peak obtained was for the bioactive component Trans-.Beta.-Ionon-5,6-Epoxide in a reaction time of 0.665 followed by 1,2-Bis (trimethyl silyl) benzene with a reaction time of 1.250. The other bioactive compounds include Propanoic acid,3,3'-dithiobis-(CAS) Dithiodi; 1,1'-bibicyclo (2.2.2) octyl-4-carboxylic acid; Trans-.Beta.-Ionon-5,6-Epoxide; 1,1,4,4-Tetra deuterio terta methyl; 2-Butanone,3-hydroxy-(CAS) Acetoin; Propanoic acid, 2-hydroxy-,ethyl ester (CAS); 5-Methoxy-1-AZA-6-Oxabicyclo(3.1.); 1,2-Propadiene (CAS) Allene; 1-Dimethyl (isopropyl) silyloxy propane; 2-Hexynoicacid; Cyclohexene,4-ethenyl-1,4-dimethyl; Bicyclo [2.2.1] heptan-2-one,1,3,3-trimethyl; 1-Propyne (CAS) Propyne; 3-Butyn-1-ol(CAS) 3-Butynol; Estragole; Phenol,2-methyl-5-(1-methylethyl)-(CAS) Ca; Ethyl1-Hexyl-4-Hydroxy-2(1H)-OX; Diethyl Phthalate; Hexa decanoic acid (CAS) Palmitic acid; trimethyl silyl derivativeof2-MO; pnz-sar and Pyrazine, tetrakis(1-methylethyl)-(CAS)TET and was obtained at different reaction time. The area and area % obtained for the bioactive component present in cranberry-herbal infusion is presented in the above table.

Bioactive compounds present are antioxidant, antimicrobial, antidiabetic, and anticancer in nature; are useful as anti-osteoporotic, antihypertensive, immunomodulatory, hypolipidemic, and anti-obesity agents; and reduce cardiovascular complications.

Estragole is one of the important bioactive compound. Estragole exhibits a dose-dependent cytotoxic activity in the monolayer culture of MCF7 cell line.

Natural bioactive agents from plants are gaining wide attention for their anticancer activities.Natural plant-based bioactive compounds can enhance the efficacy of chemotherapy, and in some cases ameliorate some of the side-effects of drugs used as chemotherapeutic agents.

MTT assay of freeze dried infusion

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm. (Alley, M. C *et al*., 1986, Mosmann *et al*., 1983).

In the present study freeze dried cranberry-herbal infusion the compound is evaluated to analyse the cytotoxicity effect on MCF-7 cell line. The concentrations of the test compound used to treat the cells is depicted in Table XI and Figures 10 and 11.

TABLE XI

MTT ASSAY OF FREEZE DRIED CRANBERRY-HERBAL INFUSION

Test compound	Cell line	Concentration treated to cell
Untreated	MCF-7	No treatment
Standard	MCF-7	5µM
Blank (C)-cisplatin	-	Only media without cell
Cranberry-herbal infusion	MCF-7	5 (31.25, 62.5, 125, 250, 500µg/ml)

The cytotoxicity activity of freeze dried cranberry-herbal infusion towards human breast cancer MCF-7 cells was tested. The results of cytotoxicity study (MTT assay) suggested that freeze dried cranberry-herbal infusion showed activity with IC50 value of **527.20µg/ml** after the treatment period of 48 hrs. The IC50 value of **527.20µg/ml** shows the **moderate** inhibitory activity. IC50 is the concentration of an inhibitor where the response (or binding) is reduced by half. So, if testing viability *via* an MTT assay, the dose of cytotoxic compound at which achieve 50% viability will be

the IC50. Plates XXIII to XXVIII depicts the freeze dried cranberry-herbal infusion activity at different concentrations.

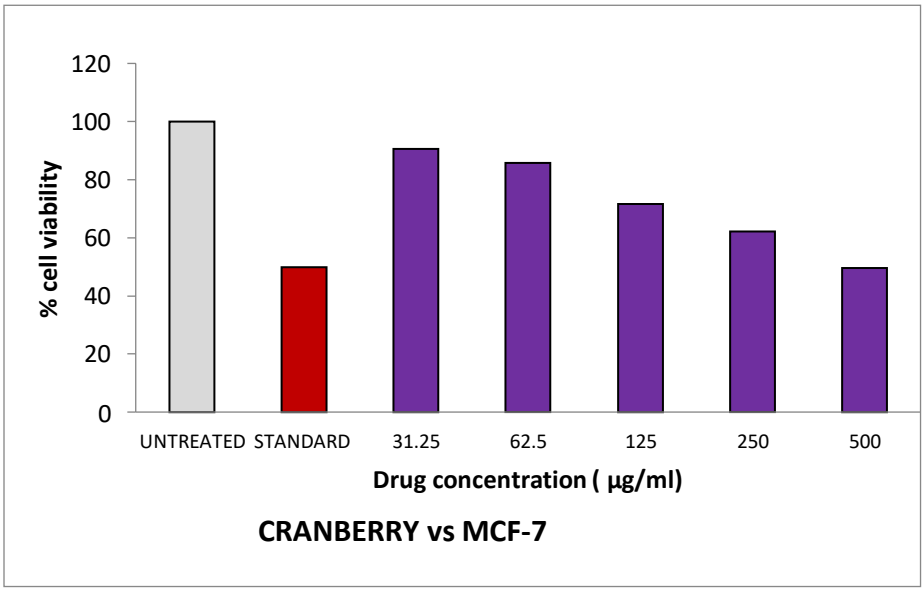


Figure 9

CELL VAIBILITY IN CRANBERRY Vs MCF-7

MTT Assay of Freeze Dried Cranberry-Herbal Infusion

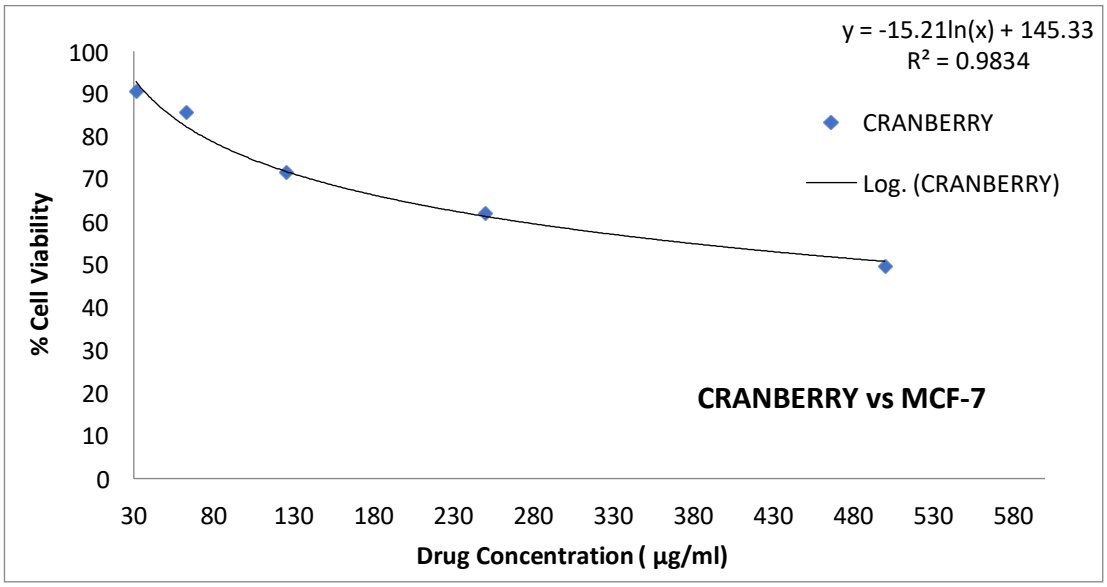
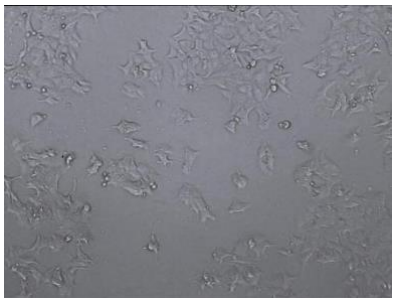


Figure 10



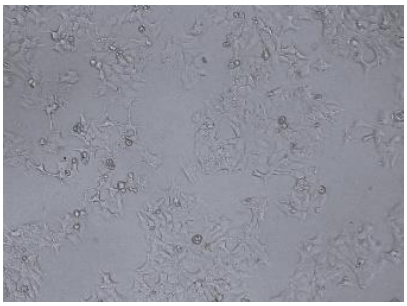
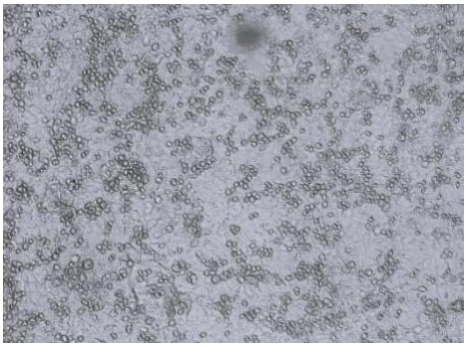


PLATE XXIII

Untreated test compound



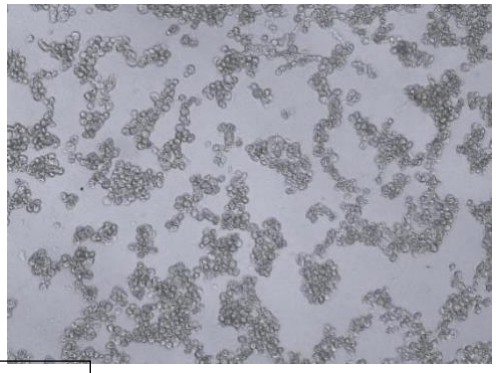
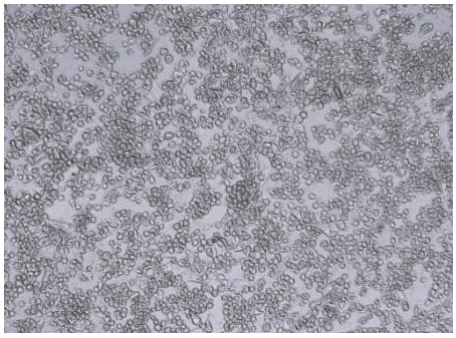


PLATE XXIV

standard test compound



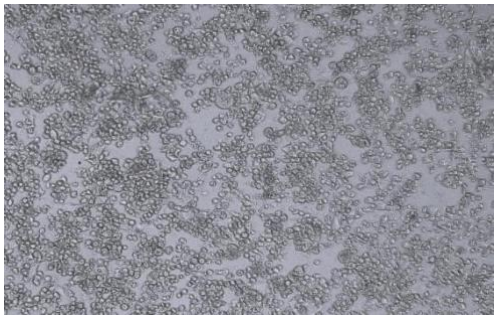
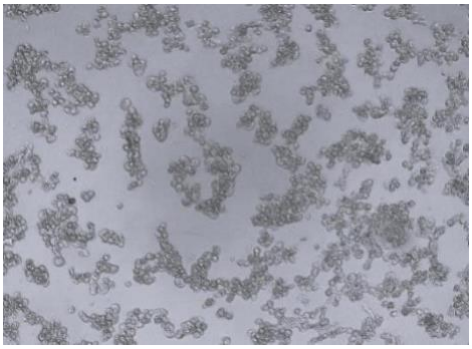


PLATE XXV

500 µg concentration



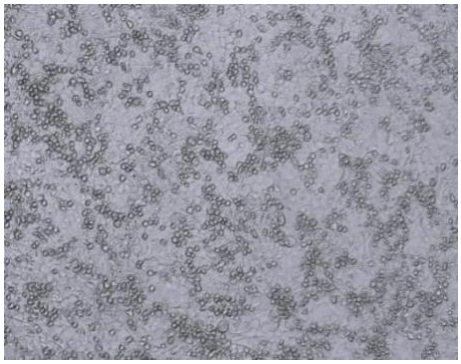
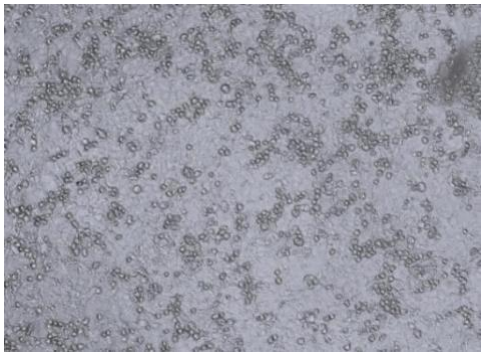


PLATE XXVI

250 µg concentration



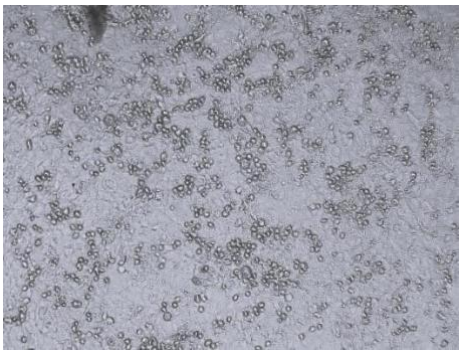
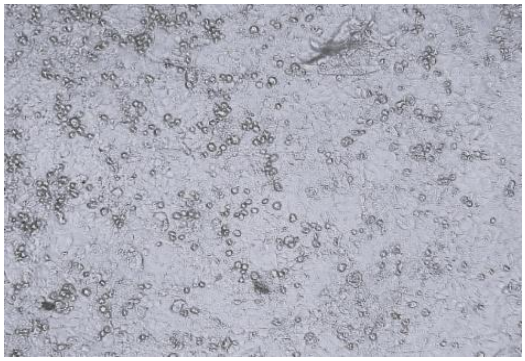


PLATE XXVII

62.5 µg cocentration



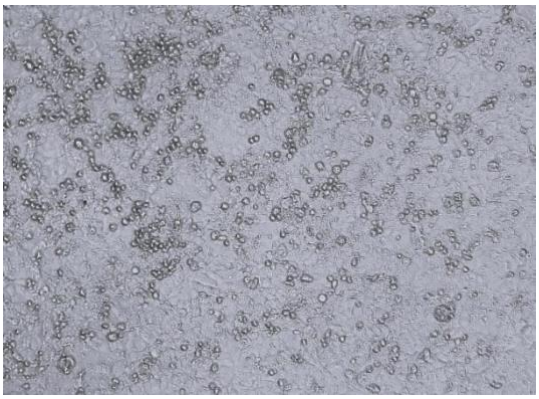


PLATE XXVIII

31.25 µg concentration

V. SUMMARY AND CONCLUSION

In recent years, herbal infusions have grown in popularity. Herbal infusions are hot beverages that can be consumed on a regular basis or for medical reasons. Herbal infusions, also known as tisanes, are extracts prepared from plants or herbs. An infusion is created by steeping an ingredient in a liquid. After that, the liquid absorbs the flavour and aroma of the soaked substance. Infusions are made by steeping herbs and fruits in hot water. Depending on the strength of the flavour, steep them for a minute to an hour. Herbal infusions are better for your health than other types of tea. Herbal infusions are an excellent method to incorporate mindfulness into your daily life. Vitamins, minerals, and antioxidants abound in fruits and herbs.

There are several methods for preserving the nutritional potential of fruits and herbs, one of which is herbal infusion in powder form. This infusion can be drunk with snacks and tea, and it can also be used to substitute caffeinated beverages. Because of its colour, flavour, taste, nutritional content, and shelf life, hygienically prepared infusions are preferred by adolescents, adults, middle-aged adults, and senior adults. Herbs have been utilised as preservatives, colourants, flavour enhancers, and even medicinal ingredients for centuries.Hence the study was undertaken with the objective of isolating the bioactive compounds present in cranberry-herbal infusion.

Cranberry -herbal infusion powder which was freeze dried was steeped into to the hot water They can be steeped from ten minutes to even a couple of hours, depending on the strongness of the flavor. The cranberry-herbal infusions are different from a regular tea as it is not brewed in a pot of boiling water. Cranberry-herbal infusions have more benefits than other types of tea.

The present study entitled **"Identification and Isolation of Bioactive Compounds in Cranberry-Herbal Infusion"** was aimed to Select and identify of herbs and fruit based on the health benefits like reducing heart disease, controlling blood pressure, prevents from cancer and has anti-inflammatory properties.

The fruit selected for the study include cranberry and the herbs include tulsi, fennel leaves and dill leaves. It was also selected and identified as it is a source of high vitamin C. The fruit and herbs was collected from the local shops of Coimbatore and within Tamil Nadu.

The formulation and standardization was carried out for cranberry-herbal infusion where for the extraction of cranberry juice both boiling method and cold pressed method was done and for extractions of herbal juices cold pressed method was done. Three variations were done and the best method was standardized. The standardized cranberry-herbal infusion was freeze dried.

The developed cranberry-herbal infusion was subjected to sensory evaluation by 30 semi-trained panel members along with standard and three different variations (Variation I, II and III) with the help of score card (9 point hedonic scale). The evaluation was done in terms of the attributes like appearance, colour, flavour, texture, taste and overall acceptability rating from like extremely to dislike extremely in the 9 point hedonic scale rating.

The analysis of antioxidant vitamin like vitamin A, C and E and minerals like calcium and manganese was analyse for the freeze dried cranberry-herbal infusion. The qualitative analysis of phytochemicals present in cranberry- herbal infusion were tannins, terpenoids, phenols, saponins, quinones, glycosides, coumarins, sterols, flavonoids and alkaloids was conducted in aqueous extracts. TotalSoluble Solids and colour intesity was also carried out. The toxins were evaluated in the cranberry herbal infusion by the method of BrineShrimp Lethality Assay.

Bioactive compounds were identified in cranberry-herbal infusion and MTT assayby Cytotoxicity method was carried to evaluate the anticancer effect of cranberry- herbal infusion on breast cancer cell line.

The salient findings of the study are summarized below:

- The total amount of cranberry juice extracted from 500g of cranberries by boiling method was 1450 ml in which the obtained residue after straining the juice from the cranberry was 99.4g.The total amount cranberry juice extracted from 500g of Cranberries by cold pressed method was 750ml in which the obtained residue after straining the juice from the cranberry was 146.9g.The total amount of Thyme leaves juice extracted from 500g of Thyme leaves by cold pressed method was 700ml in which the obtained residue after straining the juice from Thyme leaves was 296g.The total amount of Fennel juice extracted from 250g of fennel by cold pressed method was 1250ml in which the obtained residue after straining the juice from fennel was 142g.The total amountof dill

leaves juice extracted from 300g of dill leaves by cold pressed method was 200ml in which the obtained residue after straining the juice from dill leaves was 136g.

- It was observed that **boiling method** for obtaining Cranberry juice and cold-pressed method for obtaining herbal juice came out well and identified as the best method for the extraction and preparation of cranberry-herbal infusion.
- Cranberry-Herbal Infusion was prepared by mixing the extracted juice of cranberry (70ml) and herbs in the ratio Thyme (10ml), Fennel (10ml) and Dill leaves (10ml) which was total of 100ml and was standardized.
- **Sensory evaluation** of Cranberry-Herbal Infusion revealed that the appearance for standard was 7.60, variation I was 7.83, variation II was 8.66 and variation III was 7.73. The mean values obtained for colour was 7.73 for standard, 7.76 for variation I, 8.66 for variation II and 7.73 for variation III. The mean scores obtained for the flavour in Cranberry-Herbal Infusion ranged from 7.43 to 8.63, the score obtained for standard with a mean value of 7.43 followed by variation I with 7.7, variation II with 8.63 and variation III with 7.56 With regard to consistency, variation II showed the best result with a mean value of 8.56 followed by variation I with 7.93, variation III with 7.63 and standard with 7.43. The taste of the variation II scored maximum mean value of 8.58 followed by 7.2, 7.65 and 7.63 for standard, variation I and variation III respectively. The overall acceptability of Cranberry-Herbal Infusion revealed that variation II had a maximum mean score of 8.58 and was comparable with the standard of 7.44. This was followed by variation I and III with mean scores 7.76 and 7.66 respectively.
- The **overall acceptability** was analyzed statistically using-test comparing standards with Variation I, II and III. It was observed that overall acceptability was not significant when standard and variations were compared.
- It was observed from sensory evaluation that variation II was best accepted by the panel members. Hence further analysis was carried out for the best accepted variation.The best accepted variation i.e. variation II was freeze-dried. A net weight of freeze-dried cranberry herbal infusion powder weighing 20g was obtained from 600ml.

- The **vitamin A** content present in freeze dried cranberry-herbal infusion powder was **263 IU/100g.** Vitamin A helps in lowering the risk of cancer, supports bone health, promotes healthy growth and reproduction. Vitamin A protects eyes from night blindness and age-related decline.
- The **vitamin C** present in freeze dried cranberry-herbal infusion powder was **10.11 mg/g.** Vitamin C is necessary for the growth, development and repair of all body tissues. It helps in functioning of the immune system
- The **vitamin E** content present in freeze dried cranberry-herbal infusion powder was **6.1 mg/kg.** Vitamin especially those that claim to have anti-aging benefits. Vitamin E supplements may prevent coronary heart disease, support immune function, prevent inflammation, promote eye health, and lower the risk of cancer.
- The **calcium** content present in cranberry-herbal infusion was **623 mg/100g.**Calcium helps to build and maintain strong bones. Calcium helps in protecting against cancer, Diabetes and high blood pressure.
- The **manganese** content present in freeze dried cranberry-herbal infusion was **2.6 mg/100g.**Manganese helps the body form connective tissue, bones, blood clotting factors and sex hormones. It also plays a role in fat and carbohydrate metabolism, calcium absorption and blood sugar regulation. Manganese is also necessary for normal brain and nerve function.
- **Colour intensity** was analyzed for the freeze dried cranberry-herbal infusion using a colour reader The colour intensity of freeze-dried cranberry-herbal infusion powder was based on the L*A*B* values. The colour intensity of cranberry juice was 59.26*10.36*2.34* which represents white, red and yellow respectively. The colour intensity of thyme leaves juice was 59.15*-1.51*22.91* which represents white, green and yellow respectively. The colour intensity of fennel juice was 52.52*-3.34*16.50 which represents white, green and yellow. The colour intensity of dill leaves juice was 63.90*-6.23*0.60 which represents white, green and yellow respectively. The colour intensity of freeze-dried cranberry-herbal infusion was 42.40*2.79*-0.90 which represents white, red and blue respectively.
- The **Total Soluble Solids (TSS)** was analyzed for the cranberry fruit and freeze dried cranberry-herbal infusion. The sugar concentration of cranberry was analyzed using Brix Refractometer. TSS measures the sugar content in

the cranberry and it was 5 Bx° for the fresh cranberry fruit. The TSS of cranberry juice was 0 Bx°.The TSS for freeze dried cranberry-herbal infusion was also 0 Bx° as it had no sugar content in it.

- The aqueous extract of freeze dried cranberry-herbal infusion showed a strong presence of **phytochemical** alkaloids, tannins, terpenoids, saponins and glycosides. Phenols, sterols and flavonoid were moderately present in aqueous extract; while alkaloids and coumarins were weakly present and quinones were absent in aqueous extract.
- The sample Cranberry-Herbal Infusion is comparatively **less toxic** than $K_2Cr_2O_7$ which shows maximum lethality of shrimps at higher concentration. The shrimps in the sample Cranberry-Herbal Infusion is found to be very less toxic in lower as well as in higher concentration. Even after 24h, only 2- 10 shrimps are found to be mortal at lowest and highest concentration.
- The **bioactive compounds** present in freeze dried cranberry-herbal infusion revealed that the highest peak obtained is for the component Ethanol (CAS) Ethyl alcohol with reaction time 0.497. The next highest peak obtained was for the bioactive component Trans-.Beta.-Ionon-5,6-Epoxide in a reaction time of 0.665 followed by 1,2-Bis (trimethyl silyl) benzene with a reaction time of 1.250. The other bioactive compounds include Propanoic acid,3,3'-dithiobis-(CAS) Dithiodi; 1,1'-bibicyclo (2.2.2) octyl-4-carboxylic acid; Trans-.Beta.-Ionon-5,6-Epoxide; 1,1,4,4-Tetra deuterio terta methyl; 2-Butanone,3-hydroxy-(CAS) Acetoin; Propanoic acid, 2-hydroxy-,ethyl ester (CAS); 5-Methoxy-1-AZA-6-Oxabicyclo(3.1.); 1,2-Propadiene (CAS) Allene; 1-Dimethyl (isopropyl) silyloxy propane; 2-Hexynoicacid; Cyclohexene,4-ethenyl-1,4-dimethyl; Bicyclo [2.2.1] heptan-2-one,1,3,3-trimethyl; 1-Propyne (CAS) Propyne; 3-Butyn-1-ol(CAS) 3-Butynol; Estragole; Phenol,2-methyl-5-(1-methylethyl)-(CAS) Ca; Ethyl1-Hexyl-4-Hydroxy-2(1H)-OX; Diethyl Phthalate; Hexa decanoic acid (CAS) Palmitic acid; trimethyl silyl derivativeof2-MO; pnz-sar and Pyrazine, tetrakis(1-methylethyl)-(CAS)TET and was obtained at different reaction time.Estragole is one of the important bioactive compound. Estragole exhibits a dose-dependent cytotoxic activity in the monolayer culture of MCF7 cell line.
- The cytotoxicity activity of freeze dried cranberry-herbal infusion towards human breast cancer MCF-7 cells was tested. The results of cytotoxicity study (MTT assay) suggested that freeze dried cranberry-herbal infusion showed

activity with IC50 value of **527.20µg/ml** after the treatment period of 48 hrs. The IC50 value of **527.20µg/ml** shows the **moderate** inhibitory activity. IC50 is the concentration of an inhibitor where the response (or binding) is reduced by half. So, if testing viability *via* an MTT assay, the dose of cytotoxic compound at which achieve 50% viability will be the IC50. Plates XXIII to XXVIII depicts the freeze dried cranberry-herbal infusion activity at different concentrations.

It can be concluded from the present study that majority of the people prefer to consume tea and in order to consume the same kind of tea Cranberry-herbal infusion can be a good food choice as it is prepared from cranberry and other herbs like thyme leaves,dill leaves and fennel. This can a better option as it has natural flavour, natural color, no added preservatives and free from adulterants, safe for consuming and easy to prepare.It has more benefits for health compared to the tea. Variation II was accepted by the panel members and scored high when compared to other variations. Cranberry-herbal infusionis rich in nutrients such as vitamin A, calcium and vitamin E. It is also rich in phytochemicals like terpenoids, glycosides, sterols, tannins, quinones, coumarins. The toxins present in Cranberry-herbal infusion was very low. Hence it can be concluded that Cranberry-herbal is a safe and moderately effective on breast cancer cell line.

Recommendation for further research:

- Conduct clinical trials using cranberry-herbal infusion for cancer.
- Bring out the potentials for organ specific cell line studies.
- Identify the bioactive compounds responsible for anti-cancer effect and extracting same.
- Recipes other than infusion can be tried using cranberry and other herbs.
- Understand the role of cranberry –herbal infusion in other disease conditions

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Simonson, W. TAXONOMIC DIFFERENTIATION AND DISTRIBUTION STATUS OF CAMPHOR THYME THYMUS CAMPHORATUS IN THE WESTERN ALGARVE. Preface 2 Preliminary survey of bats on Quinta da Rocha 3 Two new highlights during August in the A Rocha area: 13 Purple Swamp-hen Porphyrio porphyrio and Eagle Owl Bubo bubo Partly melanistic Cattle Egret Bubulcus ibis 15, 24.

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APPENDIX I

SCORE CARD FOR SENSORY EVALUATION

NAME	
CLASS	
DATE & SIGN	

PRODUCT NAME	**V 0**	**V 1**	**V 2**	**V 3**
APPEARANCE				
COLOUR				
FLAVOUR				
CONSISTENCY				
TASTE				
OVERALL ACCEPTABILITY				
TOTAL SCORE				
SCORE				

9- Like extremely,8-Like very much,7-Like moderately,6-Like slightly,5-Neither like or dislike,4-Dislike slightly,3-Dislike moderately,2-Dislike very much,1-Dislike extremely

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam

Institute for Home Science and Higher Education for Women
(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act 1956) Re-accredited with 'A++' Grade by NAAC.Recognised by UGC Under Section 12 B
Coimbatore-641 043, Tamil Nadu, India

Chairman
Dr. SudhaRamalingam Director-Research & Innovation, Professor-Community Medicine, PSG Institute of Medical Sciences & Research, Coimbatore

Member Secretary
Dr.S.Uma Mageshwari
Professor and Head,
Department of Food Service Management & Dietetics

Members
Mr.K.Arunmoli (Legal Expert)
Dr.Subhashini K. Sripathi
Dr.A.Saraswathy (Medical Officer)
Ms.D.Kavitha
Dr.A.R.SudamaniRamasamy
Dr.G.Victoria Naomi
Dr. Judith_Justin
Dr.AnithaSubash

08th March 2022

To
Ms.N.Maalavika
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore — 641 043

Dear N.Maalavika,

Ref: Your proposal No. IHEC/21-22/FSN-12 entitled "Identification and Isolation of Bioactive Compounds in Cranberry – Herbal Infusion "resubmitted for approval of IHEC on 08.03.2022.

The Institutional Human Ethics Committee of our University hereby grants approval to your research proposal No. IHEC/21-22/ FSN-12 entitled " Identification and Isolation of Bioactive Compounds in Cranberry – Herbal Infusion "resubmitted by you. The Approval number for the same is AUW/IHEC/ FSN-21-22/XMT-12.

We wish von all the hest in your research endeavours.

Regards,

Dr.S.Uma Mageshwari
Member Secretary

Institutional Human Ethics Committee