

**FORMULATION AND INCORPORATION OF
IMMUNE BOOSTING HERBAL POWDER**

**By
M. VAISHNA DEVI
(20PFN029)**

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
FOOD SCIENCE AND NUTRITION**

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X. Balasubrahmanyam
27/5/2022
Signature of the supervisor

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Signature of the Head of the Department

CERTIFICATE

This is to certify that the thesis entitled, "FORMULATION AND INCORPORATION OF IMMUNE BOOSTING HERBAL POWDER " 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 **M. VAISHNA DEVI** with Register Number **20PFN029** 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.

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Signature of the Head of the department

DECLARATION

This is to certify that the thesis entitled, "FORMULATION AND INCORPORATION OF IMMUNE BOOSTING HERBAL POWDER" 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 M.VAISHNA DEVI with Register Number 20PFN029 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.

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INTRODUCTION

I.INTRODUCTION

Modern medicine prefers to use active elements instead of using whole plants. Pharmaceuticals can be made from phytochemicals that have been synthesized, compounded, or otherwise altered. Plants, including many that are today used as culinary herbs and spices, have been utilized as medicines since prehistoric times, but not always successfully (Sumner and Judith, 2000). Spices have been employed to combat food spoilage bacteria in the past, particularly in hot climes.

Chemical analysis changed the place of plants in medicine dramatically in the nineteenth century. A variety of therapeutic plants were used to isolate alkaloids. Around the turn of the century, the mood in the pharmacy shifted against therapeutic plants, as enzymes frequently changed the active chemicals when whole plants were dried, and alkaloids and glycosides refined from plant material began to be valued. Plant based drug development remained essential throughout the twentieth century and into the twenty-first.

Medicinal plants are employed in modern medicine and traditional medicine with the goal of maintaining health, treating a specific illness, or doing both. Medicinal plants may bring three types of benefits: substantial health benefits to people who use them as medicines; financial benefits to persons who collect, process, and sell them; and societal benefits such as job possibilities, tax revenue, and a healthier labour force. Herbal medicine and dietary supplement items have been chastised for lacking adequate standards or scientific proof to back up their claims about its contents, safety, and efficacy. Hundreds of chemicals have been discovered as a result of ethnobotany research into plants utilized by indigenous people for medicinal purposes. Herbs can aid in the prevention and management of chronic diseases such as heart disease, cancer, and diabetes. Herbs have an important part in keeping people healthy and improving their quality of life. People are focusing more on immunity booster items during the current pandemic. People are looking for items that can help them rehydrate and strengthen their immunity in order to combat the pandemic and stay safe and healthy.

Traditional medicine (TRM) is described as the sum of all knowledge, skills, and practices based on theories, beliefs, and experiences indigenous to many cultures, whether explicable or not, that are employed in the maintenance of health, as well as the prevention, diagnosis, improvement, or treatment of physical and mental illness. Herbal medicine, on the other hand, is made up of medicinal herbs, minerals, and organic materials derived from the roots, rhizome, leaf, pod, seed, bark, fruit, or even the flower of a plant. Herbal powders with medicinal value are the granulated form of a medicinal plant used as an antidote for diseases (Kamboj V.P, 2000), and they must be made according to World Health Organization guidelines.

India has a significant role to play as a supplier of herbal products, not only to meet domestic needs, but also to capitalize on the enormous export potential. Herbal medicine is in significant demand in both developed and developing countries, such as India, because of its broad biological activity, higher safety margins than synthetic medications, and lower costs (Daha S.A, *et al.*, 2001). Medicinal plants, in addition to therapeutically active compounds, play a significant part as food supplements for human health and personal care, making the medicinal plant-based industry a promising sector with vast economic growth potential. In the industrialized world, particularly in the United States and Japan, nutraceuticals (health foods) are in high demand. Traditional herbal medicines are commonly used in natural health care programmes because they are inexpensive and relatively safe. People's faith in such therapies is evident in the widespread usage of traditional herbal remedies in natural health care programmes, owing to their low cost and relative safety (Chaudhri, R.D, 1996). People's faith in such medicines is evident in the herbal market's annual sales of 450 crores.

Herbal therapy, in general, must be transferred into modern medication research and clinical trials. There has been a lot of work done in the past for natural chemical medications. The majority of these initiatives and practices, on the other hand, are founded on western medical thought. Natural microbial, plant, and animal sources account for over half of all chemical pharmaceuticals sold in western markets. Despite great achievements, there are still many challenges to conquer. Despite this, no single drug development system has been widely adopted and relied upon.

The associated issues of quality, safety, and efficacy are all linked to integrated herbal medicine. Both safety (toxicity) and efficacy are hampered by the current lack of standardized quality in herbal products. Few plants, such as ashwagandha, clove, tulsi, and ginger, have strong immune-boosting properties. This research is focused on four primary herbs that aid in improving immune function. Herbs now play a significant role in everyday life. Herbal powders are also simple to use.

Ashwagandha has long been utilized as a Rasayana in the Ayurvedic system of medicine, which dates back to 6000 BC in India (*Singh et al., 2011*). *Withania somnifera* (Aswagandha) is a well-known medicinal plant with a variety of ethnic haematological effects. Several sections of various *Withania* species have distinct actions (*Naz et al., 2009*). Ashwagandha has a powerful anti-inflammatory effect that can aid with autoimmune illnesses and skin issues. Because of its numerous rejuvenating properties on the human body, Ashwagandha is referred to as a “Royal Herb”. It is a versatile herb that affects several human body systems, including the nervous system, immunological system, energy production system, endocrine system, and reproductive system. Ashwagandha contains a variety of pharmacologically and medicinally significant components such as withanolides, sitoindosides, and other alkaloids. These compounds shield cells against oxidative stress and illness (*Sharma V, et al., 2011*).

The root of this plant was an important ingredient in over 300 formulations for treating various physiological diseases in ancient systems of medicine like as Ayurveda, Unani, and Siddha (*Tavhare, 2015*). It has emerged as a potent anticancer agent for a variety of cancers, including breast, cervical, lung, colon, prostate, and medullary thyroid cancer, and it works through a variety of mechanisms, including cell cycle arrest, apoptosis, autophagy, and suppressing various oncogenic pathways (*Palliyaguru, D.L., et al., 2016*). *Withania somnifera* has been shown to be an effective immune suppressant. (*Ziauddin et al., 1996*) found that ashwagandha increases the formation of white blood cells. In diabetic patients, oxidative stress induced by glucose homeostasis dysregulation is associated with chronic inflammation, which eventually leads to tissue damage, and the critical role of oxidative stress in the development of diabetic endothelial dysfunctions has been highlighted in numerous studies (*Rochette, et al., 2014*).

In Ayurvedic medicine, tulsi is a prominent therapeutic herb. Because of its antimicrobial properties, it is frequently utilised in the treatment of a variety of systemic disorders (Wink M, 2000). Saponins, flavonoids, triterpenoids, and tannins are all found in the stem and leaves of holy basil, and they may have biological activity. The most essential benefit stated for medicinal plant therapeutic use in many conditions is their safety, in addition to their affordability, effectiveness, and ease of availability. Tulsi juice, taken twice a day, may be beneficial to the health.

Traditional medical practitioners have used the various parts of the Tulsi plant, such as the leaves, flowers, stem, root, and seeds, as expectorants, analgesics, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, hypolipidmic, and antistress agents. Fever, bronchitis, arthritis, convulsions, and other ailments have also treated with tulsi. Patients with gastrointestinal and hepatic problems are given an aqueous decoction of Tulsi leaves. In patients with viral hepatitis, herbal preparations containing *Ocimumtenuiflorum* have been suggested to shorten the course of illness, clinical symptoms, and biochemical parameters. Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool, and -caryophyllene are some of the phytochemical elements of tulsi (Saravanan D, *et al.*, 2012). In India, an infusion of Tulsi leaves is used as a common cold cure. Along with the clove, it's used to treat fever. It also helps to reduce uric acid levels, making it a possible anti-inflammatory agent. Basil leaves are used to treat a variety of fevers. During the rainy season, when malaria and dengue fever are common, tender leaves boiled with tea work as a malaria and dengue fever preventative. Many cough syrups and expectorants contain tulsi as a key ingredient. In bronchitis and asthma, it aids in the mobilisation of mucus. Tulsi leaves are used to treat colds and flu. The leaves are a nerve tonic and memory enhancer. They aid in the evacuation of phlegm and catarrhal debris from the bronchial tube.

Clove (*Syzygiumaromaticum*) is a well-known home treatment for toothaches, especially in the form of an essential oil. The pain-relieving effect is assumed to be due to the component eugenol. Cloves also have antibacterial characteristics, which may be useful in the treatment of certain oral diseases. Clove is an analgesic (pain reliever), antimicrobial, and antispasmodic spice (Michelle robson garth, 2016).

Eugenol makes up 72–90% of the essential oil derived from cloves and is the chemical that gives cloves their distinctive perfume (Viljoen, A. M., *et al.*, 2012). Acetyl eugenol, beta-caryophyllene, vanillin, crategolic acid, tannins such bicornin, gallotannic acid, methyl salicylate (painkiller), flavonoids including eugenin, kaempferol, rhamnetin, and eugenitin, triterpenoids like oleanolic acid, stigmasterol, and campesterol (Li-Ming Bao, *et al.*, 2012).

Zingiber officinale rhizome (root) can be used to make a delightful, warming, and tasty infusion. Ginger (*Zingiber officinale*) is an anti-inflammatory spice with immune-boosting properties. The rhizome, or "root," is the portion of the plant that is utilised medicinally. Ginger may have antiviral capabilities, according to study published in the Journal of Ethnopharmacology. There are multiple pharmacologically active chemicals in ginger. Volatile chemicals (essential oils), gingerols, and shogaols are the most common forms (Li, *et al.*, 2016). Gingerols are a group of homologous phenols that contribute to the pungency of ginger. Ginger is a medicinal herb that has been used for a variety of maladies, including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches and pains, constipation, vomiting, hypertension, indigestion, dementia, fever, and infectious infections, all over the world since antiquity (Ali B.H, *et al.*, 2008).

In phytomedicine, a herb is a plant or plant part that is used to make medicine to aid in the healing process during illness or disease. Herbal teas are made entirely of one or more plant parts, such as leaves, flowers, bark, or seeds, that have been decocted or infused. Herbal teas are delicious, low-calorie, and soothing beverages. They have a lovely scent and are quite enticing.

Ginger tea is also diaphoretic, meaning it can help raise body temperature and keep warm by increasing sweating, which is beneficial when a poor immune system is present, such as when have a cold or the flu. To maximise the warming and diaphoretic qualities of ginger, it is made into heated infusion or decoction. Due to ginger's anti-inflammatory properties, it's ideal for inflammatory illnesses like arthritis or ladies with dysmenorrhoea (period discomfort) (Michelle Robson garth, 2016). Ginger is also anti-inflammatory, antiviral, and antifungal, and can be used to treat gingivitis as a mouthwash.

Herbal infusions are herbal and spice water extractions that allow the plant's goodness to be infused into the water. Teas produced with the softer components of herbs and spices are known as infusions. Instead, the procedure is employed because these plants typically include volatile compounds that are better produced with a mild heating method that might otherwise break down or release particular constituents rather than containing them within the tea (Michelle Robson garth, 2016).

After going through the literature study, as there are no much evidence based on the research carried out in the current topic, "**Formulation and Incorporation of Immune Boosting Herbal Powder**" was undertaken with the following objectives to

- a) formulate and standardize powders using culturally acceptable herbs and ingredients that boost the immunity
- b) development and standardization of immune boosting herbal powders.
- c) assess the acceptability of the immune boosting herbal powders
- d) analyse the nutrients and phytochemical present in the herbal powders qualitatively and
- e) analyse the antioxidant vitamins in the immune boosting herbal powders

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The Review of Literature pertaining to the study entitled “**Formulation and Incorporation of Immune Boosting Herbal Powders**” is presented under following sub-headings:

- A. Nutritional and medicinal values of ginger
- B. Role of clove in human nutrition and therapeutic properties.
- C. Tulsi as a medicine in human nutrition
- D. Dual role of ashwagandha in nutrition and medicine

A. Nutritional and Medicinal values of Ginger

Ginger is cultivated mainly in Asia and the tropics and, apart from its culinary function, it has been used since antiquity for a variety of conditions, including cold, fever and gastrointestinal issues, and as an appetite enhancer. It is classified by the Food and Drug Administration of the United States as a food additive, but has been studied as a cure against nausea and vomiting, as well as for arthritis (Yadav, *et al.*,2016).

Ginger root is used to alleviate and treat many common illnesses, such as headache, cold, nausea and vomiting. Numerous bio-active compounds of ginger, such as phenolics and terpenics, have been identified. The phenolic compounds are primarily gingerols, shogaols and paradols, which represent different bio-activities of ginger. In recent years, ginger has been found to possess biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Furthermore, the accumulation of research has shown that ginger has the potential to prevent and manage several diseases such as neurodegenerative diseases, cardiovascular diseases, obesity, diabetes mellitus, chemotherapeutic induced nausea and vomiting, and respiratory problems (Nile, S.H and park S.W, 2015).

Morphology and distribution of ginger - Ginger is a spice that originates in Southeast Asia's Maritime region. It's a real cultigen that doesn't exist in its natural habitat. It was among various species of ginger cultivated and utilised since ancient times among the Austronesian people, according to the most ancient evidence of its domestication. They grew bitter ginger in their garden (*Zingiber zerumbet*). The rhizomes and leaves were used to flavour food and

were also eaten raw. Mats were woven from the leaves as well. Apart from these purposes, Austronesians regarded ginger as sacred, using it in rituals for healing and seeking protection from spirits. It was also employed in the austronesian ship blessing. During the Austronesian expansion, which began around 5,000 years (BP), ginger was transported as canoe plants.

Long before any contact with other civilizations, they introduced it to the Pacific Islands. During early contact by Austronesian sailors with the Dravidian-speaking people of Sri Lanka and South India circa 3,500 years (BP)., other Southeast Asian food plants and Austronesian sailing technologies were discovered. In the first millennium CE, Austronesian voyagers brought it to Madagascar and the Comoros (Zanariah U, *et al.*,2015). By the 1st century CE, traders had brought it from India to the Middle East and the Mediterranean. During the spice trade, it was largely farmed in southern India and the Greater Sunda Islands, with peppers, cloves, and a variety of other spices.

Ginger (*Zingiber officinale*) is a most commonly used plantbased supplement and it belongs to the family of herbs that include cardamom and turmeric. Although it is often consumed for culinary purposes, it is taken by a number of patients for treatment. The strong aroma of ginger is the result of spicy ketones, including gingerol, the extract which has been primarily used in research studies. The consumed portion of ginger is the rhizome, often referred to as "ginger root," though it is not really a root. The rhizome is the horizontal stalk of the plant that causes the roots to emerge (Langner E,1998).

Nutritional value of ginger - Raw ginger is made up of 79 % water, 18 % carbs, 2% protein, and 1% fat. Uncooked ginger contains 333 kilojoules (eighty kilocalories) of food energy per hundred grammes (a well-known quantity used to compare with other foods) and contains mild amounts of vitamin B6 (12 % of the Daily Value, DV) and the nutritional minerals magnesium (12 % DV) and manganese (11 % DV), but is low in micronutrient content material. Except for manganese, floor dried ginger (9 % water) offers low content material of vital nutrients when used as a spice powder in a common serving quantity of 1 US tablespoon (five gram) (70 % DV) (www.nutritiondata.self.com).

Culinary uses of ginger - For thousands of years, ginger has been a popular spice for both healing and cooking, particularly in China and the Mediterranean. Ginger became popular in Europe about the ninth century, and it continues to rise in popularity in many parts of the world. Ginger is a common ingredient in Indian, Chinese, Korean, Thai, and other Eastern cuisines. Ginger is typically used in savoury dishes rather than sweet ones (Viestad A, 2007). The idea of utilising ginger for savoury foods has been expanding in recent years, thanks to the growing popularity of Asian and Indian recipes in other areas of the world.

It has been shown that ginger is effective against nausea and vomiting associated with pregnancy and post-surgery. There is less evidence exists to support its use for motion sickness or other kinds of nausea and vomiting. Mixed findings have been found in a few studies on ginger for the treatment of arthritis symptoms.

Ginger is a flavouring ingredient that is often used in foods and beverages. Based on the methods employed and product quality, ginger foods can be categorised into salted and non-salted categories. Based on the procedures and product features, ginger goods can be classified as salted products, chow-chow, pickled products, dry products, ale, and juice products. To help relieve discomfort, ginger oil is rubbed to the skin on occasion.

Therapeutic uses of ginger

Anti-inflammatory activity of ginger - In a clinical trial, ginger's anti-inflammatory efficacy was demonstrated by measuring pro-inflammatory enzymes including cyclo-oxygenase (COX) and lipoxygenases (LOX-5, LOX-12 and LOX-15). The reduced activity of these enzymes was corroborated by reductions in the quantities of pro-inflammatory chemicals they create, particularly eicosanoids and prostaglandin E2, after consuming 2 g of ginger powder daily for 28 days. TNF- gene activation results in the release of pro-inflammatory cytokines, which activates the transcriptional factor NF-B. NF-B activation causes the expression of additional inflammatory cytokines including COX-2, LOX-2, various chemokines, and iNOS to increase, leading to inflammation and illness. 6-gingerol has been shown to have potent anti-inflammatory properties as well as the ability to inhibit the immune system. Inhibiting NF-B activity will, in turn, reduce

inflammation and inflammation-related disorders. Ginger's natural active chemicals (gingerols and zerumbone) have been discovered to be strong inhibitors of the pro-inflammatory cytokine TNF- α and NF- κ B. Ginger can lessen muscular and joint discomfort by as much as 25%. Ginger may alleviate pain in the setting of dysmenorrhea and osteoarthritis, which is assumed to be connected to the anti-inflammatory properties of gingerol and similar chemicals (Ryan, *et al.*, 2011).

Anti-emetic activity of ginger - Ginger's anti-emetic properties are well proven in situations of pregnancy, with numerous clinical research demonstrating its usefulness. Ginger powder, at a daily dose of 1g, was effective to diminish or eradicate *Hyperemesis gravidarum* (severe type of nausea and vomiting during pregnancy) and the episodes of nausea and vomiting associated with pregnancy in eleven out of twelve trials. Ginger has also been used as an antiemetic in cases of motion and postoperative sickness. In comparison to the placebo group, the ginger-treated group had no emesis and saw a significant reduction in symptoms such as cold sweats and dizziness. However, there were a few cases of vomiting in the placebo group. Ginger can also be used in conjunction with other antiemetic medications to help prevent acute nausea resulting from chemotherapeutic drugs. It has been shown that ginger is effective against nausea and vomiting associated with pregnancy and post-surgery. There is less evidence exists to support its use for motion sickness or other kinds of nausea and vomiting. Mixed findings have been found in a few studies on ginger for the treatment of arthritis symptoms.

Anti-cancer properties of gingerol - The spicy component in ginger, [6]-gingerol, has substantial antiangiogenic activity *invitro* and *vivo* gingerol's anti-angiogenic activity ay prevent tumour growth and spread. Ginger is a powerful anti-oxidant and anti-carcinogenic food. *Invitro* ginger component influence angiogenic factor release in ovarian cancer cells, and act as a strong chemopreventive dietary intervention. Elemene, a new anticancer medication derived from the ginger plant, induces apoptosis in non-small cell lung cancer cells by triggering mitochondrial cytochrome c release. The elemene raises the amounts of cleaved caspase-9 and poly (ADP-ribose)

polymerase in cells, lowers Bcl-2 expression, promotes cytochrome c release, and activates caspase-3, -7, and -9 activity.

Enhanced enzyme activity of Glutathione Reductase (GR), Glutathione Peroxidase (GPX), Glutathione S-transferase (GST) leads to the prevention of colon carcinogenesis by ginger supplement. Colon cancer is effectively reduced with ginger. *In vivo* studies have shown that ginger and its component [6]-gingerol are effective against ovarian tumors. Ginger suppresses the inhibitors of necrosis factor kappa B (NF- κ B) and interleukin-8 (IL-8) (Choi, J.G., *et al.*, 2018).

Antioxidant properties of gingerol - Ginger has a great number of antioxidants and also aids in the decrease of lipid oxidation and disease development. Ginger extract has antioxidative properties and can scavenge superoxide anion and hydroxyl radicals, whereas gingerol inhibited the ascorbate/ferrous complex. The antioxidant and antibacterial properties of the essential oil and oleoresin were found to be substantial. In activated macrophages, 6-dehydroshogaol, 6-shogaol, and 1-dehydro-6-gingerdione have been found to be strong inhibitors of Nitric Oxide generation (Dugasani S, 2010). The presence of an unsaturated ketone molecule in 6-shogaol gives it potent antioxidant capabilities, according to an antioxidant. Phenolic compounds have potent anti-inflammatory and antioxidative characteristics, as well as significant anticarcinogenic and antimutagenic properties, and that they play a role in cancer prevention and also play a role as in scavenging of H_2O_2 , which donate electrons to H_2O_2 , thus neutralizing it to water (Kikuzaki H and Nakatani N, 2006).

Gingerol in cardiovascular diseases - The anti-inflammatory, antioxidant, antiplatelet, hypotensive, and hypolipidemic actions along with the condiment are notable. There is an apparent link between ginger and increased risk of bleeding after surgery or when taken with anticoagulant medicines like warfarin, caution should be exercised while using ginger and other herbal extracts. However, the evidence is inconclusive. An aqueous ginger extract was found to lower arterial blood pressure in a dose-dependent manner. Taking standardized ginger extract reduced aortic atherosclerotic lesion areas, plasma triglycerides and cholesterol, and LDL-associated inflammation, (LDL)-associated lipid peroxides, and LDL aggregation

(Haniadka, R., *et al.*, 2013). A daily dose of ginger can provide a significant antioxidant boost to the heart. This is due to the fact that ginger includes 12 antioxidant molecules that are more potent than vitamin E. Ginger reduces inflammation. Some people have less sensitivity to pain receptors and nerve endings. Over 75% of people reported relief from pain and swelling after ingesting ginger.

Antidiabetic Activity of ginger - Diabetes mellitus is a serious metabolic illness characterized by insulin insufficiency and/or insulin resistance, which results in an uncontrolled rise in blood glucose levels. Long-term hyperglycemia has the potential to speed up protein glycation and the production of Advanced Glycation End products (AGEs). Both 6-shogaol and 6-gingerol prevented the advancement of diabetes problems. Ginger contains a large number of anti-inflammatory chemicals. Some inhibit COX-2, a pro-inflammatory enzyme. Triglycerides, one of the key indications of heart-related illnesses such as type 2 diabetes, are reduced by ginger (Srivastava K and Mustafa T, 1992).

B. Role of Clove in human nutrition and therapeutic properties

Clove (*Syzygium aromaticum*) a valuable spice, is a member of the Mirtaceae family that has been used for centuries as a food preservative and medication due to its antimicrobial and antioxidant properties. *Syzygium aromaticum* is the largest genus of Mirtaceae family, comprising of about 1200 to 1800 species of flowering plants, which are widely distributed in tropical and subtropical areas (I.E. Cock and Cheesman. M, 2018). Cloves contain significant amounts of volatile oil (used to flavour foods and pharmaceuticals), which is primarily contained in the aerial portions of plants (Z. Arshad, *et al.*, 2014). Cloves contain significant amounts of volatile oil (used to flavour foods and pharmaceutical products), which is mainly contained in airborne parts of plants.

Cloves are the fragrant flower buds native to Indonesia's Maluku Islands (or Moluccas) and are widely used as a spice. Due to varying harvest seasons in different nations, cloves are accessible all year. The clove tree is an evergreen with big leaves and crimson flowers arranged in terminal clusters that grows up to 8–12 metres (26–39 feet) tall (A.Y. Al-Maskri,*et al*, 2019). When the flower buds are ready to be harvested, they start out pale,

turn green, and finally turn vivid red. Cloves have a lengthy calyx that ends in four spreading sepals, as well as four unopened petals that create a little central ball, and are harvested when they are 1.5–2 cm (0.59–0.79 in) long.

Medicinal value of clove oil - Clove oil is produced by plants via secondary metabolic pathways, and can be extracted from buds (bud oil), flower stalks (stem oil), and leaves (leaf oil) via hydrodistillation, steam distillation, or dry distillation (Riyanto, *et al.*, 2016). Essential oils and their derivatives, such as oleoresin, are commercially advantageous and widely used in the health, food, and cosmetics industries, as well as food preservatives, pesticides, and fumigants (Xu, *et al.*, 2016).

The primary constituent of clove oil is eugenol, which has antiseptic and analgesic properties (Riyanto, *et al.*, 2016), anti-inflammatory properties, anti-oxidant properties, anti-fungal (Pinto, *et al.*, 2009), anti-bacterial anti-viral (Rathinam dan Viswanathan, 2018), anti-cancer, and widely used in the treatment of teeth and gums (Pulikottil and Nath, 2015). The climatic conditions in which the varieties have grown can affect the content of essential oils produced. Ambon Island, where cloves of various varieties have been grown, and climatic conditions that can influence the content of essential oils produced.

Morphological characteristics, composition, and concentration of clove essential oil components extracted from buds, flower stalks, and leaves varied knowing the components of clove essential oil is important for identifying its potential uses; however, information on the composition and concentration of essential oil varieties is still limited (Chicca, *et al.*, 2014). Balinese spouses are wise to incorporate cloves in their boreh. A molecule in clove oil has anaesthetic properties, according to animal testing conducted at the University of Florence in Italy. To put it another way, it reduces the sensation of pain. Clove oil contains eugenol, which stimulates the stomach lining to make more protective mucous, which helps to cure ulcers, as the Balinese have known for centuries (M.A. Hanif, *et al.*, 2019).

Nutritional value of clove - Clove is rich in vitamin and minerals. Whereas, manganese is a mineral that is necessary for brain function and for the formation of strong bones. Cloves are high in antioxidants, as well as a number of vital vitamins and minerals. Antioxidants are molecules that help to

minimise oxidative stress, which can lead to chronic disease. Eugenol was found to be five times more effective than vitamin E in preventing oxidative damage produced by free radicals. Cloves also include fibre as well as minerals such as potassium, calcium, magnesium, and iron. Just a few of tablespoons can provide with 10% of daily requirements (Rahmani A.H and shabrmi, 2014). That's more than many well-known multivitamins provide. Clove is high in calcium, hydrochloric acid, iron, phosphorus, salt, potassium, vitamin A, and vitamin C, among other minerals.

Culinary uses of clove - Cloves are widely used in spice blends for meat rubs and marinades, such as Chinese five-spice powder and garam masala, which are both popular. They stud full baked hams and pots of German braised red cabbage, and they're one of many spices used to prepare Vietnamese pho. This aromatic spice is commonly used to flavour hot beverages such as mulled wine, masala chai tea, and hot apple cider.

Sweet meals like stewed apples and pears, pumpkin pie, and gingerbread are another option. Cloves are widely used to add sweetness and spice to various sauces, jams, and pickling combinations, and are a key ingredient in Worcestershire sauce. Before serving, whole cloves are usually removed from beverages, sauces, and other preparations. Clove pods, powder, and oil have long been a popular food addition, thanks to their pungent flavour and scent. Because ground cloves are easier to blend with other ingredients than whole cloves, they are commonly used as a spice (Amini, *et al.*, 2016). Cloves are a popular component in many meals around the world, with a shelf life of six to twelve months. Clove oil is used to flavour tea, coffee, and other beverages such as wine, while clove powder and pods are mostly employed in the creation of dishes and confectioneries. Clove is a unique specialty in the wine market that is used to enhance the scent and flavour of numerous types of wine.

Therapeutic uses of clove - There is evidence that clove oil containing eugenol is useful for toothache pain and other types of pain, Eugenol mixed with zinc oxide was effective as an analgesic for alveolar osteitis. Studies to see if it works as a fever reducer, a mosquito repellent, or to prevent premature ejaculation have come up empty. Cloves or clove oil have yet to be demonstrated to lower blood sugar levels. The US Food and Drug

Administration has not approved the use of clove for any medicinal reason, and it may produce side effects if taken orally by those with liver disease, blood coagulation and immune system issues, or food allergies (Gay-Escoda C, *et al.*, 2015).

Cloves are low in vitamins, with the exception of vitamin K. The importance of vitamin K in the body is often overlooked by doctors. It can aid in the prevention of liver cancer. Vitamin K also destroyed leukaemia, pancreatic cancer, other cancers, leukemia, pancreatic cancer, and ovarian cancer cells were all destroyed by vitamin K.

This is accomplished by instructing cancer to "self-destruct." Cloves might also help to maintain a healthy blood sugar level. Researchers discovered that persons with the greatest levels of vitamin K had improved insulin sensitivity and lower blood sugar than those with the lowest levels of vitamin K.

Antioxidant activity of Eugenol - Eugenol's Pharmacological Properties
Eugenol is thought to be responsible for the pharmaceutical functions of essential oils extracted from various plants. The free radical scavenging activity of eugenol, prevention of reactive oxygen and nitrogen species (ROS) generation, DNA and protein damage, and elevation of cellular anti-oxidant potency have all been proposed as reasons for its functional activity in various trials (Kamatou, G.P, 2012).

Eugenol has been shown to be effective against a variety of lifestyle-related threats such as nervous disorders, digestive complications, reproductive issues, blood cholesterol irregularity, hypertension, elevated blood glucose levels, microbial infections, inflammatory actions, and carcinogenesis. Eugenol, a powerful phenolic component of clove oil, is primarily responsible for its antioxidant and free radical scavenging properties. Eugenol's antioxidant activity can be determined by forming complexes with reduced metals. The potent inhibitory effect of iso-eugenol and eugenol on lipid peroxidation is attributed to the elimination of free radicals and the formation of iron–oxygen chelate complexes by keeping iron and copper in a reduced state, respectively. (Cortés-Rojas, *et al.*, 2014).

Oral health and clove oil - Clove oil has been used to alleviate tooth pain in traditional Indian and Chinese medicine for millennia. Eugenol is found in

clove oil. Eugenol and magnesium oxide were used to make a filler substance in 1837. Later, zinc oxide was used to replace the magnesium oxide, resulting in ZOE (Zinc Oxide Eugenol), which is still commonly used as a temporary infill cement. Eugenol has been one of many essential oil components utilized in root canal therapy, periodontal therapy, and the treatment of abscesses since the 19th century. Clove oil is most commonly used in dental care. Because of the oil's germicidal characteristics, it can be used to treat dental pain, toothaches, sore gums, and mouth ulcers. Clove oil includes eugenol, a chemical that has been utilized in dentistry for many years. Gargles with diluted clove oil can assist with throat irritation. Clove oil's distinctive aroma aids in the elimination of foul breath. Clove oil is found in a variety of dental and pharmaceutical products, including mouthwashes and tooth pastes (M. Ito, and K. M. 2005).

Eugenol, a polyphenol found in clove oil – It has an antibacterial activity against a variety of Gram-positive (*Enterococcus faecalis*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis*) and Gram-negative (*E. coli*, Prote In Gram negative and Gram positive bacteria, eugenol destroys the cell membrane and cell wall, causing cell lysis and the release of intracellular fluid, as well as lipid and protein contents.

Since the 13th century, clove has been used as an analgesic for toothache, joint discomfort, and antispasmodic, with Eugenol being the major component responsible for this effect. The activation of calcium and chloride channels in ganglion cells is thought to be the mechanism that evolved. The analgesic effect of clove is aided by Eugenol's voltage-dependent effects on sodium and calcium channels, as well as receptors expressed in the trigeminal ganglion. Clove has analgesic properties due to its function as a capsaicin agonist (Kaur D., and Chandrul, K., 2017).

Clove extract has been shown to slow tumour development and induce cancer cell death. Eugenol has potent anti-lung cancer properties. Eugenol may be regarded a possible chemotherapeutic drug for human lung cancer for the following reasons: a modest dose of eugenol significantly reduced lung cancer cell viability, and eugenol hindered lung cancer cell metastasis. As a

result, when paired with its antiproliferative properties, eugenol may be a great treatment for preventing lung cancer growth and metastasis.

C. Tulsi as a medicine in human nutrition

It is widely available and grown throughout India, botanically called *Ocimum sanctum*, Tulsi belongs to the *Lamiaceae* family of plants and is one of the popular remedies for asthma and cough (Pattanayak, *et al.*, 2010). It is a ramified, fragrant and upright and a mature plant reaches a height of approximately 75-90 cm. Its leaves are almost circular and up to 5 cm long with margin, *i.e.* whole or toothed. The flowers are small and purple to reddish; they occur in small, compact clusters or cylindrical spikes. Fruits are small, yellowish or reddish in colour. The various parts of the plant are traditionally used in ayurvedic and siddha systems for processing multiple foods like infection, skin disease, hepatic disorder, common cold and cough, malarial fever and as an antidote for snake bite and scorpion sting (Godhwani, *et al.*, 1988).

In India, holy basil is a widespread herb. It is antibacterial, antifungal, and anti-malarial, and has been used as an air purifier and anti-malarial in Indian houses from ancient times (Singh S, Majumdar DK, 1999). Its stem and leaves are powdered and used as medication for balancing blood glucose, maintaining a healthy digestive system, encouraging the effective use of oxygen, increasing the efficacy of numerous therapeutic procedures, and so on. Tulsi (*Ocimum tenuiflorum*) in the diet can assist with not only appearance but also bodily and mental wellness, according to an Ayurveda specialist. Taking Tulsi also known as "the queen of herbs" has significant benefits to physical and mental health.

Morphology and distribution of Tulsi - Tulsi, also known as Tulassi, Manjari, Krishna Tulsi, Trittavu, Tulshi, and Thulsi, is the sacred plant of India. In English, the plant is known as Holy Basil. *Ocimum sanctum* is one of the most common and revered household plants in India. The plant is commonly grown in gardens and also near temples. The plant's medicinal properties are mentioned not only in Ayurveda and Siddha, but also in Greek, Roman, and Unani systems of medicine (Prakash.J, *et al.*, 2002). It is an erect, heavily branched subshrub that grows to be about 30-60 cm tall, with hairy stems and simple opposite green leaves that are strongly scented.

Petioles are present on the leaves, which are ovate and up to 5 cm long. Petioled leaves are ovate, up to 5 cm long, and usually slightly toothed. They are simple, petioled, with an ovate blade up to 5 cm (2 in) long, which usually has a slightly toothed margin; they are strongly scented and have a decussate phyllotaxy. The purplish flowers are placed in close whorls on elongated racemes (Kothari.S, 2005).

The presence of essential or volatile oils is responsible for *Ocimum sanctum's* distinct aromatic odour. The aromatic volatile oil is primarily composed of phenols, terpenes, and aldehydes. Several studies have shown that chemical constituents vary due to edaphic and geographical factors (BakkaliF,2006). In addition to oil, the plant contains alkaloids, glycosides, saponines, and tannins. The leaf contains the highest concentration of volatile oils. The leaf of *Ocimum sanctum* contains 0.7 % volatile oil, which is composed of approximately 71% eugenol and 20% methyl eugenol (Patil KS, 2010). Among the biologically active chemical components are alkaloids (ashwagandhine, cuscohygrin, anahygrin, tropine, and so on) and steroid compounds (including ergostane type steroidallactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone, and so on). Saponins containing an additional acyl group (sitoindoside VII and VIII) and withanolides containing a glucose at carbon 27 are also constituents (sitoindoside IX and X).

Nutritional value of Tulsi - Tulsi is rich in vitamin and minerals, except for vitamin K, it is a good source of most nutrients. Basil also contains phytochemicals that have antioxidant, anti-inflammatory, and other health benefits (Padalia, *et al.*,2011).

Culinary uses of Tulsi - Tomatoes, salads, eggplant, meat spices, stuffing, soups, sauces, and other foods benefit from the use of basil. One of basil's most popular uses is pesto, a creamy green sauce. Crushed basil, garlic, parmesan cheese, olive oil, and pine nuts are usual ingredients, however dairy-free variants are also available. It is used as a dip or a spread for sandwiches. Other herbs and spices that go well with basil include garlic, marjoram, mustard, oregano, paprika, parsley, pepper, rosemary, and sage. It's used to flavour everything from veggies to sauces to pizzas and cheeses all around the world. Thai cuisine uses a lot of Tulsi in its dishes as well. It can

be used to make pesto and can be used to salads, desserts, stir fries, and tea. Aqueous extract of Tulsi was put to a soybean product named "Tofu" to extend its shelf life. (Anbarasu K and vijayalakshmi G, 2007).The shelf life of 'Tofu' was increased from 3-4 days to 7-8 days.

Therapeutic uses of Tulsi

Antiinflammatory properties - In acute (carrageenan-induced pedal oedema) and chronic (croton oil-induced granuloma and exudate development) inflammations in rats, methanolic extract (500 mg/kg) and aqueous solution of *Ocimum sanctum* displayed analgesic, antipyretic, and anti-inflammatory actions. It helps by blocking both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, the fixed oil and linolenic acid have strong anti-inflammatory effect against PGE₂, leukotriene, and arachidonic acid caused paw oedema in rats (Singh S and Majumdar DK, et al., 1987).

Antimicrobial properties - *Klesbiella*, *E. coli*, *Proteus*, and *Staphylococcus aureus* were all inhibited by Aqueous of *Ocimum sanctum*, while *Vibrio cholerae* was inhibited by alcoholic of *Ocimum sanctum* (GeetaVasudevan DM, et al.,2001). *Ocimum sanctum*'s AIE was also discovered to be effective against multidrug-resistant, *S. aureus* strains that were also resistant to common beta lactam antibiotics (Auil F, et al.,2005). *Ocimum sanctum* was also proven to be effective against resistant strains of *Neisseriagonorrhoea* (Shoken P et al., 2005). The antibacterial activity of *Ocimum sanctum* fixed oil against *Bacillus pumilus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* was good. The presence of mdroelinolenic acid in *Ocimum sanctum* fixed oil may contribute to its antibacterial properties (Singh S, et al.,2005).

Memory enhancing properties - In AIE from dried whole plant of *Ocimum sanctum* reduced the amnesic effect of scopolamine (0.4 mg/kg) as well as aging-induced memory deficits. The exteroceptive behavioural model was the passive avoidance paradigm. Step-down latency and acetylcholinesterase inhibitions were dramatically increased by *Ocimum sanctum* extract. *Ocimum sanctum* can be used to treat cognitive diseases including dementia and Alzheimer's disease (Singh S, et al., 2006).

Hepatoprotective properties - Oral treatment of a hydro-ethanolic extract of *Ocimum sanctum* leaves at 200 mg/kg protected from paracetamol-induced

liver injury. *Ocimum sanctum* was found to be efficacious in preventing liver damage caused by carbon tetrachloride (0.2 ml/100 g, subcutaneously).

Antidiabetic properties - In normal glucose fed hyperglycemic and streptozotocin-induced diabetic rats, oral treatment of *Ocimum sanctum* extract resulted in a significant reduction in blood sugar levels (Chattopadhyay RR, *et al.*,1993). A randomised, placebo-controlled, cross-over single blind human experiment found a substantial reduction in fasting and postprandial blood glucose levels of 17.6% and 7.3 percent, respectively. The glucose levels in urine followed a similar pattern (P.Agrawal, *et al.*,1996) Furthermore, *Ocimum sanctum* has aldose reductase activity, which may aid in the reduction of diabetes problems like as cataracts, retinopathy, and so on.(Halder N, *et al.*,2003).

Antiarthritic properties - The anti-arthritic activity of *Ocimum sanctum* fixed oil was tested in the presence of formaldehyde. The diameter of the inflamed paw was greatly reduced by the fixed oil. The arthritic symptoms in rats improved significantly after 10 days of intraperitoneal treatment of the fixed oil. At 3 ml/kg, the anti-arthritic efficacy was comparable to aspirin at 100 mg/kg, ip41. Carrageenan was blocked by the fixed oil, and inflammation was produced by inflammatory mediators (e.g., serotonin, histamine, bradykinin, and PGE2). The oil's ability to prevent any inflammatory response involving these mediators is natural. The findings show that inflammation models, such as adjuvant and turpentine oil-induced joint oedema in rats, may have anti-arthritic properties (Singh S, *et al.*, 1996).

Tulsi helps boost immunity - Vitamin C and zinc are abundant in tulsi. As a result, it functions as a natural immunity booster and helps to keep illnesses at bay. It has powerful antibacterial, antiviral, and antifungal capabilities, protecting from a wide range of ailments. Tulsi leaf extract boosts the immune system by increasing the activity of T helper cells and natural killer cells. Tulsi's antibacterial and antiviral qualities aid in the battle against illnesses, lowering fever. Periodic fevers can be cured by mixing fresh Tulsi juice with black pepper powder. Ocimumosides A and B are chemicals found in tulsi. The neurotransmitters serotonin and dopamine in the brain are balanced by these chemicals, which relieve stress. Tulsi's anti-inflammatory qualities help to lower blood pressure and inflammation. Tulsi contains camphene, cineole,

and eugenol, which help to relieve colds and chest congestion. Bronchitis, asthma, influenza, cough, and cold can all be treated with Tulsi leaf juice combined with honey and ginger. Tulsi's phytochemicals have potent antioxidant effects. As a result, they aid in the prevention of skin, liver, oral, and lung cancers. Tulsi leaves might aid with indigestion and appetite loss. They're also utilised to alleviate bloating and flatulence (Agrawal P, *et al.*, 1996).

The antifatigue activity of a 70 percent alcoholic extract of *Ocimum sanctum* L. was investigated in an aqueous suspension. Swimming time, body weight change, lipid peroxidation, Lactic Acid (LA), glycogen, and blood biochemical indicators such as hemoglobin (Hb percent), Blood Urea Nitrogen (BUN), and Creatine Kinase (CK) were all tested as biomarkers of physical exhaustion. The optimum concentration to combat fatigue would be 300 mg kg⁻¹ weight of *Ocimum sanctum* leaf extract. *Ocimum sanctum* is a great source of secondary metabolites and has a lot of medical applications.

These metabolites are not required for the organism's survival, yet they are extremely important to humans. In the human body, secondary metabolites perform a variety of protective activities. Secondary metabolites from plants can stimulate the immune system, protect the body from free radicals, and destroy harmful bacteria, among other things. When tested on rat abdomen skin, it was discovered that transdermal medication delivery utilising a combination of Tulsi oil and turpentine oil delivered substantially more medicine than synthetic combinations such as isopropylene and propylene glycol (Charoo NA, 2008).

D. Dual role of Ashwagandha in nutrition and medicine

The roots of the plant are categorized as rasayanas, which are renowned for promoting health and longevity by increasing the defense against illness, stop the aging process, revitalize the body in debilitating conditions, enhance the individual's ability to withstand negative environmental factors and create a sense of mental well-being (M.A. Weiner and J. Weiner, 1994).

Withania somnifera, also known as ashwagandha or winter cherry, is a *Solanaceae* (nightshade) family annual evergreen shrub that grows in India, the Middle East, and parts of Africa. Several other species in the *Withania*

genus have flowers that are morphologically similar to this. Despite the fact that it is used as a medicinal herb in Ayurveda and is offered as a dietary supplement in many countries, there is inadequate scientific data to suggest that it is safe or helpful for treating any ailment. The flowers are tiny, bell-shaped, and green. The fruit is orange-red when fully ripe. It has antibacterial, antioxidant, bleaching, emollient, and humectant properties (Ahmad MK, *et al.*, 2010).

The biologically active chemical components mainly alkaloids (ashwagandhine, cuscohygrin, anahygrin, tropine, etc.) and steroid compounds (including ergostane type steroidallactones, withaferin A, withanolides A-y, withasomniferin-A, withasomidienone, withasomniferols A-C, withanone, etc.) are among the biologically active chemical components. Saponins with an additional acyl group (sitoindoside VII and VIII) and withanolides with a glucose at carbon 27 are also constituents (sitoindoside IX and X). The dried root, known as amukkara, is used as a component of formulations indicated for treatment of conditions such as oligospermia, lancinating pain, loss of body strength, anaemia, and convulsions in Siddha medicine, a Dravidian system of medicine that originated in the southeastern Indian state of Tamil Nadu and is now also practised in neighbouring states of Karnataka, Kerala, and Andhra Pradesh, as well as parts of Malaysia (Poswal, F.S., *et al.*, 2019).

Nutritional value of Ashwagandha - Ashwagandha root powder (per 100g): Moisture 7.45 %, ash 4.41 g, protein 3.9 g, fat 0.3 g, crude fiber 32.3 g, energy 245 kcal, carbohydrate was 49.9 g, iron 3.3 mg, calcium 23 mg, total carotene 75.7 g, and vitamin C 5.8 mg/100 g.

Culinary uses of Ashwagandha - Ashwagandha root extract is available in capsules and tinctures, although powder is the most popular form. Powdered ashwagandha root is mixed with ghee, honey, or water and consumed or used topically to swollen joints or as part of an Ayurvedic skincare routine. Ashwagandha powder used in sweet pastries, hot beverages, and smoothies to balance out the earthy, bitter flavour. The powdered dried root of ashwagandha is used as a component of preparations in countries where the Ayurvedic system of medicine is officially recognized and practiced (for example, India, Bangladesh, Bhutan, Malaysia, Nepal, and Sri Lanka) (any

wasting or atrophic disease, weakness, diseases due to vatadosha). The dried mature root, known as "asgard," is used as a component of medicinal formulations to treat leucorrhoea, spermatorrhoea, decreased viscosity of semen, sexual debility, lumbago, and arthritis in countries where the Unani system of medicine is officially recognised and practised (e.g., Bangladesh, India, Malaysia, Pakistan, and Sri Lanka). Ashwagandha is frequently used as a component of majoon or halwa, which are compound Unani medications. These dishes are made with pre-made and powdered ingredients. These concoctions are produced with honey and prepared and powdered botanicals, producing in a soft or semi-solid consistency similar to halva, a popular dessert.

Therapeutic uses of Ashwagandha

Antioxidant effect - Brain and nervous system are high in lipids and iron, both of which are known to contribute to the generation of reactive oxygen species, they are more vulnerable to free radical damage than other tissues. Normal ageing and neurodegenerative disorders such as epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases may be linked to free radical damage to neural tissue (A. Chakrabarti, *et al.*,1997). Chronic stress (CS) can cause a variety of negative physiological effects, such as cognitive impairment, immune suppression, sexual dysfunction, stomach ulceration, glucose homeostasis problems, and changes in plasma corticosterone levels. Antioxidant defense, as measured by the enzymes SOD, CAT, and ascorbic acid, increased dramatically, but lipid peroxidation decreased significantly. When compared to the usual medicine ranitidine, *Withania somnifera* prevented stress-induced stomach ulcers more efficiently (A. Bhattacharya, *et al.*,2003).

Anxiety and depression - The bioactive *Withaniasomnifera*, extracted from *Withania somnifera* roots, was tested in rats for its anxiolytic and depressive properties. *Withania somnifera* was given orally once daily for 5 days and the results were compared to those generated by the benzodiazepine lorazepam and the tricyclic antidepressant imipramine for anxiolytic activity. In Ayurveda, the use of *Withania somnifera* as a mood stabiliser in clinical anxiety and despair (A. Bhattacharya, *et al.*, 2000) was notable.

Anti-tumor properties - In a search of *Withania somnifera* root extract on caused skin cancer in mice given *Withania somnifera* before and during exposure to the skin cancer inducing agent 7,12-dimethylbenz[a]anthracene, the chemopreventive effect was established. When compared to the control group, there was a significant reduction in the incidence and average number of skin lesions (A.K. Dinda, *et al.*, 2002). Tumors, inflammation, arthritis, asthma, and hypertension are all treated with *Withania somnifera* in the Ayurvedic system of treatment. The roots and leaves of this plant have revealed bioactive withanolides after chemical analysis. Withanolides have been shown to inhibit cyclooxygenase enzymes, lipid peroxidation, and tumour cell proliferation in previous investigations. Activation of nuclear factor-kappaB (NF-kappaB) regulates several genes involved in cellular proliferation, carcinogenesis, metastasis, and inflammation (B.B. Aggarwal, *et al.*, 2006).

Cardiovascular protection –*Withania somnifera* may be useful as a general tonic, due in part to its beneficial effects on the cardiopulmonary system (N.S. Dhalla, *et al.*, 1981). The hypotensive effect was mainly due to autonomic ganglion blocking action and that a depressant action on the higher cerebral centers also contributed to the hypotension (C.L. Malhotra, *et al.*, 2004).

Hypolipidemic effect - In hypercholesteremic, *Withania somnifera* root powder reduced total lipids, cholesterol, and triglycerides. The plasma HDL-cholesterol levels, HMG-CoA reductase activity, and bile acid content in the liver all increased significantly (N.P. Visavadiya and A.V. Narasimhacharya, 2006).

Immunomodulation and hematopoiesis - The role of *Withania somnifera* as an immunomodulator has received a lot of attention. The total white blood cell count was increased in a mouse study using *Withania somnifera* root extract. In addition, as compared to a control group, this extract suppressed delayed-type hypersensitivity reactions and increased macrophage phagocytic activity (N.P. Visavadiya and A.V. Narasimhacharya, 2006).

Anti-inflammatory properties *Withania somnifera* in the cyclooxygenase inhibitor oxyphenbutazone kept glucose absorption at a normal level. Both medicines have anti-inflammatory properties. In comparable tests, ¹⁴C-leucine absorption from the jejunum. Inhibition of cyclooxygenase may be implicated in the mechanism of action of ashwagandha.

General medicinal properties of ashwagandha - Ashwagandha may function as a pain reliever by inhibiting the transmission of pain signals through the central nervous system. It's also possible that it has anti-inflammatory qualities. When compared to the sedative and anxiety medication lorazepam, ashwagandha may have a soothing effect on anxiety symptoms (Mohanty I, *et al.*, 2004). The ability of ashwagandha to slow or prevent the loss of brain function in persons with neurodegenerative diseases like Alzheimer's, Huntington's, and Parkinson's disease. In some malignancies, Ashwagandha may be able to inhibit cell development. Ashwagandha root extract has been shown to boost cardiorespiratory endurance and thus heart health. Diarrhea, skin blistering and colouring, sedation, liver injury, thyrotoxicosis, elevated testosterone levels, and miscarriage are all possible side effects.

METHODOLOGY

III. METHODOLOGY

The methodology followed for the present study entitled "**Formulation and Incorporation of Immune Boosting Herbal Powders**" is presented as follows:

- A. Identification and preparation of immune boosting herbs
- B. Development and standardization of herbal powder
- C. Sensory evaluation of herbal powder
- D. Nutrient, phytochemical and toxicity analysis of herbal powder
- E. Incorporation of herbal powder in food products
- F. Statistical analysis and interpretation of the results

A. Identification and preparation of immune boosting herbs –

Identification of the herb

The identification of immune boosting herbs is based on the components present in each individual herb which helps to increase the immunity of the individual. The herbs identified for development and standardization of immune boosting herbal powder was ginger, clove, tulsi and ashwagandha.

Ginger (*Zingiber officinale*) – Ginger is a root or rhizome that comes under the family of *Zingiberaceae*, a common spice used for spicing the tea. In Ayurvedic medicine the presence of active component *gingerol* act as a perfect immune booster. It has both anti-bacterial and anti-inflammatory properties which help to fight against infection.

Clove (*Syzygium aromaticum*) – Clove is the dried flower of the clove tree that comes under the family of *Myrtaceae* with the scientific name of *Syzygium aromaticum*. Clove contains multiple compounds which act as anti-inflammatory properties. Eugenol is the most important component which reduce the inflammatory response in the body. These compounds help body to fight against free radicals.

Tulsi (*Ocimum sanctum*) – Tulsi is the fresh herb comes under the family *Lamiaceae* with the scientific name of *Ocimum sanctum*. Vitamin C and A, phytonutrients are great antioxidants and protect the damages caused by free radicals.

Ashwagandha (*Withania somnifera*) – Ashwagandha is a dry ingredient which is commonly used as ayurvedic medicine, comes under the family of

Solanaceae with a scientific name of *Withania somnifera*. Ashwagandha is also known as Indian ginseng. It increases immune system, reduces anxiety, relieves stress, increase blood production, stimulates the thyroid gland and protects the body from infection.

Thus, the four herbs that are easily available and having immune boosting properties as cited in literature namely ginger, clove, tulsi and ashwagandha has been selected for the present study.

Preparation of herbal powder – Powders are in the dried form and has less or no moisture content. Availability of fresh herbs is difficult. Hence herbs in the dried form were thought for the preparation of herbal powders and also dried powders has long shelf life. Shade drying is the process used to dry the leafy substances and for herbs. Shade drying method utilizes day light as a heating source. The selected herbs namely ginger, clove, and ashwagandha are purchased from the local market in a large quantities. The fresh tulsi leaves are purchased from local flower market in large quantities.

Ginger (*Zingibebr officinale*) – The preparation of Ginger (*Zingibebr officinale*) powder is based on shade drying. The fresh ginger is soaked and cleaned to remove the dirt particle in the ginger. Then it is drained and cut into slices. The slices are dried in the sunlight to remove the complete moisture content in the ginger. The dried ginger is then grind in the mixie. Then powder is collected and sieved to get a fine herbal powder.

Clove (*Syzygium aromaticum*) – The preparation of clove (*Syzygium aromaticum*) is based on shade drying. The dry clove was dried in the sunlight to remove the moisture content if any. The dried clove is then grind in the mixie. The powder was collected and sieved to get herbal clove powder.

Tulsi (*Ocimum sanctum*) – The preparation of Tulsi (*Ocimum sanctum*) is based on shade drying. The fresh tulsi was soaked in water and cleaned to remove the dirt particle. This was repeated thrice to ensure through cleaning of the tulsi leaves. Then water is drained, leaves are picked separately and dried in the sunlight to remove the complete moisture content in the tulsi. The dried tulsi is then grind in the mixie. The powder was collected and sieved to get a fine tulsi powder.

Ashwagandha (*Withania somnifera*) – The preparation of Ashwagandha (*Withania somnifera*) is based on shade drying. The dry ashwagandha was

dried in the sunlight to remove the moisture content. The dried ashwagandha is then ground in the mixer. The powder was collected and sieved to get a fine powder of the herb.

B. Development and Standardization of Herbal Powder

The shade dried powders of herbs namely ginger, tulsi, clove and ashwagandha are used for the development and standardization of herbal powders. The herbal powder was mixed in different proportions for standardization. The herbal powder was standardized by making infusion for 2g of powder in 200ml of water. The standardization is carried out by three different combinations and is given in Table I.

TABLE I
INGREDIENTS USED IN STANDARDIZATION OF HERBAL POWDER

Ingredients	Variation I	Variation II	Variation III
Tulsi	1 g	1 g	1 g
Ginger	0.5 g	0.5 g	0.7 g
Clove	0.3 g	0.25 g	0.2 g
Ashwagandha	0.2 g	0.25 g	0.1 g
Brown sugar	10 g	10 g	10 g

All the dried herbal powders were mixed thoroughly in the mentioned proportions and sieved twice or thrice to ensure proper thorough mixing of the herbs. This also ensures the fineness of the herbal powder.

Preparation of standard herbal powder – The standard product used for comparing the three variations for sensory evaluation was lemon tea. The lemon tea was prepared with the help of 2g tea dust available in the market and was made into infusion. The lemon was squeezed into it.

Preparation of variation I – The variation I was prepared using 1g of tulsi, 0.2g of ashwagandha, 0.3 g of clove, 0.5 g of ginger and 10g of brown sugar. Measured quantities of the herbal powder was mixed in 125 ml of water and allowed to boil for 3^{1/2} minutes. The infusion is ready for sensory evaluation.

Preparation of variation II – The variation II contains about 1g of tulsi, each 0.25g of ashwagandha and clove, 0.5 g of ginger and 10g of brown sugar measured. The ingredients are mixed in 125ml of water. Allowed to boil for 3 ^{1/2} minutes. The decoction is thus infused in the water.

Preparation of variation III – The variation III has about 1g of tulsi, 0.1g of ashwagandha, 0.2g of clove, 0.7 g of ginger and 10g of brown sugar measured. All the herbal powders are mixed in 125 ml of water. It was allowed to boil for 3 ^{1/2} minutes for the extract to get infused into the water.

C. Sensory evaluation of herbal powder

The sensory evaluation of herbal powder was carried out to know the acceptance level of herbal powder during incorporation and also helps to know scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (appearance, texture, taste, colour and flavour) for the purpose of evaluating consumer products. The discipline requires panels of human assessors, on whom the products are tested, and recording the responses made by them (Sukanya Wichchukit and Michael O'Mahony, 2015). A score card is a tool that aids in evaluating by providing right 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-Like extremely, 8-Like very much, 7-Moderately, 6-Like slightly, 5-Neither like nor dislike, 4-Dislike, 3-Dislike moderately, 2-Dislike very much, 1-Dislike extremely was used in the score card. Panel members are the group testers chosen to participate in sensory test who are requested to assess food quality of samples presented for evaluation (Laura Nicolas, *et al.*, 2010).

Thirty semi-trained panel members were 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 in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

The standard used for the sensory evaluation was lemon tea. The variations I, II and III was prepared using herbal powder. The herbal infusion was presented to the 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 (Juyun Limand Tomomi Fujimaru, 2010). The sensory evaluation of the developed infusion was done in the mid-morning between 10.30 am and 12:00 noon. The portion sizes for all the products was kept consistent and uniform.

During the sensory evaluation of the herbal infusion each panel member was permitted to take their time for evaluating the product that was presented to them. The finished items was neatly organised into standard, variation I, variation II, and variation III and delivered to the semi-trained panel members. A pen and an evaluation form was given to the semi-trained panel members. Between tastings, the evaluator was given a glass of water to rinse her 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 which obtained the highest totaland mean score was considered to be the best acceptable infusion. The sensory evaluation of herbal infusion is given in Plate 1.

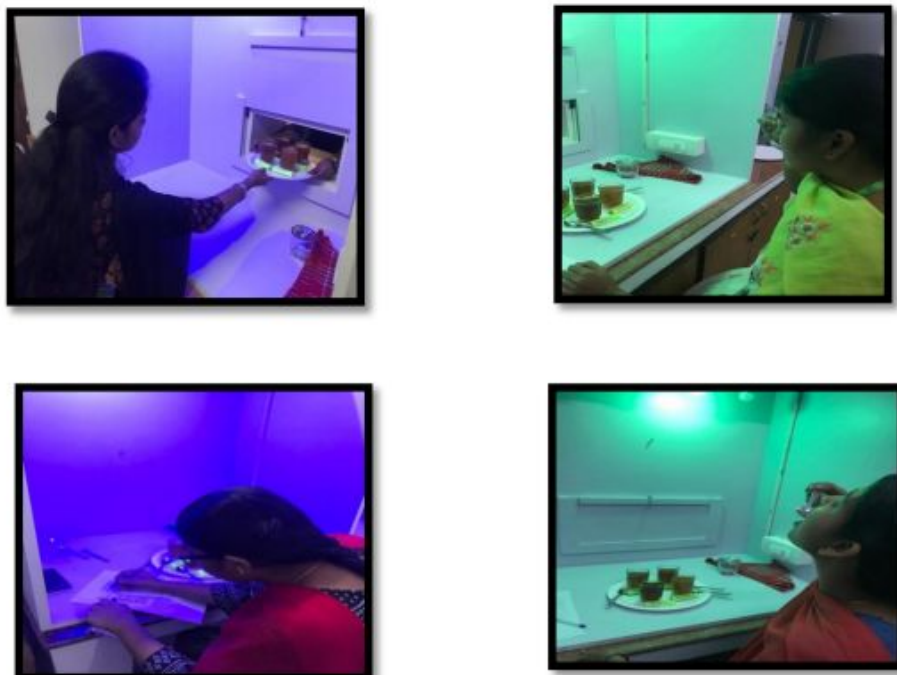


Plate 1

SENSORY EVALUATION OF HERBAL INFUSION

D. Nutrient, phytochemical and toxicity analysis of herbal powder

Nutrient analysis of the herbal powder

Nutrient analysis is carried out to know the qualitative and quantitative amount of nutrient present in the herbal powder. The nutrient analysis carried out for herbal powder are ash, vitamin A, B, C, E, K and minerals namely calcium, iron, magnesium, phosphorus, potassium, zinc. The analysis of vitamins and minerals are given in Plate 2.

Preparation of Ash - The herbal powder was made into ash by continuous heating, the substance gets charred which can be used for the determination of minerals present (AOAC, 1990).

Analysis of vitamins

Estimation of Vitamin A – The estimation of vitamin A is carried out by HPLC procedure. The sample is saponified with ethanolic potassium hydroxide solution and vitamin A is extracted into light petroleum. The light petroleum is removed by evaporation and the residue is dissolved in 2-propanol. The vitamin A concentration in the 2-propanol extract is determined by reverse-phase liquid chromatography using conditions that give a single peak for all retinol isomers.

Estimation of Vitamin B – The estimation of vitamin B is carried out by HPLC method. The estimation is carried out using four different concentration levels (5µg/ml, 10µg/ml, 20µg/ml and 40µg/ml) which were prepared from standard solutions of each by diluting with HPLC water. Then 20µl from each diluted solution was injected into HPLC using auto-sampler and the analyses was monitored at 210nm and repeated three times. The average peak areas were plotted against concentrations. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation, slope and intercept values.

Estimation of Vitamin C – The estimation of vitamin C is carried out by titration method. Vitamin C is a good reducing agent and it reduces the dye 2,6 dichlorophenolindophenol. In this reaction, the ascorbic acid itself is oxidised to dehydro ascorbic acid. In the absence of interfering substances, the capacity of an extract of the sample to reduce a standard solution of the dye as determined by titration is directly proportional to the vitamin C content.

Oxalic acid is not only used to reduce the pH of the extracting medium, thereby establishing vitamin C but also form complexes with metals like copper thereby preventing the catalytic oxidation of vitamin (AOAC, 1990).

Estimation of Vitamin E – Determination of vitamin E in food is carried out by colorimetric procedure for determining total tocopherols, based on their reaction with iron(III) chloride and bathophenanthroline (4,7-diphenyl-1,10-phenanthroline), and chromatographic method for determining individual tocopherols. Techniques for the removal of interfering substances such as sterols and tocopherol dimers are given and results obtained by both methods for the vitamin E contents of some uncooked foods are compared.

Estimation of Vitamin K – Vitamin K was determined by HPLC with fluorescence detection after post column zinc reduction. The detection was performed at 246 nm (excitation) and 430 nm (emission). The internal standard and 2 mL of ethanol was added to 500 µL of serum. The mixture was extracted with 4 mL of hexane, and solid phase extraction was then used.

Analysis of minerals

Estimation of Calcium – Calcium is determined by precipitating it as calcium oxalate and titrating the oxalate solution in dilute sulphuric acid against standard potassium permanganate (AOAC, 1990).

Estimation of Iron – The food sample is oxidized with ignition or oxidation. Iron as ferric iron reacts with ammonium thiocyanate or with potassium thiocyanate to give ferric thiocyanate which is red in colour. The colour which is a measure of the concentration is measured calorimetrically (AOAC, 1990).

Estimation of Phosphorus – When the ash solution is treated with ammonium molybdate, phosphomolybdic acid is formed. Phosphomolybdic acid is reduced by the addition of 1,2,4 Amino Naphthol Sulphonic Acid (ANSA) reagent to produce a blue colour which is apparently a mixture of oxides of molybdenum. The intensity of the colour developed is the measure of phosphorus present (AOAC, 1990).

Estimation of Potassium – Quantitative determination of the level of potassium in samples was done using spectrophotometric method. The absorbance of the product was read at 515nm. The qualitative test was performed directly on a portion of each sample using 2ml of 0.01M

promethazine and 0.6ml of 12M hydrochloric acid. The change in colour of each sample to pink indicates the presence of potassium.

Estimation of Magnesium – The estimation of magnesium is carried out by taking 25 ml sample of dry at low flame in evaporating dish and ash it in a muffle furnace. Dissolved the ash in HCl and added 50 ml of water. Allowed to stand on water bath for few minutes and filtered in 250 ml beaker. Washed the insoluble residue with hot water and collected the washing in same beaker. Adjusted the pH of solution to 4.4-4.6 by acetic acid drop by drop and boiled the solution hot added, saturated ammonium oxalate solution till the precipitate is formed. Heated to boiling the solution and allowed to stand for 3 hours or longer. Decanted the clear solution through an ash less filter paper. Poured 25 ml of hot water on the precipitate and again decanted the clear solution through filter paper. Dissolved any precipitate remaining on filter paper by washing with hot dil. HCl into the original beaker while boiling ammonium hydroxide and a little saturated ammonium oxalate solution was added into it. Allowed to stand for 3 hours or longer. Filtered through the same filter paper and washed with hot water. Washed the filter paper with dil. H₂SO₄ acid and titrate at a temperature not less than 70°C with standard KMnO₄ solution.

Estimation of Zinc – The contents of zinc in foods was measured by Atomic Absorption Spectrophotometer, (Hernandez, *et al.*, 2004). A 5g sample was placed in a previously weighed porcelain crucible and heated. The resulting white ash was weighed, dissolved in 3ml of concentrated nitric acid and diluted with distilled water in a 25ml calibrated flask. The solution then was used to determine zinc. The standard stock solution of zinc was prepared from AAS grade chemicals.

Estimation of Crude Fibre – To estimate the crude fibre accurately about 2-5 g of the moisture-free sample after determining the total solid content was weighed and extracted for about one hour with petroleum ether, using a Soxhlet apparatus. The fat free material was transferred to 1L flask. 200 ml of dilute sulphuric acid in a beaker was taken and boiled. Then transferred the whole of the boiling acid to the flask containing the fat-free material and immediately connected to the flask with a water-cooled reflux condenser and heated so that the contents of the flask begin to boil within one minute. The

flask should be rotated frequently, taking care not to allow the material to stick to the sides of the flask, and not to keep the material out of contact with the acid. Boiling was continued for exactly 30 minutes. Removed the flask, and filtered through fine linen (about 18 threads to a centimeter) held in a funnel, and washed with boiling water until the washings are no longer acidic to litmus. Some quantity of sodium hydroxide solution added to boiling under a reflux condenser. The residue was washed on the linen into the flask with 200 ml of boiling sodium hydroxide solution. Immediately the flask was connected with the reflux condenser and boiled for exactly 30 minutes. Removed the flask and immediately filter through the filtering cloth. The residue was washed first with hot water and then with 15ml of ethyl alcohol 95 percent by volume. Dried the crucible fibre and contents at $105 \pm 2^\circ\text{C}$ in an air-oven to constant mass. It was cooled and weighed. Incinerated the contents of the crucible fibre in an electric muffle furnace at $600 \pm 20^\circ\text{C}$ until all the carbonaceous matter was burnt. Then the crucible fibre containing the ash in a desiccator was weighed.

$$\text{Crude fibre} = \frac{10000 (M_1 - M_2)}{M \times (100 - W)}$$



Preparation of ash



Estimation of vitamin C



Estimation of vitamin E.



Estimation of calcium

Plate 2

ANALYSIS OF VITAMINS AND MINERALS

Phytochemical analysis of herbal powder

Phytochemicals are bioactive constituents that sustain or promote health which are broadly described as phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, polyphenols, flavonoids, isoflavonoids and anthocyanidins. The phytochemical analysis in Aqueous extract are given in Plate 3.

Preparation of phytochemical extracts

Aqueous extract – The sample was used to prepare an infusion in hot (95°C) distilled water. The infusion was left overnight under refrigeration at 4°C to prevent any possible contamination. After 24 hours the extract was kept in rotary shaker at 100 rpm for an hour and filtered with Whatman No. 1 filter paper (Doughari et al., 2012).

The phytochemicals present in the sample was thus analyzed in aqueous extract using both ethanol and methanol as solvents.

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

Terpenoids- To 0.5 ml of the extract, 2ml of the 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 plant 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).

Saponins (Foam test)- Five millilitre sample extract was dissolved in 2.5ml 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 the extract, 1ml of the concentrated sulphuric acid was added. Red colour formation indicates the presence of quinone compound (AOAC, 2005).

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

Coumarins- To 2 ml of the test solution, a few drops of alcoholic sodium hydroxide were added. Appearance of yellow colour indicates the presence of coumarin (AOAC, 2005).

Sterols (Sulphuric acid Test) - To the plant extracts 2 ml of chloroform was added. 2 ml of concentrated sulphuric acid was added by the sides of the test tube and observed for red colour at the lower chloroform layer (Shah and Shah, 2015).



Plate 3

PHYTOCHEMICAL ANALYSIS IN HERBAL POWDER

Toxicity test

The toxins present in the infusion was determined 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 (*Artemiasalina*) has been utilised by many researchers and has proven to be a useful tool in screening various chemical compounds found in various bioactivities. After 24 hours the number of survival of nauplii was counted and percentage of mortality was determined. The toxicity of herbal powder was analysed using brine shrimp is given in Plate 4.

Preparation of Samples - The given sample (Toxicity A) was weighed and dissolved in water to get 1mg/ml stock solution. The sample (Toxicity A) of different volume 250, 500, 1000, 1500, 2000 μ l is added to each beaker containing saline solution.

Preparation of samples-The given sample (herbal powder) was weighed and dissolved in water to get 1mg/ml stock solution. Then the sample (herbal powder) of different volume 100, 250, 500, 1000, 1500 mg is added to each beaker containing saline solution respectively.

Procedure :30 shrimps were introduced into the sample solution of various concentration. The movement of shrimps 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 shrimps was calculated after 24 hours. For each of the sample, 30 shrimps were added to 25 ml of the solution. The mortality of the shrimps was monitored as that of blank and positive control.

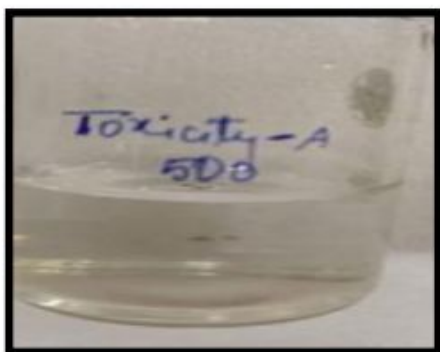


Plate 4

TOXICITY IN HERBAL POWDER

E. Incorporation of herbal powder in food products

The variation of the herbal powder that was best accepted by the sensory panel members was incorporated into food recipes and evaluated the acceptability of the product. The finalised herbal powder was incorporated into different food products like soups (tomato soup, clear vegetable soup, spicy mushroom soup), tea and coffee. The sensory evaluation of incorporation of herbal powder in food products.

Standardization and incorporation of herbal powder in soups – The herbal powder was incorporated into prepared soups like tomato soup, clear vegetable soup and spicy mushroom soup.

Preparation of tomato soup - The tomato soup was prepared by blanching the fresh tomatoes and peeled the skin of the blanched tomatoes. Heated the oil in a pan, fried onions and added blanched tomatoes. Added 1 cup of water,

salt and pressure cooked for 1 whistle. After it cooled, it was blended to a puree. Strained the blended mixture in a muslin cloth to remove seeds and skin. Then, cooked for a few seconds on low flame. Kept on stirring and soup was ready to serve.

Incorporation of herbal powder - Standardized herbal powder was incorporate into the soup at three different measurements *i.e* 2g, 3g and 4g. These variations was scored sensorily.

Preparation of clear vegetable soup - The clear vegetable soup was prepared by chopping the vegetable into pieces and pressure cooked for 1 whistle with added 1 cup of water. Strained the extract. Heated the pan with little amount of oil, added the extract and heated for 4 minutes on low flame. Kept on stirring and soup was ready to serve.

Incorporation of herbal powder - The standardized herbal powder was incorporated into the soup with three different variations *i.e* 2g, 3g and 4g. These variations was scored sensorily.

Preparation of spicy mushroom soup -The spicy mushroom soup was prepared by chopping the mushroom, garlic, onion and green chilli into pieces and pressure cooked for 1 whistle with addition of 1 cup of water. Strained the extract. Heated the pan with little amount of oil and then added the extract to the pan and boiled for 4 minutes on low flame. Kept on stirring and soup was ready to serve.

Incorporation of herbal powder - Then standardized herbal powder was incorporated into the spicy mushroom soup with three different variations *i.e* 2g, 3g and 4g. These variations was scored sensorily.

Preparation of tea - The tea was prepared by boiling water in a pan, then added sugar and tea powder into it. Boiled for 3-4 minutes in a medium flame. Added milk and boiled over for 6-7 minutes. Strained the tea.

Preparation of coffee - The coffee was prepared by boiling milk in a pan, then added sugar into it. Boiled for 3-4 minutes in a medium flame until it rises. Mixed 1 tsp of instant coffee per cup and added boiled milk into it. Coffee is ready to serve.

To the prepare tea and coffee, the herbal powder was added at the end of 2g, 3g and 4g each and evaluated for acceptability (Plate 5). Variation I contains 2g, variation II contains 3g, and variation III contains 4g of herbal powder.



Plate 5
SENSORY EVALUATION OF INCORPORATED
HERBAL POWDER IN FOOD PRODUCTS

F. Statistical analysis and interpretation of the results

The data was collected, consolidated, tabulated and analyzed statistically to evaluate the sensory characteristics and nutrient content of the herbal powders in the infusion. The research design of the study is presented in Figure 1.

Ethical clearance

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 -21-22/XPD/28 and the IHEC approved form is appended in the Appendix I.

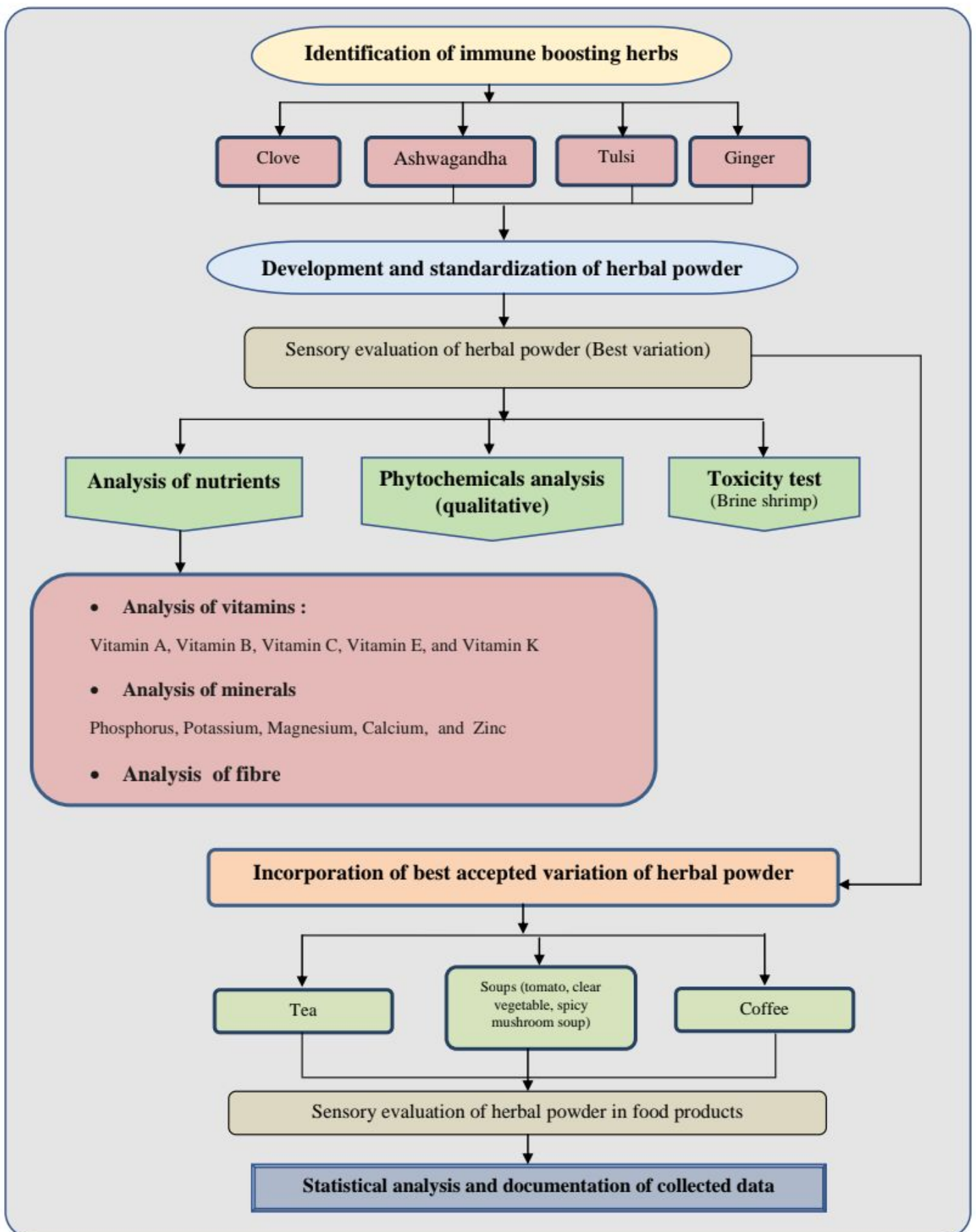


Figure 1
Research design

RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

The Results and Discussion pertaining to the study entitled “**Formulation and Incorporation of Immune Boosting Herbal Powder**” is presented under following sub-headings:

- A. Sensory characteristics of herbal infusion
 - B. Vitamins and minerals in the herbal powder
 - C. Phytochemicals in the herbal powder
 - D. Toxicity in the herbal powder
 - E. Sensory characteristics of herbal powder incorporated into food products
- A. Sensory characteristics of herbal infusion**

The sensory evaluation of the herbal infusion was carried out with the help of semi-trained panel members. The panel members are the group testers. The scores as given by the semi-trained panel members are calculated using statistical analysis. The mean and standard deviation for the sensory evaluation of herbal infusion was calculated and it is mentioned in Table II and Figure 2.

TABLE II
SENSORY EVALUATION OF HERBAL INFUSION

Characters	Standard	Variation I	Variation II	Variation III
Colour	8.83 ± 0.38	8.73 ± 0.45	8.07 ± 0.87	8.40 ± 0.81
Taste	8.83 ± 0.38	8.77 ± 0.43	8.23 ± 0.86	8.62 ± 0.68
Appearance	8.67 ± 0.48	8.60 ± 0.50	8.07 ± 0.83	8.20 ± 0.83
Consistency	8.67 ± 0.48	8.70 ± 0.47	8.23 ± 0.90	8.50 ± 0.78
Flavour	8.73 ± 0.45	8.67 ± 0.47	8.07 ± 0.87	8.13 ± 0.80
Overall acceptance	8.77 ± 0.43	8.70 ± 0.47	8.17 ± 0.87	8.37 ± 0.81
t test		3.051	14.351	5.720
Significant		0.028**	0.0 ^{NS}	0.002**

** - Significant at 5% level, ^{NS} – Not significant

The sensory evaluation of herbal infusion showed that the mean values of the color was 8.83 for standard, 8.73 for variation I, 8.07 for variation II and 8.40 for variation III. The taste obtained for standard was 8.83, variation I was

8.77, variation II was 8.23 and variation III was 8.62. The mean value obtained for appearance for standard was 8.83, variation I was 8.60, variation II was 8.07 and variation III was 8.20. The consistency for herbal infusion was obtained as 8.67 for standard, 8.70 for variation I, 8.23 for variation II and 8.50 for variation III. The mean obtained for flavour was 8.73 for standard, 8.67 for variation I, 8.07 for variation II and 8.13 for variation III. The overall acceptance for the standard was 8.77, variation I was 8.70, variation II was 8.17 and variation III was 8.37.

The sensory evaluation of herbal infusion variation I was much better in colour, taste, appearance, consistency, flavour and overall acceptance on comparing with variation II and III. The variation I was compared with standard (lemon tea). The variation I sensory evaluation value was slightly different from standard but it was better as standard. Meanwhile, The variation III was better than variation II in all aspects.

The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The first comparison was carried out for standard and variation I. The 't' test value for standard vs Variation I was 3.051 and it was significant at 5% level. The comparison was carried out for standard and variation II. The 't' test value for standard vs Variation II was 14.351 and it was not significant. The 't' test value for standard vs Variation III was 5.720 and it was statistically significant at 5% level.

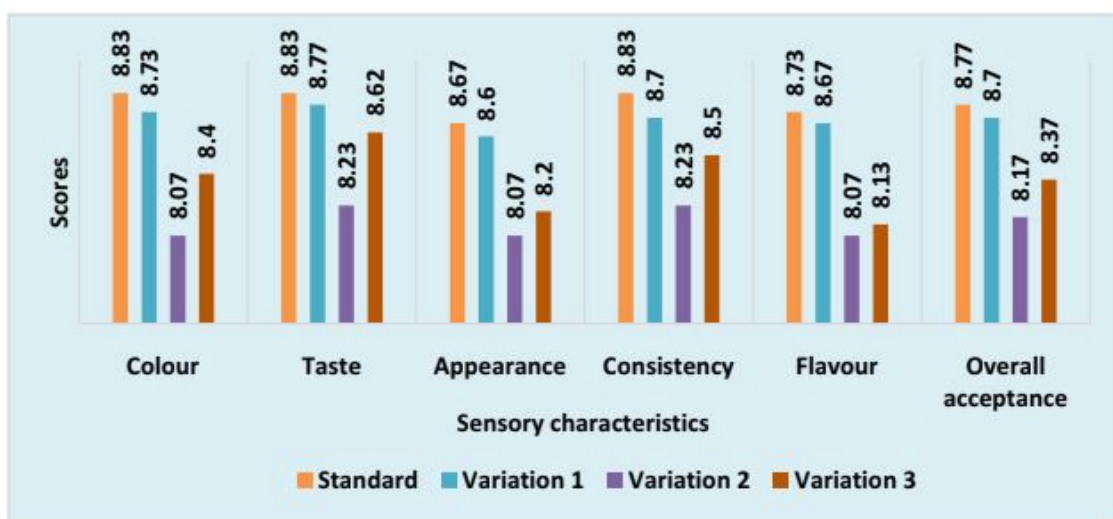


Figure 2

SENSORY CHARACTERISTICS OF HERBAL INFUSION

The vitamins and minerals analysis and phytochemical was analysed for the best accepted variation. It was observed from sensory analysis that variation I was best accepted and hence all the tests were carried out for variation I.

B. Vitamins and minerals in the herbal powder

The nutrient analysis was carried out to know the quantity of nutrients present in the herbal powder. The nutrients present helps to ensure the quantity of consumption of the herbal powder on regular basis. The presence of vitamins and minerals helps to justify that it is an immune boosting herbal powder.

Presence of vitamin content of the herbal powder

The estimation of vitamins like antioxidant vitamin A, B, C, E and K was analyzed to know the presence of immune boosting compound in herbal powder. Table III and Figure 3 gives the vitamins present in the herbal powder.

TABLE III

ESTIMATION OF ANTIOXIDANT VITAMINS IN THE HERBAL POWDER

Vitamins	RDA (ICMR, 2022)	Values
Vitamin A	2333 IU/d	5429.49 IU/100g
Vitamin B complex	400 mg/d	89.34 mg/100g
Vitamin C	65 mg/d	125.54 mg/100g
Vitamin E	0.8 mg/g	233.70 mg/100g
Vitamin K	0.055 mg/g	2.12 mg/100g

The estimation of vitamin A was carried out by HPLC method. The vitamin A in the herbal powder was 5429.49 IU /100g. Compared with other

vitamins, vitamin A was rich in herbal powder. The vitamin A helps to boost the immunity in the body, growth and cell division.

The estimation of vitamin B complex was carried out by HPLC method. In this method, total B complex vitamin was estimated. The vitamin B complex present in the herbal powder was 89.34 per mg/100g. The vitamin B helps to increase the brain function and growth of red blood cells.

The estimation of vitamin C was carried out by titration method. The vitamin C present in the herbal powder was 125.54 per mg/100g. Vitamin C helps to prevent iron deficiency and also increase the immune function in the body.

The estimation of vitamin E was carried out by colorimetric procedure. This helps to know the total tocopherols in the herbal powder. The tocopherols present in the herbal powder was 233.70 per mg/100g. Vitamin E helps to prevent inflammation, coronary heart disease and support immune function.

The estimation of vitamin K was carried out by HPLC method with fluorescence detector. The vitamin K present in herbal powder was 2.12 per mg/100g. Vitamin K makes various proteins that are needed for blood clotting and the building of bones.

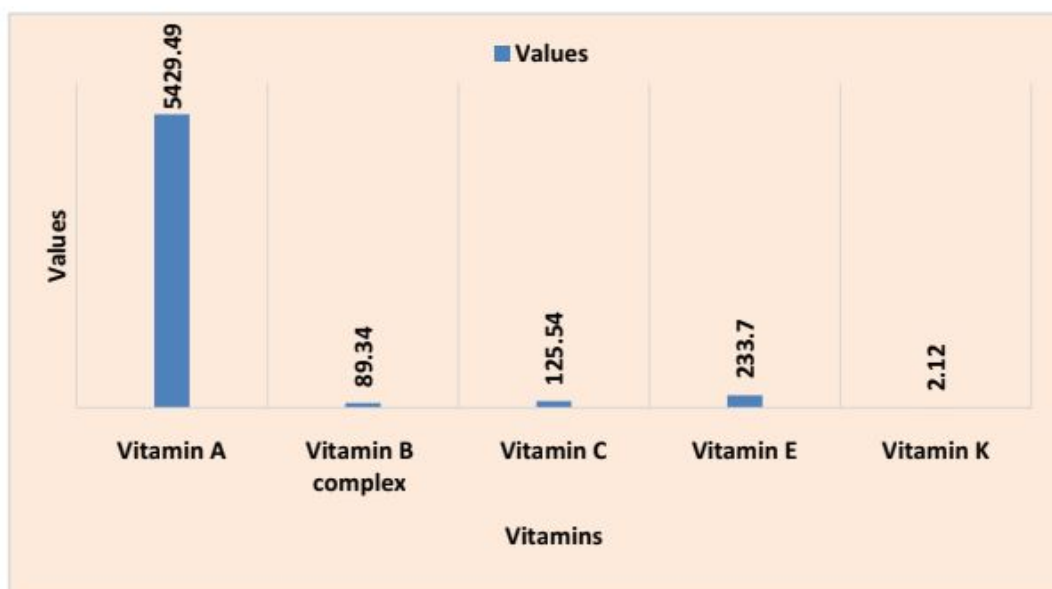


Figure 3

VITAMINS IN THE HERBAL POWDER

Mineral content of the herbal powder

The estimation of minerals like calcium, iron, magnesium, phosphorus, potassium and zinc was analysed to understand the presence of trace minerals in the herbal powder. The minerals present in the herbal powder is given in Table IV and Figure 4.

TABLE IV
ESTIMATION OF MINERALS IN THE HERBAL POWDER

Minerals	RDA (ICMR, 2022)	Values (mg/100g)
Calcium	800 (mg/100g)	712.22 (mg/100g)
Iron	15 (mg/100g)	0.16(mg/100g)
Magnesium	4 (mg/100g)	252.23(mg/100g)
Phosphorus	1000 (mg/100g)	2.16(mg/100g)
Potassium	3500(mg/100g)	82.42(mg/100g)
Zinc	11.0 (mg/100g)	15.29(mg/100g)

The estimation of calcium in herbal powder was carried out by titration method. The calcium present in the herbal powder was 712.22 per mg/100g. The calcium helps to build the bone and teeth.

The estimation of iron was carried out by colorimetric procedure. The iron present in herbal powder was 0.16 per mg/100g. Iron helps to increase the hemoglobin in the blood.

The estimation of magnesium is carried out and the presence of magnesium in herbal powder was 252.23 per mg/100g. Magnesium helps the body to normalize the blood pressure.

The estimation of phosphorus was carried out and the presence of phosphorus in the herbal powder was 2.16 per mg/100g.

The estimation of potassium reveals the presence of phosphorus in the herbal powder was 82.42 per mg/100g.

The estimation of zinc was carried out by atomic spectrometer. The zinc present in the herbal powder was 15.29 per mg/100g.

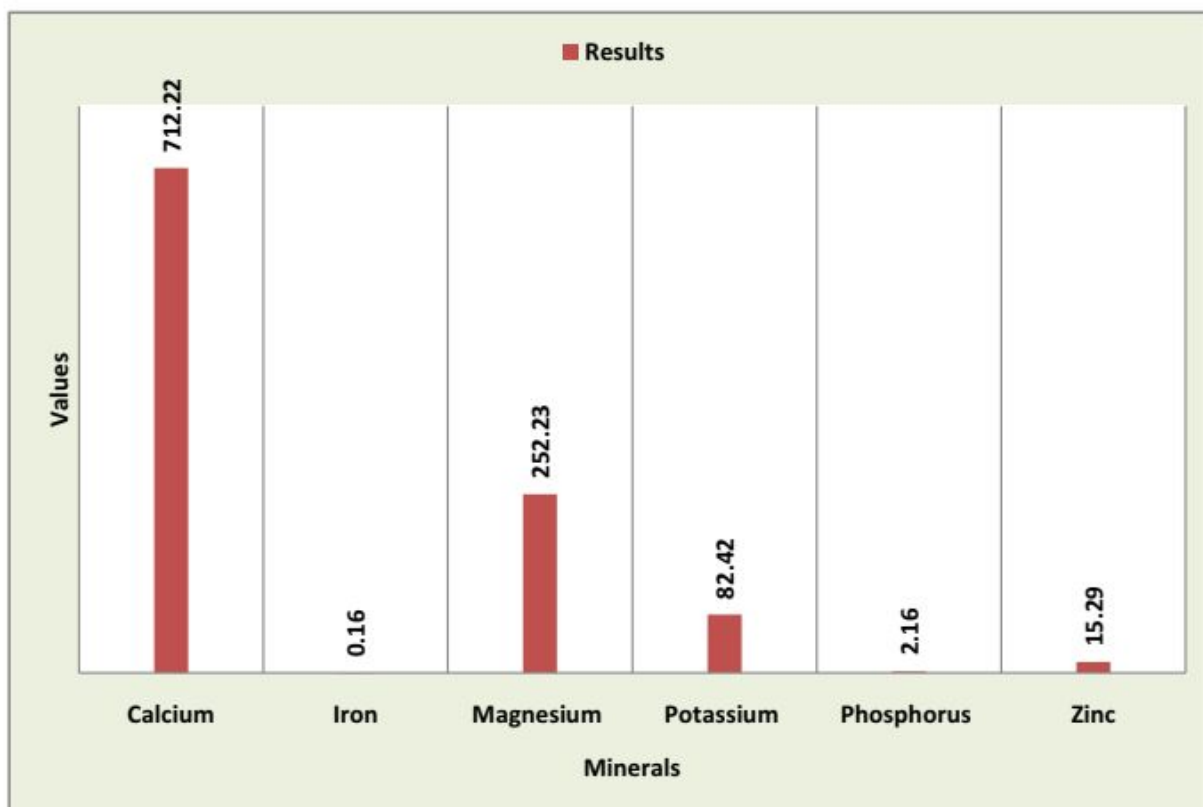


Figure 4
MINERALS IN THE HERBAL POWDER

C. Phytochemicals in the herbal powder

The qualitative phytochemical screening was carried out to know the presence and absence of certain phytochemicals in the herbal powder. The Table V gives the phytochemicals present in the herbal powder qualitatively.

TABLE V
PHYTOCHEMICALS CONTENT OF THE HERBAL POWDER

Phytochemical	Aqueous extract
Tannins	+++
Terpenoids	+
Phenols	+++
Saponins	++
Quinones	-
Glycosides	+++
Coumarins	-
Sterols	-
Flavanoids	+++
Alkaloids	++

+++ Strongly present, ++ moderately present, + weakly present, - absent

The phytochemical screening carried out for the herbal powder revealed that tannins, flavanoid, phenols and glycosides are strongly present due to the presence of tulsi, clove and ashwagandha. Whereas, saponins and alkaloids was moderately present in herbal powder due to the presence of tulsi, ashwagandha and ginger. The phytochemicals like terpenoids and coumarins are weakly present in the herbal powder. The phytochemicals like sterols and quinones are absent in phytochemical screening of herbal powder.

D. Toxicity in the herbal powder

Toxicity study was carried out in Bharat Ratna Prof. CNR Rao Research Centre, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. Table VI gives the toxicity and mortality of Brine shrimp in the herbal powder.

TABLE VI
TOXICITY IN THE HERBAL POWDER

Sample code	Concentration ($\mu\text{g}/\text{mg}$)	Mortality of Brine shrimp (no. of shrimps dead) (h)					
		1	2	4	6	24	% Mortality (in 24 hours)
Toxicity A	250	0	0	2	2	8	27
	500	0	0	3	3	9	30
	1000	0	0	2	3	11	37
	1500	0	0	0	1	11	37
	2000	0	0	0	1	11	37
Control $\text{K}_2\text{Cr}_2\text{O}_7$	1(mg/ml)	30	-	-	-	-	100
Blank	Saline water	0	0	0	0	0	0

The sample Toxicity A is comparatively less toxic than $\text{K}_2\text{Cr}_2\text{O}_7$ which shows maximum lethality of shrimps at higher concentration. The shrimps in the sample is found to be very less toxic in lower as well as in higher concentration. Even after 24 hours, only 8 - 11 shrimps are found to be mortal at lowest and highest concentration.

E. Sensory evaluation of herbal powder incorporated into food products

Incorporation of herbal powder in tomato soup

The herbal powder was incorporated into tomato soup at concentrations 2g, 3g and 4g. The sensory evaluation for the incorporated tomato soup was carried out with the help of semi-trained panel members. The scores as given by the semi-trained panel members are calculated using statistical analysis. The mean and standard deviation for the sensory evaluation of Incorporation of herbal powder in tomato soup was calculated and is mentioned in Table VII and Figure 5.

TABLE VII
SENSORY EVALUATION OF HERBAL POWDER INCORPORATED
TOMATO SOUP

Characters	Standard	Variation I	Variation II	Variation III
Colour	8.77 ± 0.43	8.60 ± 0.50	8.20 ± 0.76	7.73 ± 0.98
Taste	8.70 ± 0.47	8.73 ± 0.45	8.37 ± 0.81	8.27 ± 0.83
Appearance	8.63 ± 0.50	8.67 ± 0.48	8.20 ± 0.82	8.31 ± 0.86
Consistency	8.83 ± 0.38	8.73 ± 0.45	8.20 ± 0.85	8.20 ± 0.85
Flavour	8.73 ± 0.45	8.70 ± 0.47	8.23 ± 0.82	8.10 ± 0.85
Overall acceptance	8.77 ± 0.43	8.77 ± 0.43	8.30 ± 0.84	7.83 ± 0.85
t test (standard vs variations)		1.147	12.092	5.811
Significant		0.038**	0.0 ^{NS}	0.002**

** - Significant at 5% level, ^{NS} – Not significant

The sensory evaluation of incorporation of herbal powder in tomato soup revealed that the mean scores for color was 8.77 for standard, 8.60 for variation I, 8.20 for variation II and 7.73 for variation III. The taste obtained for standard was 8.70, variation I was 8.73, variation II was 8.37 and variation III was 8.27. The appearance for standard was 8.63, variation I was 8.67, variation II was 8.20 and variation III was 8.31. The consistency for incorporation of herbal powder in tomato soup was obtained as 8.67 for standard, 8.70 for variation I, 8.23 for variation II and 8.50 for variation III. The mean values obtained for flavour was 8.73 for standard, 8.70 for variation I, 8.23 for variation II and 8.10 for variation III. The overall acceptance for the standard was 8.77, variation I was 8.70, variation II was 8.30 and variation III was 7.83.

The sensory evaluation of herbal powder in tomato soup variation I was much better in colour, taste, appearance, consistency, flavour and overall acceptance on comparing with variation II and III. The variation I was compared with standard. The variation I sensory evaluation value was slightly different from standard but variation I was better in colour and appearance. Meanwhile, The variation II was better than variation III in all aspects.

The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The first comparison was carried out for standard and variation I. The 't' test value for standard vs Variation I was 1.147 and it was significant at 5% statistically. The comparison was carried out for standard and variation II. The 't' test value for standard vs Variation II was 12.092 and it was not significant. The 't' test value for standard vs Variation III was 5.811 and it was significant at 5% level.

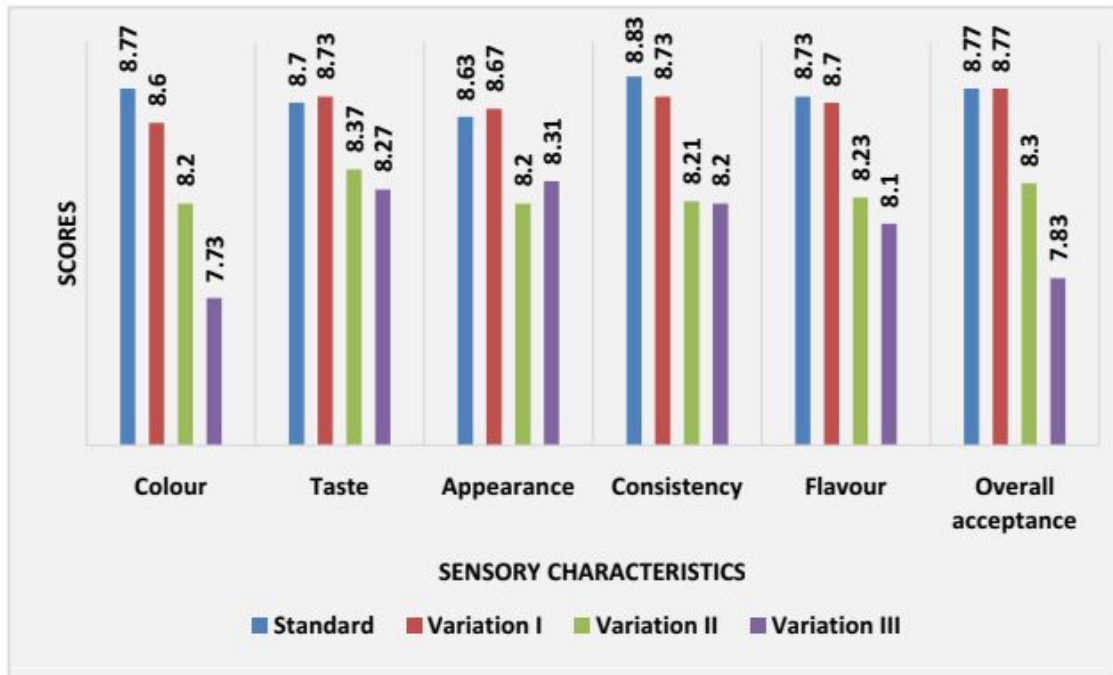


Figure 5

SENSORY EVALUATION OF HERBAL POWDER INCORPORATED TOMATO SOUP

Incorporation of herbal powder into clear vegetable soup

The sensory evaluation for the herbal powder incorporated clear vegetable soup was carried out with the help of semi-trained panel members. The scores as given by the semi-trained panel members are calculated using statistical analysis. The mean and standard deviation for the sensory evaluation of incorporation of herbal powder in clear vegetable soup was calculated and is mentioned in Table VIII and Figure 6.

TABLE VIII
SENSORY EVALUATION OF HERBAL POWDER INCORPORATED
CLEAR VEGETABLE SOUP

Characters	Standard	Variation I	Variation II	Variation III
Colour	8.60 ± 0.50	8.73 ± 0.45	7.97 ± 0.81	7.73 ± 0.98
Taste	8.73 ± 0.45	8.77 ± 0.43	8.12 ± 0.83	6.97 ± 0.96
Appearance	8.67 ± 0.48	8.60 ± 0.50	8.03 ± 0.81	7.70 ± 0.88
Consistency	8.73 ± 0.45	8.70 ± 0.47	8.20 ± 0.89	7.63 ± 0.99
Flavour	8.70 ± 0.47	8.67 ± 0.48	8.03 ± 0.85	7.67 ± 0.99
Overall acceptance	8.77 ± 0.43	8.70 ± 0.46	8.17 ± 0.87	7.30 ± 0.99
T test		0.158	31.556	8.577
Significant		0.088**	0.00 ^{NS}	0.00 ^{NS}

** - Significant at 5% level, ^{NS} – Not significant

The sensory evaluation of incorporation of herbal powder in clear vegetable soup showed that the mean values for color was 8.60 for standard, 8.73 for variation I, 7.97 for variation II and 7.73 for variation III. The taste obtained for standard was 8.73, variation I was 8.77, variation II was 8.12 and variation III was 6.97. The appearance for standard was 8.67, variation I was 8.60, variation II was 8.03 and variation III was 7.70. The consistency for incorporation of herbal powder in clear vegetable soup was obtained as 8.73 for standard, 8.70 for variation I, 8.20 for variation II and 7.63 for variation III. The mean obtained for flavour was 8.70 for standard, 8.67 for variation I, 8.03 for variation II and 7.67 for variation III. The overall acceptance for the standard was 8.77, variation I was 8.70, variation II was 8.17 and variation III was 7.30.

The sensory evaluation of herbal powder in clear vegetable soup variation I was much better in colour, taste, appearance, consistency, flavour and overall acceptance on comparing with variation II and III. The variation I was compared with standard. The variation I sensory evaluation value was slightly different from

standard but variation I was better in colour and taste. Meanwhile, The variation II was better than variation III in all aspects.

The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The first comparison was carried out for standard and variation I. The 't' test value for standard vs Variation I was 0.158 and it was significant at 5% level statistically. The comparison was carried out for standard and variation II. The 't' test value for standard vs Variation II was 31.556 and it was not significant. The 't' test value for standard vs Variation III was 8.577 and it was not significant.

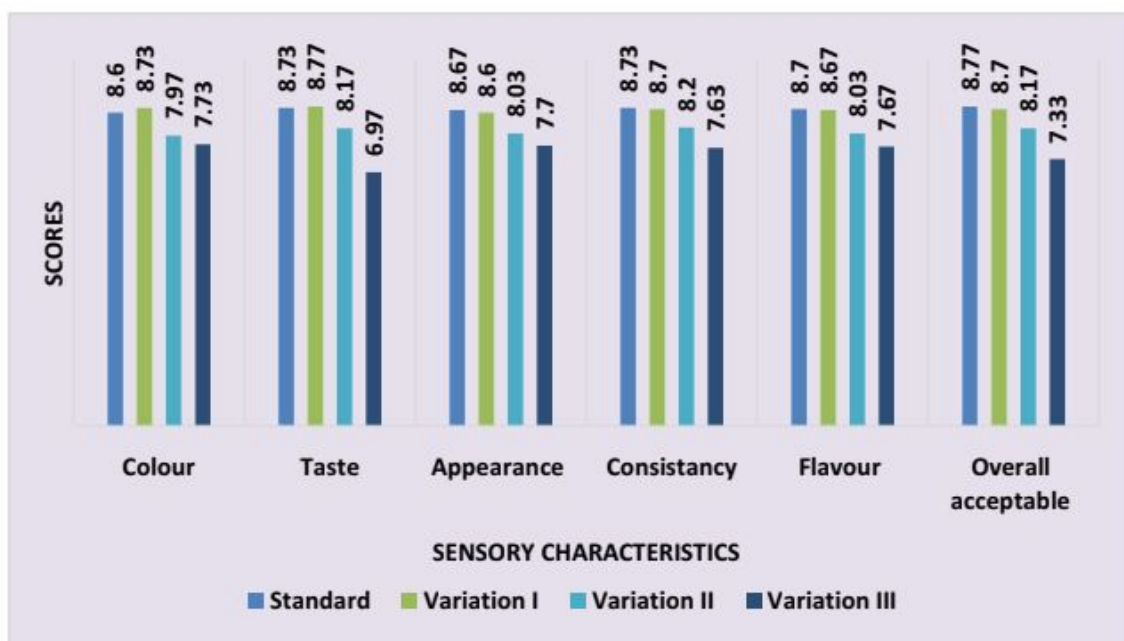


Figure 6

SENSORY EVALUATION OF HERBAL POWDER INCORPORATED CLEAR VEGETABLE SOUP

Incorporation of herbal powder in spicy mushroom soup

The sensory evaluation for the incorporated spicy vegetable soup was carried out with the help of semi-trained panel members. The scores given by the semi-trained panel members are calculated using statistical analysis. The mean and standard deviation for the sensory evaluation of Incorporation of herbal powder in spicy mushroom soup was calculated and it is mentioned in Table IX and Figure 7.

TABLE IX

**SENSORY EVALUATION OF HERBAL POWDER INCORPORATED
SPICY MUSHROOM SOUP**

Characters	Standard	Variation I	Variation II	Variation III
Colour	8.73 ± 0.45	8.50 ± 0.63	7.87 ± 0.86	7.73 ± 0.98
Taste	8.77 ± 0.43	8.70 ± 0.47	8.27 ± 0.83	8.07 ± 0.98
Appearance	8.60 ± 0.50	8.60 ± 0.56	8.13 ± 0.86	8.13 ± 0.86
Consistency	8.70 ± 0.47	8.60 ± 0.62	8.20 ± 0.85	8.10 ± 0.92
Flavour	8.67 ± 0.48	8.57 ± 0.63	8.10 ± 0.85	8.10 ± 0.85
Overall acceptance	8.70 ± 0.47	8.63 ± 0.62	7.80 ± 0.95	7.73 ± 0.98
T value		3.080	7.985	8.02
Significant		0.027 ^{**}	0.00 ^{NS}	0.00 ^{NS}

****** - Significant at 5% level, ^{NS} – Not significant

The sensory evaluation of incorporation of herbal powder in spicy mushroom soup was evaluated. The mean value obtained for color was 8.73 for standard, 8.50 for variation I, 7.87 for variation II and 7.73 for variation III. The taste obtained for standard was 8.77, variation I was 8.70, variation II was 8.27 and variation III was 8.07. The appearance for standard was 8.60, variation I was 8.60, variation II was 8.13 and variation III was 8.13. The consistency for incorporation of herbal powder in spicy mushroom soup was obtained as 8.70 for standard, 8.60 for variation I, 8.20 for variation II and 8.10 for variation III. The mean obtained for flavour was 8.67 for standard, 8.57 for variation I, 8.10 for variation II and 8.10 for variation III. The overall acceptance for the standard was 8.70, variation I was 8.63, variation II was 7.80 and variation III was 7.73.

The sensory evaluation of herbal powder in spicy mushroom soup variation I was much better in colour, taste, appearance, consistency, flavour and overall acceptance on comparing with variation II and III. The variation I was compared with standard. The variation I sensory evaluation value was slightly different from standard but variation I was better in appearance. Meanwhile, The variation II was better than variation III in all aspects.

The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The first comparison was carried out for standard and variation I. The 't' test value for standard vs Variation I was 3.080 and it was significant at 5% level. The comparison was carried out for standard and variation II. The 't' test value for standard vs Variation II was 7.985 and it was not significant. The 't' test value for standard vs Variation III was 8.02 and it was not significant.

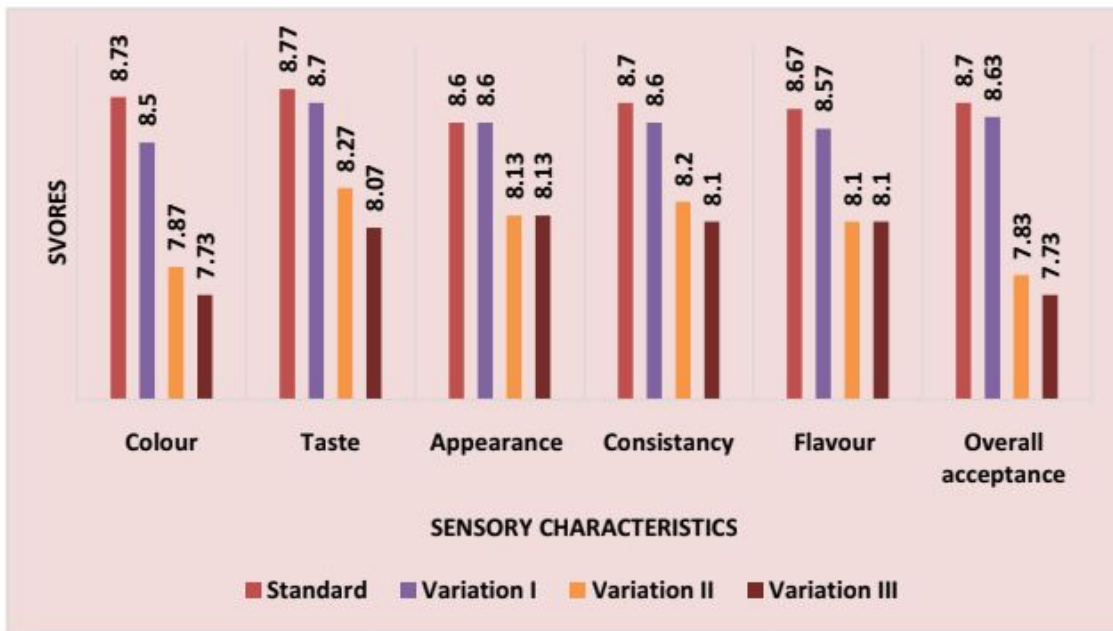


Figure 7

SENSORY EVALUATION OF HERBAL POWDER INCORPORATED SPICY MUSHROOM SOUP

Incorporation of herbal powder into tea

The standardized herbal powder was incorporated into tea. The sensory evaluation for the incorporated tea was carried out with the help of semi-trained panel members. The scores as given by the semi-trained panel members are calculated using statistical analysis. The mean and standard deviation for the sensory evaluation of Incorporation of herbal powder in tea was calculated and it is mentioned in Table X and Figure 8.

TABLE X**SENSORY EVALUATION OF HERBAL POWDER INCORPORATED TEA**

Characters	Standard	Variation I	Variation II	Variation III
Colour	8.83 ± 0.38	8.73 ± 0.38	8.33 ± 0.96	7.63 ± 0.99
Taste	8.83 ± 0.38	8.70 ± 0.46	7.73 ± 0.05	7.83 ± 0.99
Appearance	8.67 ± 0.48	8.57 ± 0.50	8.13 ± 0.94	7.90 ± 0.99
Consistency	8.83 ± 0.37	8.67 ± 0.48	8.23 ± 0.97	7.97 ± 0.96
Flavour	8.73 ± 0.45	8.57 ± 0.50	8.20 ± 0.99	8.03 ± 0.85
Overall acceptance	8.76 ± 0.43	8.63 ± 0.49	8.37 ± 0.89	7.53 ± 0.90
T test		11.867	4.075	10.606
Significant		0.00 ^{NS}	0.010 ^{**}	0.00 ^{NS}

** - Significant at 5% level, ^{NS} – Not significant

The sensory evaluation of incorporation of herbal powder in tea revealed that the mean values for color was 8.83 for standard, 8.73 for variation I, 8.33 for variation II and 7.63 for variation III. The taste obtained for standard was 8.83, variation I was 8.70, variation II was 7.73, and variation III was 7.83. The appearance for standard was 8.67, variation I was 8.57, variation II was 8.13 and variation III was 7.90. The consistency for incorporation of herbal powder in tea was obtained as 8.83 for standard, 8.67 for variation I, 8.23 for variation II and 7.97 for variation III. The mean obtained for flavour was 8.73 for standard, 8.57 for variation I, 8.20 for variation II and 8.03 for variation III. The overall acceptance for the standard was 8.76, variation I was 8.63, variation II was 8.37 and variation III was 7.53.

The sensory evaluation of herbal powder in tea, variation I was much better in colour, taste, appearance, consistency, flavour and overall acceptance on comparing with variation II and III. The variation I was compared with standard. The variation I sensory evaluation value was slightly different from standard but variation I was better in appearance. Meanwhile, The variation II was better than variation III in all aspects.

The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The first comparison was carried out for standard and variation I. The 't' test value for standard vs Variation I was 11.867 and it was not significant. The comparison was carried out for standard and variation II. The 't' test value for standard vs Variation II was 4.075 and it was significant at 5% level. The 't' test value for standard vs Variation III was 10.606 and it was not significant.

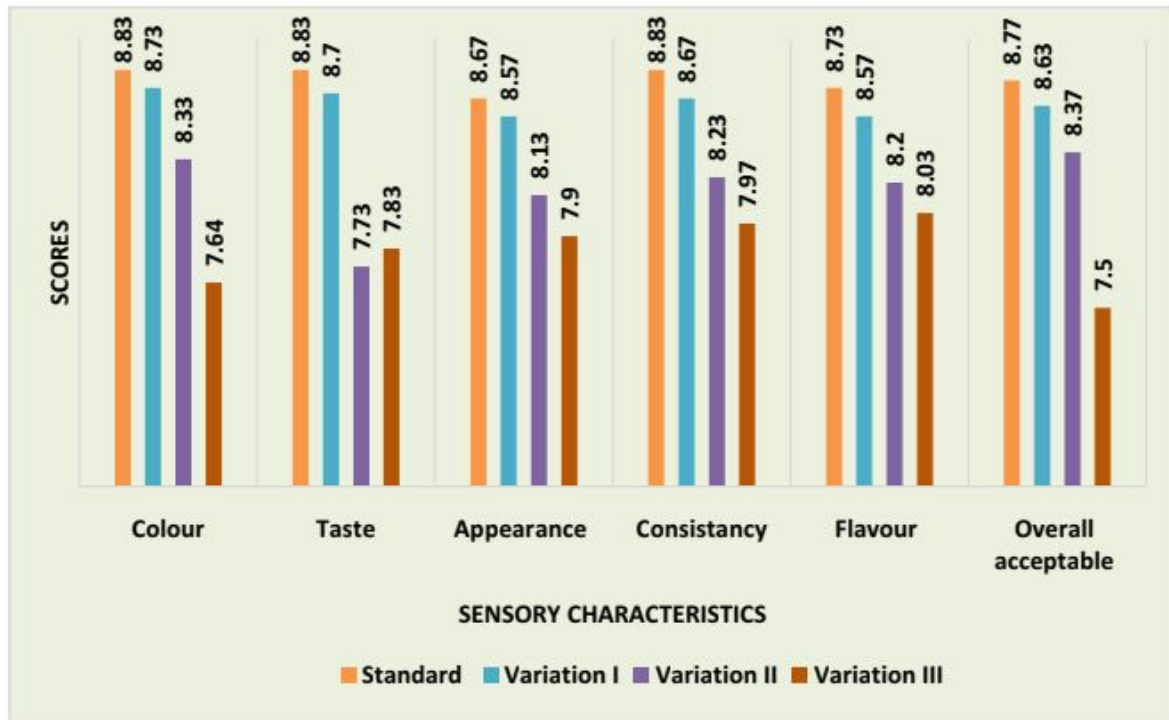


Figure 8

SENSORY EVALUATION OF HERBAL POWDER INCORPORATED TEA

Incorporation of herbal powder in coffee

The standardized herbal powder was incorporated into coffee. The sensory evaluation for the incorporated coffee with herbal powder was carried out with the help of semi-trained panel members. The scores are as given by the semi-trained panel members are calculated using statistical analysis. The mean and standard deviation for the sensory evaluation of Incorporation of herbal powder in coffee was calculated and it is mentioned in Table XI and Figure 9.

TABLE XI

SENSORY EVALUATION OF HERBAL POWDER INCORPORATED COFFEE

Characters	Standard	Variation I	Variation II	Variation III
Colour	8.76 ± 0.43	8.70 ± 0.47	7.90 ± 0.99	7.63 ± 0.96
Taste	8.73 ± 0.45	8.67 ± 0.48	8.63 ± 0.49	7.93 ± 0.98
Appearance	8.63 ± 0.49	8.57 ± 0.50	8.33 ± 0.66	8.07 ± 0.87
Consistency	8.63 ± 0.49	8.67 ± 0.48	8.10 ± 0.99	8.03 ± 0.93
Flavour	8.60 ± 0.50	8.57 ± 0.50	8.33 ± 0.72	8.03 ± 0.85
Overall acceptance	8.67 ± 0.48	8.63 ± 0.49	8.27 ± 0.64	7.60 ± 0.89
t test		2.206	3.824	7.50
Significant		0.078**	0.012**	0.001**

** - Significant at 5% level, ^{NS} – Not significant

The sensory evaluation of incorporation of herbal powder in coffee showed that the mean values for color was 8.76 for standard, 8.70 for variation I, 7.90 for variation II and 7.63 for variation III. The taste obtained for standard was 8.73, variation I was 8.67, variation II was 8.63, and variation III was 7.93. The appearance for standard was 8.63, variation I was 8.57, variation II was 8.33 and variation III was 8.07. The consistency for incorporation of herbal powder in coffee as obtained as 8.63 for standard, 8.67 for variation I, 8.10 for variation II and 8.03 for variation III. The mean obtained for flavour was 8.73 for standard, 8.57 for variation I, 8.20 for variation II and 8.03 for variation III. The overall acceptance for the standard was 8.67, variation I was 8.63, variation II was 8.27 and variation III was 7.60.

The sensory evaluation of herbal powder in tea, variation I was much better in colour, taste, appearance, consistency, flavour and overall acceptance on comparing with variation II and III. The variation I was compared with standard. The variation I sensory evaluation value was slightly different from standard but variation I was better in appearance. Meanwhile, the variation II was better than variation III in all aspects.

The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The first comparison was carried out for standard and variation I. The 't' test value for standard vs Variation I was 2.206 and it was significant at 5% level statistically. The comparison was carried out for standard and variation II. The 't' test value for standard vs Variation II was 3.824 and it was significant at 5% level statistically. The 't' test value for standard vs Variation III was 7.50 and it was significant at 5% level statistically.

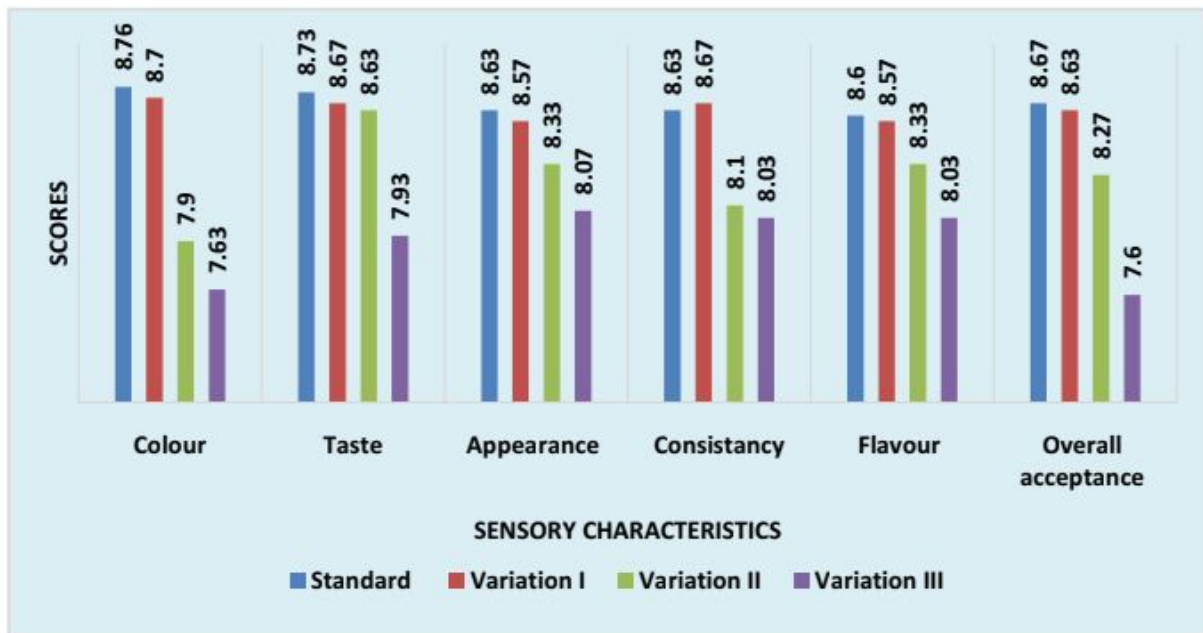


Figure 9

SENSORY EVALUATION OF HERBAL POWDER INCORPORATED COFFEE

It can be inferred from the sensory evaluation of the immune boosting herbal powder developed *i.e.* variation I was accepted by the panel members and was significant at 5% level statistically and variation I was rich in vitamin namely A, C, E, K and minerals namely calcium, magnesium and zinc. These vitamins and minerals enhance the immune system and it act as a immune booster for the body.

The estimation of vitamins like antioxidant vitamin A,B,C,E and K was analysed to know the presence of immune boosting compound in herbal powder. The vitamin A in the herbal powder was 5429.49 IU /100g. The vitamin B complex present in the herbal powder was 89.34 per mg/100g. The vitamin C present in the herbal powder was 125.54 per mg/100g. The tocopherols present in the herbal

powder was 233.70 per mg/100g. The vitamin K present in herbal powder was 2.12 per mg/100g.

The estimation of minerals revealed that calcium present in the herbal powder was 712.22 per mg/100g. The iron present in herbal powder was 0.16 per mg/100g. The estimation of magnesium is carried out and the presence of magnesium in herbal powder was 252.23 per mg/100g. The estimation of phosphorus was carried out and the presence of phosphorus in the herbal powder was 2.16 per mg/100g. The estimation of potassium reveals the presence of phosphorus in the herbal powder was 82.42 per mg/100g. The zinc present in the herbal powder was 15.29 per mg/100g.

The phytochemical screening carried out for the herbal powder revealed that tannins, flavanoid, phenols and glycosides are strongly present due to the presence of tulsi, clove and ashwagandha. Whereas, sponins and alkaloids was moderately present in herbal powder due to the presence of tulsi, ashwagandha and ginger. The phytochemicals like terpenoids and coumarins are weakly present in the herbal powder. The phytochemicals like sterols and quinones are absent in phytochemical screening of herbal powder.

The herbal powder is comparatively less toxic than $K_2Cr_2O_7$ which shows maximum lethality of shrimps at higher concentration. The shrimps in the sample is found to be very less toxic in lower as well as in higher concentration.

The sensory evaluation was carried out in food products namely soups (tomato, clear vegetable soup and spicy mushroom), coffee and tea. Among three different variations, variation I was much better in overall acceptance in all the food products. So 2g of herbal powder was much suitable for 200 ml of soups, 2g of herbal powder was much suitable for 125 ml of coffee and tea. But the variation II and III produces a strong extract effect on food products.

SUMMARY AND CONCLUSION

V.SUMMARY AND CONCLUSION

Medicinal plants are employed in modern medicine and traditional medicine with the goal of maintaining health, treating a specific illness, or doing both. Herbal medicine and dietary supplement items have been chastised for lacking adequate standards or scientific proof to back up their claims about its contents, safety, and efficacy. India has a significant role to play as a supplier of herbal products, not only to meet domestic needs, but also to capitalize on the enormous export potential. Herbal medicine is in significant demand in both developed and developing countries, such as India, because of its broad biological activity, higher safety margins than synthetic medications, and lower costs.

Herbal therapy, in general, must be transferred into modern medication research and clinical trials. The associated issues of quality, safety, and efficacy are all linked to integrated herbal medicine. Both safety (toxicity) and efficacy are hampered by the current lack of standardized quality in herbal products. Few plants, such as ashwagandha, clove, tulsi, and ginger, have strong immune-boosting properties. This research is focused on four primary herbs that aid in improving immune function. Herbs now play a significant role in everyday life. Herbal powders are also simple to use.

Herbs have an important part in keeping people healthy and improving their quality of life. People are focusing more on immunity booster items during the current pandemic. People are looking for items that can help them rehydrate and strengthen their immunity in order to combat the pandemic and stay safe and healthy. The present study entitled "**Formulation and Incorporation of Immune Boosting Herbal Powder**" was aimed at evaluation of vitamins, minerals, phytochemicals and toxic substances present in immune boosting herbal powder.

The herbs identified for development and standardization of immune boosting herbal powder was ginger, clove, tulsi and ashwagandha. Three variations using different concentrations of selected herbs was developed and standardized. The 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 of the recipes by rating from like extremely to dislike extremely in the 9-point hedonic scale rating.

The analysis of vitamins in the herbal powder was carried out namely vitamin A, B, C, E, and K was carried out using standard procedures. The estimation of minerals in the herbal powder namely calcium, iron, magnesium, phosphorus, potassium and zinc was analysed. The qualitative analysis of phytochemical present in herbal powder such as tannins, terpenoids, phenols, saponins, quinones, glycosides, coumarins, sterols, flavonoids and alkaloids was analysed in aqueous solution. Toxicity study was carried out in Bharat Ratna Prof. CNR Rao Research Centre, Avinashilingam Institute for Home Science and Higher education for Women - Coimbatore.

The salient findings of the study are summarized below:

- ✓ The **sensory evaluation** of herbal infusion the color was 8.83 for standard, 8.73 for variation I, 8.07 for variation II and 8.40 for variation III. The taste obtained for standard was 8.83, variation I was 8.77, variation II was 8.23 and variation III was 8.62. The mean value obtained for appearance for standard was 8.83, variation I was 8.60, variation II was 8.07 and variation III was 8.20. The consistency for herbal infusion was obtained as 8.67 for standard, 8.70 for variation I, 8.23 for variation II and 8.50 for variation III. The mean obtained for flavour was 8.73 for standard, 8.67 for variation I, 8.07 for variation II and 8.13 for variation III. The overall acceptance for the standard was 8.77, variation I was 8.70, variation II was 8.17 and variation III was 8.37. The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The 't' test value for standard vs Variation I was 3.051 and it was significant at 5% level. The 't' test value for standard vs Variation II was 14.351 and it was not significant. But was most accepted by the panelists. The 't' test value for standard vs Variation III was 5.720 and it was statistically significant at 5% level.
- ✓ The vitamins and minerals analysis and phytochemical was analysed for the best acceptable variation. It was observed from sensory analysis that **variation I** was best accepted and hence all the tests were carried out for variation I.
- ✓ The estimation of **vitamins** like antioxidant vitamin A, B, C, E and K was analysed to know the presence of immune boosting compound in herbal powder. The vitamin A in the herbal powder was 5429.49 IU /100g. The

vitamin B complex present in the herbal powder was 89.34 per mg/100g. The vitamin C present in the herbal powder was 125.54 per mg/100g. The tocopherols present in the herbal powder was 233.70 per mg/100g. The vitamin K present in herbal powder was 2.12 per mg/100g.

- ✓ The estimation of **minerals** like calcium, iron, magnesium, phosphorus, potassium and zinc was analysed to understand the presence of trace minerals in the herbal powder. The calcium present in the herbal powder was 712.22 per mg/100g. The iron present in herbal powder was 0.16 per mg/100g. The estimation of magnesium is carried out and the presence of magnesium in herbal powder was 252.23 per mg/100g. The estimation of phosphorus was carried out and the presence of phosphorus in the herbal powder was 2.16 per mg/100g. The estimation of potassium reveals the presence of phosphorus in the herbal powder was 82.42 per mg/100g. The zinc present in the herbal powder was 15.29 per mg/100g.
- ✓ The **phytochemical** screening carried out for the herbal powder revealed that tannins, flavanoid, phenols and glycosides are strongly present due to the presence of tulsi, clove and ashwagandha. Whereas, sponins and alkaloids was moderately present in herbal powder due to the presence of tulsi, ashwagandha and ginger. The phytochemicals like terpenoids and coumarins are weakly present in the herbal powder. The phytochemicals like sterols and quinones are absent in phytochemical screening of herbal powder.
- ✓ The herbal powder is comparatively **less toxic** than $K_2Cr_2O_7$ which shows maximum lethality of shrimps at higher concentration. The shrimps in the sample is found to be very less toxic in lower as well as in higher concentration.
- ✓ The developed immune boosting herbal powder was incorporated into recipes to find out the suitability and acceptability of the herbal powder. Hence recipes namely tomato soup, clear vegetable soup and spicy mushroom soup, tea and coffee was identified for incorporation of immune boosting herbal powder and the acceptability was evaluated.
- ✓ The sensory evaluation of incorporation of herbal powder in **tomato soup** reveals that the mean score for color was 8.77 for standard, 8.60 for variation I, 8.20 for variation II and 7.73 for variation III. The taste obtained for standard was 8.70, variation I was 8.73, variation II was 8.37 and variation III was

8.27. The appearance for standard was 8.63, variation I was 8.67, variation II was 8.20 and variation III was 8.31. The consistency for incorporated herbal powder in tomato soup was obtained as 8.67 for standard, 8.70 for variation I, 8.23 for variation II and 8.50 for variation III. The mean obtained for flavour was 8.73 for standard, 8.70 for variation I, 8.23 for variation II and 8.10 for variation III. The overall acceptance for the standard was 8.77, variation I was 8.70, variation II was 8.30 and variation III was 7.83. The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The 't' test value for standard vs Variation I was 1.147 and it was significant at 5%. The 't' test value for standard vs Variation III was 5.811 and it was significant at 5% level.

- ✓ The sensory evaluation of incorporation of herbal powder in **clear vegetable soup** showed that the mean value for color was 8.60 for standard, 8.73 for variation I, 7.97 for variation II and 7.73 for variation III. The taste obtained for standard was 8.73, variation I was 8.77, variation II was 8.12 and variation III was 6.97. The appearance for standard was 8.67, variation I was 8.60, variation II was 8.03 and variation III was 7.70. The consistency for incorporation of herbal powder in clear vegetable soup was obtained as 8.73 for standard, 8.70 for variation I, 8.20 for variation II and 7.63 for variation III. The mean obtained for flavour was 8.70 for standard, 8.67 for variation I, 8.03 for variation II and 7.67 for variation III. The overall acceptance for the standard was 8.77, variation I was 8.70, variation II was 8.17 and variation III was 7.30. The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The 't' test value for standard vs Variation I was 0.158 and it was significant at 5% statistically. The 't' test value for standard vs Variation II was 31.556 and it was not significant. The 't' test value for standard vs Variation III was 8.577 and it was not significant.
- ✓ The sensory evaluation of incorporation of herbal powder in **spicy mushroom soup**. The mean value obtained for color was 8.73 for standard, 8.50 for variation I, 7.87 for variation II and 7.73 for variation III. The taste obtained for standard was 8.77, variation I was 8.70, variation II was 8.27 and variation III was 8.07. The appearance for standard was 8.60, variation I was 8.60, variation II was 8.13 and variation III was 8.13. The consistency for incorporation of herbal powder in spicy mushroom soup was obtained as 8.70

for standard, 8.60 for variation I, 8.20 for variation II and 8.10 for variation III. The mean obtained for flavour was 8.67 for standard, 8.57 for variation I, 8.10 for variation II and 8.10 for variation III. The overall acceptance for the standard was 8.70, variation I was 8.63, variation II was 7.80 and variation III was 7.73. The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The 't' test value for standard vs Variation I was 3.080 and it was significant at 5% level. The comparison was carried out for standard and variation II. The 't' test value for standard vs Variation II was 7.985 and it was not significant. The 't' test value for standard vs Variation III was 8.02 and it was not significant.

- ✓ The sensory evaluation of incorporation of herbal powder in **tea** revealed that the mean values for color was 8.83 for standard, 8.73 for variation I, 8.33 for variation II and 7.63 for variation III. The taste obtained for standard was 8.83, variation I was 8.70, variation II was 7.73, and variation III was 7.83. The appearance for standard was 8.67, variation I was 8.57, variation II was 8.13 and variation III was 7.90. The consistency for incorporation of herbal powder in tea was obtained as 8.83 for standard, 8.67 for variation I, 8.23 for variation II and 7.97 for variation III. The mean obtained for flavour was 8.73 for standard, 8.57 for variation I, 8.20 for variation II and 8.03 for variation III. The overall acceptance for the standard was 8.76, variation I was 8.63, variation II was 8.37 and variation III was 7.53. The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The 't' test value for standard vs Variation I was 11.867 and it was not significant. The 't' test value for standard vs Variation II was 4.075 and it was significant at 5% level. The 't' test value for standard vs Variation III was 10.606 and it was not significant.
- ✓ The sensory evaluation of incorporation of herbal powder in **coffee** showed that the mean values for color was 8.76 for standard, 8.70 for variation I, 7.90 for variation II and 7.63 for variation III. The taste obtained for standard was 8.73, variation I was 8.67, variation II was 8.63, and variation III was 7.93. The appearance for standard was 8.63, variation I was 8.57, variation II was 8.33 and variation III was 8.07. The consistency for incorporation of herbal powder in coffee as obtained as 8.63 for standard, 8.67 for variation I, 8.10 for variation II and 8.03 for variation III. The mean obtained for flavour was 8.73 for standard, 8.57 for variation I, 8.20 for variation II and 8.03 for variation III.

The overall acceptance for the standard was 8.67, variation I was 8.63, variation II was 8.27 and variation III was 7.60. The one sample paired 't' test was carried out to compare the standard and variation I, II and III. The 't' test value for standard vs Variation I was 2.206 and it was significant at 5% level statistically. The 't' test value for standard vs Variation II was 3.824 and it was significant at 5% level statistically. The 't' test value for standard vs Variation III was 7.50 and it was significant at 5% level statistically.

- ✓ The sensory evaluation was carried out in food products namely soups (tomato, clear vegetable soup and spicy mushroom), coffee and tea. Among three different variations, **variation I** was much better in overall acceptances in all the food products. So **2g of immune boosting herbal powder was much accepted for 200 ml of soup, 2g of herbal powder was much suitable for 125 ml of coffee and tea.** But the variation II produces strong extract effect on food products and variation III produces strong extract effect on food products.

It can be inferred from the sensory evaluation of the immune boosting herbal powder developed *i.e.* variation I was accepted by the panel members and was significant at 5% level statistically and variation I was rich in vitamin namely A, C, E, K and minerals namely calcium, magnesium and zinc. These vitamins and minerals enhance the immune system and it act as an immune booster for the body. It is rich in phytochemicals like tannins, flavanoid, phenols and glycosides due to the presence of tulsi, clove and ashwagandha. The toxicity of the herbal powder is comparatively less toxic. The shrimps in the sample is found to be very less toxic in lower as well as in higher concentration. During the sensory evaluation when incorporated into soups, tea and coffee, variation I was much better in overall acceptances in all the food products. So 2g of herbal powder was much suitable for 200 ml of soups, 2g of herbal powder was much suitable for 125 ml of coffee and tea.

Hence the developed immune boosting herbal powder would help to boost the immunity of the community particularly during post covid period. It can be consumed by all age groups. The developed immune boosting herbal powder can be consumed as such or by incorporating into other recipes. The powder do not have any side

effects and the herbs selected are easily available in the local markets and few in home kitchen garden too.

Recommendations for further research:

- ✓ Incorporate herbal powder into different food products
- ✓ Identification of other immune boosting herbs
- ✓ Herbal capsule can be prepared for easy consumption
- ✓ Study the effect of herbal powder for disease conditions

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BIBLIOGRAPHY

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APPENDIX

APPENDIX – I
ETHICAL CLEARANCE

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.Sudha Ramalingam
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.Vaishna Devi.M
Department of Food Science and Nutrition
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear Vaishna Devi.M,

Ref: Your proposal No. IHEC/21-22/FSN-28 entitled
"Formulation of Immune Boosting Herbal Powders" resubmitted for
approval to IHEC on 02.03.2021.

The Institutional Human Ethics Committee of our University
hereby grants approval to your research proposal No. IHEC/21-22/
FSN-28 entitled "Formulation of Immune Boosting Herbal Powders"
resubmitted by you. The Approval number for the same is
AUW/IHEC/FSN-21-22/XPD-28.

We wish you all the best in your research endeavours.

Regards,

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



APPENDIX – II

SCORE CARD

SENSORY EVALUATION OF HERBAL INFUSION

Name	
Class	
Date and sign	

CHARACTERS	STANDARD	VARIATION I	VARIATION II	VARIATION III
APPEARANCE				
COLOUR				
TEXTURE				
FLAVOUR				
TASTE				
OVERALL ACCEPTANCE				
TOTAL SCORE				
AVERAGE				

Hedonic scale -

9- like extremely, 8- like very much, 7- like moderately, 6- Like slightly,

5- Neither like nor dislike, 4- dislike slightly, 3- dislike moderately, 2- dislike very much, 1 dislike extremely.

APPENDIX – III

SCORE CARD

(SENSORY EVALUATION OF HERBAL POWDER INCORPORATED INTO FOOD PRODUCTS)

Name	
Class	
Date and sign	

Product – I (Tomato soup)

CHARACTERS	STANDARD	VARIATION I	VARIATION II	VARIATION III
APPEARANCE				
COLOUR				
TEXTURE				
FLAVOUR				
TASTE				
OVERALL ACCEPTANCE				
TOTAL SCORE				
AVERAGE				

Product – II (Clear vegetable soup)

CHARACTERS	STANDARD	VARIATION I	VARIATION II	VARIATION III
APPEARANCE				
COLOUR				
TEXTURE				
FLAVOUR				
TASTE				
OVERALL ACCEPTANCE				
TOTAL SCORE				
AVERAGE				

Product – III (spicy mushroom soup)

CHARACTERS	STANDARD	VARIATION I	VARIATION II	VARIATION III
APPEARANCE				
COLOUR				
TEXTURE				
FLAVOUR				
TASTE				
OVERALL ACCEPTANCE				
TOTAL SCORE				
AVERAGE				

Product – IV (Tea)

CHARACTERS	STANDARD	VARIATION I	VARIATION II	VARIATION III
APPEARANCE				
COLOUR				
TEXTURE				
FLAVOUR				
TASTE				
OVERALL ACCEPTANCE				
TOTAL SCORE				
AVERAGE				

Product – V (coffee)

CHARACTERS	STANDARD	VARIATION I	VARIATION II	VARIATION III
APPEARANCE				
COLOUR				
TEXTURE				
FLAVOUR				
TASTE				
OVERALL ACCEPTANCE				
TOTAL SCORE				
AVERAGE				

Hedonic scale -

9- like extremely, 8- like very much, 7- like moderately, 6- Like slightly,
5- Neither like nor dislike, 4- dislike slightly, 3- dislike moderately, 2- dislike very much, 1 dislike extremely.

APPENDIX – IV

TOXICITY ANALYSIS



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

Bharat Ratna Prof.CNR Rao Research Centre

Results of Brine Shrimp Lethality Assay

Number of Samples submitted: 1

Sample Code – Toxicity A

Preparation of Samples

The given sample (Toxicity A) was weighed and dissolved in water to get 1mg/ml stock solution. The sample (Toxicity A) of different volume 250, 500, 1000, 1500, 2000 μ l is added to each beaker containing saline solution.

Procedure

- 🔦 30 shrimps \longrightarrow 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.

N= 30.

S. No	Sample Code	Concentration (µg/ml)	Mortality of Brine shrimp (no. of shrimps dead) (h)					% Mortality (at 24h)
			1	2	4	6	24	
1.	Toxicity A	250	0	0	2	2	8	27
		500	0	0	3	3	9	30
		1000	0	0	2	3	11	37
		1500	0	0	0	1	11	37
		2000	0	0	0	1	11	37
2.	Control $K_2Cr_2O_7$	1 (mg/ml)	30	-	-	-	-	100
3.	Blank	Saline water	0	0	0	0	0	0

Attached are the images of sample (Toxicity A) and the shrimps present in it respectively.

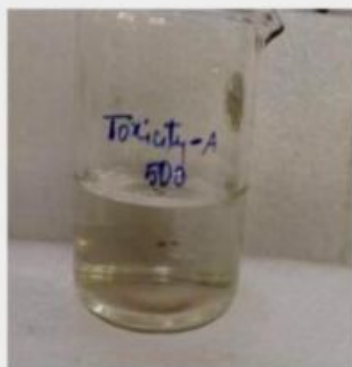


Fig. 1

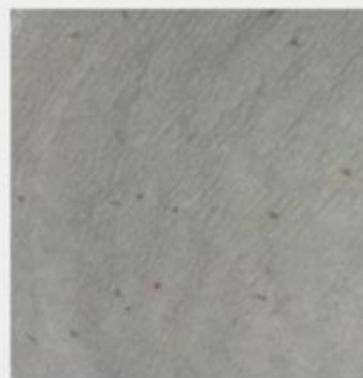
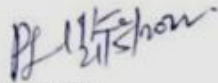


Fig. 1 a

- ✚ The sample Toxicity A is comparatively less toxic than $K_2Cr_2O_7$ which shows maximum lethality of shrimps at higher concentration.
- ✚ The shrimps in the sample is found to be very less toxic in lower as well as in higher concentration.
- ✚ Even after 24h, only 8- 11 shrimps are found to be mortal at lowest and highest concentration.

The results of Brine Shrimp Lethality Assay of sample submitted by M. Vaishna Devi, Food Science and Nutrition of our Institution are given in the above table and photographs.

Attested by



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