Phytochemical Analysis, Antioxidant And Antimicrobial Activity of *Piper nigrum, Myristica fragrans*, and *Cinnamomum zeylanicum.*

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DECLARATION

I hereby declare that the data presented in this Dissertation entitled, **"Phytochemical Analysis, Antioxidant And Antimicrobial Activity of *Piper nigrum*, *Myristica fragrans*, and *Cinnamomum zeylanicum*"** is based on the results of investigations carried out by me in the **Botany Discipline** at the **School of Biological Sciences and Biotechnology, Goa University** under the supervision of **Dr. Rupali Bhandari** and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the Dissertation.

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Date: 10/04/2023

Place: Goa University

CERTIFICATE

This is to certify that the dissertation report "**Phytochemical Analysis, Antioxidant And Antimicrobial Activity of *Piper nigrum*, *Myristica fragrans*, and *Cinnamomum zeylanicum***" is a bonafide work carried out by **Mr. Pandurang Dharma Bhagat** under my supervision in partial fulfilment of the requirements for the award of the degree of **Master's Degree of Science** in the **Discipline of Botany** at the **School of Biological Sciences and Biotechnology, Goa University.**

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ABSTRACT

Piper nigrum, Myristica fragrans, and *Cinnamomum zeylanicum* are the most important spices used historically for household purposes and possess antioxidant activity. The ethanolic, methanolic, and acetone extract with the concentration of 0.25, 0.5, and 1.0 μg /mL leaves of *Piper nigrum, Myristica fragrans,* and *Cinnamomum zeylanicum.* The extracts were used to study the total phenolic content, total flavonoid content, antioxidant activity, antibacterial activity, and antifungal activities. The TPC and TFC were highest in the acetone extract of *Cinnamomum zeylanicum,* followed by *Piper nigrum* and *Myristica fragrans*. Various extracts at different concentrations showed antibacterial activity. However, the acetonic extract at 1 mg/mL of *Piper nigrum*, *Myristica fragrans,* and *Cinnamomum zeylanicum* showed the highest antibacterial activity against the gram-positive and gram-negative bacteria, *Bacillus cereus* and *Escherichia coli* in comparison to other extracts. Similar results were observed in antifungal activity against the fungus *Aspergillus niger.*

The antioxidant activity studied for the various concentration showed a positive result; however, the maximum activity was studied in the acetone extract of *Piper nigrum*, *Myristica fragrans,* and *Cinnamomum zeylanicum.* The study provides a promising approach that the leaves of *Piper nigrum, Myristica fragrans,* and *Cinnamomum zeylanicum* are a source of phenolic compounds with natural antioxidant and antibacterial activities.

1. INTRODUCTION

The relationship between man and nature has always been close; from his earliest beginnings, man has known the plant world's advantages and exploited them to feed himself, heal himself, and survive. Man's first knowledge of plants was passed down through the generations. Our nation is rich and diverse in medicinal plant species due to its geographic position and environment. Several herbal guides that explained how to employ medicinal plants for healing were written during the Middle Ages. The earliest civilisations were aware of several plants, and for thousands of years, people used them. Also, modern research has validated its efficacy in managing various disorders. Many medicinal plants were known to the ancient Chinese, Egyptians, Indians, Greeks, Romans, and Old Slavs, wherein other countries also benefited from the transfer of that information.

Many plant species employed in herbalism are included under the umbrella term "medicinal plant" ("herbology" or "herbal medicine"). It involves both the study of and the use of plants for therapeutic purposes. The Roman word "herba" and the old French word "herbe" are the origins of the word "herb." Today, the term "herb" is used to describe any component of a plant, including the fruit, seed, stem, bark, flower, leaf, stigma, or root of a non-woody plant. Before, only non-woody plants, such as those from trees and bushes, were called "herbs."

Furthermore, these plants are also used in food, flavonoids, medicine, perfume, and spiritual practices to be utilised as medicine. Medicinal plants are gifts of nature for humans; there are evidential epics that it has been used from the earlier historical era. According to the World Health Organization, plants can synthesise a wide variety of chemical compounds used to treat various diseases (Lai and Roy, 2004). Medicinal plants are of great importance to the health of individuals and communities. The therapeutic value of plants lies in some chemical substances that produce a definite physiological action on the human body. Historically, medicinal plants have been essential

as sources of pharmacological lead compounds. Because early humans used plants to heal their illnesses due to instinct, taste, and experience, the history of medicinal plants predates that of humans. The classification of medicinal plants is one difficulty that their evolution must overcome. Taxonomists have developed various plant categorisation methods over the years, including morphologic classification, anatomical classification, and chemotaxonomic classification. Due to their reputation for safety and lack of side effects, herbal medicinal plants are a popular choice for treatment. They have a more significant advantage over chemically processed items and synthetic treatments since they are in harmony with nature. Ayurvedic herbs, as opposed to other pharmaceuticals and medications, are known to treat illness from the source, helping to keep you well and fit over time.

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for various ailments. Progress over the centuries toward a better understanding of plant-derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that medicine does what it is claimed to do, and the other has been the identification by chemical analysis of the active compound in the plant (Holiman, 1989). According to the world health organisation (WHO), more than 80% of the world's population relies on traditional medicines for their primary healthcare needs.

Spices and herbs have been used for centuries for culinary and medicinal purposes. Spices not only enhance the flavour, aroma, and colour of food and beverages, but they can also protect from acute and chronic diseases. More Americans are considering using spices and herbs for medicinal and therapeutic/remedy use, especially for various chronic conditions. There is ample evidence that spices and herbs possess antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, glucose-lowering, and cholesterol-lowering activities and properties that affect cognition and

mood. Researchers over the past decade have reported on the diverse range of health properties they possess via their bioactive constituents, including sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins, especially flavonoids and polyphenols. Spices and herbs such as clove, rosemary, sage, oregano, and cinnamon are excellent sources of antioxidants with their high content of phenolic compounds. Frequent consumption of spicy foods was also linked to a lower risk of death from cancer and ischemic heart and respiratory system diseases.

Products of secondary metabolism found in plants, including herbs and spices, include phenolics, phenolic acids, quinones, flavonoids, and tannins (Lai and Roy, 2004). Many of these phytochemicals are abundant antioxidants and act as plant defences against insects and pathogenic organisms (Dean and Ritchie, 1987). Several studies have found a strong relationship between the quantity of phenolic components in particular herbs and spice preparations and antibacterial activity.

The largest producer, consumer, and exporter of spices is India. Bio-active antimicrobial chemicals are said to be abundant in the sign of spices. Many food items are stabilised using spices to prevent degradation. Spices played a significant role in treating critical bodily ailments, particularly in Ayurveda. In most of their compositions, homoeopathic medicine has used spices as one of the main ingredients. Spices are defined as plant substances of native or exotic origin, aromatic or with strong taste, used to enhance the flavour of foods. Spices include leaves (coriander, mint), buds (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stems (cinnamon), rhizomes (ginger), and other plants.

According to Lambert et al. (2001), these bioactive compounds may exert their antimicrobial effects through a variety of mechanisms, including changes in the synthesis of DNA and RNA, disruption of protein translocation, cell wall degradation, disruption of the cytoplasmic

membrane, leakage of cellular components, alteration of fatty acid and phospholipid constituents, and leakage of cellular components (Shan et al., 2007). Mixing spice and herb extracts will probably have synergistic antibacterial effects against food deterioration and harmful microorganisms.

Garlic, ajwain, black pepper, clove, ginger, cumin, and caraway are some spices frequently used in Indian cuisine and traditional medicine. Due to its antibacterial qualities, garlic is commonly used to treat several infectious disorders. Eugenol is the primary ingredient in cloves, which has antibacterial and local anaesthetic properties, making it helpful in treating toothaches. Curries, pickles, sauces, and other dishes frequently incorporate traditional Indian spices and herbs like cumin, black cumin, mustard, fenugreek, ajwain, curry leaf, nutmeg, and henna. Some spices are also recognised to possess certain antibacterial or ethnomedicinal qualities.

Phytochemicals are a large group of naturally occurring chemicals that give plants colour, flavour, aroma, and texture. A potent class of compounds called phytochemicals, especially those with plant origins, are derived from natural resources. Phytosterols, flavonoids, terpenoids, saponins, alkaloids, carotenoids, aromatic acids, organic acids, essential oils, and protease inhibitors are the main categories of phytochemicals. The presence of metabolites in plants acts as a defence mechanism against infectious conditions since they possess qualities including antibacterial, anti-inflammatory, anthelmintic, anticarcinogenic, antigenotoxic, antiproliferative, antimutagenic, and antioxidative. In addition to cell lines and animal cancer models, they have demonstrated chemopreventive and chemotherapeutic properties; some are currently undergoing phase I and II clinical trials. The term “phytochemicals” comes from the Greek word for the plant and refers to non-nutritive elements of a plant-based diet with significant antimutagenic and anticarcinogenic characteristics. Phytochemicals play various roles in the treatment and prevention of cancer

(Bathaie et al., 2015). Phytochemicals are bioactive substances derived from plants. Since the plant that produces them may not have much need for them, they are regarded as secondary metabolites. Every element of the plant body, including the bark, leaves, stem, roots, flowers, fruits, and seeds, are naturally synthesised to produce these active ingredients.

The potential health benefits of dietary phytochemicals like flavonoids, flavanols quercetin, isoflavones genistein, daidzein, and other polyphenolic substances like the stilbene resveratrol are of great interest. Chocolate, cocoa, black tea, onions, green tea, red wine, grape juice, berries, fruit, and soy are foods high in polyphenols. Through a variety of mechanisms, such as effects on gene expression, regulatory microRNA, post-translational modification, and modulation of cell signalling pathways, phytochemicals may have positive health effects, including protection against cardiovascular disease, cancer, and loss of cognitive function and possibly even increase longevity (Wiseman, 2013).

Examination of the medicinal plants' phytochemical qualities was utilised to identify and separate the medication, lead compounds, and parts from the plant's parts. The qualities of the phytochemicals in plants can be used to pinpoint their specific biological activity. Leaves, roots, stem barks, and fruits comprised the majority of plant parts employed to examine the phytochemical characteristics (Agidew, 2022).

1.1. Plant Phytochemicals

1.1.1. Alkaloids

Alkaloids are metabolic byproducts derived from amino acids and are one of the plants' most essential and significant components. Alkaloids have been removed from various plant parts using a variety of solvents, including ethanol, methanol, chloroform, acetone, hexane, petroleum ether,

ethyl acetate, and aqueous (water). With the help of these solvents, phytochemical components can be extracted from the leaves, roots, stem bark, and fruits of medicinal plants (Ullah et al., 2014).

1.1.2 Flavonoids

Plants contain flavonoids, a broad category of polyphenol chemicals with a benzoyl-pyrone structure. The phenylpropanoid pathway is responsible for their synthesis. Flavonoids and other secondary phenolic metabolites are primarily responsible for various pharmacological activities (Mahomoodally et al., 2005). Flavonoids are hydroxylated phenolic substances known to be synthesised by plants in response to microbial infection (Dixon et al., 1983). Flavonoids are members of a class of natural compounds that have recently been the subject of considerable scientific and therapeutic interest. Flavonoids constitute the largest class of phenolic compounds with more than 3,000 structures. These consist of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle (hydroxylated phenolic substances) (Li et al., 2012). These are known to be synthesised by plants in response to microbial infection, and they are antimicrobial substances against a wide array of microorganisms in vitro.

1.1.3. Tannins

A complex, big polyphenol-type biomolecule with enough hydroxyls and other appropriate groups, like carboxyl, to form powerful complexes with various macromolecules is commonly referred to as tannin (Navarrete, 2013). Tannins are generally used in tanning and as healing agents in inflammation, burn, piles, and gonorrhoea (Boroushaki et al., 2016).

1.1.4. Saponins

The plant kingdom is home to a significant collection of secondary metabolites known as saponins. Phytochemicals called saponins are primarily present in most vegetables, legumes, and herbs (Francis et al., 2002; Haralampidis et al., 2002).

1.1.5. Steroids

Sterol is a naturally occurring or artificially produced chemically active element that resembles a hormone. A steroid is one of many chemical compounds categorised by a specific carbon structure. Prednisone, cortisone, vitamin D, and various sex hormones, such as testosterone and estradiol, are medications that reduce edema and inflammation (Hill et al., 2007).

1.1.6. Terpenoids

The most common natural products are likely terpenoids, tiny molecules plants produce. Terpenoids exhibit remarkable pharmacological properties, including antiviral, antibacterial, antimalarial, anti-inflammatory, cholesterol synthesis inhibition, and anti-cancer properties (Boroushaki et al., 2016).

1.1.7.Phenolic compounds

Phenolic compounds are secondary metabolites produced in plants' shikimic acid pathway and pentose phosphate through phenylpropanoid metabolisation (Lin et al., 2016).

1.2. Antioxidant activity

Antioxidant compounds that restrain or delay the oxidation of molecules by reacting with free radicals are known as antioxidants. In the biological system, antioxidants can be defined as substances that, when present in low concentration with an oxidisable substrate, would significantly prevent substrate oxidation (Halliwell et al., 1996). The oxidisable substrate could be

a molecule in food or biological materials, including carbohydrates, DNA, lipids, and proteins (Shenoy and Shirwaikar, 2002). Free radicals are responsible for ageing, and their presence in excess constitutes the cause of various human diseases. Different studies showed that antioxidant substances that scavenge free radicals are essential in preventing free radical-induced diseases (Ismail and Hong, 2002).

The medicinal value of Spices, which include leaves (coriander, mint), buds (clove), bulbs (garlic, onion), fruits (red chilli, black pepper), stem (cinnamon), rhizomes (ginger), star anise, cinnamon (bark) and other plant parts, have been defined as plant substances from the indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods. Herbs and spices were used during the Middle Ages for flavouring, food preservation, and medicinal purposes. Only a small percentage of plant species have been investigated phytochemically, and the fraction submitted to biological screening is even smaller. Several studies have attributed spices and herbs' antimicrobial, antioxidant, and pharmaceutical properties to their phenolic compounds. However, the actual role of spices and herbs in maintaining health, explicitly protecting against the development of chronic, non-communicable diseases, is currently unclear.

1.3. Selected Plant Species

1.3.1. Cinnamon

The three species of cinnamon: *Cinnamomum verum*, *Cinnamomum zeylanicum* and *Cinnamomum cassia*, are utilised extensively in the food, fragrance, and medicine industries. The active ingredients in essential oils include cinnamic and cinnamyl aldehydes, cinnamyl acetate mucus, tannin, eugenol, caryophyllene, cinnamyl acetate mucus, and carbohydrate. They perform various biological tasks, such as operating as an antioxidant and antibacterial, anti-inflammatory,

antidiabetic, and anti-tumour properties. It has the potential to be anti-inflammatory, antimicrobial, antioxidant, anti-tumour, cardiovascular, cholesterol-lowering, hypoglycemia, and lipid-lowering. It also has activities against neurological disorders like Parkinson's and Alzheimer's disease, immunomodulatory effects, wound healing, anti-HIV, anti-anxiety, and antidepressant properties. Moreover, cinnamon has historically been used as a tooth powder to alleviate issues with dental and oral infections (Rao and Gan, 2014).

Cinnamon extracts' ability to fight cancer is associated with changes in angiogenesis and CD+ 8 T cell effector activity (Kwon et al., 2010). Cinnamon lowers the risk of hyperglycemia and inflammation by reducing the activity of the -glycosidase enzyme, restricting glucose absorption, and promoting glycogen synthesis (Kirkham et al., 2009). Moreover, cinnamon polyphenols raise SOD (Superoxide Dismutase) and GSH-Px (Glutathione Peroxidase) activity, decrease malondialdehyde (MDA) levels, and improve glucose tolerance in diabetic rats' pancreatic glands (Liao, 2019).

Cinnamon's primary antidiabetic action is concentrated on the ability of this extract's water-soluble component to aid insulin signalling. It lessens tyrosine phosphatase activity while increasing the autophosphorylation of insulin receptors (an enzyme that in vitro activates insulin receptors). The above findings lead to an improvement in insulin sensitivity (Rafehi et al., 2012). Moreover, it contains a phenolic component that functions as an antioxidant while also preventing the development of the glycation process's final result due to its capacity for trapping reactive oxygen (ROS) and reactive carbonyl species (RCS) (Verdini et al., 2020).

1.3.2. Nutmeg

Myristica fragrans, or nutmeg, is a pulverised spice from the Myristicaceae family. Macelignan, carvacrol, myristicin, -caryophyllene, -pinene, p-cymene, and eugenol are some of the main bioactive compounds found in nutmeg. Nutmeg is used in medicinal formulations for dysentery, flatulence, stomach ache, nausea, vomiting, rheumatism, sciatica, malaria, and the early stages of leprosy. Inferential evidence supports the depressive, aphrodisiac, antibacterial, upper antioxidant, memory-enhancing, and hepatoprotective effects of *M. fragrans* in treating hyperlipidemia and hypercholesterolemia. Several pharmacologically active chemicals with aphrodisiac, hepatoprotective, antibacterial, antidiabetic, antioxidant, and anti-cancer effects have been found in nutmeg essential oil and its fractions.

1.3.3. Black pepper

Black pepper (*Piper nigrum* L.), known as the "king of spices," has been studied for its biological qualities and bioactive compounds. It may be utilised in food, medicine, and fragrances. The liver's enzymatic drug biotransformation processes are modulated by piperine. It actively inhibits UDP-glucuronyl transferase and the aryl hydrocarbon hydroxylase in the liver and the intestine. Piperine promised to increase the bioavailability of a variety of medicinal medications as well as phytochemicals. Piperine may have antimutagenic and anti-tumour effects. Piperine has been discovered to increase the oral bioavailability of numerous medications, vaccines, and minerals by blocking several metabolising enzymes. It is also known to improve fertility and cognitive function.

Amide compounds, piperidine, pyrrolidines, and traces of safrole are other phytochemicals that are found (Gulati et al., 2021). Piperine has been employed as a natural bioenhancer to boost the

therapeutic effects of other medications. Its ability to operate on a metabolising enzyme, improve medication delivery, impact the blood supply to the gastrointestinal tract (GIT), and increase membrane fluidity have all been used to demonstrate its bioenhancer properties (Wadhwa et al., 2014).

Very little information is available on various spices' phytochemicals, antioxidants, secondary metabolites, and pharmacological applications. Because of the need for new alternative bioactive compounds with different biochemical activities, spices could potentially be a great source. This study used different extraction solvents to study three spices for their bioactivity, antibacterial activity, and antifungal activity.

OBJECTIVES

This study aims to understand the effects of three extraction solvents on three spices' antifungal and antibacterial activity. The quantitative and qualitative analyses of phenolic compounds, flavonoids, antioxidants, and pigments were studied by TLC and spectrophotometric methods. The detailed objectives are as follows:

- To extract the bioactive compounds like total phenolics and flavonoids from spices using three extraction solvents.
- To quantify the amount of Total Phenolic Content and Total Flavonoid Content in spices' methanolic, ethanolic, and acetone extracts.
- To investigate the antioxidant activity of spices' methanolic, ethanolic, and acetone extracts using a DPPH radical scavenging assay.
- To evaluate the antifungal and antibacterial activity of spices' methanolic, ethanolic, and acetone extracts on *Aspergillus* sp. and two bacterial strains (*Escherichia coli* and *Bacillus cereus*).
- To carry out TLC profiling and UV-VIS spectra for the spices' phenolic and flavonoid.

2. MATERIALS AND METHODS

2.1. Collection Of Samples

Leaf samples of three different plant species, namely *Piper nigrum*, *Myristica fragrans*, and *Cinnamomum zeylanicum*, were collected from a local farm in Goa, India. The leaves were washed with sterile distilled water and air-dried in the shade for 48 hours.

2.2. Preparation Of Extracts

The dried leaf samples were grounded to make a fine powder using liquid nitrogen. Ten grams of powder was soaked in 100 mL of respective solvents, viz. ethanol, methanol, and acetone, to prepare an extract by solvent extraction. The sample was kept dark for 24 h with intermittent shaking for better extraction. After incubation, the solution was filtered with Whatman's filter paper No. 4 (20-25 μm), retaining hygienic conditions. The ethanol, methanol, and acetone extracts were then concentrated using a rotary evaporator at 36˚C. Finally, working solutions were prepared as 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL (Sobuj et al., 2021)

2.3. Quantitative Analysis

2.3.1. Determination of Total Phenolic Content (TPC)

Total Phenolic content was estimated by Folin Ciocalteu's method. Standard Gallic acid solution was prepared by dissolving 10 mg in 10 mL of distilled water (1 mg/ mL). The total phenolics were expressed as mg of Gallic acid equivalents (GAE)/ g of extract. Various concentrations of gallic acid solutions in distilled water (0.25, 0.5, and 1 mg/ mL) were prepared from the standard solution. The concentration of 1mg/mL of leaf extracts was prepared in respective solvents (ethanol, methanol, and acetone), and concentrations of 0.25, 0.5 and 1 mg/ mL were prepared

from the stock solution. 200 µL of each sample was added into test tubes, and the final volume was made to 500 µL using distilled water. Then 150 µL of 10-fold dilute Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 2 mL of 7.5% sodium carbonate was added. The test tubes were covered with aluminium foil and incubated for 1 hour at room temperature. After incubation, absorbance was read at 760 nm spectrophotometrically. All determinations were performed in triplicate. Folin Ciocalteu's reagent, sensitive to reducing compounds including polyphenols, produced a blue colour upon reaction measured spectrophotometrically. The calibration curve was plotted using standard gallic acid (Mongkolsilp et al., 2004).

2.3.2. Determination of Total Flavanoid Content (TFC)

Total flavonoid content was estimated by aluminium chloride colourimetric assay. The total flavonoids were calculated as mg of quercetin equivalent per gram. The standard quercetin solution was prepared by dissolving 10 mg in 10 mL 80% methanol (1 mg/mL) and prepared concentrations of 0.25, 0.5, and 1 µg / mL. Concentrations of 1mg/mL of algal extracts were prepared in respective solvents ethanol, methanol and acetone, and 0.25, 0.5, and 1 µg / mL concentrations were prepared from the stock solution using the respective solvent. 500 µL of the sample was added to a test tube with 1.5 mL 95% ethanol (v/v). To the test tube, 0.1mL of 10% aluminium chloride was added, and after 5 min, 0.1 mL of 1M potassium acetate was added, and the total volume was made up to 5 mL with distilled water. The solution was mixed well and incubated at room temperature for 30 min. The absorbance of the reaction was measured at 420 nm spectrophotometrically. All determinations were performed in triplicate. The calibration curve was plotted using standard quercetin (Shraim et al., 2021).

2.4. Determination Of Antioxidant Activity

2.4.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

Free radical scavenging activity of ethanolic, methanolic, and acetonic extracts of *Piper nigrum*, *Myristica fragrans* Houtt. and *Cinnamomum verum* was determined according to the DPPH methods (Ahmed et al., 2013; Brand-Williams et al., 1995). The hydrogen atom donating ability of the algal extracts was determined by the colouration of the ethanol solution of (DPPH). DPPH produces a violet/ purple colour in ethanol solution and fades to shades of yellow in the presence of antioxidants. The stock solution of DPPH radical, 24 mg /100 mL in ethanol, was prepared and stored at -20˚C until further use. A working solution was prepared by diluting the stock solution of DPPH with ethanol in 10 mL/45 mL ethanol. Stock solutions of algal extracts were prepared in methanol and ethanol with a 3 mg/ mL concentration. Different dilutions of 0.25 µg /mL, 0.5 µg /mL, and 1 µg / mL were made from this. The test mixture contained 3 mL of DPPH working solution and 1 mL of a sample. The mixture was incubated at room temperature for 30 min in the dark. The absorbance of each sample was recorded at 517 nm. For a negative control, 1 mL of methanol and ethanol was added instead of leaf extract. Ascorbic acid 10 mg/10 mL distilled water was used as a positive control. Each extract was analysed in triplicate. % Inhibition curves were made, and IC_{50} values were calculated for all samples. The percentage of inhibition was calculated by using the formula:

$$\% \text{ Inhibition} = \{(A \text{ blank} - A \text{ sample})/ A \text{ blank}\} \text{ X } 100$$

A blank is the absorbance of the negative control, and *A* sample is the absorbance of the sample/ standard (Ahmed et al., 2013; Brand-Williams et al., 1995).

2.5. Determination Of Antimicrobial Activity

2.5.1. Preparation of sterile disc

Whatman's No. 1 filter paper was punched into 6 mm disc form and then sterilised by autoclaving; each sterile disc was soaked in different concentrations (0.25, 0.5 and 1 mg/mL) extracts for 5 minutes and then was allowed to air dry.

2.5.2. Antibacterial assay

The bacterial *Escherichia coli* and *Bacillus cereus* strains were obtained from the Department of Microbiology, PES's College of Arts and Science, Farmagudi, and was maintained in nutrient agar slants at 4˚C for experimental studies.

2.5.3. Antifungal assay

The fungal strain *Aspergillus niger* was obtained from the Goa University fungal culture collection lab (GUFCC), Botany Discipline, Goa University, and was maintained in a malt-extract agar (MAE) slant at room temperature.

2.5.4. Antimicrobial assay using disc diffusion method:

The antibacterial activity of ethanolic, methanolic and acetone extracts of *Piper nigrum*, *Myristica fragans* and *Cinnamomum zeylanicum* was carried out using the disc diffusion method were compared with the commercially available antibiotics Ampicillin (10 mg/mL). Sterile Potato Mueller Hinton Agar (MHA) was poured into sterile Petri plates; after solidification, 100 µL of bacterial *Escherichia coli* and *Bacillus cereus* inoculum was spread plate onto their respective plates. The extract was loaded onto different filter paper discs prepared from Whatman filter No.

1 paper. The dried discs were placed in the centre of each marked region (according to respective concentration) on the plates using sterile forceps. The disc was then placed on the agar medium containing the cultures and incubated for 48 hours at room temperature. The antibiotic discs (Ampicillin) were placed in the centre of the plates using sterile forceps as a positive control. After incubation, the diameter of the inhibitory zones formed around each disc was recorded (Karsha and Lakshmi, 2010).

2.5.5. Antifungal activity of commercially available antibiotics

The antifungal activity of leaf extracts of the ethanolic, methanolic and acetone extract of *Piper nigrum*, *Myristica fragans* and *Cinnamomum zeylanicum* on fungal (*Aspergillus niger*) strain were compared with the commercially available antibiotics Ketoconazole (10mg/mL) using well diffusion method. Malt plates were prepared, and the test organisms were spread plate onto their respective agar plates. The well was filled with 0.25, 0.5, and 1 mg/mL plant extracts using a micropipette and incubated for 48 hours at room temperature. The antibiotic Ketoconazole was put in the well in the centre of the plates using a micropipette, which served as a positive control. After incubation, the diameter of the inhibitory zones formed around each well was noted.

2.6. Spectrophotometric Analysis of Extracts

The methanolic and ethanolic extracts of *Padina sp.* and *Sargassum sp.* were examined under UV and visible light for proximate analysis using UV-VIS Spectrophotometer. The extracts were diluted to 1:9 with the respective solvents and scanned in the wavelength ranging from 190-700 nm. The characteristic peaks were detected.

2.7. Thin Layer Chromatography and UV-VIS Spectra

2.7.1. TLC of Phenolic acids

Pre-coated TLC Silica Aluminium Plates (Merck) were used. For this experiment, the solvent systems used was Toluene: Acetone: Formic acid (4.5:4.5:1), The methanolic, ethanolic and acetone extracts of *Piper nigrum*, *Myristica fragrans*, and *Cinnamomum zeylanicum* were loaded as concentrated bands, 1.5-2 cm from the edge of their respective TLC plate, and allowed to dry. The plates, with dried samples, were gently lowered into the chromatography chamber, closed and left to develop. The plates were removed from the chromatography chamber when the solvent front had travelled 3/4 of the plate's length. The position of the solvent front was immediately marked with a soft pencil. Phenolic acids were detected using an ultraviolet trans-illuminator (Long LV-365 nm). For a better resolution of the separated phenolic acids and photographic records, the chromatogram was then exposed to ammonia fumes for 10 minutes. The retention factor (R) values of the different bands were then calculated using the equation (Wagner and Bladt, 1996),

2.7.2. TLC of Flavonoids

Pre-coated TLC Silica Aluminium plates (Merck) were used. For this investigation, the solvent systems used were Methanol: Chloroform: Hexane (7:2:1). The methanolic, ethanolic and acetone extracts of *Piper nigrum*, *Myristica fragrans*, and *Cinnamomum zeylanicum* were loaded as concentrated spots, 1.5-2 cm from the edge of their respective TLC plate and allowed to dry. The plates with dried samples were gently lowered into the chromatography chamber, closed and left to develop. The plates were removed from the chromatography chamber when the solvent front had travelled 3/4" of the plate's length. The position of the solvent front was immediately marked with a soft pencil. Flavonoids were detected using an ultraviolet trans-illuminator (Long UV

365nm). For a better resolution of the separated flavonoids and photographic records, the chromatogram was then exposed to ammonia fumes for 10 minutes. The retention factor (R) values of the different bands were then calculated using the equation (Wagner and Bladt, 1996).

3. RESULTS

3.1. Total Phenolic Content (TPC)

The TPC results in ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* are shown in **Table1** and **Fig.3.** The ethanolic extract of *Cinnamomum zeylanicum* showed higher TFC than the ethanolic extracts of *Piper nigrum* and *Myristica fragrans*. Further, the methanolic extract of *Piper nigrum* also showed higher TFC than the other methanolic extracts of *Myristica fragrans and Cinnamomum zeylanicum* The acetone extract of *Cinnamomum zeylanicum* showed higher TFC than *Piper nigrum* and *Myristica fragrans*.

3.2. Total Flavonoid Content (TFC)

The result obtained for TFC in ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* is depicted in **Table 2**. and **Fig 5.** The ethanolic extract of *Myristica fragrans* showed higher TPC than the methanolic extracts of *Piper nigrum* and *Cinnamomum zeylanicum* Further, the methanolic extract of *Cinnamomum zeylanicum* also showed higher TPC than *Piper nigrum* and *Myristica fragrans.* Likewise, the acetone extract of *Cinnamomum zeylanicum* showed higher total phenolic content (TPC) than *Piper nigrum* and *Myristica fragrans.*

3.3. Antioxidant assay

3.3.1. DPPH scavenging activity

Antioxidant compounds like phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxyl, thus inhibiting the oxidative mechanism leading

to degenerative diseases. DPPH scavenging activity of ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* are shown in **Tables 4, 5, 6** and **Fig 6.** The result of the per cent scavenging activity of ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* are compared with the standard L- Ascorbic acid. It was observed that % scavenging activity increases with increased concentration of extracts. Data showed higher per cent scavenging activity in the ethanolic extract of *Cinnamomum zeylanicum* compared to the *Piper nigrum and Myristica fragrans.* Similarly, The methanolic extract of *Piper nigrum* showed a high % scavenging activity compared to *Myristica fragrans* and *Cinnamomum zeylanicum* extracts. Further, the acetone extract of *Myristica fragrans* showed a high % scavenging activity compared to *Piper nigrum* and *Cinnamomum zeylanicum* The lower the IC_{50} value, the higher the antioxidant activity. It was observed that the acetone extracts of *Cinnamomum zeylanicum* showed higher antioxidant activity (IC_{50} = 75 µg/mL). The acetone extract of *Piper nigrum* showed higher antioxidant activity (IC_{50} = 110 µg/mL). The acetone extract of *Myristica fragrans* showed higher antioxidant activity (IC_{50} = 80 µg/mL) (**Table 3** and **Fig 6**.).

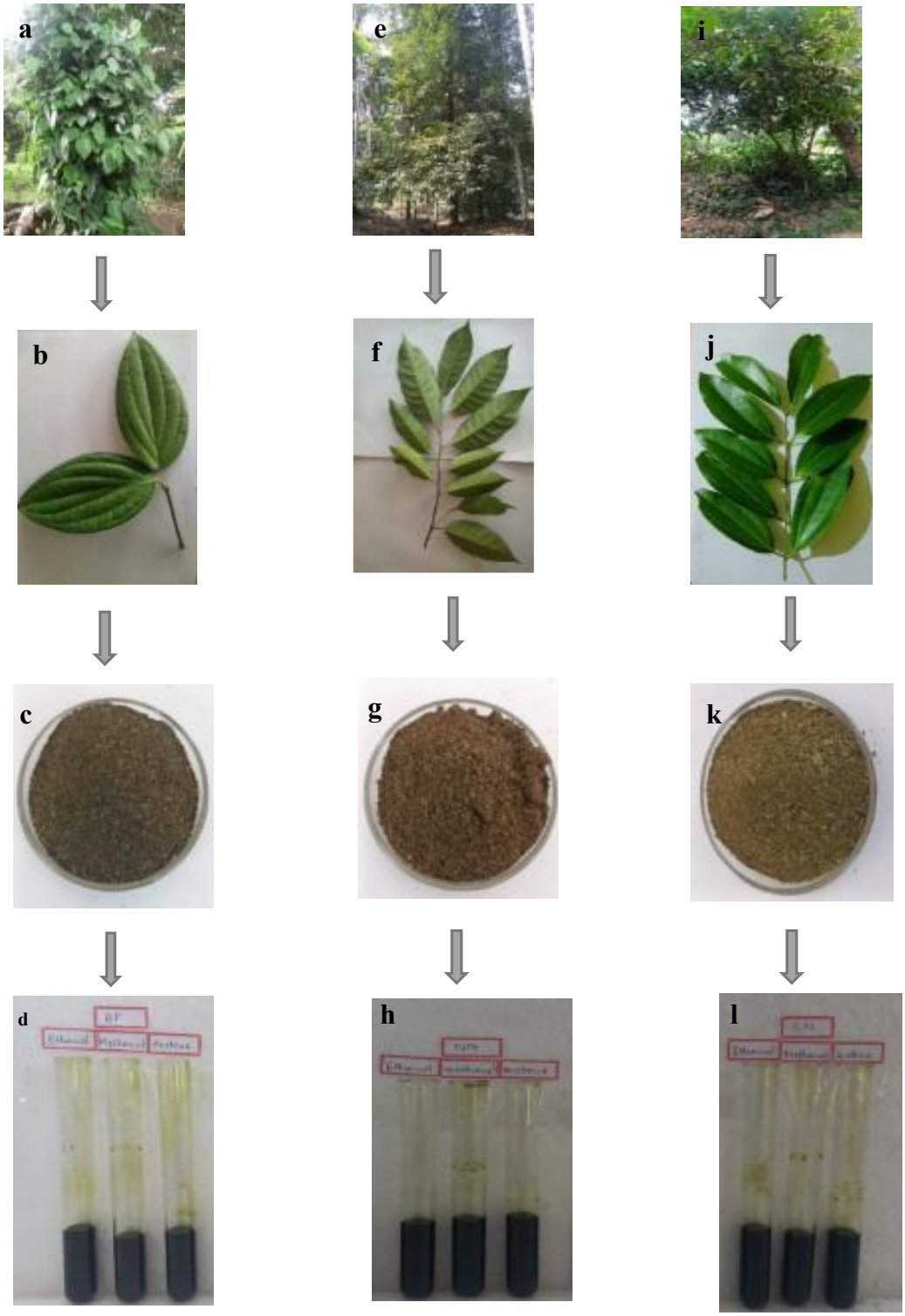


Fig. 1: Flow chart depicting habitat (**a,e,i**), Leaf structure (**b,f,j**), ground sample (**c,g,k**), ethanolic, methanolic and acetone extract (**d,h,l**) of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* respectively.

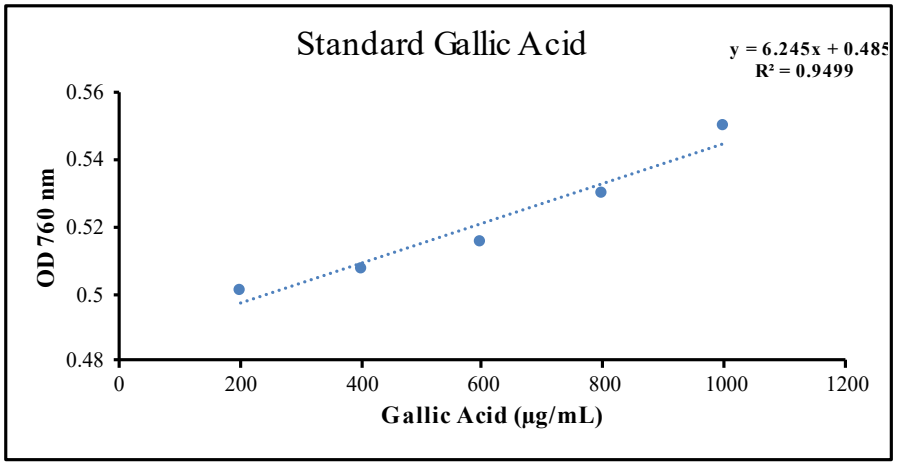


Fig. 2: Calibration Curve for Gallic acid for determination of Total Phenolic Content (mg of Gallicacid equivalent (GAE)/g of extract) at varying concentrations

Table 1: The amount of Total Phenolic Content (TPC) in Etahnolic, Methanolic and Acetone extracts of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* . Data represent mean values ± Standard Deviation (n=3). Note: GAE: Gallic Acid Equivalent.

Solvents	**Concentration (µg/mL)**	**Total Phenolic Content (mg of GAE/g of extract)**		
		Piper nigrum	*Myristicafragrans*	*Cinnamomum zeylanicum.*
Ethanol	250	160±30	110±20	150±20
	500	480±20	320±30	350±10
	1000	615±20	545±40	650±20
Methanol	250	90±10	260±20	180±10
	500	300±30	450±30	560±30
	1000	520±20	500±40	480±40
Acetone	250	210±10	210±10	260±30
	500	410±20	500±20	600±40
	1000	830±20	780±40	880±20

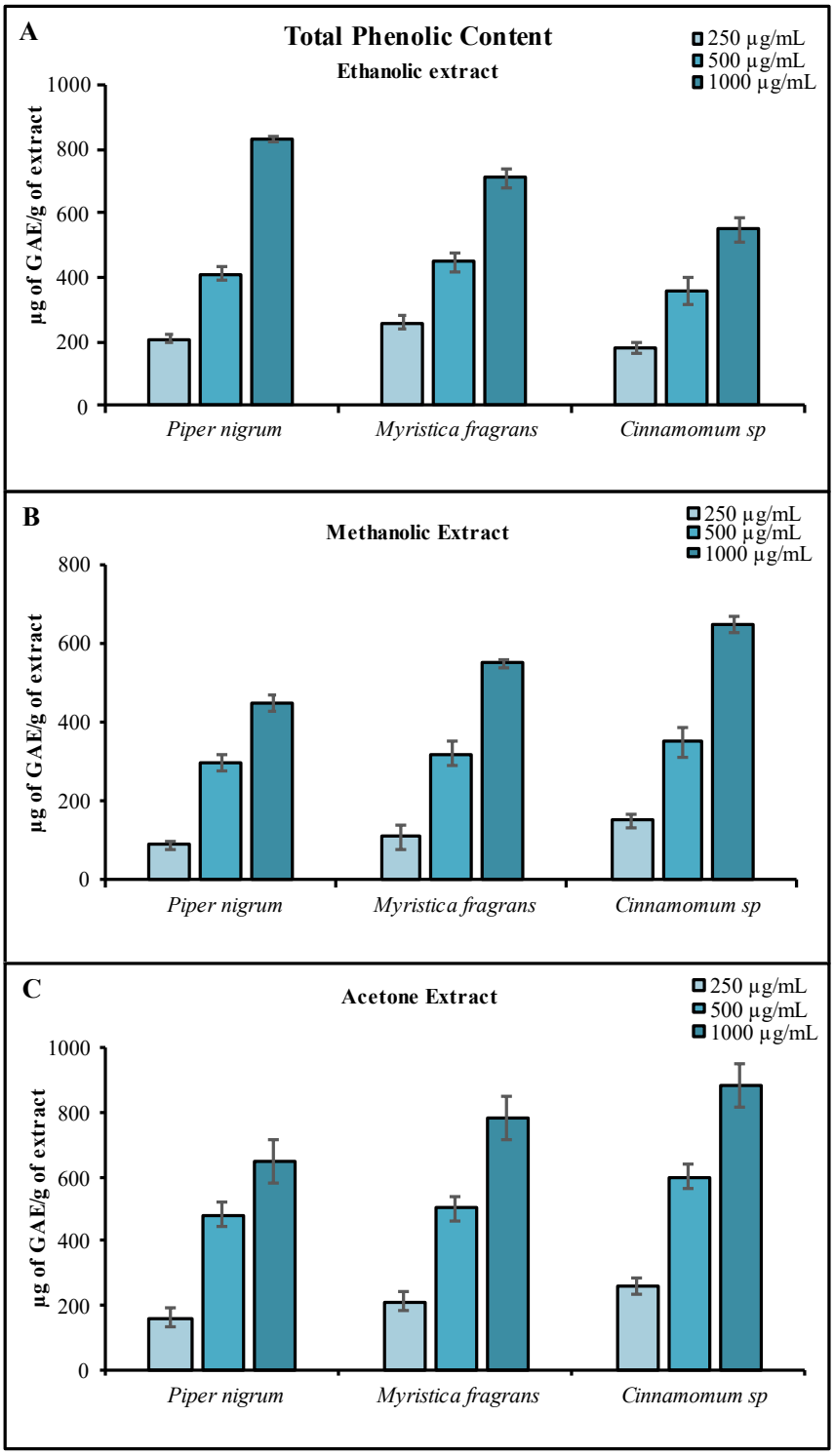


Fig. 3: Determination of Total Phenolic Content (TPC) in mg of Gallic acid equivalent (GAE)/g of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum .;* **A-** Ethanolic extract **, B-** Methanolic extract **, C-** Acetone extract **.** Bars represent mean values ± SD (n=3).

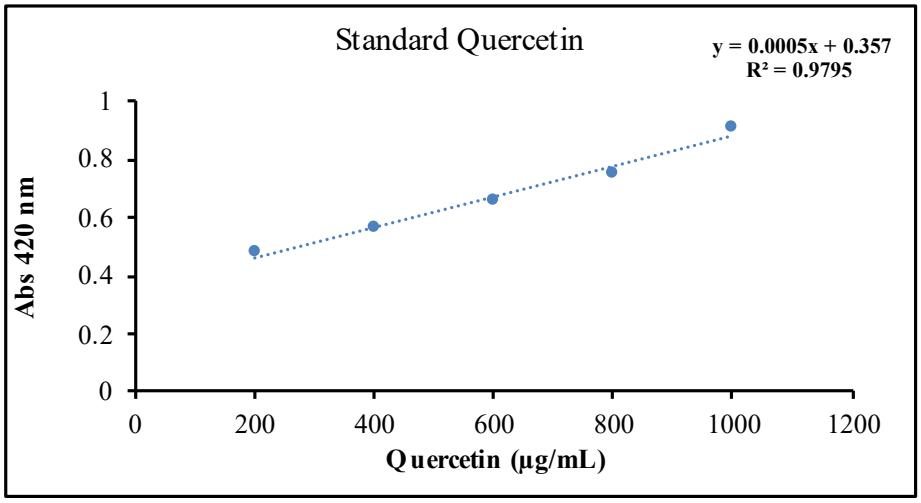


Fig. 4: Calibration Curve for Quercetin for determination of Total Flavanoid Content (TFC at varying concentrations

Table 2: The amount of Total Flavanoid Content (TFC) in Ethanolic, Methanolic and Acetone extracts of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* . Data represent mean values ± Standard Deviation (n=3). Note: QE: Quercetin Equivalent

Solvents	Concentration (µg/mL)	Total Flavonoid Content (µg of QE/g of extract)		
		Pipernigrum	*Myristicafragrans*	*Cinnamomum zeylanicum.*
Ethanol	250	210±30	180±30	170±30
	500	360±20	450±30	470±40
	1000	610±30	780±20	760±20
Methanol	250	140±20	150±40	170±30
	500	230±30	250±30	470±20
	1000	740±20	650±20	760±30
Acetone	250	200±30	120±30	230±20
	500	350±40	400±30	760±20
	1000	830±20	820±20	950±30

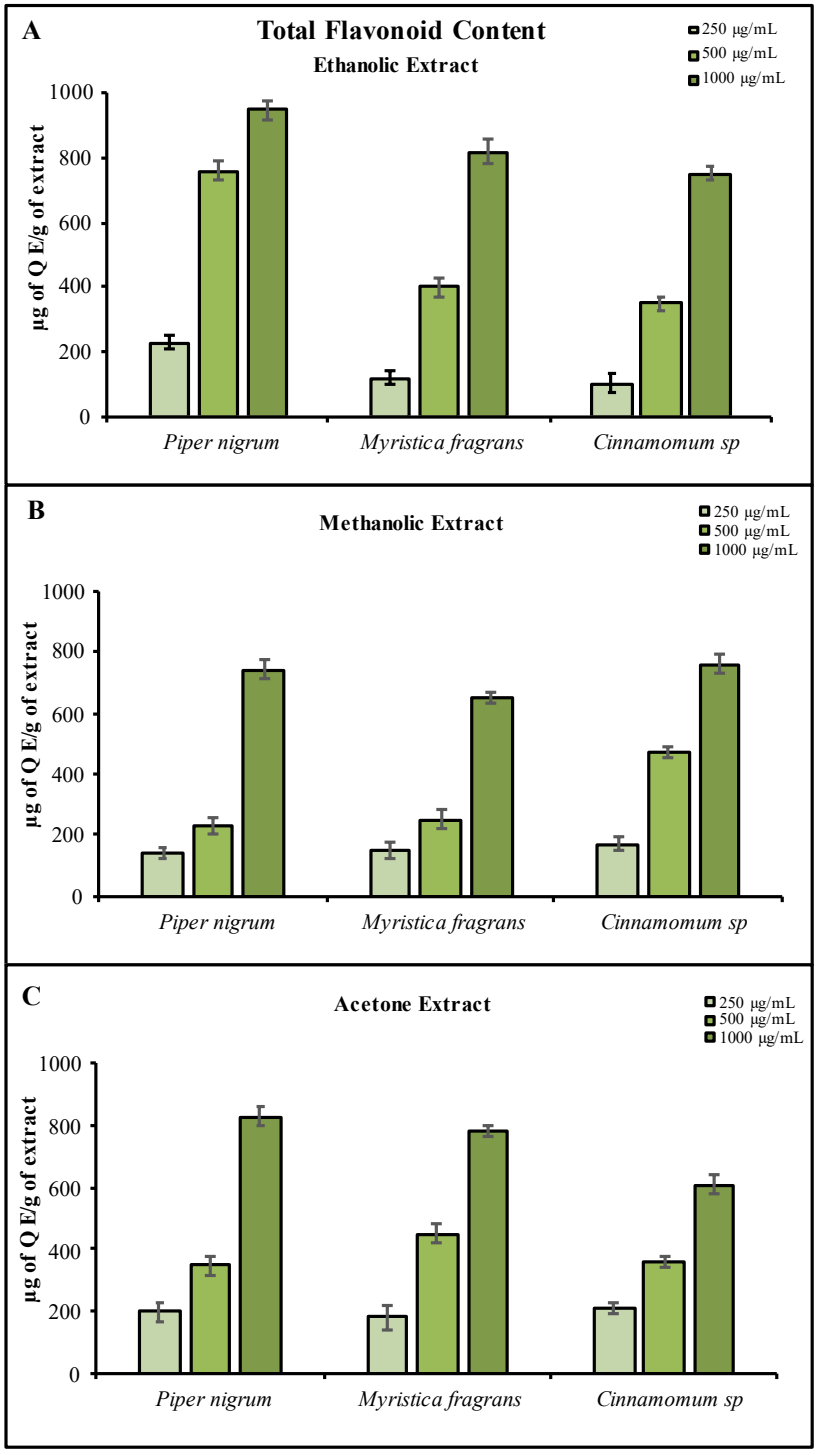


Fig. 5: Determination of Total Flavanoid Content (TFC) in mg of QE/g of extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum;* **A**- Ethanolic extract, **B**- Methanolic extract, **C**- Acetone extract. Bars represent mean values ± SD (n=3).

Table 3: Antioxidant activity:IC_{50} values of Ethanolic, Methanolic and Acetone extracts of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* with L-Ascorbic acid as standard.

Sr. No.	Samples/Standard	IC_{50} Values (µg/mL)		
		Ethanolic extract	Methanolic extract	Acetone extract
1.	*Piper nigrum*	120	135	110
2.	*Myristica fragrans*	110	120	80
3.	*Cinnamomum zeylanicum* .	95	110	75
4.	L-Ascorbic acid (Standard)	60		

Table 4: Antioxidant activity: DPPH free radical scavenging by ascorbic acid and Ethanolic extract of *Piper nigrum, Myristica fragrans* and *Cinnamomum zeylanicum* **.** Data represent mean values ± Standard Deviation (n=3).

Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Ethanolic extract		
			Piper nigrum	*Myristica fragrans*	*Cinnamomum zeylanicum*
1.	250	80 ± 5	60±5	50±8	60±5
2.	500	120 ± 5	101±5	98±10	98±6
3.	1000	160 ± 5	112±10	130±11	125±10

Table 5: Antioxidant activity: DPPH free radical scavenging by ascorbic acid and Methanolic extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* **.** Data represent mean values ± Standard Deviation (n=3).

Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Methanolic extract		
			Piper nigrum	*Myristica fragrans*	*Cinnamomum zeylanicum* .
1.	250	80 ± 5	58±5	45±8	54±5
2.	500	120 ± 5	98±5	110±6	89±6
3.	1000	160 ± 5	112±6	130±7	120±4

Table 6: Antioxidant activity: DPPH free radical scavenging by ascorbic acid and Acetone extract of *Piper nigrum, Myristica fragrans* and *Cinnamomum zeylanicum* **.** Data represent mean values ± Standard Deviation (n=3).

Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Acetone extract		
			Piper nigrum	*Myristica fragrans*	*Cinnamomum zeylanicum*
1.	250	80 ± 5	50±6	70±8	60±5
2.	500	120 ± 5	102±7	108±6	102±6
3.	1000	160 ± 5	130±5	145±7	150±4

3.4. Antimicrobial activity

3.4.1. Antibacterial assay

The antibacterial activity of ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* for *Escherichia coli* and *Bacillus cereus* is shown in **Table 7** and **Fig 7**.

For *Piper nigrum*, acetone extract of 1 mg/mL showed high antibacterial activity with a 0.83 cm inhibition zone against *Escherichia coli;* methanolic extract of the same concentration showed high activity with a 0.85 cm zone of inhibition for *Bacillus cereus*. With the same concentration of *Myristica fragrans,* the acetone extract showed high antibacterial activity at 0.72 cm against *Escherichia coli.* Moreover, the acetone extract showed elevated antibacterial activity with a 0.78 cm zone of inhibition against *Bacillus cereus.* Furthermore, the acetone extract of *Cinnamomum zeylanicum* of 1 mg/mL showed antibacterial activity, i.e., 0.76 cm and 0.86 cm, for *Escherichia coli*. And *Bacillus cereus*, respectively.

3.4.2. Antifungal assay

The antifungal activity of ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* for *Escherichia coli* and *Bacillus cereus* is shown in **Table 8** and **Fig 8**.

For *Piper nigrum*, 1 mg/mL of the acetone extract showed high antifungal activity of 1.37 cm for *Aspergillus niger* acetone extract with 1 mg/mL concentration for *Myristica fragrans* showed antifungal activity with a 1.67 cm zone of growth inhibition against *Aspergillus niger*. Similarly,

with 1 mg/mL of the acetone extract, *Cinnamomum zeylanicum* showed high antifungal activity with a 1.76 cm zone of clearance against *Aspergillus niger.*

3.5. Thin layer chromatography (TLC)

Thin layer chromatography (TLC) and UV-VIS spectra were carried out to separate phenolic acids and flavonoids in ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* phenolic acids and flavonoids were identified based on retention factor.

3.5.1. Separation of phenolic acids

Thin layer chromatography (TLC) separation of Phenolic acids was carried out, and It was observed that Phenolic compounds were separated using a Solvent system Toluene: Acetone: Formic acid (4.5:4.5:1) after exposure to ammonia fumes (**Fig 9.**)

In the ethanolic extract, the phenolic compounds identified in Piper nigrum were Orcinol, 4-methyl Resorcinol, Hydroquinone, Catechol, pyrogallol, Vanillic, p-hydroxybenzoic, and Salicylic acid. Resorcinol, 4-methyl Resorcinol, Catechol,p-hydroxybenzoic, and syringic are in the methanolic extract. In acetone extract, Gentisic, Catechol, Protocatechuic, Pholoroglucilol, Pyrogallol, and Salicylic acid, As mentioned in (**Tables 9,10** and **11.).**

In *Myristica fragrans,* the ethanolic extracts showed Catechole, 2-Methylresorcinol, p-hydroxybenzoic, and Salicylic acid. In the methanolic extract, Syrengic, p-hydroxybenzoic, 4-methyl Resorcinol, Vanillic, p-hydroxybenzoic and in acetone extract 4-methyl Resorcinol, Syringic, 2-methyl resorcinol, and Resorcinol.

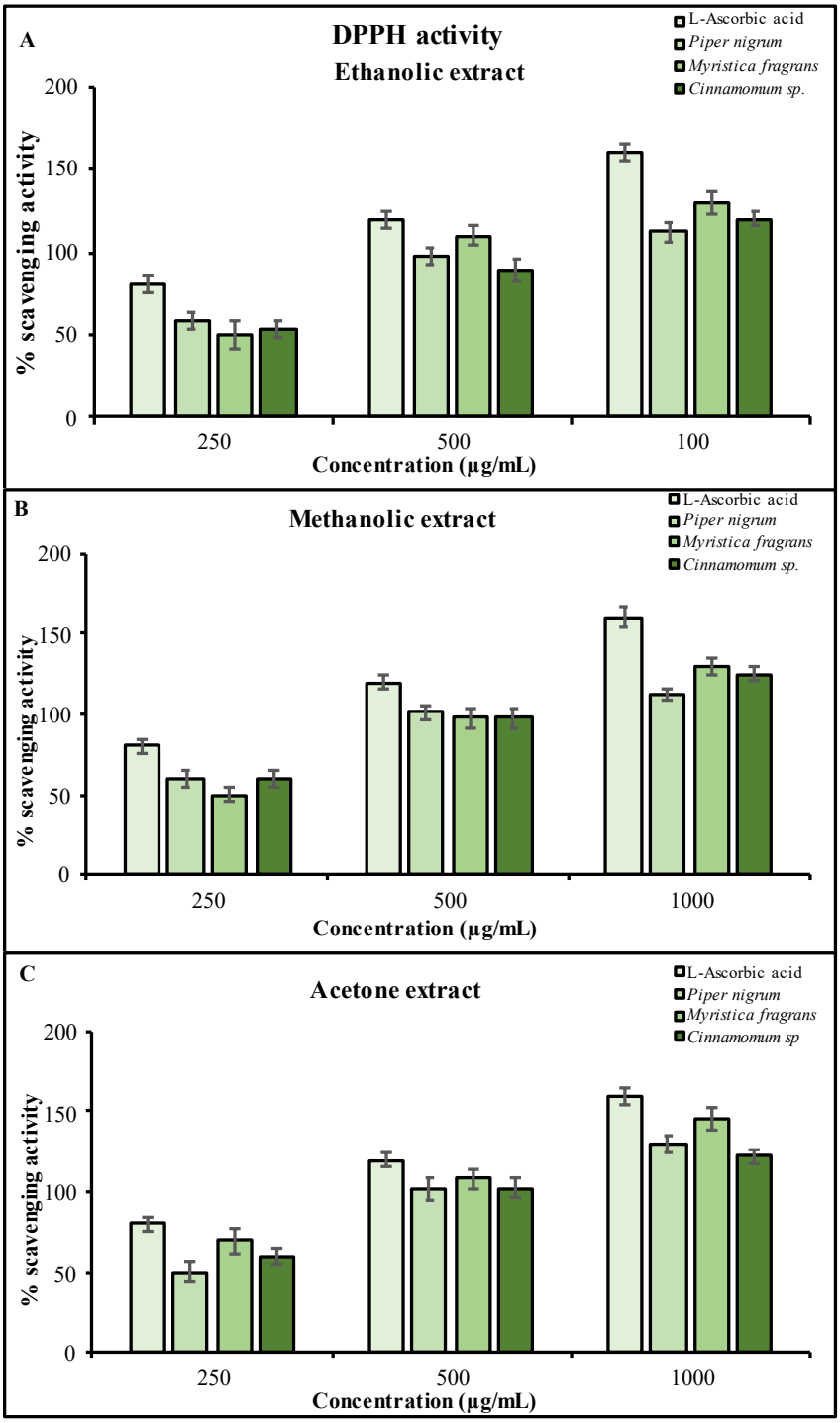


Fig. 6: Antioxidant activity- DPPH free radical scavenging activity of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum;* **A-** Ethanolic Extract ; **B-** Methanolic extract**, C-** Acetone extract**.** Bars represent mean values ± SD (n=3).

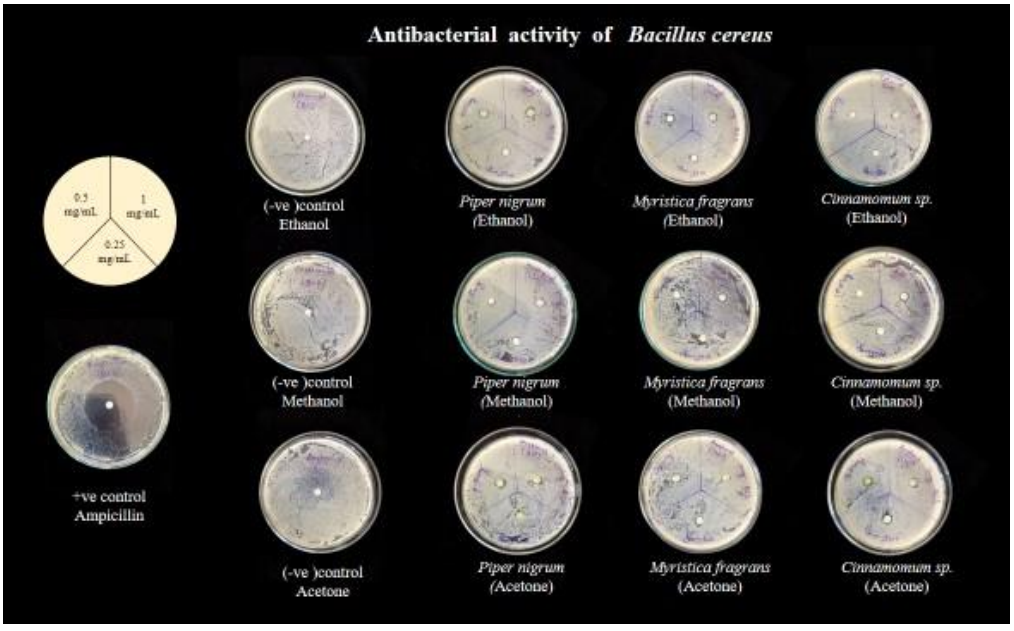


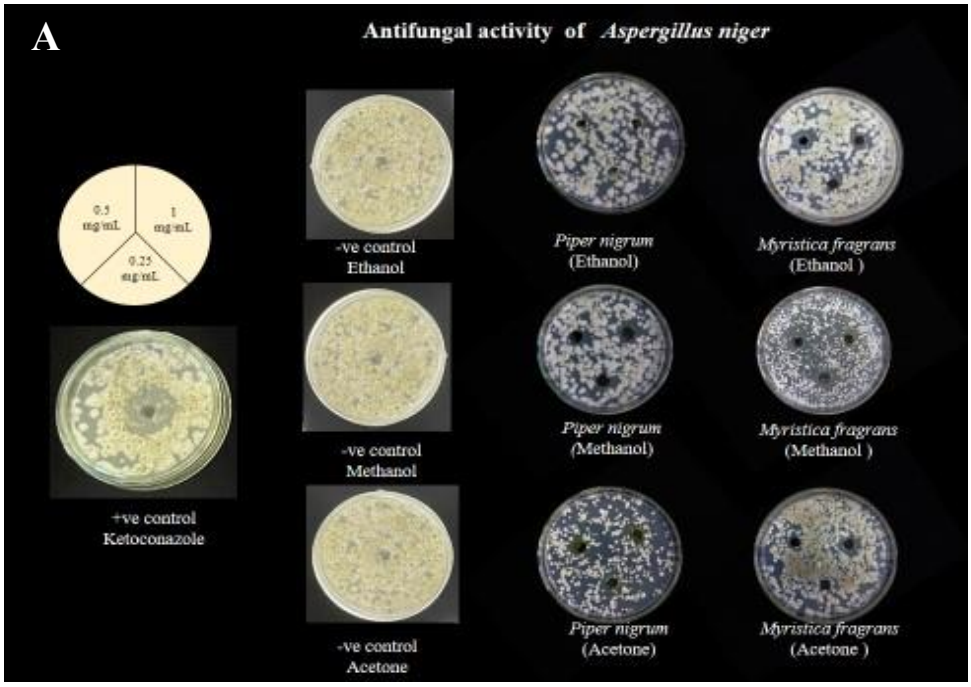
Fig.7: Antibacterial activity of E thanolic, Methanolic and Acetone extracts of against; **A-** *Escherichia coli* and **B-***Bacillus cereus* of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum.*

Table 7: Antibacterial assay- Inhibition zone of *Escherichia coli* and *Bacillus cereus* in the presence of different concentrations of Ethanolic, Methanolic and Acetone extracts of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* .. Data represent mean values ± Standard Deviation (n=3).

Leaf samples	Extraction solvents	Zone of inhibition for Bacterial strains					
		Escherichia coli			*Bacillus cereus*		
		0.25 mg/mL	0.5 mg/mL	1 mg/mL	0.25 mg/mL	0.5 mg/mL	1 mg/mL
Piper nigrum	Ethanol	0.62±0.02	0.69±0.01	0.72±0.02	0.61±0.01	0.65±0.01	0.82±0.01
	Methanol	0.62±0.01	0.71±0.01	0.81±0.02	0.62±.01	0.70±0.02	0.83±0.00
	Acetone	0.66±0.02	0.75±0.01	0.83±0.2	0.67±0.01	0.73±0.01	0.85±0.00
Myristica fragrans	Ethanol	0.61±0.01	0.62±0.02	0.68±0.02	0.65±0.00	0.68±0.01	0.71±0.28
	Methanol	0.63±0.02	0.63±0.01	0.65±0.00	0.64±0.01	0.67±0.00	0.70±0.01
	Acetone	0.63±0.02	0.67±0.01	0.72±0.03	0.70±0.01	0.75±0.01	0.78±0.01
Cinnamomum zeylanicum .	Ethanol	0.61±0.01	0.64±0.01	0.63±0.01	0.60±0.01	0.65±0.00	0.79±0.01
	Methanol	0.62±0.00	0.70±0.01	0.75±0.00	0.64±0.00	0.70±0.01	0.75±0.00
	Acetone	0.62±0.02	0.71±0.01	0.76±0.01	0.70±0.00	0.71±0.01	0.86±0.01
+ve control (Ampicillin)		4±0.81			4.67±0.94		
-ve control	Ethanol	0.65±0.03			0.73±0.01		
	Methanol	-			-		
	Acetone	-			-		

Table 8: Antifungal assay- Inhibition zone of *Aspergillus niger* in the presence of different concentrations of ethanolic, methanolic and acetone extracts of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* . Data represent mean values ± Standard Deviation (n=3).

Leaf samples	**Solvent Extracts**	**Zone of inhibition**		
		Aspergillus niger		
		0.25 mg/mL	0.5 mg/mL	1 mg/mL
Piper nigrum	Ethanol	1.1±0.11	1.2±0.00	1.36±0.11
	Methanol	1.06±0.05	1.21±0.11	1.3±0.11
	Acetone	1.16±0.05	1.23±0.05	1.37±0.17
Myristica fragrans	Ethanol	1.23±0.05	1.5±0.05	1.66±0.11
	Methanol	1.26±0.05	1.46±0.05	1.67±0.06
	Acetone	1.33±0.05	1.53±0.11	1.7±0.05
Cinnamomum zeylanicum	Ethanol	1.1±0.05	1.3±0.00	1.43±0.05
	Methanol	1.06±0.05	1.43±0.05	1.66±0.05
	Acetone	1.13±0.11	1.4±0.10	1.76±0.10
+ve control (Ketoconazole)		2.7±0.94		
-ve control	Ethanol	-		
	Methanol	-		
	Acetone	-		



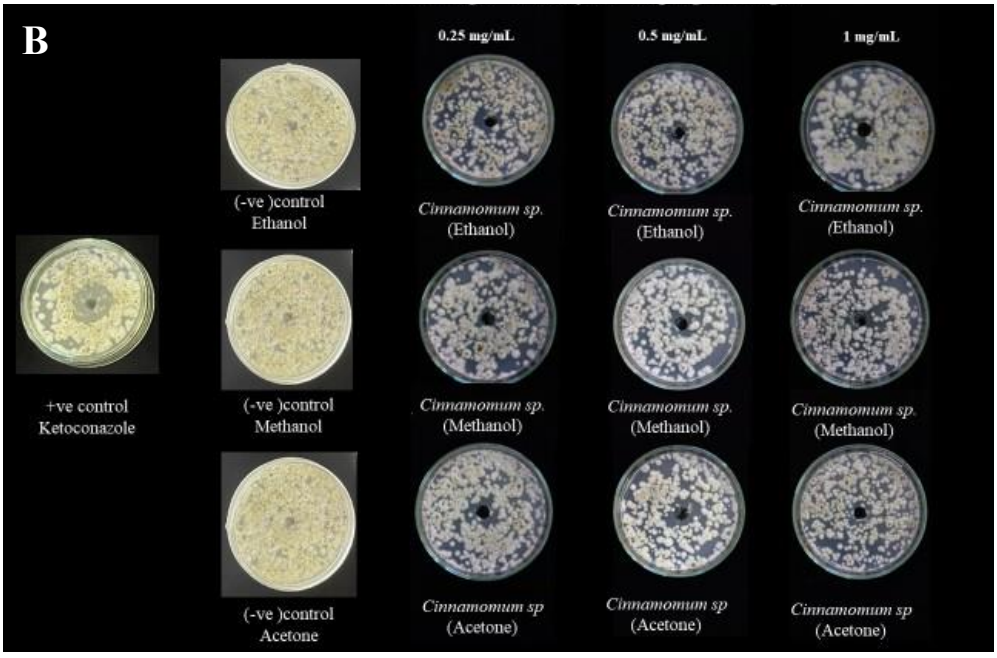


Fig 8: Antifungal activity: **A-**E thanolic, Methanolic and Acetone extracts of *Piper nigrum, Myristica fragrans* and **B-** E thanolic, Methanolic and Acetone extracts of *Cinnamomumzeylanicum* against *Aspergillus niger*

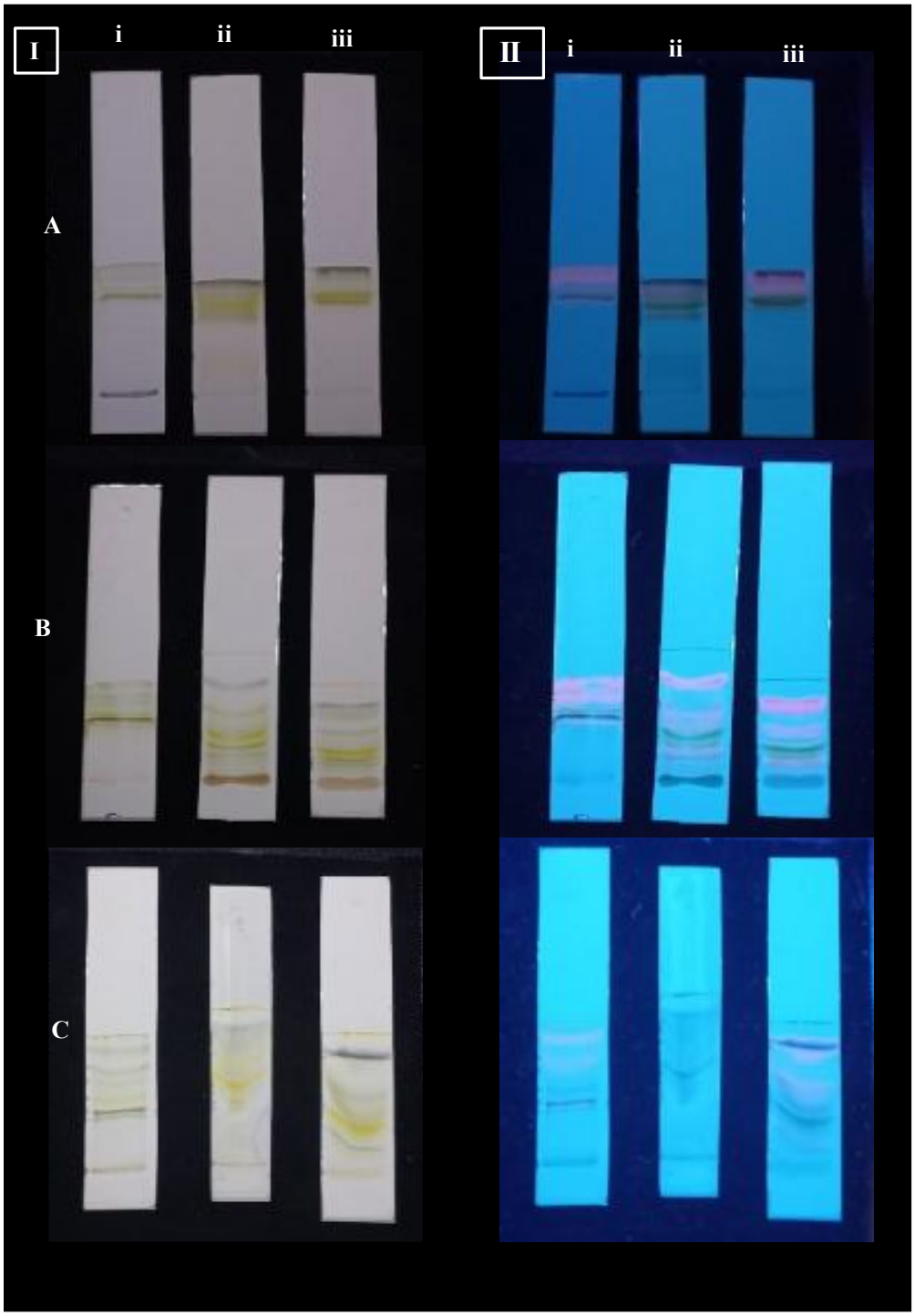


Fig. 9: Separation of Phenolic compounds using Thin Layer Chromatography (TLC): **I- Visible light; II- UV light; A-** Ethanolic extract*;* **B-** Methanolic extract ; **C-** Acetone extract of **i-** *Myristica fragrans***; ii-** *Piper nigrum***;** and **iii-** *Cinnamomum zeylanicum*

In *Cinnamomum zeylanicum*, the ethanolic extract showed Catechol, pyrogallol, Resorcinol, Syringic, and Salicylic. In the methanolic extract, 4-methyl Resorcinol, Gallic acid, Syringic, 2-methyl resorcinol, Catechol, and acetone extract, 4-methyl Resorcinol, Gentisic, p-hydroxybenzoic, and Vanillic.

In UV-VIS spectra, the phenolic compounds identified were Orcinol, 4-methyl Resorcinol at 283 nm, and Catechole, 2-Methylresorcinol at 279 nm.

3.5.2. Separation of Flavonoid compounds

Thin layer chromatography (TLC) separation of Flavonoids was carried out. It was observed that Flavonoids were separated using Solvent system Methanol: Chloroform: Hexane (7; 2; 1) after exposure to ammonia fumes (**Fig 10.**).

In the ethanolic extract, the flavonoid compounds identified in *Piper nigrum* were Orientin, Isovitexin, Kaempferol, and Chrysoeriol. In the methanolic extract, Isovitexin, Luteolin, and Apigenin. In acetone extract, Quercetin, Isorhamnetin, and Chrysoeriol (**Tables 12, 13** and **14.**).

In *Myristica fragrans*, the ethanolic extracts showed Azaleatin, Quercetin, Luteolin, Apigenin, and Kayaflavone. In the methanolic extract, Alaleatin, Isovitexin, Isorhamnetin, Apigenin, Kayaflavone and in the acetone extract, Isovitexin, Apigeinin Kayaflavone.

Similarly, In *Cinnamomum zeylanicum*, the ethanolic extract showed, Orientil, Azaleatin, Luteolin, and Kayaflavone. In the methanolic extract, Quercetin, Isorhamnetin, Chrysoeriol, Apigenin, Kayaflavone, and acetone extract Quercetin, Isorhamnetin, Chrysoeriol, Apigenin, Kayaflavone. In UV-VIS spectra, the Flavonoid compounds were not identified.

Table 9: Rf values and spectral properties of Phenolic compounds showing respective colouration in Ethanolic extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* .

Sr. No.	Ethanolic plant extract	Rf	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max} nm	Phenolic compounds
1	*Piper nigrum*	0.60	Brick red	Brick red	283	Orcinol, 4-methyl resorcinol
		0.69	Yellow	-	-	hydroquinone
		0.72	Light brown	-	-	Catechol, pyrogallol
		0.81	Blue	-	-	Vanillic , p-hydroxybenzoic , Salicylic
2	*Myristica fragrans*	0.72	Light brown	Bluish pink	279	Catechole , 2-Methylresorcinol
		0.80	Light brown	Blue	-	p-hydroxybenzoic
		0.88	Brown	Blue	-	Salicylic
3	*Cinnamomum zeylanicum* .	0.71	Light brown	Brown	-	Catechol, pyrogallol
		0.74	Light Brown	Orange	-	Resorcinol
		0.77	Pale yellow	Bluish -pink	-	Syringic
		0.88	Brown	Blue	-	Salicylic

Table 10: Rf values and spectral properties of Phenolic compounds showing respective colouration in Methanolic extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* .

Sr. No.	Methanolic plant extract	Rf	Colourof spot under visible light	Colourof spot under long UV at 365nm	λ_{max} nm	Phenolic compounds
1	*Piper nigrum*	0.16 0.27 0.37 0.56 0.75	Dark brown Light yellow Light brown Yellow Pale yellow	Dark brown - Bluish-pink Pink Orange	- - - - -	Resorcinol 4-methyl resorcinol Catechol p-hydroxybenzoic syringic
2	*Myristica fragrans*	0.53 0.56 0.63 0.73 0.80	Pale yellow Yellow Light yellow Blue Yellow	Bluish pink Pink - - Pink	- - - - -	Syrengic p-hydroxybenzoic 4-methyl resorcinol Vanillic p-hydroxybenzoic
3	*Cinnamomum zeylanicum*	0.24 0.41 0.51 0.65 0.72	Light yellow Blue Pale yellow Light brown Light brown	- Blue Bluish-pink Bluish-pink Bluish-pink	- - - - -	4-methyl resorcinol Gallic acid Syringic 2-methyl resorcinol Catechol

Table 11: Rf values and spectral properties of Phenolic compounds showing respective colouration in Acetone extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* .

Sr. No.	Acetone plant extract	Rf	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max} nm	Phenolic compounds
1	*Piper nigrum*	0.32 0.36 0.43 0.47 0.71 0.84	Olive green Light brown Olive green Light green Light brown Brown	Grey Bluish -pink Light blue - Brown Blue	- - - - - -	Gentisic Catechol Protocatechuic Pholoroglucilol Pyrogallol Salicylic
2	*Myristica fragrans*	0.23 0.51 0.64 0.74	Light yellow Pale yellow Light brown Dark brown	- Bluish -pink Bluish -pink Dark brown	- - - -	4-methyl resorcinol Syringic 2-methyl resorcinol Resorcinol
3	*Cinnamomum zeylanicum* .	0.25 0.30 0.55 0.82	Light yellow Olive green Yellow Blue	- Grey Pink -	- -	4-methyl resorcinol Gentisic p-hydroxybenzoic Vanillic

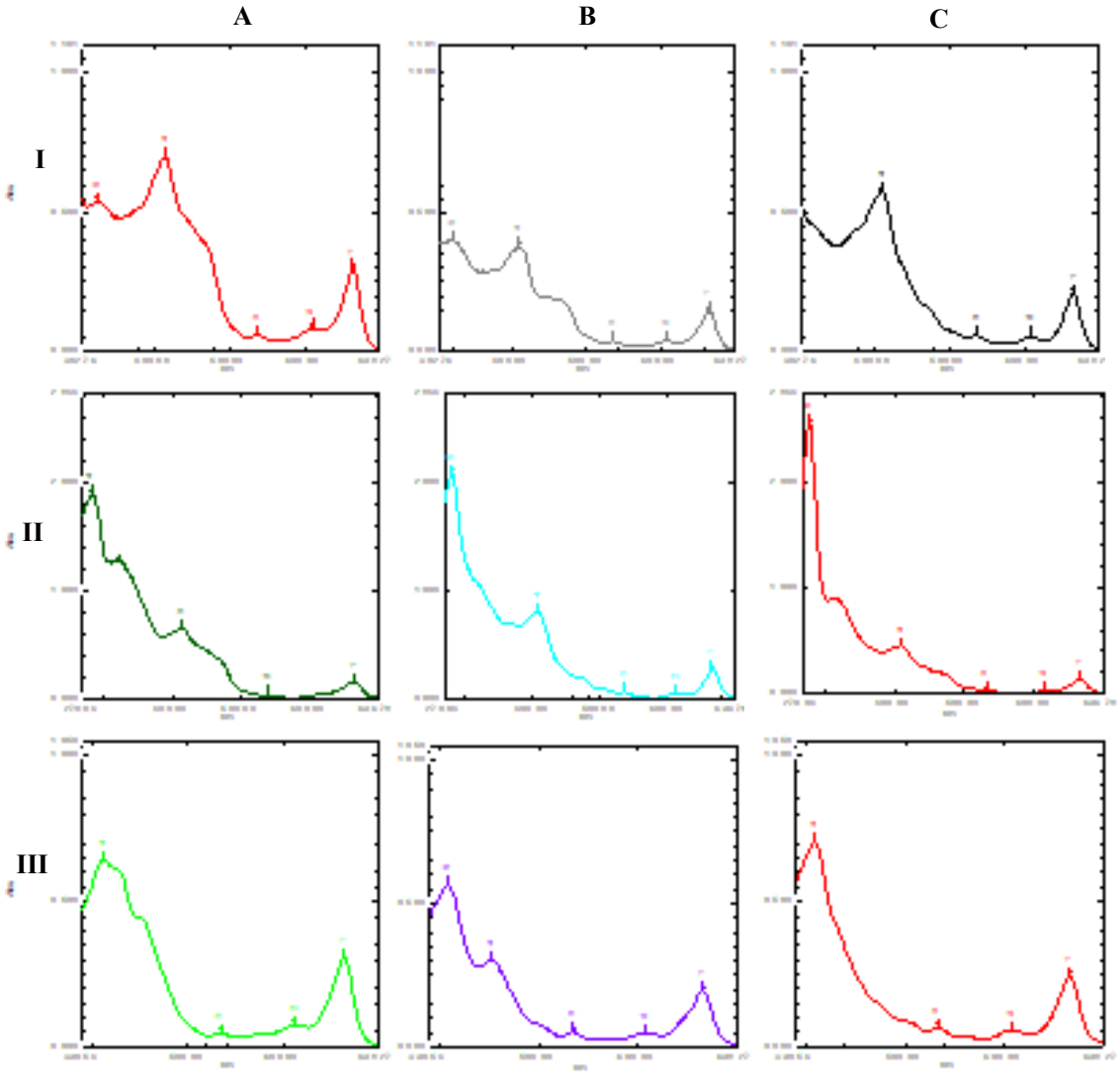


Fig. 10: UV-VIS spectra (190-700 nm) of **I-** Ethanolic extract; **II-** Methanolic extract **, III-** Acetone extract ; Where **A-** *Piper nigrum,* **B-** *Myristica fragrans and* **C-** *Cinnamomum zeylanicum* .

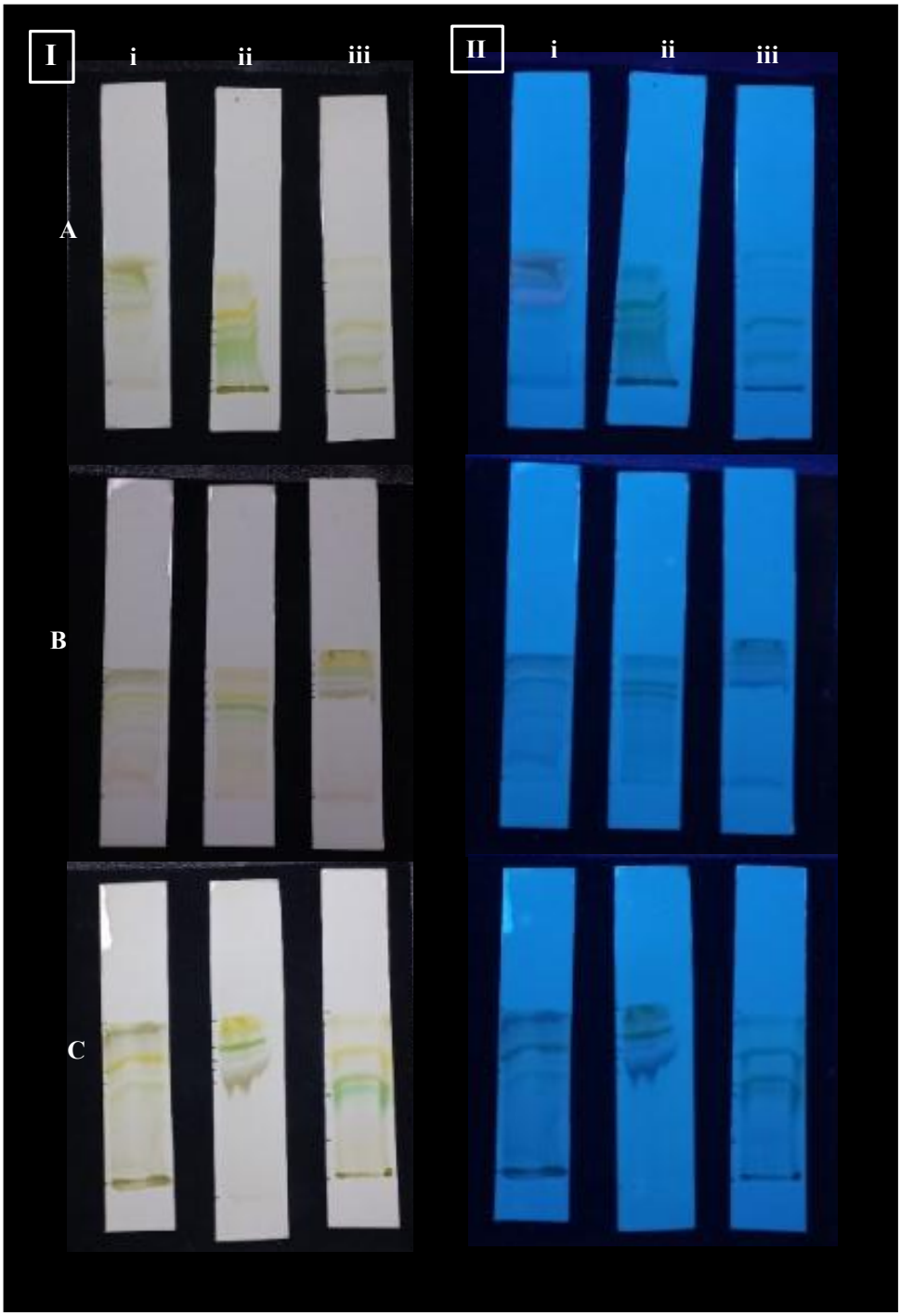


Fig. 11: Separation of Flavanoids using Thin Layer Chromatography (TLC): **I- Visible light; II- UV light; A-** Ethanolic extract; **B-** Methanolic extract; **C-** Acetone extract of **i-** *Myristica fragrans*; **ii-** *Piper nigrum*; and **iii-** *Cinnamomum zeylanicum*

Table 12: Rf values and spectral properties of Flavonoid showing respective colouration in Ethanolic extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* .

Sr. No.	Ethanolic extract	Rf	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max} nm	Flavanoids compounds
1	*Piper nigrum*	0.33 0.48 0.57 0.82	Dull ochre Dull ochre Bright yellow Dull ochre	Dull ochre Dull ochre Bright yellow Dull ochre	- - - -	Orientin Isovitexin Kaempferol Chrysoeriol
2	*Myristica fragrans*	0.48 0.70 0.78 0.89 0.94	Fluorescent yellow Bright yellow Dull ochre Dull ochre Dull brown	Fluorescent yellow Bright yellow Dull ochre Dull ochre Dull brown	- - - -	Azaleatin Quercetin Luteolin Apigenin Kayaflavone
3	*Cinnamomum zeylanicum* .	0.33 0.46 0.76 0.92	Dull ochre Fluorescent yellow Dull ochre Dull brown	Dull ochre Fluorescent yellow Dull ochre Dull brown	- - - -	Orientil Azaleatin Luteolin Kayaflavone

Table 13: Rf values and spectral properties of Flavonoid compounds showing respective colouration in Methanolic extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* .

Sr. No.	Methanolic extract	Rf	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max} nm	Flavanoids compounds
1	*Piper nigrum*	0.60 0.80 0.88	Dull ochre Dull ochre Dull ochre	Dull ochre Dull ocher Dull ochre	- - -	Isovitexin Luteolin Apigenin
2	*Myristica fragrans*	0.48 0.60 0.74 0.88 0.94	Fluorescent yellow Dull ochre Bright yellow Dull ochre Dull brown	Fluorescent yellow Dull ochre Bright yellow Dull ochre Dull brown	- - - -	Azaleatin Isovitexin Isorhamnetin Apigenin Kayaflavone
3	*Cinnamomum zeylanicum* .	0.65 0.75 0.80 0.85 0.97	Bright yellow Bright yellow Dull ochre Dull ochre Dull brown	Bright yellow Bright yellow Dull ochre Dull ochre Dull brown	- - - - -	Quercetin Isorhamnetin Chrysoeriol Apigenin Kayaflavone

Table 14: Rf values and spectral properties of Flavonoid compounds showing respective colouration in Acetone extract of *Piper nigrum, Myristica fragrans and Cinnamomum zeylanicum* .

Sr. No.	Acetone plant extract	Rf	Colour of spot under visible light	Colour of spot under long UV at 365nm	λ_{max} nm	Flavanoids compounds
1	***Piper nigrum***	0.62 0.74 0.82	Bright yellow Bright yellow Dull ochre	Bright yellow Bright yellow Dull ochre	- - -	Quercetin Isorhamnetin Chrysoeriol
2	***Myristica fragrans***	0.53 0.57 0.91	Dull ochre Dull ochre Dull brown	Dull ochre Dull ochre Dull brown	- - -	Isovitexin Apigeinin Kayaflavone
3	***Cinnamomum zeylanicum .***	0.50 0.56 0.93	Fluorescent yellow Dull ochre Dull brown	Fluorescent yellow Dull ochre Dull brown	- - -	Azaleatin Isovitexin Kayaflavone

4. DISCUSSION

4.1. Total Phenolic Content

The total phenolic substances and flavonoids are plants' most critical bioactive constituents and may have vast potential to be an essential source of phytomedicine. Phenolic compounds contained in plants have redox properties, allowing them to act as antioxidants (Baba and Malik, 2015). Phenols are important plant compounds that can destroy radicals because they contain hydroxyl groups and give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals; hence, they play an important role in antioxidant activity. Therefore, determining the quantity of phenolic compounds is crucial to determining plant extracts' antioxidant capacity (Hatano et al., 1989). The present study tested the ethanolic, methanolic, and acetone extracts for their total phenolic content (TPC) in *Piper nigrum, Myristica fragrans*, and *Cinnamomum zeylanicum*, respectively. The results showed acetone extract of *Piper nigrum, Myristica fragrans*, and *Cinnamomum zeylanicum* had higher total phenolic content (TPC) than the ethanolic extract and methanolic extracts (**Table1** and **Fig.2.**). However, these findings were supported by previous studies of Chavan et al. (2013), Dailey and Vuong (2015). Do et al. (2014) which reported that different extraction solvents significantly affect the extraction yields of TPC in *Salacia chinensis*, *Macadamia tetraphylla*, *Limnophila aromatica*. Dailey and Vuong (2015) reported that 50% acetone with water was the best solvent for the extraction of TPC from macadamia skin. Furthermore, Do et al. (2014) found that absolute ethanol and acetone were the best extraction solvents for TPC from *Limnophila aromatica*, which reported that extraction solvents significantly affected TPC. The variation can also be explained by the different polarities of compounds that were selectively more soluble in different solvents.

4.2. Total Flavonoid Content

Flavonoids are the most diverse and widespread polyphenolic compounds with a broad spectrum of biological activities, including radical scavaging properties found in plants for average growth and development and defence against infection and injury (Harborne and Williams, 2000). Flavonoids, including flavones, flavonols, and condensed tannins, are secondary plant metabolites, the antioxidant activity of which depends on free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and act as antioxidants *in vivo* (Geetha et al., 2003; Shimoi et al., 1989). The present study observed that the total flavonoid content (TFC) was higher in the acetone extract of *Piper nigrum, Myristica fragrans,* and *Cinnamomum zeylanicum* compared to their ethanolic and methanolic extract. Similar results were seen in *Phoenix dactylifera* and *Limnophila aromatica.* The recovery of flavonoid contents in different samples is influenced by the polarity of extracting solvents and the solubility of this compound in the solvent used for the extraction of the process where acetone extract showed maximum extraction of total flavonoid content (TFC) (Kchaou et al., 2013; Do et al., 2014).

4.3. Antioxidant activity

A linear correlation between antioxidant activity and the content of polyphenols has been reported by Katalinic et al. (2006). The free radical scavenging activity and concentration-dependent radical scavenging activity were observed in DPPH methods. A positive correlation coefficient between the total phenolic content and DPPH assay of plant extracts obtained from ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) was reported by Maizura et al. (2011). The results of the antioxidant assay using DPPH correlated with the results of total phenolic content (TPC). The

results observed were similar to the total phenolic contents (TPC). The acetone extract showed *the highest antioxidant activity of Piper nigrum, Myristica fragrans, and Cinnamomum zeylanicum* (**Table3** and **Fig.5.**). Shanmugapriya et al. (2012) reported high antioxidant activity of acetone extract compared to the other solvent extracts in *Piper nigrum.* Singh (2020) reported similar results for the bark of *Cinnamomum zeylanicum* in different solvents, where acetone extract showed the maximum radical scavenging activity compared to the other extracts. The acetone extract of Nutmeg (*Myristica fragrans*) showed the maximum antioxidant activity compared to methanolic and ethanolic extracts (Gupta et al., 2013).

4.4. Antimicrobial Activity

Our studies showed that all three extracts, namely ethanolic, methanolic, and acetone, showed successful antibacterial activity against *Escherichia coli* and *Bacillus cereus*. Among all three solvents, acetone extracts showed the maximum antibacterial activity, followed by methanolic and ethanolic extracts. This can be supported by a study by Ateş and Turgay (2003), who reported a successful inhibition against the bacterial strain *Bacillus cereus* using acetone extract of *Cinnamomum zeylanicum* Ahmed et al. (2020) reported a similar activity against *Escherichia coli* with *Cinnamomum* extract, which stated that essential oils from cinnamon exhibited antimicrobial effects against selected food-borne and food spoilage bacteria, which can be attributed to the presence of the principle bioactive components, particularly cinnamaldehyde. Similarly, ethanolic, methanolic, and acetone extracts showed successful antifungal activity against *Aspergillus niger;* similar to the bacterial strains, the acetone extracts showed the highest antifungal activity, followed by methanolic and then ethanolic extracts. Amongst the three spices, the acetone extracts of *Cinnamomum zeylanicum* showed the highest inhibition against *Aspergillus niger.* Such

observations were also recorded in the study done by Ankita et al. (2013). The antimicrobial activity shown by the cinnamon extracts may also be due to the presence of cinnamaldehyde. According to Aneja et al. (2009), this aromatic aldehyde, cinnamaldehyde, is rich in Cinnamon bark; however, our results obtained from the leaf extract can be correlated with the bark extract of the *Cinnamomum zeylanicum* selected.

4.5. Thin Layer Chromatography and UV-VIS Spectra

The phenolic compounds identified in the ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans,* and *Cinnamomum zeylanicum* are Orcinol, 4-methyl Resorcinol, Hydroquinone, Catechol, pyrogallol, Vanillic, p-hydroxybenzoic, Salicylic acid, Resorcinol, p-hydroxybenzoic, syringic acid, Gentisic, Protocatechuic, Pholoroglucilol 2-methyl resorcinol, p-hydroxybenzoic, Gallic acid. The flavonoid compounds identified in the ethanolic, methanolic, and acetone extracts of *Piper nigrum, Myristica fragrans,* and *Cinnamomum zeylanicum* were Orientin, Isovitexin, Kaempferol, Chrysoeriol, Luteolin, Apigenin, Quercetin, Isorhamnetin, Azaleatin, Quercetin,., Alaleatin, Isorhamnetin, Apigenin, and Kayaflavone. Ali *et al*. (2021) state that Phenolic acids are found ubiquitously in herbs and spices and are widely reported for their potential prospective, including antioxidant, antimicrobial, anti-inflammatory, antimutagenic, and anti-cancer. At the same time, flavonoids are the most abundant class of secondary plant metabolites, used for pharmaceutical, medicinal, and cosmetic applications due to their antioxidative, anti-inflammatory, anticarcinogenic, and antimutagenic properties. Phenolic compounds in plants may act as antioxidants Shori, (2022). Due to this, the extracts showed a positive effect on antioxidant and antimicrobial activities.

5. Conclusion

Spices are an excellent source of vitamins A and C, phenolic compounds which are essential antioxidants for various plant defence responses. Due to the presence of phenolic compounds, it has a more potent antimicrobial and antioxidant activity. Flavonoids play a vital role in scavenging free radicals, and these phytoconstituents can be focused on for investigating many biological and antioxidant activities. We used three extracts, of which acetone extracts showed higher values for TPC, TFC, and DPPH assay and also for antimicrobial activity. The acetone extract of *Cinnamomum zeylanicum* showed higher values for all the tests compared to other two extracts of *Piper nigrum* and *Myristica fragrans*. Acetone extracts are rich in phytochemicals due to acetone being a suitable solvent due to its ability to dissolve polar and nonpolar substances, while other solvents can only dissolve one or the other. Acetone's chemical makeup includes polar and nonpolar elements, which means acetone can be used with both organic and inorganic substances. Antioxidant properties can be used as an easily accessible source of natural antioxidants, as a possible food supplement, and in the pharmaceutical industries.

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