T.K.M. College of Arts and Science
Kollam- 5
Department of Biochemistry

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STUDY ON SELECTED NUTRITIONAL PARAMETERS IN *MENTHA SPICATA*

PROJECT REPORT

Dissertation to the University of Kerala in partial fulfilment of the requirement for the award of the Degree of Bachelor of Science in Biochemistry.

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Phytochemical Screening and Antioxidant Activities of aqueous extract of *Mentha spicata*

DECLARATION

We hereby declare that the project titled **'Phytochemical Screening and Antioxidant Activities of aqueous extract of *Mentha spicata'*** is based on the original work carried out by us under the supervision of Ms Soumya S, Assistant Professor, Department of Biochemistry, T.K.M. College of Arts and Science, Kollam.

We also declare that the project report hasn't been submitted either partly or completely for the award of any other degree or diploma or other similar titles of any other University/Institution in India or aboard

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CERTIFICATE

This is to certify that the dissertation entitled **'Phytochemical Screening and Antioxidant Activities of aqueous extract of *Mentha spicata*'** submitted to the University of Kerala in partial fulfilment of the requirements for the award of the degree of bachelor of science in biochemistry, is a record of original research work carried out by the following candidates under my guidance and supervision.

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It is also certified that no part thereof has been presented for the award of any other degree or diploma or other similar titles of any other university.

Ms Soumya S

Project guide

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CONTENTS

1. INTRODUCTION

Ever since ancient times, people looked for drugs in nature in search of rescue from their diseases. In the beginning, medicinal plants use was instinctive as in the case with animals (Stojanoski N,1999). Plant-derived compounds have played an important role in treating and preventing human diseases.

Medicinal plants are an important source of life-saving drugs for humans, especially in developing countries. More than 80% of the world's population in developing countries depend primarily on herbal medicine for basic health care. The issue is associated with the use of synthetic drugs, antibiotics, and the renewed interest in the use of plant-based drugs. Researchers on medicinal plants and their traditional medicinal plant use have increased in different regions of the world over the past few decades. It is important to document indigenous traditional knowledge through ethnobotanical studies to conserve and utilise biological resources. Plants can be used in their original or advanced form. Numerous biologically active substances are known to contain medicinal plants that have been isolated from plants and applied based on ethnobotanical expertise and approved drugs from medicinal plants (Carney et al., 1999).

Plant synthesize a vast range of organic compounds that are tragically classified as primary and secondary metabolites. Primary metabolites are compounds like lipids, amino acids, phytosterols, and organic acids. They have an essential role associated with photosynthesis, respiration, growth, and development. Secondary metabolites are mainly flavonoids, phenolic compounds, terpenoids, nitrogen-containing alkaloids, and Sulphur containing compounds. They are of interest because of their use as dyes, fibres, ailments, flavouring agents, drugs, and perfumes and they are viewed as potential sources and new natural drugs, antibiotics, insecticides, and herbicides (Croteau et al., 2000; DeWitt 2002).

Herbs are plants valued for their medicinal and aromatic properties and are often grown and harvested for these unique properties. In most parts of the world, herbs are grown mainly as field crops or on the scale as catch crops among vegetables (Brown D, 1999). Herbs are used for flavouring food, culinary preparation, perfumery, cosmetics, beauty, and body care (peter,2001). Herbs are rich in volatile oil which gives a pleasant aroma. They also have alkaloids and glycosides which have great pharmaceutical effects. Essential oils have been extensively investigated for their activity against several fungi, plant and human pathogens, bacteria, and harmful microorganisms (kalimba and Juneja,2003).

Mentha, a member of the Labiatae family, originates from eastern Asia and there are two major forms, *Mentha piperita* (peppermint) and *Mentha spicata* Labiatae (spearmint). The historical use of Mentha is not different from its use in modern herbal medicine. Mentha can be used for common cold, cough, sinusitis, fever, bronchitis, nausea, vomiting, indigestion, loss of appetite, and intestinal colic (Starbuck,2001).

Spearmint is a species of mint native to North Africa, Egypt, and Morocco. Spearmint is a common constituent of the Indian eastern Asia diet. It is a perennial herb and is grown commercially worldwide. Fresh and dried leaves from spearmint are used to make teas and aromatic agents (Alishtahyeh et al., 2019). Commonly, the fresh leaves are used as raw vegetables or flavouring herbs, whereas the dried leaves are traditionally used for herbal tea or medicine. Mint possesses several biological effects, including antioxidant, anti-inflammatory, anti-cancer, and antimicrobial activities (Tang et al.,2016; Zaia et al., 2016; Wang et al., 2018). The biological activities of spearmint are significantly correlated with their total phenol flavonoid content (Manosroi et al.,2006; Santos et al., 2014)

Mentha spicata is used to treat gastrointestinal, respiratory, bad breath, carminative, anti-spasmodic, diuretic, and sedative agents (Mabboubi, 2021). Spearmint leaves are used to strengthen the stomach and are helpful for symptoms of dyspepsia (Bahaeian et al., 2015). Spearmint oil is a flavouring agent used in the preparation of chewing gum, cosmetics, and toothpaste (Mahboubi,2021).

1.1 LOCAL NAME

- Garden mint
- Common mint
- Lamb mint
- Mackerel mint
- Our lady's mint
- Spearmint
- Sage of Bethlehem
- Field mint/wild mint
- Japanese mint

1.2 BOTANICAL CLASSIFICATION

Kingdom	Plantae
Phylum	Magnoliophyte
Class	Magnoliopsida
Subclass	Asteridae
Order	Lamiales
Family	Lamiaceae
Genus	*Mentha*
Species	*spicata*

Fig no 1: spearmint

Fig no 2: whole plant of spearmint

1.3 BOTANICAL DESCRIPTION

Mints are hardy perennials which spread by underground runners. They may become troublesome weeds in the garden if not tended and controlled, months thrive in semi-shade and rich moist soil. All mints have a square stem with simple leaves growing in opposite pairs. Spearmint leaves are about two inches long, bright green, oblong or lance-shaped, veined, and somewhat wrinkled with unevenly toothed margins. The upper leaves are sessile and the lower leaves have a short stalk. The herb is unbranched and grows in thick clumps in moist areas along roadsides, near streams, and in low meadows and pastures where it may reach a height of two to three feet. The flowers form in a cluster in the leaf axils at the tip of the purple or green stem taping nearly to a point. One or more flowering stems flank the central spike. Blooms are a pale to deep violet colour and bloom in July and August. The small tubular flower each have two long and two short stamens. the brown seeds are tiny and round (Taylor, Suzi,2002)

Spearmint contains volatile oil, flavonoid thymosin, caffeic acid derivatives, rosmaric acid carvone, and limonene. Spearmint is a distinctive pungent aroma that is attributed to the primary constituent of the volatile oil, the chemical carvone (Imai et al., 2001)

According to the united states department of agriculture (USDA), spearmint is regarded as a massive weed only in Tennessee and other parts of the south. However, in the northern plain's states and parts of the mid-west, spearmint was raised as a cash crop. It is presently on the list of the fifty top cash crops in the united states.

Fig no 3: Flower of spearmint

Fig no 4: Leaves of spearmint

1.4 GEOGRAPHICAL DISTRIBUTION

Spearmint and peppermint are cultivated commercially for use in flavourings and medicines. They are also cultivated in gardens throughout much of the world as food herbs and medicinal herbs (Lawrence 2006, Flores 2008, Abbaszadeh 2009). In North America, spearmint grows in moist to wet disturbed areas, aquatic sites, stream banks, swamps, ditches, and meadows (Klinkenberg 2010, Ling 2010).

Native and current distribution: Spearmint is native to the Balkan Peninsula and Turkey, and it has been naturalized throughout much of Europe, the Mediterranean region, and Southwest Asia (Kokkini and Vokou 1989, Flores 2008). It has been introduced to North America, Japan, Australia, and New Zealand (Mito and Uesugi 2004, GBIF New Zealand 2010, USDA 2010, Western Australian Herbarium 2010). Spearmint grows in all states of the U.S. except North Dakota, and it is present throughout much of Canada. Peppermint grows in 44 states of the U.S. (USDA 2010). Neither spearmint nor peppermint has been documented from arctic regions. Spearmint has been documented in Alaska's Pacific Maritime and Interior Boreal ecogeographic regions (Hulten 1968, UAM 2010). Peppermint has escaped from cultivation in Alaska, but no specific locations are known (Hulten 1968).

1.5 CHEMICAL COMPOSITION

The composition of the essential oil of M. spectra is presented in the table below. Thirty-four compounds were identified, representing 99.9% of the total compounds. The oil contains 50.6% oxygenated monoterpenes, 45.1% monoterpene hydrocarbons, and 2.8% of sesquiterpene hydrocarbons. The main constituents were carvone (40.8% ± 1.23%) and limonene (20.8% ± 1.12%), followed by 1,8-cineole (17.0% ± 0.60%), β-pinene (2.2% ± 0.25%), cis-dihydrochalcone (1.9% ± 0.49%), and dihydrocarveol (1.7% ± 0.31%). The oil yield of this Tunisian variety of spearmint was 1.1% and it can be described as the carvone/limonene chemotype. There is a large variation in the chemical composition of M. spectra, wild as well as cultivated, around the world. Indeed, a series of chemotypes have been described in previous studies, with the prevalence of pulegone, carvone, linalool, piperine, piperine oxide, menthone/iso menthone, pulegone/menthone/iso menthone, and pulegone/piperine (Baser K.H.C. et al.,1999)

1.6 EDIBLE USES

In terms of food uses, spearmint is used in the food, confectionery, and chewing gum industries (Abo-zeid et al.,1992). Spearmint contributes to food preservation (Seherer R et al.,2013) and imparts food tastes and aroma. Spearmint is used in Iran as a flavouring agent in food products such as cheese and dough (Songh R et al.,2015). Besides, spearmint is added in Indian and Italian cuisine either in fresh or dried form, to fish and shellfish plates before or after cooking (Kizil S et al.,2010) owing to the antioxidant, antiradical and chelating properties possesses the incorporation of spearmint in food can help people to maintain the equilibrium of redox status in the organism as well as to improve safety and effect on human well-being (Snoussi M et al.,2015). Spearmint has been used broadly in cosmetics and soap (Igoumenids et al.,2016) as well as a toothpaste breath freshener, and antiseptic mouth rinse.

1.7 MEDICINAL USES

Spearmint has established that a wide variety of traditional medications is used in different cultures. Spearmint has been used in traditional Iranian medicine since ancient times for the management of diarrhoea, antidote, indigestion, intestinal weakness, abdominal pain, cold, influenza, sinusitis headache, and flatulence. Traditional Iranian medical practitioners have recommended that M. spicata leaves are used to treat digestive disorders and flatulence (Babaeian et al., 2015).

Both fresh and dried spearmint plants are widely used in a variety of applications (Lawrence B M et al.,2006). Since ancient times, both western and eastern cultures have practised spearmint as medical and aromatic plants (Park KJ et al.,2002). In terms of biological uses, spearmint acts as insecticides (Samarth RM and Kumar A et al.,2003), antispasmodics, and antiplatelets (Tognolini M et al., 2006). Moreover, spearmint is used as an antimicrobial (Sulieman AME et al.,2011) and antioxidant agent (Mata AT et al.,2007).

In terms of medical uses, spearmint is considered a herbal medicine in folkloric remedies for treating colds and flu, respiratory tract problems, gastralgia, haemorrhoids, and stomach aches (Tetik et al.,2013; Asekun et al.,2007; Kanatt S et al.,2007). Spearmint is extracted in the form of oil and is regularly used in medicine (Abu-zeid et al.,1992; Bensabah et al., 2013), states that spearmint leaves are generally taken as a tea in which it is carminative properties can help to treat digestive disorders fever and minor ailments (Peter KV et al.,2006).

1.8 PHARMACOLOGICAL PROPERTIES

1.8.1 Antimicrobial Activity

Mentha acts as a counterirritant and analgesic with the ability to reduce pain and improve blood flow to the affected area. For these reasons mint, essential oils are well studied due to their antibacterial activities against both Gram-negative and Gram-positive ones and can be useful as a substitute for some antibiotics and combat the antimicrobial bacterial resistance (Horvath P, Koscova J et al.,2017).

Mint essential oil contains phenolic compounds such as α-pinene, citronellol and methyl eugenol, which have antimicrobial activity against a code range of microorganisms and antioxidant activity. For these reasons, MEOs are widely used as food additives and in pharmaceutical industries, because they are considered potent film addictive that helps in preventing lipid oxidation and microbial spoilage of foods (Akhter R et al., 2019). Addiction to mint essential oil (MEO) into gelatin-based edible films with effective inhibition of microbial growth on the film surface (Scartazzini L et al., 2019). Moreover, MEOs are also used both in agriculture to fight bacterial and fungal diseases (Githaiga BM et al., 2018) and to give other examples and in aquaculture as an additive in fish food to increase immune defences, but also as sedative and anaesthetic for farmed fish (Aydin B and Barbas Lal et al., 2020)

MEOs have antibacterial effects against a wide range of pathogenic microorganisms in humans, fish and vegetables also. MEO's antibacterial activity is linked to their chemical composition, rich in pulegone, menthone, menthol, carvone, 1,8 cineol limonene and β caryophyllene and phenolic compounds also such as α-pene, citronellol and methyl eugenol. The most used method to test the antimicrobial activity of essential oils is the disc diffusion method. The determination of minimum inhibitory concentration (MIC) and the vapour phase method (Sartoratto A et al., 2004). The use of MEOs and tin general of essential oils is very important because being natural substances and therefore easily biodegradable, it could be a promising alternative to synthetic material to fight the increasingly common bacterial infections (Aydin B and Barbas Lal et al., 2020).

1.8.2 Antioxidant Activity

Essential oils are liquid mixtures of volatile compounds that are commonly collected through steam distillation of aromatic plants. Essential oils have been reported for their beneficial biological functions including antiviral, antimicrobial, anti-inflammatory as well as antioxidant effects (Baydar NG et al., 2004; Dundar Eet al., 2008; Liu Et al.,2012).

Mentha (mint) is a genus of an aromatic perennial herb belonging to the Lamiaceae family (Arzani et al., 2007). The two species of spearmints, 'Scotch' spearmint and 'Native' spearmint are among the most important crops in essential oil production worldwide and have been widely used as flavour food, toothpaste, pharmaceuticals and cosmetics (Bensabab F et al., 2013). Owing to the abundant content of phenolic compounds (Riachi L. G, De Maria CA et al., 2015 ad Naidu JR et al., 2012), aqueous extracts and essential oils from mint plants are potential natural antioxidants (Dorman H J et al., 2003 and Zheljazkov V.Det al., 2010). However, many studies that have assessed the antioxidant activities of mint essential oils have exclusively relied on chemical-based assays where the effectiveness of mint essential oils in the prevention of oxidative stress at the cellular level or in a living organism has not been characterised. Spearmint is a temperate plant and requires long days for the deposition of essential oil constituents. Therefore, geographical site and leaf maturity at harvest considerably affect the chemical composition of mint essential oils and their bioactivities including antioxidant capacity (Buleandra M et al., 2016 Hussain AI et al 2010 and Chauhan R S et al., 2008). A wide range of carvone content was reported for essentials oils of spearmint harvested from 26 sites in the Northwest Himalayan region of India (Chauhan RS et al.,2008). In essential oil, it is believed that some unsaturated terpenes, monocyclic, terpenes and monoterpenes contribute to antioxidant activity (Clark RJ et al., 1984)

Antioxidants are natural chemical compounds found in plants that help protect against and repair damage caused by free radicals, which are harmful molecules that can lead to oxidative stress. Oxidative strength has been linked to several chronic conditions including heart disease, cancer, and diabetes (Buleandra M et al., 2016 Hussain AI et al., 2010). Spearmint contains a large number of antioxidant compounds, including rosmarinic acid, and flavones like limonene and menthol (Mata AT et al., 2007). Two tablespoons (11 grams) of spearmint also provides 2% of the Reference Daily Intake (RDI) for vitamin C, another potent antioxidant (Arzani A et al., 2007).

According to research, spearmint shows excellent antioxidant activity against free radicals. In one study, extract from this herb prevented fat oxidation in meat was as effective as the synthetic antioxidant BHT (Singh R et al., 2015)

1.8.3 Anti-Inflammatory Activity

Spearmint essential oil (SEO) is rich in carvone and limonene and has a refreshing aroma in comparison with peppermint essential oil (PEO) is perceived to be a milder option for tropical application in individuals with sensitive skin. This could be one of the reasons for the increased popularity of SEO in skincare beauty and healthcare products. Studies in various models have demonstrated its therapeutic potential which includes antiproliferative, antibacterial, antifungal, anticonvulsant and antiemetic activities (Fitsiou E et al.,2016 Tayarani Najarian et al 2013). However, studies on the effects of SEO on human skin cells are scarce. Although the anti-inflammatory properties of limonene a major active component of SEO have been demonstrated in both pre-clinical and clinical models to our knowledge. It is investigated the biological activity of a commercially available SEO in a validated human dermal fibroblast cell culture lone designed to model the pathology of chronic inflammation (Kunkel EJ, Dea M, Ebens A, Hytopoulos E Melrose J et al., 2004 and Bergamini G, Bell K Shomamura S Kelener T Cansfoelel et al., 2012). It is analysed that the impact of SEO on 17 important protein biomarkers is closely related to the inflammatory and tissue remodelling pathways.

SEO showed robust antiproliferative effects in diseased human skin cells and significantly inhibited the increased production of two pro-inflammatory biomarkers such as Vascular Cell Adhesion Molecule I (VCAM I) and Interferon Inducible T cell, a chemoattractant (ITAC). SEO significantly modulated global gene expression and altered signalling pathways many of which are critical in the inflammatory and tissue remodelling processes (Kunkel EJ, Dea M, Ebens A, Hytopoulos E, Metrose J et al., 2004 and Bergamin G Bell K Shinamur S Werner T Canfield A et al.,2012). SEO was a promising candidate for use in anti-inflammatory skin products (De Sousa DP, Camargo EA, Oliveira FS, De Almeida et al.,2010).

1.8.4 Insecticidal Activity

Mint is also known to exhibit insecticidal activity against a wide variety of insects. Mint has been used as insecticide mainly in the form of essential oils (Kumar et al.,2011). *Mentha spicata* oil demonstrated insecticidal properties against adults of Rhyzopertha dominance, in contact and fumigation bioassays and repelling (Brahmi et al.,2016).

1.8.5 Cytotoxicity

Several studies have indicated that Mentha plants contain constituents with cytotoxic properties that may find use in developing anticancer agents. *Mentha spicata* methanolic and aqueous extracts showed anti-proliferative effects against various cancer cell lines in-vitro (Sharma et al.,2014). Spearmint and peppermint methanolic extract significantly inhibited colon cancer cell growth (Yi and Ywetztein et al.,2011)

2. REVIEW OF LITERATURE

Medicinal plants are useful for healing as well as for curing human diseases because of the presence of phytochemical constituents (Nostro A, Germand MP, et al.,2000). Phytochemicals are naturally occurring in medicinal plants, leaves, vegetables and roots that have defence mechanisms and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids and phenolic compounds (Krishnaian D et al.,2007). Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, and anti-viral antibacterial activities (Mahato SB, Sen S et al.,1997). Terpenoids are very important in attracting useful mites and consuming herbivorous insects (Kappers IF et al(2005) et al., 2013). Alkaloids are used as anaesthetic agents and are found in medicinal plants (HérouartD et al.,1988). The Momordica charantia belongs to the Cucurbitaceae family and it has common names such as bitter melon, karela and bitter gourd. More than a thousand herbal products of Momordica charantia are used for the treatment of diabetic patients and are also helpful in lowering glucose levels in the blood (Marles RJ et al.,1995). The bioactive constituents are present in Momordica charantia that is charantosides, momordin and goyaglycosides. It also includes terpenoids constituents such as momordicinin, momordenol, momordicin-28, momordicilin and momordol(Begum S et al.,1997). Morus nigra is the botanical name of the mulberry and it belongs to the family Moraceae. Mulberries have shown various biological properties such as anti-inflammatory activities (Kim Ha et al., 1999). Guava is the common name of the Psidium guajava and it belongs to the family Myrtaceae. Its phytochemical study shows that its extracts have more than twenty compounds (Osman AM et al., 1974). Prunus persica belongs to the Rosaceae family. It is used as a medicinal plant in African countries and this medicinal plant has shown strong anti-fungal activities (Caccini DRU Tonini G et al.,2002). Pomegranate is the common name of the Punica granatum (PG) and belongs to the family Lythraceae. It has much medical significance and used as medicine for centuries (Orak HH et al.,2011).

Recent studies have investigated that pomegranates are used for the treatment of several diseases e.g., diabetes, dysentery, diarrhoea, cough, asthma, bleeding disorders, bronchitis, fever, AIDS, inflammation, ulcers, malaria, prostate cancer, hypertension, atherosclerosis, hyperlipidemia, male infertility, infant brain ischemia and obesity. Fagonia cretica (Zygophyllaceae) is one of the plants which are locally used in Pakistan as a cure for snakebite (Pannwar AQ et al., 2007). Acacia nilotica is a member of the Leguminosae family. The subfamily

of the Acacia nilotica is Mimosoideae (Arena JPM et al.,1983). Luffa cylindrica is the botanical name of the sponge gourds and belongs to the Cucurbitaceae family. The fruits of this plant have flat seeds and are black which are enclosed by a group of fibres (Stephens JM et al.,2003). Medicinal and nutritional properties are the characteristics of Luffa cylindrical and seeds of this plant are used for curing asthma, fever and sinusitis (Sashikala GO et al., 2009). Morus alba is included in the Moraceae family. Their leaves and fruits are used for curing prematurely grey hair. Its root bark is used by humans for more than 4 thousand years (Yogisha S et al., 2009). Ficus palmate is included in the family of Moraceae and is used as a dry vegetable. It is an herbaceous perennial plant. Its leaves have hypotensive actions (Ayinde BE et al., 2007).

Medicinal plants are the richest source of raw materials for the synthesis of traditional as well as modern medicine all over the world. But unfortunately, still, their scientific role has not been identified in a large number of medicinal plants (Tsakala O et al.,2006). The knowledge of medicinal plants has been possessed from generation to generation by ancient people and new knowledge added to it by the next generation. Gradually, a group of people have been trying continuously each generation to collect medicinal plants and use them for the treatment of various types of diseases but unfortunately, many of them had not been explored scientifically. The disease is controlled by pharmacotherapy. It is observed that the many phytoconstituents present in the plant which have most essential because most drugs have been synthesized (Ghani A et al.,1990). Such Phytochemicals are plant-derived chemical constitutes that are not essential nutrients but have important properties such as protective or preventive diseases properties (Ahmed F et al., 2009). Phytochemicals are antioxidants (Wong SK et al.,2009), antibacterial antifungal (Khan M, Wasstlew SW et al.,1987), anti-inflammatory, and antidiabetic (Singh N et al.,2007). People still have been facing the problem that few drugs are scarce, expensive common for a man so the study of medicinal plants is most important, scientifically and identification of these plants for the treatment of various types of disease. It is possible to increase the formation of less costly and effective plants derived drugs from plants materials (Zamble A et al.,2006).\

In recent years, plant metabolomics has been successfully applied to metabolite profiling of different extract samples, thus bridging the knowledge gap between plant genotypes and phenotypes (De Vos et al., 2007; Creydt and Fischer, 2017). Progress in plant metabolomics techniques has made it possible to detect several hundred metabolites and to reliably compare differences and similarities between samples. Current metabolomics techniques utilize mass spectrometry (MS) coupled to gas or liquid chromatography, which achieves rapid metabolite analysis and has the advantages of allowing for high throughput and effective identification. Moreover, high-

performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry (HPLC-QTOF-MS) using electrospray ionization is a particularly well accepted platform for untargeted metabolite profiling in plant extracts (Su et al., 2018). In addition, to providing a comprehensive chemical profile of plants, chemometric methods are used for the classification and identification of metabolites and to define similarities and differences among varieties (Lubes and Goodarzi, 2017). There is, however, very little information reported on the metabolite differences between fresh and dried spearmint leaves. Besides, some scientific evidence indicates that several biochemical modifications and inter-conversions may occur during processing steps (Martins et al., 2016). Especially in traditional Chinese medicine, drying is considered to be the crucial step due to limiting enzymatic degradation and microbial growth while preserving the active principle content. Post-harvest fresh plant materials would exhibit a series of anti-dehydration mechanisms including production of related secondary metabolites at the early stage of dehydration (Li et al., 2012; Yuan et al., 2015). Given the beneficial health effects of spearmint, more detailed study of its phytochemical composition is worthwhile. The current study presents an untargeted comparative metabolomics approach utilizing HPLC-QTOF-MS high-throughput analytical technology together with principal component analysis (PCA) to provide insights into the effects of the drying process on the examined spearmint species.

Although a range of bioactive compounds in herbs and spices has been studied for antioxidant and anti-inflammatory properties *in vitro* and *in vivo* animal models, the present challenge lies in integrating this knowledge to ascertain whether these effects can be observed in humans. Since Huh et al. (2010) first described a lung-on-a-chip at the Wyss Institute, various organ-on-a-chip models have been developed to mimic the microenvironment and basic functions of tissues, and have become a novel drug screening platform. Those models yield reliable predictions and can be used to address problems that cannot ethically be investigated in humans, and for which available animal models have poor homology with humans. However, to date, there have been few reports on bioanalysis of active components from natural products through organ-on-a-chip systems. In this study, a kidney-on-a-chip was developed to investigate the nephrotoxicity of kaempferol, a bioactive metabolite from spearmint. Microfluidic techniques were introduced to mimic the continuous transport of nutrients and waste to and from the chip. To obtain a native extracellular matrix and functional tissue-tissue interfaces, GelMA, a product of gelatin methacrylate (Yue et al., 2015), was used for cell encapsulation, owing to its advantages of allowing for three-dimensional (3D) culturing and direct real-time visualization of cell conditions.

3. OBJECTIVES

- Collection, and authentication of the plant *Mentha spicata*.
- Preparation of aqueous extract of the shade dried plant.
- To carry out the Phytochemical Screening of the aqueous extract of *Mentha spicata.*
- To determine the total antioxidant activity of the aqueous extract.

4. MATERIALS AND METHODS

4.1 Collection of *Mentha Spicata.*

Mentha spicata plants were collected from Kollam, Kerala.

4.2 Extraction

The collected plant was dried in shade and after a week dried material was made as fine powder by mechanical grinding. About 100mg of the powdered leaf was mixed in 100ml of distilled water and subjected to 48 hours of occasional stirring at room temperature. After two days the extract was filtered with a cotton cloth and preserved in n refrigerator for future usage.

4.3 Reagents

1. **Mayer's reagent**: is an alkaloidal precipitating reagent used for the detection of alkaloids in natural products. Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and potassium iodide (5.00 g) in water (100.0 ml).
2. **Wagner's Reagent:** 2.5 gm iodine is dissolved in 12.5 gm of potassium iodide (KI 2); add 250 ml of water to produce a solution.
3. **Dragendroff reagent:** DR is a solution of potassium bismuth iodide composed of basic bismuth nitrate ($Bi(NO_3)_3$), tartaric acid, and potassium iodide (KI), and when contact with alkaloids DR produces an orange or orange-red precipitate
4. **Lead Acetate Solution:** It is prepared by dissolving 40g Pb (CH3COO)2·3H2O in water, adding 0.5 mL CH3COOH, and diluting to 100 mL.
5. **NaOH solution:** To make 1 M NaOH solution, you have to dissolve 40.00 g of sodium hydroxide pellets in 250 mL distilled water and then make up the solution to 1 litre.
6. **Ferric Chloride Solution:**

   By dissolving iron ore in HCl (hydrochloric acid)

   $$Fe_3O_4 + 8HCl \rightarrow FeCl_2 + 2FeCl_3 + 4H_2O$$

   By oxidizing iron (II) chloride with chlorine (Cl) $2\,FeCl_2 + Cl_2 \rightarrow 2\,FeCl_3$ by oxidizing iron (II) chloride with oxygen.
7. **Potassium dichromate reagent:** It is produced industrially by reacting potassium chloride (KCl) with sodium dichromate ($K_2Cr_2O_7$).
8. **Sodium nitroprusside reagent:** Diluted preparation is stable for 24 hours at room temperature (must be protected from light).

4.4 Phytochemical Screening Tests

4.4.1 Test for Alkaloids

a) **Mayer's test:** To 1ml of extract added 2ml of Mayer's reagent (Potassium Mercuric Iodide). The formation of a cream coloured precipitate indicated the presence of alkaloids.

b) **Wagner's test**: To 1ml of extract added 2ml of Wagner's reagent (Iodine in potassium iodide). The formation of brown/ reddish precipitate indicates the presence of alkaloids.

c) **Dragendroff's test:** To 1ml of extract added 1 ml of Dragengroff's reagent (solution of potassium bismuth iodide) was added. The formation of an orange-red precipitate indicates the presence of alkaloids.

4.4.2 Test for Flavonoids

a) **Lead acetate test:** Extract was treated with a few drops of sodium hydroxide solution. The formation of intense yellow colour, which becomes colourless with the addition of dilute acid, indicated the presence of flavonoids.

b) The extract was treated with sodium hydroxide, the formation of yellow colour indicated the presence of flavonoids.

c) Few drops of 1% ammonia were added to 1 ml of sample, the formation of yellow colour indicated the presence of flavonoids.

4.4.3 Test for Phenolic Compounds and Tannins

a) 1 ml of the test solution was mixed with basic lead acetate solution and the formation of white precipitate indicated the presence of tannins and phenolic compounds.

b) To 1ml of the extract, ferric chloride solution was added. The formation of a dark blue or greenish-black colour product confirmed the presence of phenolic compounds and tannins.

c) Strong potassium dichromate solution was added to the test extract, yellow colour precipitate confirmed the presence of tannin and phenolic compounds.

4.4.4 Test for Glycosides

a) **Legal's test:** 1 ml of the extract was treated with sodium nitroprusside in pyridine and sodium hydroxide. The formation of pink to the blood-red colour indicated the presence of cardiac glycosides.

b) 5ml of aqueous extract was mixed with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution carefully added to 1 ml of concentrated Sulfuric acid (The concentrated sulphuric acid is seen underneath the mixture). The formation of the brown ring indicates the presence of glycosides.

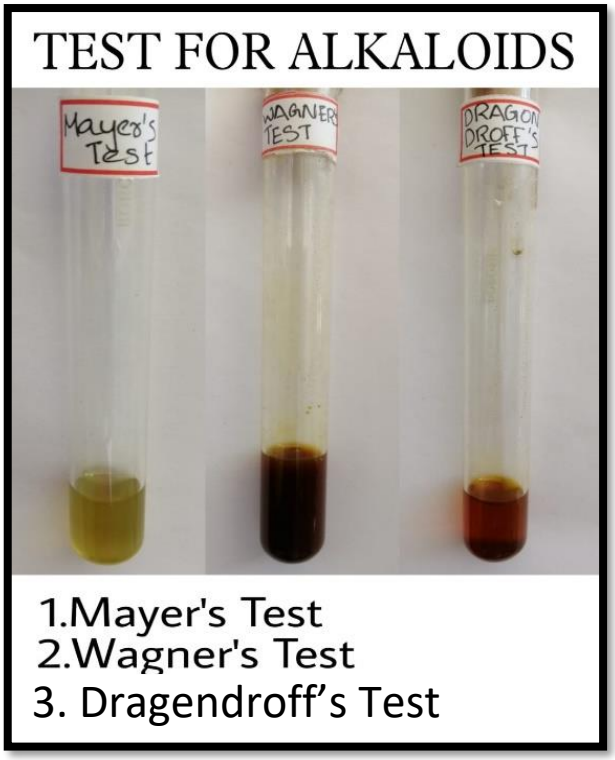
4.4.5 Test for Saponins

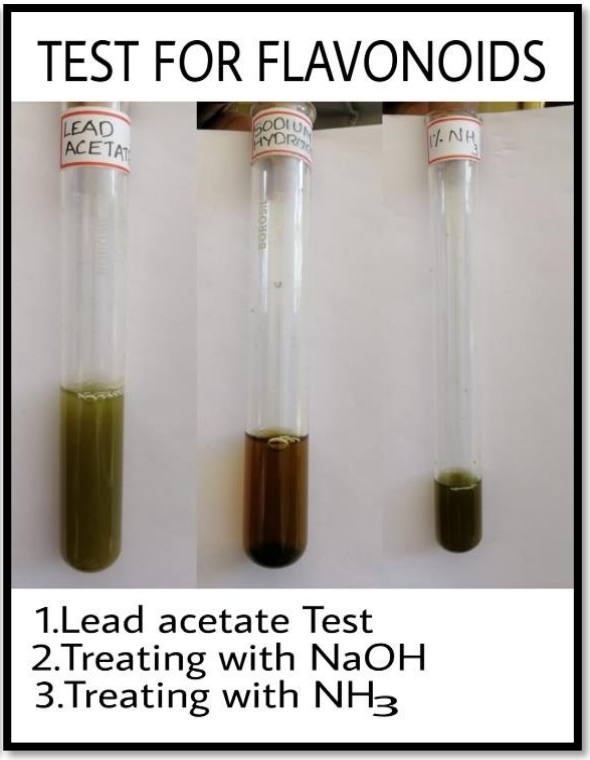
b) **Froth test:** 1ml of the extract was diluted to 20 ml with water and was shaken in a graduated cylinder for 15 min. Ring indicated the presence of cardiac glycosides.

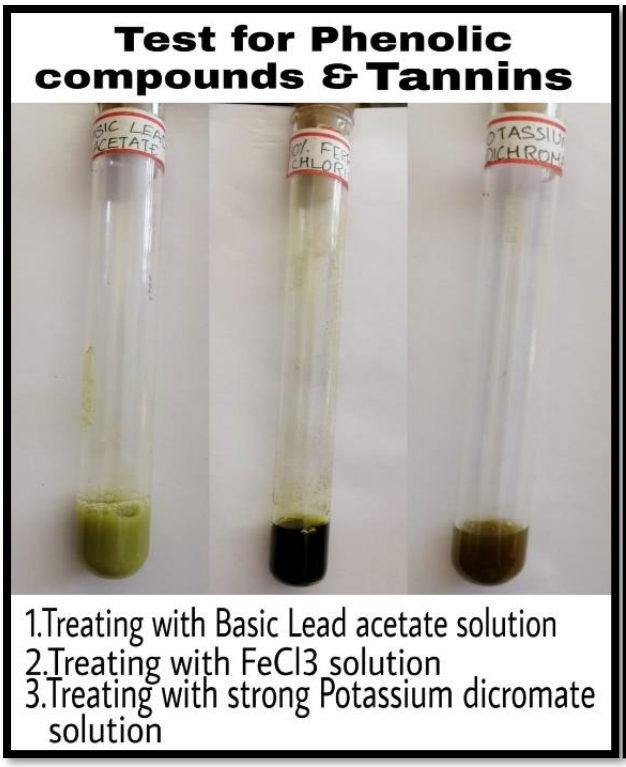
c) **Foam test:** 0.5g of extract was shaken with 2ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

4.4.6 Test for Terpenoids

a) 5ml of the extract was added with 2ml of chloroform and 3ml of concentrated Sulfuric acid was added along the sides of all test tubes. The appearance of a brownish-red colour indicates the presence of terpenoids.


TEST FOR ALKALOIDS
Mayer's Test
WAGNER TEST
DRAGON DROFF'S TEST
1.Mayer's Test
2.Wagner's Test
3. Dragendroff's Test

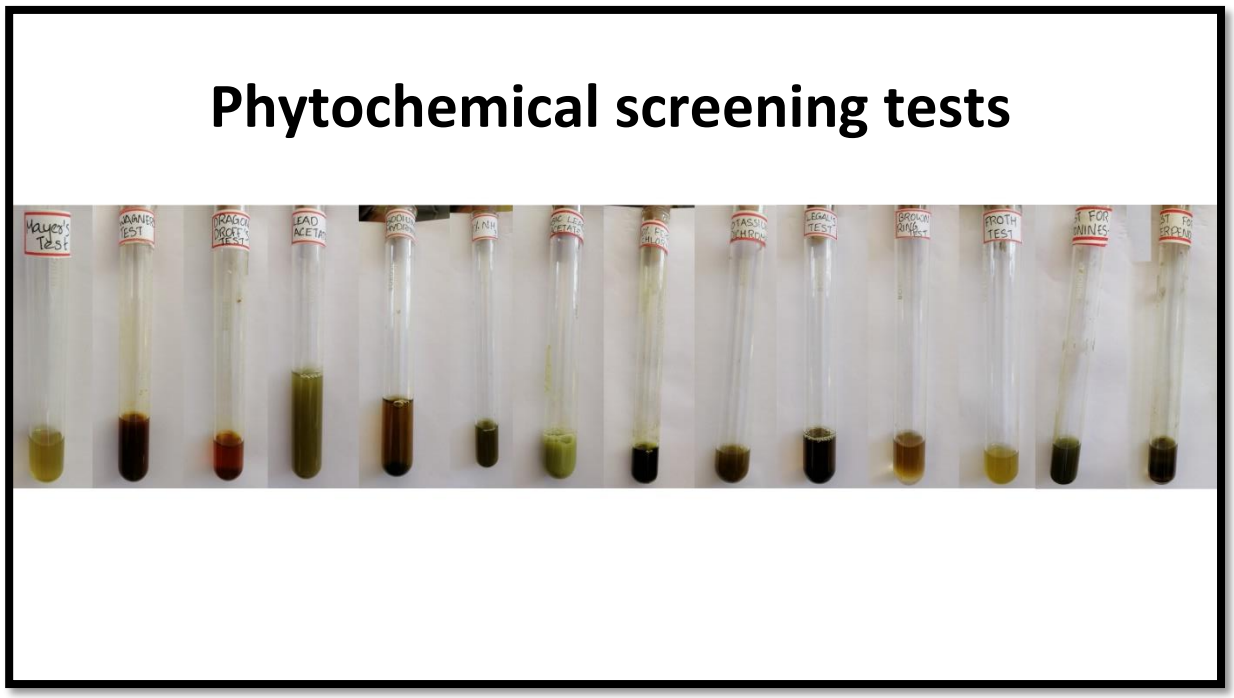

TEST FOR FLAVONOIDS
LEAD ACETA
1.Lead acetate Test
2.Treating with NaOH
3.Treating with NH3


Test for Phenolic compounds & Tannins
1.Treating with Basic Lead acetate solution
2.Treating with FeCl3 solution
3.Treating with strong Potassium dicromate solution


Test for Glycosides
LEGAL'S TEST
BROWN RING TEST
1.Legal's Test
2.Brown ring Test


Test for Saponins
FROTH TEST
Froth Test
[1 & 2]


Test for Triterpenoids & Steroids
1.Treating with Chloroform & Conc. H2SO4


Phytochemical screening tests

5. RESULTS AND DISCUSSION

The powdered leaves of *Mentha spicata* were extracted with water. The resultant cold extracted aqueous solution was subjected to screening of various phytochemical constituents. The table shown below indicates the presence and absence of various phytoconstituents in the aqueous extracts of *Mentha spicata* leaves. It revealed the presence of alkaloids, flavonoids, phenols, and terpenoids.

Table no:1- Phytoconstituents of the aqueous extract of *Mentha spicata*.

SL.NO	NAME OF TEST	RESULT
1.	Test for alkaloids	+ +
2.	Test for flavonoids	+ +
3.	Test for phenolic compounds and tannins	+ +
4.	Test for glycosides	-
5.	Test for saponins	-
6.	Test for terpenoids	++

+ + Indicates the Presence of Phytoconstituents;

-Indicates the Absence of Phytoconstituents

6. DETERMINATION OF TOTAL PHENOLIC CONTENT

The total phenolic content in plant extract was determined by using the colorimeteric method. Folin' Ciocalteau reagent and Na_2CO_3 are required for this method. Gallic acid was taken as standard. In an acid medium, the phenolic compounds react with sodium tungstate and sodium molybdate in the Folin's reagent to form blue colour, which was read at 650 nm.

5 test tubes marked S_1 to S_5 were taken and 0.2-1 ml of standard solution were added. All the tubes were made up of 1 ml of distilled water. Blank was prepared by taking 1ml of distilled water. 0.4 ml of Folin's Ciocalteau reagent was added to the test tubes and mixed well. All the tubes were kept at room temperature for 5 minutes. Then 4 ml of sodium carbonate was added. 100 mg sample is grinded with 10 ml distilled water it is then centrifuged at 2000rpm for 10 minutes. 1ml supernatant w made up to 5 ml with distilled water. 0.5 ml of this solution was taken as a test.

A standard graph was plotted by taking the concentration of the standard (gallic acid) on the X-axis and optical density along the Y-axis. From the graph, the total phenolic content was determined.

Table no 2: Standard graph valves of Phenolic Content

Sl no	The concentration of the standard (µg)	Optical density at 650nm
B	0	0
S_1	8	0.18
S_2	16	0.34
S_3	24	0.58
S_4	32	0.63
S_5	40	0.8
T_1	-	0.49
T_2	-	0.5

Fig no 5: Calibration Curve of Gallic Acid

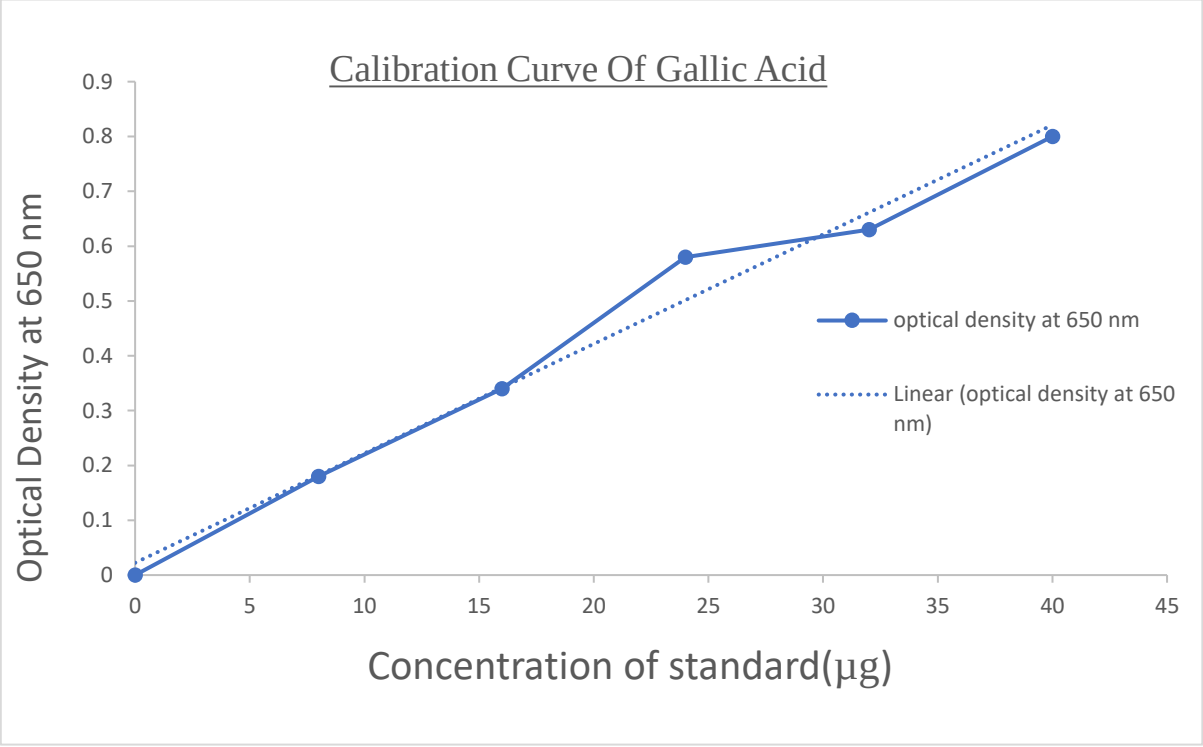


Table no 3: Test valves of phenolic content

Plant extract	OD valves	Gallic acid equivalence per ml of extract
0.5	0.5	48±0.73 µg/ml

Total phenolic content in the plant extract of Mentha spicata = 48±0.73 µg/ml

9. ESTIMATION OF TOTAL ANTIOXIDANT ACTIVITY

The antioxidant activity of the extract was calculated by the phosphomolybdenum method. The assay is based on the reduction of Mo (IV) to Mo(V) by the extract and subsequent formation of a green colour phosphate Mo(V) complex at acidic P^H. 5 test tubes marked S_1 to S_5 were taken and 0.2-1 ml of standard solution were added. The phosphomolybdenum reagent was prepared by mixing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Blank was prepared by 0.1 g of plant sample ground in 10ml distilled water. Centrifuge at 2000rpm for 10 minutes. Then 1 ml stock diluted to 5ml distilled water. From this, 0.5 ml was taken as a test. The sample was incubated at 95ºC for 90 minutes. The absorbance of the solution was measured at 690 nm against blank after cooling.

A standard graph was plotted by taking the concentration of ascorbic acid on the X-axis, and optical density on the Y-axis. From the graph total antioxidant activity was determined.

Table no 4: Standard graph valves of Total Antioxidant Activity

Sl no	The concentration of the standard (µg)	Optical density at 690nm
B	0	0
S_1	8	0.11
S_2	16	0.20
S_3	24	0.31
S_4	32	0.44
S_5	40	0.55

Fig no: 2 Calibration Curve of Ascorbic Acid

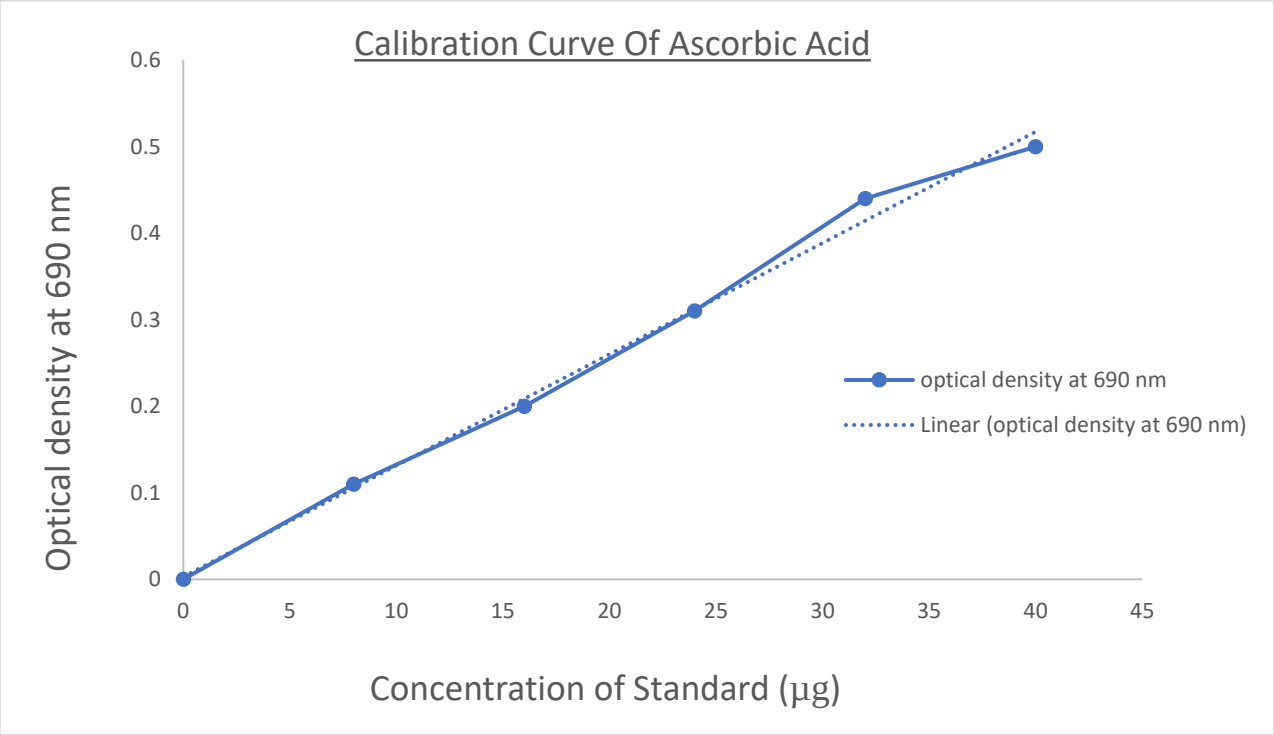


Table no 5: Test valves of Antioxidant Activity

Plant extract	OD valves	Ascorbic acid equivalence per ml of extract
0.5	0.20	16.2±0.37 µg/ml

Total antioxidant content in the plant extract of *Mentha spicata* =16.2±0.37 µg/ml

10. SUMMARY AND CONCLUSION

Phytochemicals are chemical compounds produced by plants. Due to the presence of active phytochemical compounds in plants, they are a potent source of human health. Phytochemical compounds are responsible for their various pharmacological activities. Based on the results obtained, many phytochemicals compounds like alkaloids, flavonoids, phenols, and terpenoids are detected in the leaves of *Mentha spicata*. These phytochemicals are responsible for antioxidant, anti-inflammatory, antimicrobial, insecticidal and cytotoxic activities of *Mentha spicata.* Due to quality and safety concerns, studies on the toxicological effects of various parts of *Mentha spicata* should be conducted. Bioactive compounds present in *Mentha spicata* make them useful for treating different ailments and have the potential of providing useful drugs for human use. Therefore, further work aims toward the isolation and purification of the active components present in mint and tracing out its pharmacological activities.

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