QUALITATIVE SCREENING AND SPECTROSCOPIC EVALUATION OF THERAPEUTIC PHYTOCHEMICALS IN SELECTED MEDICINAL HERBS

THESIS

Submitted for partial fulfillment of the requirements for the degree of

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Submitted by

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DECLARATION

I, Abhishek , 24/MSCBIO/66, hereby, certify that the work which is being presented in the thesis entitled "**Evaluation Of Phytochemical Profiles And Bioactive Compounds In Selected Medicinal Plants Using Qualitative And Spectroscopic Techniques**" in partial fulfilment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2024 to 2026 under the supervision of Dr. Navneeta Bhardvaja. The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Candidate's Signature

This is to certify that the student has incorporated all the corrections suggested by the examiner in the thesis and the statement mailed by the candidate is correct to the best of our knowledge.

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I, Harshita, 24/MSCBIO/63, hereby, certify that the work which is being presented in the thesis entitled "**Evaluation Of Phytochemical Profiles And Bioactive Compounds In Selected Medicinal Plants Using Qualitative And Spectroscopic Techniques"** in partial fulfilment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2024 to 2026 under the supervision of Dr. Navneeta Bhardvaja. The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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CERTIFICATE BY THE SUPERVISOR

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ABSTRACT

Plant derived compounds have been a topic of continued scientific scrutiny globally, especially in the era of increased antimicrobial resistance and the growing epidemic of chronic non-communicable diseases. This dissertation discusses the phytochemical composition and medicinal significance of two widely used medicinal plants: *Bougainvillea spectabilis* Willd. (Nyctaginaceae), *Thymus vulgaris* L. (Lamiaceae), *Rosmarinus officinalis* L and Harsingar or Parijat *Nyctanthes arbor-tristis* — through a comprehensive review of current literature.

Bougainvillea spectabilis is particularly rich in a large number of secondary metabolites including betalains (betacyanins and betaxanthins), flavonoids (such as quercetin and kaempferol), the insulin sensitizing agent pinitol, hydrolysable tannins and gallic acid. Experimental studies have shown that these constituents work synergistically to exhibit antioxidant, anti-inflammatory, antidiabetic and antimicrobial properties, with well-defined molecular mechanisms. *Thymus vulgaris* has a similarly interesting phytochemical profile, with the phenolic monoterpenes thymol and carvacrol, polyphenolic acid rosmarinic acid, and flavones luteolin and apigenin. These compounds have been extensively studied for their pharmacological activities that have substantiated the plant's efficacy as an antimicrobial, anti-fungal, antioxidant and anti-inflammatory. Various parts of the plant such as leaves, flowers, seeds, bark and stems are employed for treating several health problems. Its therapeutic importance is mainly attributed to the presence of bioactive compounds like flavonoids, glycosides, alkaloids, tannins, phenolics and essential oil

One of the key goals of this work is to establish a mechanistic correlation based on experimental data between the specific phytochemicals identified in these 2 plants and the molecular pathways where they are acting on disease processes. The review shows that both species have coherent and scientifically established pharmacological mechanisms for the traditional and contemporary applications, providing scientific backing for ongoing research and possible drug development.

Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

1.1 GENERAL INTRODUCTION

Medicinal plants contain biologically active compounds that are used for the prevention and treatment of several diseases. These plant-based compounds possess antioxidant, antimicrobial, anti-inflammatory, antiviral, anticancer, and antidiabetic activities. Flavonoids, phenols, alkaloids, tannins, terpenoids, steroids, and saponins are the major secondary metabolites that are responsible for the medicinal properties. These compounds are formed during secondary metabolism and act as a defence mechanism of plants to environmental stress, microbial attack, insects, and other noxious conditions. According to the World Health Organization (WHO), a large part of the world's population still depends on traditional herbal medicine for primary healthcare. Medicinal plants and herbal products are used directly or indirectly by more than 80% of the population in developing countries for health benefits. Herbal medicines are important because they provide natural alternatives for disease management and play a significant role in pharmaceutical research and drug discovery. Medicinal plants are rich sources of nutraceutical compounds such as vitamins, carotenoids, flavonoids, essential oils and phenolic compounds. These bioactive compounds possess potent antioxidant properties and play an important role in reducing oxidative stress and prevention of chronic disorders such as cardiovascular diseases, cancer, diabetes, inflammation and neurodegenerative diseases. Due to these therapeutic benefits, medicinal plants are gaining increasing interest for development of novel drugs and healthcare products. In recent times, use of medicinal plants has gained significant importance in scientific studies due to increased awareness regarding the adverse effects and resistance of synthetic drugs. Therefore, scientific evaluation and phytochemical screening of medicinal plants is important for identification of biologically active compounds and to substantiate their medicinal uses. Phytochemical studies are useful to understand the therapeutic potential of plants and assist in utilizations of plants in pharmaceutical, nutraceutical and biomedical applications. Secondary plant metabolites have underpinned human medicine across diverse civilisations for thousands of years. Contemporary drug discovery continues to draw heavily from plant chemistry; a systematic analysis of newly approved therapeutic agents between 1981 and 2014 found that more than half were either directly derived from, or structurally inspired by, natural products [1][2]. This enduring relevance reflects the structural diversity of plant-derived molecules and their evolutionary optimisation for interaction with biological macromolecules. Against the backdrop of increasing antibiotic resistance, rising prevalence of metabolic disorders such as type 2 diabetes mellitus, and the limitations of existing synthetic therapies, the systematic investigation of medicinal plant chemistry presents a scientifically compelling and clinically relevant avenue of research.

Bougainvillea spectabilis is a vigorous woody climber originating from South America and now widely cultivated across tropical and subtropical regions of Asia, Africa, and the Indian subcontinent. Despite its ornamental prominence, the plant carries substantial ethnomedicinal significance. In Brazilian folk practice, its bract and leaf preparations are administered for respiratory ailments; in Ayurvedic medicine, the plant is employed for diabetes management and liver disorders; and in West African traditional practice, topical leaf preparations are used for wound healing[3], [4]

Thymus vulgaris L. is a perennial aromatic subshrub of the Lamiaceae family with deep roots in European and Mediterranean medicine dating back over three millennia. It is formally recognised in the European Pharmacopoeia as a bronchospasmolytic and expectorant agent, and its essential oil is included in multiple national formularies for antiseptic applications[5]

Rosmarinus officinalis L., also called Salvia rosmarinus, is an evergreen aromatic herb of the family Lamiaceae. The stems are woody and can be either erect or trailing depending on the cultivar. The leaves are narrow and needle like with slightly recurved margins, giving them a tough leathery look. The upper surface of the leaves is dark green, while the undersurface is a lighter shade of green. The flowers are small, showy, usually blue or lavender, but white and pink forms are available. The flowers are borne in clusters and are adapted to insect pollination, particularly by bees. It is widely distributed in the Mediterranean region and cultivated all over the world for its medicinal and culinary uses.[6]

Harsingar or Parijat *Nyctanthes arbor-tristis* is a popular medicinal plant of traditional systems of medicine like, Ayurveda, Unani and Siddha. Various parts of the plant such as leaves, flowers, seeds, bark and stems are employed for treating several health problems. Its therapeutic importance is mainly attributed to the presence of bioactive compounds like flavonoids, glycosides, alkaloids, tannins, phenolics and essential oil.[7], [8]

The present review brings together phytochemical and pharmacological evidence on both species with the specific aim of connecting identified bioactive constituents to experimentally characterised molecular mechanisms. Rather than cataloguing biological activities in isolation, this work seeks to explain why these plants exhibit the activities they do — by tracing each therapeutic effect to the compound class responsible and to the cellular or biochemical target involved. This mechanistic orientation distinguishes the current review from purely descriptive accounts and provides a foundation for rational drug development from these plant sources.

1.2 SECONDARY METABOLITES

Primary metabolites are organic compounds that are required for normal growth, development and reproduction of plants they are directly involved in important biological processes such as cell division, respiration, photosynthesis and energy production. They include carbohydrates, proteins, amino acids, nucleic acids and intermediates of metabolic pathways such as glycolysis and Krebs (citric acid) cycle. Primary metabolites are usually similar in all living organisms and are needed for survival. However, secondary metabolites are organic compounds that are not directly involved in primary processes that sustain life but that play an important role in plant defence, adaptation and interaction with the environment. The compounds are synthesized by secondary metabolic pathways and are the main contributors to the medicinal properties of the plants. Secondary metabolites are involved in plant defence against pathogens, herbivores, environmental stress and other external factors.

Secondary metabolites of plants are divided into different groups based on their chemical structure. These classes are well studied due to their pharmacological and therapeutic importance. The major classes of secondary metabolites are:

1. Phenols
2. Alkaloids
3. Saponins
4. Terpenes
5. Lipids
6. Carbohydrates

All the classes of secondary metabolites have different structural characteristics, distribution in plants and biological effects. These compounds have a variety of pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory and anticancer activities making them of great importance in the study of medicinal plants

1.3 SIGNIFICANCE OF PHYTOCHEMICAL ANALYSIS

Phytochemical analysis forms the chemical fundamentals to the biological effect of medicinal plants. Phytochemical screening tests can be used to identify the major secondary metabolite classes present in a plant (alkaloids, flavonoids, terpenoids, tannins, saponins, glycosides, steroids and phenolic acids) and more detailed chromatographic and spectroscopic analyses can be used to identify and quantify individual compounds [9] It is a prerequisite to understand the phytochemical composition to define structure-activity relationships and to elucidate the pharmacological properties of a plant in molecular terms.

Secondary metabolites of plants are not synthesized for growth or development but for the protection against adverse environmental factors (herbivores, pathogens, abiotic conditions). Many of these molecules have therapeutic potential because of their interaction with the biochemistry of animals. Phenolic compounds chelate metal ions and scavenge reactive oxygen species (ROS); terpenoids disrupt microbial cell membranes; alkaloids inhibit enzymes and flavonoids regulate inflammatory signalling pathways. The elucidation of these interactions at the mechanistic level links a traditional knowledge and evidence-based medicine [10]

The objectives of the Project are:

1. Quantitative estimation of Flavonoid, terpenoid, quinones, fats and oils in plants
    i. *Bougainvillea spectabilis*
    ii. *Thymus vulgaris*
    iii. *Nyctanthes arbor-tristis*
    iv. *Rosmarinus officinalis*

2. Qualitative estimation of Flavonoid, terpenoid, quinones, fats and oils in plants
    i. *Bougainvillea spectabilis*
    ii. *Thymus vulgaris*
    iii. *Nyctanthes arbor-tristis*
    iv. *Rosmarinus officinalis*

1.4 ORGANISATION OF THESIS

The following thesis titled as is a reviewed information gathered from various research and review articles. The thesis focuses on the estimation of secondary metabolites in two different medicinal plants. The thesis also contains information about the therapeutic mechanism of these secondary metabolites in disease control.

Chapter 1 provides a brief introduction to the medicinal plants, and the secondary metabolites produced the plants and their therapeutic effects against diseases in humans.

Chapter 2 is the review of literature section which contains a summary of all the information obtained from the different research papers and review articles which contains experimental and reviewed data on the two medicinal plants and therapeutic effects of the secondary metabolites present in them.

Chapter 3 puts forward the methodology used in this project for the extraction process, techniques used for estimation of different secondary metabolites,

Chapter 4 contains results, discussions and conclusion of the project

CHAPTER 2: REVIEW OF LITERATURE

2.1 *Bougainvillea spectabilis*

Bougainvillea spectabilis Willd. is a vigorous, woody climber of the family Nyctaginaceae, native to South America and introduced throughout tropical Asia, Africa and India. It has large bracts that are not actually petals, but are modified leaves, which contain small tubular flowers, and which concentrate the most characteristic secondary metabolites of the plant. Bract and leaf decoctions are used in folk medicine in Brazil for cough and bronchitis, in Ayurvedic medicine for hepatic complaints, diabetes, and inflammatory conditions, and in West African healing for treating infected wounds [3], [11]

Phytochemicals studied on B. spectabilis are related to the presence of water-soluble, nitrogen containing pigments, called betalains, which are limited in occurrence within the plant kingdom to the Caryophyllales order. The bracts are red violet color due to betacyanins (mainly betanin and isobetanin) and yellow orange color due to betaxanthins (indicaxanthin, vulgaxanthin I). Both subclasses share the betalamic acid chromophore, which has a high degree of extended conjugation, allowing it to exhibit high electron-donating capacity [12], [13]. HPLC-DAD has quantified total betalain content of bract extracts as 34-118mg BE/100g FW [14]. Flavonoids, particularly quercetin, kaempferol and their glycosides (rutin and quercetin-3-O-glucoside), are regularly found in extracts from leaves and bracts and total flavonoids range from 12.3 to 38.7 mg quercetin equivalent (QE)/g DW. Pharmacologically important compounds were isolated from the leaves and stem bark of the plant and identified as inositol derivative pinitol (3-O-methyl-D-chiro-inositol) by GC-MS, which was found to exhibit insulin-mimetic activity [15]. The chemical profile includes hydrolysable tannins, gallic acid, saponins and indole-type alkaloids.

2.2 Phytochemical profile of *Bougainvillea spectabilis*

2.2.1 Betalains

Betalains, a group of water-soluble, nitrogen-containing pigments that occur only in plants of the order Caryophyllales (including Nyctaginaceae), are the most characteristic and structurally unique phytochemicals of B. spectabilis. Betalains are divided into red-violet betacyanins (e.g. betanin, isobetanin) and yellow-orange betaxanthins (e.g. indicaxanthin, vulgaxanthin). The structural basis is the betalamic acid chromophore; it bestows excellent electron-donating ability [12]. HPLC-DAD and LC-MS analysis of B. spectabilis bract pigments reveal the presence of betacyanin-type pigment compounds as the major ones [13], [14]

2.2.2 Flavonoids

The flavonoids have been isolated from different parts of B. spectabilis. HPLC analysis of leaf and bract extracts have identified quercetin and kaempferol and its glycosidic derivatives (3-O-glucoside of quercetin and kaempferol-3-O-rutinoside) in the extracts [11]. Another compound, rutin (quercetin-3-O-rutinoside), has also been found. These compounds play an important role in the antioxidant and anti-inflammatory activities of the plant.[16]

2.2.3 Alkaloids and Pinitol

A few alkaloids such as bougainvillin and a small amount of indole type compounds were reported in B. spectabilis (Jawad et al., 2012). Pinitol (3-O-methyl-D-chiro-inositol) is a derivative of inositol that has been documented as insulin-mimetic and antidiabetic [15] and is thus of pharmacological interest. Pinitol has been detected in extracts of the leaves and stems of B. spectabilis and is thought to be one of the main compounds responsible for the hypoglycaemic effect.

2.2.4 Tannins, Saponins, and Phenolic Acids

Hydrolysable tannins, saponins, terpenoids and steroids are always detected in Bougainvillea spectabilis extracts from both qualitative and quantitative phytochemical screening tests. The extracts from bracts have been found to contain gallic acid, a phenolic acid with strong antioxidant and hepatoprotective properties [3]. The total phenolic content (TPC) of leaf methanol extracts ranges from 18.4 to 62.7 mg of gallic acid equivalent (GAE)/g of dry weight depending on extraction solvent and plant part [14]

2.3 *Thymus vulgaris*

Thymus vulgaris L. Monograph status in the European Pharmacopoeia (10th ed.), British Pharmacopoeia and German Commission E (monograph) for treatment of bronchitis and upper respiratory catarrh is held by the species of Lamiaceae found in the western Mediterranean basin. It has been used medicinally since ancient Egypt, in embalming, and as an antiseptic in ancient Greece, and is still used in modern phytomedicine (Bhatt et al., 2020). Thymol (2-isopropyl-5-methylphenol) and carvacrol (5-isopropyl-2-methylphenol) are phenolic monoterpenes that comprise 30–80% of the essential oil composition, identified by GC-MS [17], obtained through the hydrodistillation method with a yield of 0.8–2.6% (v/w). Other monoterpenes include p-cymene (5-30%), γ-terpinene (2-10%), linalool and borneol.

T. vulgaris contains a significantly higher amount of rosmarinic acid (RA), an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (3,4-DHPL), than the other Lamiaceae species in the polar fraction, with 6–34 mg/g dry weight [18]. Two non-volatile triterpenoids, ursolic acid and oleanolic acid, are distributed over the leaf surface wax, and flavones, luteolin and luteolin 7-O-glucoside, apigenin, naringenin and eriodictyol are consistently detected by HPLC and LC-MS. The minor constituents are tannins, saponins and flavanones.

2.4 Phytochemical profile of *Thymus vulgaris*

2.4.1 Essential Oil: Thymol and Carvacrol

The essential oil from steam distillation of aerial parts of Thymus vulgaris is present in the dried plant at 0.8-2.6%. The major compounds of the oil consistently detected by GC-MS analysis are thymol (2-isopropyl-5-methylphenol) and carvacrol (5-isopropyl-2-methylphenol), which respectively occur in 30-80% of the total oil composition, depending on the chemotype and geographical origin [17]. Other monoterpenes found are p-cymene (5–30%), γ-terpinene (2–10%), linalool and borneol. Thymol is the most extensively investigated bioactive component of thyme and holds a major share of the antimicrobial and antifungal activity of this herb.

2.4.2 Polyphenolic Acids

Thymus vulgaris contains an exceptional amount of hydroxycinnamic acids. The major phenolic acid found in the leaf extracts is rosmarinic acid (RA) (6–34 mg/g dry weight) and accounts for much of the antioxidant and anti-inflammatory activity in the plant[18]. Other compounds have also been found, including caffeic acid, chlorogenic acid and salvianolic acids. They are biosynthesized by the phenylpropanoid pathway and contain the characteristic catechol functionality which is responsible for their strong radical scavenging and chelating properties.

2.4.3 Flavonoids

Thymus vulgaris extracts have been analyzed by HPLC and a number of flavone and flavonol aglycones and glycosides have been found. The main components of flavonoids are luteolin and luteolin 7-O-glucoside (apigenin), naringenin, and eriodictyol [19]. They are scattered throughout the leaves and flowering tops. They tend to be at higher concentration at early flowering. As for particular interest, luteolin has a potent anti-inflammatory, anticancer and neuroprotective activity.

2.4.4 Terpenoids and Other Constituents

In addition to the volatile monoterpenes, Thymus vulgaris also contains diterpenoids such as oleanolic acid, ursolic acid, which have been reported as having anti-inflammatory and hepatoprotective properties. Ursolic acid, a pentacyclic triterpenoid, binds to the NF-κB pathway and has been proven to have antitumour activity. Thyme is also low in flavanones, tannins and saponins.

2.5 *Nyctanthes arbor-tristis*

Nyctanthes arbor-tristis L. Harshingar, also known as Night Jasmine or Parijat in Sanskrit (family Oleaceae), is a small deciduous shrub or tree found all over the Indian subcontinent, Southeast Asia and parts of Nepal and Pakistan. The white flowers with orange-red corollas are fragrant and open at night, dropping to the ground before dawn. It is extensively used in Ayurvedic medicine for centuries, for treatment of fever (malaria, dengue), rheumatoid arthritis, skin diseases, and sciatica, and is mentioned in classical literature such as the Charaka Samhita and Ashtanga Hridayam. In Unani system of medicine, leaves are useful in bilious fevers, liver disorders [20]. It has a high national importance in India as state flower of West Bengal and Kanchanaburi Province of Thailand.[8]

Chemical studies of the leaves, flowers, stem bark and seeds of N. arbor-tristis have yielded a wide range of secondary metabolites. The seeds and flowers contain the most pharmacologically important compounds, namely iridoid glycosides namely nyctanthoside A, B and C which are esterified iridoids derived from 8-epiloganic acid and have been isolated and characterised by NMR and mass spectrometry [21] The flavonoids astragalin (kaempferol-3-O-glucoside) and nicotiflorin (kaempferol-3-O-rutinoside) are the main flavonoids found in the leaves by HPLC-UV and LC-MS/MS fragmentation pattern [22]. The leaves are a significant source of the sugar alcohol, biosynthetically important mannitol. Nyctanthin, a carotenoid-derived pigment (crocetin di-β-D-gentiobioside), structurally similar to the active principles of saffron is present in the orange corollar tube. The presence of tannins, oleanolic acid, ursolic acid, β-sitosterol and phenolic glycosides, such as syringin and verbascoside are

also reported [7], [8]. The main volatile components of the leaf essential oil are linalool, geraniol and β-ionone identified by GC-MS.

2.6 Phytochemical profile of *Nyctanthes arbor-tristis*

2.6.1 Iridoid Glycosides

Phytochemicals of N. arbor-tristis with pharmacological potential include iridoid glycosides such as arbortristoside A, arbortristoside B, arbortristoside C and nyctanthoside. The glycosides are isolated and characterised by HPLC and NMR spectroscopy in the leaves and flowers that are made of monoterpene [23]. Of particular interest is the antiparasitic activity of arbortristosides, known against Leishmania donovani and Plasmodium falciparum, thus justifying the use of the plant for the treatment of fever and parasitic diseases. The iridoid skeleton is basically the cyclopentane ring fused to a pyran ring, responsible for the interaction with parasitic enzymes.

2.6.2 Flavonoids and Phenolic Acids

The extracts from the leaves and flowers of N. arbor-tristis contain several flavonoids, such as quercetin, astragalin (kaempferol-3-O-glucoside), nicotiflorin and rutin [24]. The above flavonoids have been noted for the high antioxidant and anti-inflammatory effects of the plant. The Folin–Ciocalteu method showed that the methanol leaf extract contains 24.3–58.7 mg GAE/g dry weight total phenolics. High performance liquid chromatography (HPLC) of extracts of the leaves revealed the presence of caffeic acid, p-coumaric acid, and chlorogenic acid, which serve as the basis for an antioxidant mechanism of action in the same way as other Oleaceae. Geraniol, linalool and β-ionone are the main volatile constituents of the essential oil of the flower, which has been characterised by GC-MS.

2.6.3 Terpenoids, Alkaloids.

The phytochemical analysis of N. arbor-tristis is always found to contain triterpenoids (oleanolic acid, ursolic acid, β-sitosterol), tannins, saponins and mannitol. Fixed oil of the seeds is rich in triterpenoid acid ‘nyctanthic acid’ and oleic acid. The bark contains only trace amounts of alkaloids. The seed extract is known to have a mechanism of antiparasitic action that disrupts the integrity of the membranes of protozoans and the glycyrrhizin-type saponins are thought to play a role in this action [23]. Friedelin and lupeol, pentacyclic triterpenoids with well characterized anti-inflammatory activity based on their modulation of the NF-κB pathway, have been isolated from the bark.[25]

2.7 *Rosmarinus officinalis*

Rosmarinus officinalis L. (syn. Salvia rosmarinus Schleid. belongs to the Lamiaceae family and is a perennial evergreen aromatic shrub that is native to the Mediterranean basin and is used today throughout the world as a culinary herb, flavoring agent, cosmetic ingredient and medicinal plant. It has characteristic morphological features such as needle-like leaves and blue-violet flowers. Traditionally *Rosmarinus officinalis* has been used in the Mediterranean region to improve memory, to aid in the relief of headache, to treat gastrointestinal complaints, and as a topical antiseptic and hair tonic [6]. The plant is officially recognized in the European Pharmacopoeia and the Commission E for its carminative, spasmolytic and circulatory stimulating properties. In the Unani Tibb system of medicine of South Asia, it is used as brain tonic (muqawi-e-dimagh) and for rheumatic pain.

R. officinalis is one of the richest plant sources of phenolic diterpene antioxidants. The most abundant lipophilic antioxidants, found in leaf extracts, are the abietane-type diterpenes, carnosic acid and its oxidation product carnosol, which are unique to Lamiaceae subfamily Nepetoideae and confer the remarkable ability of *Rosmarinus officinalis* to stabilize food products through oxidative reactions [6]. *Thymus vulgaris* , which belongs to the Lamiaceae family, is very different, with the essential oil having 1,8-cineole (eucalyptol; 15–55%), camphor (10–25%), α-pinene (10–25%) and camphene as the major components as determined by GC-MS analysis. The triterpenoids that are consistent constituents of the non-volatile fraction are ursolic acid and oleanolic acid.

2.8 Phytochemical profile of *Rosmarinus officinalis*

2.8.1 Essential Oil: 1,8-Cineole, Camphor, α-Pinene.

The essential oil of S. rosmarinus, which is extracted by hydrodistillation of aerial parts, usually makes up 1.0–2.5% of the dried plant material. The characteristic components are 1,8-cineole (eucalyptol, 15–55%) and camphor (5–25%) and α-pinene (10–25%) and borneol (2–10%) and camphene and β-pinene, the proportions of which vary according to geographical origin and chemotype. Three chemotype groups are defined: the 1,8-cineole (Mediterranean coast), the camphor (Spain) and the α-pinene/β-pinene (Corsica). The main bronchodilatory and mucolytic component is 1,8-cineole, accounting for the traditional applications of rosemary in respiratory diseases.

2.8.2 Diterpenic Phenols: Carnosic Acid and Carnosol

The most characteristic and studied non-volatile phytochemicals of rosemary are carnosic acid and its oxidized form, carnosol. Carnosic acid is a lipophilic antioxidant and present in 1.5–6.0% of the dry leaf weight and it is considered as the major antioxidant of rosemary (del Baò et al., 2006). It has an ortho-dihydroxy group on the phenolic A-ring that gives it a remarkable radical-scavenging capacity, around 60-600 times greater than α-tocopherol (vitamin E) per mole. Carnosic acid has a special "intelligent" antioxidant activity; it is only slightly active at normal redox level in antioxidant reactions but gradually gets oxidized to carnosol, rosmanol and epirosmanol as the oxidative stress increases, it acts as a sacrificial antioxidant reservoir (Aruoma et al., 1992). Carnosic acid and carnosol are strong activators of the Nrf2/Keap1 pathway and a strong induction of phase II detoxification enzymes.

2.8.3 *Rosmarinic Acid, Flavonoids, and Other Polyphenols*

The rosmarinic acid is one of the abundant water-soluble polyphenols in rosemary plants, and it is found in the plant at concentrations of 0.5–2.5% of the dry leaf mass, like in Thymus vulgaris. The biosynthesis, structure (an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid) and antioxidant mechanisms are the same as those found for thyme S. rosmarinus also contains the flavonoids luteolin, apigenin, diosmin, hesperidin, genkwanin and nepitrin, and the diterpenoid acids rosmaridiphenol and rosmanol (Pintore et al., 2002). The anti-inflammatory and anti-cancer properties of rosemary are also due to the presence of significant amounts of ursolic acid and oleanolic acid (both pentacyclic triterpenoids shared with Thymus vulgaris).

2.9 Secondary Metabolites: Classification, Biosynthesis, and Structural Features

The synthesis and structures of secondary metabolites are discussed in this chapter. The structures and biosynthesis of secondary metabolites is discussed in this chapter.

Secondary metabolites are small organic molecules that are not required for plant primary growth and reproduction but provide plants with an adaptive benefit—defence against herbivores and pathogens, attraction of pollinator insects and tolerance of abiotic stresses. Secondary metabolites are usually species- or genus-specific in their occurrence and are synthesized by specific enzyme pathways which diverge from primary metabolic intermediates. Their diversity of structure is enormous (200,000+ secondary metabolites have been described from the plant kingdom, and many interact with animal and microbial biology in ways that are pharmacologically exploitable) [26]. The four major groups of pharmacologically important secondary metabolites are described below.

2.9.1 Phenolic Compounds

Phenolic compounds are the largest and most diverse group of plant secondary metabolites, including simple phenols, phenolic acids and coumarins, lignans, stilbenes and the much larger sub-family of flavonoids. They all contain a structural unit that consists of at least one aromatic ring with one or more hydroxyl group(s) attached to it. Biosynthetically, phenolics are synthesized by two convergent pathways: The shikimate pathway, which starts with phosphoenolpyruvate (PEP) and erythrose-4-phosphate that produce chorismate and then the aromatic amino acids phenylalanine and tyrosine; and, in a few instances, the polyketide pathway. The committed step of the phenylpropanoid pathway is the deamination of phenylalanine to trans-cinnamic acid, which is then hydroxylated, methylated and modified in different ways to produce the different phenylpropanoid derivatives such as coumarins, hydroxycinnamic acids (caffeic acid, ferulic acid, sinapic acid), and monolignols (Harborne, 1998).

The phenolic acids which have pharmacological significance in the four plant species studied are rosmarinic acid (the predominant of them in T. vulgaris and R. officinalis) and gallic acid (identified in B. spectabilis bracts) and chlorogenic acid (identified in R. officinalis). Caffeic acid derivatives share a catechol group, two hydroxyl groups adjacent on the aromatic ring, which is directly involved in hydrogen atom transfer and metal chelation that give the compounds their radical-scavenging antioxidant activity. Hydrolysable tannins of B. spectabilis and N. arbor-tristis are polymeric phenolics which are either gallic acid or ellagic acid based, with glucose as an ester group, and which provide antimicrobial and astringent properties by complexing proteins.

2.9.2 Terpenoids

Terpenoids (isoprenoids) are the largest single class of plant secondary metabolites by number of known structures (more than 80,000). They are synthesized in two parallel biosynthetic pathways, one in the cytosol and endoplasmic reticulum (mevalonate pathway or MVA pathway) and the other in the plastid (2-C-methyl-D-erythritol-4-phosphate pathway or DXP pathway). The two pathways converge at isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP) which are converted to geranyl diphosphate (GPP, precursor to monoterpenes, C_{10} diphosphate), farnesyl diphosphate (FPP, precursor to sesquiterpenes and triterpenes, C_{15} diphosphate), and geranylgeranyl diphosphate (GGPP,

precursor to diterpenes, C_{20} diphosphate) by prenyltransferases [26]. These prenyl diphosphate building blocks are cyclized and rearranged to the various skeletal classes by terpene synthases.

Terpenoids are present in several classes in the four plants investigated. T. vulgaris and R. officinalis contain volatile essential oil fractions which are called monoterpenes and include thymol, carvacrol, p-cymene, γ-terpinene, 1,8-cineole, α-pinene and camphor. The abietane-type diterpenes, carnosic acid and carnosol (C_{20}), are the most abundant antioxidants in *Rosmarinus officinalis* that are unique to the Lamiaceae. All four plants contain allopurinol, pentacyclic triterpenoids (C_{30}) such as ursolic acid and oleanolic acid, which have anti-inflammatory and anti-cancer activities. The iridoids found in N. arbor-tristis (arbortristoside A–C) are monoterpenoid glycosides that are synthesized via geraniol as a key intermediate and then cyclised and oxidised by cytochrome P450 enzymes (Saxena et al., 1984).

2.9.3 Alkaloids

Nitrogen-containing secondary metabolites where one or more N atom is in a ring system are generally basic in reaction and are termed as alkaloids. They are biosynthesized from amino acid precursors (mainly tyrosine, tryptophan, lysine, ornithine and phenylalanine) by enzymes such as decarboxylases, oxidases, and cytochrome P450 mono-oxygenases. Alkaloids can be divided into sub-classes according to their nitrogen ring structure, as pyrrolizidines (ornithine derived), indole alkaloids (tryptophan derived), isoquinolines (tyrosine derived) and quinolizidines (lysine derived) (Harborne, 1998). Many of the alkaloids are biosynthetically formed by the decarboxylation of the amino acid precursor by an aromatic L-amino acid decarboxylase (e.g., tyrosine to tyramine; tryptophan to tryptamine).

Alkaloids were not the major class of secondary metabolites but have been reported in the four plants studied. A few amounts of indole-type alkaloids are reported in B. spectabilis (Jawad et. al, 2012). The iridoid glycosides of N. arbor-tristis do not belong to that class of alkaloids but share a biosynthetic intermediate with some monoterpene indole alkaloids, at the secologanin stage, which demonstrates the metabolic interconnection between terpenoid and alkaloid pathways. The alkaloids present in crude plant extracts are responsible for the antimicrobial and analgesic activity observed and have a wide range of structures, which make them important leads in pharmaceutical development.

In this connection one should mention betalains found in *Bougainvillea spectabilis*, which are nitrogen-containing pigments, biosynthetically different from the alkaloids, but structurally as complex. Betalain biosynthesis involves the conversion of tyrosine to DOPA-dioxygenase-catalysed ring-opening product betalamic acid, which in turn reacts non-enzymatically with amino acids and amines to yield betaxanthins and with cyclo-DOPA glucoside to produce betacyanins [12]. It is interesting to note that this pathway is not found in Caryophyllales, which also lack the anthocyanin biosynthetic pathway, and that Betalain pathway is an independent solution to the problem of pigmentation, as there is no plant known to produce both.

2.9.4 Flavonoids

Flavonoids are a group of structurally organized phenylpropanoids with a C_6-C_3-C_6 structure: two phenyl rings (A and B) linked by a three-carbon bridge (C-ring) often containing an oxygen atom. They are biosynthesized by a combined pathway in which one molecule of malonyl-CoA (from the acetate-malonate pathway) is condensed with one molecule of p-coumaroyl-CoA (from the phenylpropanoid pathway) by the enzyme chalcone synthase (CHS) to form the

universal flavonoid precursor, naringenin chalcone. Chalcone isomerase (CHI) catalyzes the conversion to naringenin, a flavanone, which gives rise to the major sub-classes of flavonoids: flavone synthase activity leads to the conversion to flavones; flavanone 3-hydroxylase and flavonol synthase activity leads to the conversion to dihydroflavonols and flavonols, respectively; and dihydroflavonol 4-reductase activity leads to the conversion to anthocyanidins [27].

The hydroxylation of the B-ring and the character of the substituents at certain positions have a pharmacological significance. Quercetin (3,3',4',5,7-pentahydroxy) as glycosides in B. spectabilis and N. arbor-tristis possesses antioxidant, anti-inflammatory, and antidiabetic properties by its catechol B-ring (radical scavenging), C2=C3 double bond (planar geometry that facilitates π-stacking interactions with protein binding sites), and 3-OH group (metal chelation). The major flavone of T. vulgaris and R. officinalis possesses potent anti-inflammatory activity by inhibiting the STAT3 and NF-κB pathways, luteolin (3',4',5,7-tetrahydroxy).Luteolin (3',4',5,7-tetrahydroxy) is the major flavone of T. vulgaris and R. officinalis, which lacks the 3-hydroxyl of quercetin but retains the catechol B-ring and the 5,7-hydroxylation pattern of the A-ring, both of which contribute to potent anti-inflammatory activity by inhibiting the STAT3 and NF-κB pathways. Kaempferol (4',5,7-trihydroxy) found in B. spectabilis and N. arbor-tristis contains a single hydroxyl in the B-ring instead of the catechol system, and has somewhat less radical scavenging activity, but is still an anticancer and antiproliferative compound. The major flavonoid compound of N. arbor-tristis is astragalin (kaempferol-3-O-glucoside) which has been reported to have strong anti-inflammatory and antiparasitic effects in experimental models [22].

2.10 Extraction Method for Plants Secondary Metabolites

The method used for the extraction of bioactive secondary metabolites from plant matrix is a critical factor in the recovery process. There are a variety of techniques that have different physical and chemical principles, and vary in effectiveness, selectivity, solvent use, waste heat, and applicability to various classes of compounds. This method selection thus affects the phytochemical profile of a product obtained and consequently, the biological activities detected in the corresponding assays [28]. Therefore, knowledge of extraction principles is essential for the interpretation of data collected from any medicinal plant study.

2.10.1 Maceration

The oldest and easiest process of extraction is maceration. The finely ground or powdered plant material is soaked in a solvent, usually methanol, ethanol or water, at room temperature for a specified period (24 to 72 hours) with repeated shaking or stirring. Soluble constituents are dissolved by diffusion of the solvent into the plant cell walls and move into the bulk solvent. The mixture after the soaking period is filtered and the marc (plant material) can be re-extracted and maximised with fresh solvent. Certain glycosides and betalains from *Bougainvillea spectabilis* are among the thermolabile compounds which are suitable for maceration. It is time consuming, however, and usually has lower extraction efficiency than other techniques, and uses large amounts of solvent. In comparative studies, N. arbor-tristis leaves consistently recovered flavonoid content of 12–28 mg QE/g in ethanol macerates while the total phenolic content in methanol macerates of B. spectabilis bracts were reported as 24.7–62.7 mg GAE/g [7].

2.10.2 Soxhlet Extraction

The Soxhlet extraction uses a special glass extraction apparatus that is made up of a distillation flask, extraction chamber, and condenser. The plant material is placed in a porous thimble in a chamber, and the solvent vapour evaporates from the heated flask, condenses and drips down onto the plant material, dissolving compounds, which are then concentrated and siphoned back into the flask when the chamber is full. This cycle is repeated indefinitely with the plant material being exposed to fresh solvent each time. The Soxhlet extraction method is a common reference method in phytochemical research, where high extraction efficiency and good reproducibility are obtained. The solvents are applied in order increasing in polarity, in order to get fractions enriched in compounds of increasing polarity (serial exhaustive extraction). The optimal extraction methods are Soxhlet extraction for the recovery of terpenoids and lipophilic diterpenes (such as carnosic acid, carnosol) in the hexane or chloroform fraction and methanol extraction for the recovery of polar phenolics and flavonoids [6]. The only major drawback is long exposure to heat that can cause some thermolabile constituents, such as volatile terpenes and some glycosides to break down.

2.10.3 Influence of Solvent Polarity on Metabolite Recovery

Polarity of the solvent is the most important factor in all extraction methods that determines the classes of secondary metabolites recovered. Non-polar solvents (hexane, petroleum ether) dissolve terpenoids, fixed oils, waxes and lipophilic diterpenes (carnosic acid). Flavonoid aglycones, moderately polar alkaloids, and some phenolic acids are more soluble in intermediate-polarity solvents (ethyl acetate, chloroform). High polarity solvents (methanol, ethanol, water) can dissolve a broad range of phenolic compounds, flavonoid glycosides, tannins, betalains and water-soluble alkaloids. Chemical composition for each extract fraction is therefore dictated by the 'like dissolves like' principle (polarity matching) and needs to be considered when developing extraction protocols for target chemical classes.

2.11 Spectroscopic analysis of Secondary Metabolites

To identify and quantify the secondary metabolites contained in complex plant extracts, a set of complementary analytical methods are needed. Spectroscopic methods (UV-Vis absorption, FTIR) can be used to obtain structural and functional group information of isolated or semi-purified compounds, identify and quantify complex mixtures. Below is the review of the application of these methods to the four plants under study.

2.11.1 UV-Vis Spectrophotometry

The quantitative estimation of total phenolic content (TPC) is done by Folin-Ciocalteu assay (absorbance at 765 nm, calibrated against gallic acid), total flavonoid content (TFC) is done by aluminium chloride colorimetric method (absorbance at 415–510 nm, calibrated against quercetin or rutin) and total betalain content (betacyanins at 535 nm, betaxanthins at 480 nm). In B. spectabilis, bract methanol extracts exhibited strong absorption peak at 535 nm which corresponds to the absorption of betacyanins (betanin: λ_{max} 536 nm) and an additional peak at 480 nm for betaxanthins [14]. The UV absorption of the extract ranges from 240–280 nm (Band II, A-ring) and 350–380 nm (Band I, B-ring) and is caused by flavonoids. The rosmarinic acid is characterized by its UV absorption at 328 nm, which is typical for extracts of T. vulgaris, hence it can be quantified rapidly. The total phenolic content of the leaf methanol extracts of R. officinalis has been reported at 35.2–89.4 mg GAE/g of dry matter, which corresponds to the sum of carnosic acid, rosmarinic acid and flavonoids [6]. The TPC of the leaf ethanol extracts of N. arbor-tristis ranges from 18–45 mg GAE/g, while the TFC ranges from 12–28 mg QE/g, depending on the extraction solvent and plant part used (Rani et al., 2012).

2.11.2 Fourier-Transform Infrared Spectroscopy

FTIR spectroscopy gives a molecular fingerprint of the plant extracts as it is based on the characteristic absorption bands that are associated with the vibrational modes of the functional groups. The presence of phenolic hydroxyl groups is reflected by the presence of broad O-H stretching bands in the 3200–3600 cm^{-1} region, which are present in all four plants in their extracts. The C=O carbonyl stretches around 1,690–1,730 cm^{-1} is typical of carboxylic acid groups present in phenolic acids (rosmarinic, gallic, carnosic) and the ester carbonyl of carnosic occurs at ~1,720 cm^{-1}, and the lactone carbonyl of carnosol at 1,745 cm^{-1}. The presence of aromatic C=C ring stretching vibrations in the region of 1,500–1,600 cm-1 is indicative of the presence of the flavonoid and phenylpropanoid frameworks in all four plants.

FTIR analysis of the bract extracts shows the presence of characteristic peaks at 3420 cm-1 (O-H stretch), 1626 cm-1 (C=N stretch of betalamic acid chromophore, which is responsible for the difference between betalains and anthocyanins), 1384 cm-1 (C-N stretch) and 1038 cm-1 (C-O-C of glycosidic bonds in betanin) for B. spectabilis (Reshmi et al., 2012). The C=N stretch is a dependable diagnostic feature of the betalain compounds occurring around 1,620 – 1,640 cm-1. The essential oil-depleted extracts of T. vulgaris exhibit high absorbance values at 3,380 cm^{-1} (phenolic O-H), 1,707 cm^{-1} (rosmarinic acid C=O), 1,599 cm^{-1} (aromatic C=C) and 1,263 cm^{-1} (C-O of phenol ether), typical of a rosmarinic acid-rich profile (Petersen & Simmonds, 2003). The extracts of R. officinalis have a characteristic FTIR spectrum with a broad O-H hydrogen bond at 3,450 cm-1, C=O (carboxylic acid) at 1,718 cm-1, and a characteristic conjugated diene system at 1,590-1,620 cm-1. The peaks at 3430 cm-1 (O-H), 1657 cm-1 (conjugated C=O of flavone carbonyl), 1522 cm-1 (aromatic ring) and 1074 cm-1

(C-O-C of glycoside linkages in astragalin and nyctanthoside) were observed in the FTIR spectrum of the extracts of leaves of N. arbor-tristis [22].

2.12 Mechanism of Phytochemical Action Corelated to Plant Compound

2.12.1 Antioxidant Mechanisms

Pathogenesis of cardiovascular diseases, neurodegeneration, diabetes and cancer is attributed to oxidative stress, the imbalance between the production of reactive oxygen species (ROS) and the antioxidant defence [29]. Phytochemicals do this by binding directly to the radicals, by chelating with metal ions, and by up-regulating the Nrf2/HO-1 pathway. Quercetin and kaempferol from B. spectabilis and N. arbor-tristis have been found to donate hydrogen atoms to neutralise •OH (peroxyl radicals) and chelate Fe2+/Fe3+ to prevent •OH from being generated from the Fenton reaction. Betanin obtained from B. spectabilis activates the Nrf2/ARE signalling pathway, leading to increased expression of HO-1 in a more complex manner than just radical scavenging. Both T. vulgaris and R. officinalis contain rosmarinic acid, which has two catechol groups for the ability of a single molecule to sequentially scavenge two ROS equivalents [18] and performs better than mono-catechol phenolics on a molar basis. The mechanism of action of Carnosic acid of R. officinalis is unique, since it is a pro-antioxidant that is converted to carnosol by ROS and then to rosmaquinone and rosmadial, each of which is an antioxidant equivalent to one ROS consumed during the conversion process [6]. The DPPH IC_{50} values for the four plants were: B. spectabilis 12.4–48.6 μg/mL, T. vulgaris thymol 73 μg/mL, R. officinalis methanol extract 8.3–22.7 μg/mL, and N. arbor-tristis leaf ethanol extract 34–68 μg/mL [6], [7], [14]

2.12.2 Anti-inflammatory Mechanisms

The inflammatory transcriptional programme is regulated by the NF-κB pathway. Quercetin (B. spectabilis, N. arbor-tristis) inhibits IκB kinase (IKK) to inhibit the NF-κB nuclear translocation and the transcription of COX-2, iNOS and cytokines. Luteolin (T. vulgaris, R. officinalis) also inhibits phosphorylation of STAT3 and MAPK/ERK signalling. *Thymus vulgaris* and *Rosmarinus officinalis* contain rosmarinic acid which helps prevent the activation of complement and allergic reactions to IgE. Carnosol, which is found in R. officinalis, is a natural inhibitor of both 5-LOX (IC_{50}~1.3 μM) and COX-2 and thus inhibits both the production of prostaglandins and leukotrienes (Andrade et al., 2018). Astragalin from N. arbor-tristis inhibits the production of TNF-α and IL-6 in RAW264.7 macrophages and has anti-arthritic activity in adjuvant-induced arthritis rat models [22]. The plant T. vulgaris, when the expression of COX-2 gene is reduced in macrophages cells at sub-MIC concentrations [30].

2.12.3 Antimicrobial Mechanisms

The disruption of the bacterial cytoplasmic membrane by intercalation into the phospholipid bilayer, dissipation of the proton motive force and leakage of ions gives T. vulgaris the ability to kill both gram-positive and gram-negative bacteria with MIC values of 0.05–2.0 mg/mL [31]. 1,8-Cineole and α-pinene of essential oil of R. officinalis exerts the same effect on the fungal ergosterol membranes and exhibit synergistic antifungal activity against C. albicans. Bacterial surface proteins are precipitated by tannins and gallic acid from B. spectabilis, which affects cell wall synthesis [3]. Iridoid glycosides, specifically the arbortristoside compounds of N. arbor-tristis, have been shown to have antifungal and antiparasitic activity, likely through their inhibitory effect on glycolytic enzyme activity in parasites (Siddiqui et al., 2014).

2.12.4 Antidiabetic Mechanisms

Pinitol from B. spectabilis also induces GLUT4 translocation and enhances peripheral glucose uptake, without the requirement of insulin, and leads to significant glycaemic reduction in diabetic STZ rats at 100 mg/kg [32]. Quercetin is a potent inhibitor of intestinal α-glucosidase (IC_{50} = 1.92 μM), which slows down the absorption of glucose. *Thymus vulgaris* and *Rosmarinus officinalis* contain rosmarinic acid, which disrupts the activity of PTP1B and enhances the endogenous insulin signalling pathway (Kim et al., 2010). The beneficial effect of carnosic acid on insulin sensitivity in adipocytes is also obtained by the insulin sensitizing action of the thiazolidinedione class of antidiabetic drugs [6]. Each of the four plants has an antidiabetic profile with multiple mechanisms of action, which makes them promising for polypharmacological approaches for the management of metabolic diseases.

CHAPTER 3: MATERIALS AND METHODOLGY

3.1 Plant extraction and sample preparation

The plants used in this project were taken from nearby parks and some from the market to get plants grown in their natural habitats and plants grown specifically. *Nyctanthes arbor-tristis* leaves, *Rosmarinus officinalis* leaves and stem, *Thymus vulgaris* leaves and stalk, and *Bougainvillea spectabilis* leaves, stem and flowers were all shade dried for two weeks until all the parts of the plant dried completely. Fresh sample of same parts of these plants obtained on the day of experiment were also taken to do a comparative analysis on the results of quantitative phytochemical analysis experiments.

For the extraction of phytochemicals, a fine powder was made of the dried plants sample of which 1 gram was put in 10 ml of distilled water, boiled over a water bath and set aside for 15 mins. This process was repeated for all the plant samples for extraction.

For fresh sample the plant parts were arranged on the day of experiment which were macerated using mortar and pestle loosen with some DW. 5 grams of this macerated pulp was added to 10 ml of cold DW and kept aside for 15 minutes.

3.2 Qualitative estimation of Flavonoids

Flavonoids produce intense yellow coloration in alkaline medium which disappears upon addition of acid (Kokate, 1994).

Preparation: plant extract, 2% sodium hydroxide, dil. Sulfuric acid

Procedure: 1ml of the prepared plant extract was taken in which 2ml of 2% sodium hydroxide was added giving out yellow colour. Few drops of diluted sulfuric acid were added to this turning the solution colourless indicating presence of flavonoids.

Observation: A colourless solution was observed

3.3 Qualitative estimation of Terpenoids

Salkowski test was performed for the qualitative estimation of terpenoids. Terpenoids react with concentrated sulfuric acid to form reddish-brown coloration (Raaman, 2006).

Preparation: plant extract, chloroform, conc. Sulfuric acid

Procedure: 2ml of chloroform was added to 2 ml of plant extract which was heated over a water bath till the chloroform was evaporated, after which 2ml of conc. Sulfuric acid was added and heated again for 2 mins.

Observation: A greyish-black colour solution was formed indicating the presence of terpenoids.

3.4 Qualitative estimation of Fats and oils

The fats and oils in plants extract reacts with copper sulphate and sodium hydroxide to give a blue coloured solution indicating its presence.

Preparation: plant extract, 1% copper sulphate solution, sodium hydroxide

Procedure: 1ml of freshly prepared 1% copper sulphate solution was added to 1ml of prepared plant extract to which sodium hydroxide was added dropwise

Observation: no colour change was observed, and the solution remained blue fats and oils were confirmed.

3.5 Qualitative estimation of Quinones

Preparation: plant extract, sodium hydroxide

Procedure: 1ml of dil. Sodium hydroxide was added to 1ml of plant extract

Observation: the colour changes to blue green or red it indicates presence of quinones.

3.6 Qualitative estimation of Alkaloid

Wagner test is performed to determine the presence of alkaloids

Preparation: Wagner's reagent, plant extract

Procedure: Few drops of Wagner's reagent are added to the plant extract

Observation: reddish brown ppt.

3.7 Qualitative estimation of Saponin

Preparation: plant extract

Procedure: 2ml of plant extract was shaken vigorously for 30 seconds in a test tube, if froth formation was observed it indicates presence of saponin

Observation: froth formation

3.8 Qualitative estimation of Phenol

Preparation: plant extract, 5% ferric chloride solution

Procedure: 1ml of plant extract was taken in a test tube to which few drops of 5% ferric chloride was added

Observation: the solution turned black

3.9 Qualitative estimation of Tannins

Preparation: plant extract, 0.5% ferric chloride solution

Procedure: few drops of 0.5% ferric chloride was added to 1ml of plant extract

Observation: blue black coloured solution was observed

3.10 Quantitative estimation of Phenols

Preparation: 1:10 Fc reagent, sodium carbonate, plant extract

Procedure: 1mg of dried plant sample was added to 5ml of fc reagent, after 3-4 mins 4 ml sodium carbonate was added to the solution and incubated in dark for 30 minutes. After incubation the observation the absorbance was measured at 765nm.

3.11 Quantitative estimation of Alkaloids

Preparation: ethanolic plant extract

Procedure: 0.1g of dried plant sample was added to 10 ml of ethanol left aside for 48 hours the absorbance was taken at 460nm

Chapter 4: RESULTS

4.1 Qualitative analysis of aqueous solution of dry extract

a. Test for Alkaloids

Bougainvillea spectabilis stem and *Thymus vulgaris* leaves have shown more positive result with dark red-brown colour change in the solution. *Rosmarinus officinalis* leaf, *Thymus vulgaris* stem, *Bougainvillea spectabilis* flower, *Nyctanthes arbor-tristis* leaf have shown the presence of alkaloids. Whereas stem of *Nyctanthes arbor-tristis* and *Rosmarinus officinalis* and *Bougainvillea spectabilis* leaf show no colour change that is negative result.

b. Test for Phenols

Phenolic compounds were detected in *Rosmarinus officinalis* leaf, *Thymus vulgaris* stem, *Bougainvillea spectabilis* stem and flower, and *Nyctanthes arbor-tristis* leaf and stem. Also, no reaction was observed in *Thymus vulgaris* leaf and *Bougainvillea spectabilis* leaf.

c. Test for Flavonoids

Aqueous solution turned completely colourless indicating strong presence of flavonoids in the leaves of *Rosmarinus officinalis*, *Thymus vulgaris* and *Nyctanthes arbor-tristis*. Stems of *Rosmarinus officinalis*, *Thymus vulgaris*, *Nyctanthes arbor-tristis* and the leaf of *Bougainvillea spectabilis* also shows the presence of flavonoids. However, flavonoids were absent in *Bougainvillea spectabilis* stem and flower extracts.

d. Test for Tannins

A prominent black coloration was observed in leaves of *Rosmarinus officinalis* and *Nyctanthes arbor-tristis*, stem of *Rosmarinus officinalis*, *Thymus vulgaris* and *Bougainvillea spectabilis* indicating strong presence of tannins. Also, *Thymus vulgaris* leaf, *Bougainvillea spectabilis* flower, and *Nyctanthes arbor-tristis* stem confirms the presence of tannins. No coloration was observed in *Bougainvillea spectabilis* leaf.

e. Test for Saponins

Persistent frothing was observed in *Rosmarinus officinalis* leaf and stem, *Thymus vulgaris* leaf, *Bougainvillea spectabilis* leaf, stem and flower extracts indicating the presence of saponins. No froth formation was observed in *Thymus vulgaris* stem and *Nyctanthes arbor-tristis* extracts.

f. Test for Terpenoids

Red to dark brown colour development indicating presence of terpenoids was observed in almost all extracts including *Rosmarinus officinalis*, *Thymus vulgaris*, *Bougainvillea spectabilis* and *Nyctanthes arbor-tristis* except *Nyctanthes arbor-tristis* stem where it was absent.

g. Test for Quinones

Quinones were observed in *Rosmarinus officinalis* stem, *Thymus vulgaris* stem, and *Bougainvillea spectabilis* stem and flower extracts. They were absent in *Rosmarinus officinalis* leaf, *Thymus vulgaris* leaf, *Bougainvillea spectabilis* leaf and *Nyctanthes arbor-tristis* extracts.

h. Test for Fats and oils (glycerine)

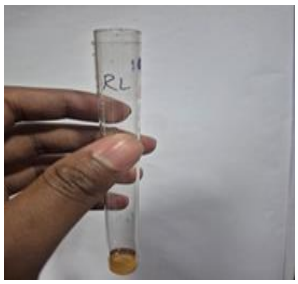
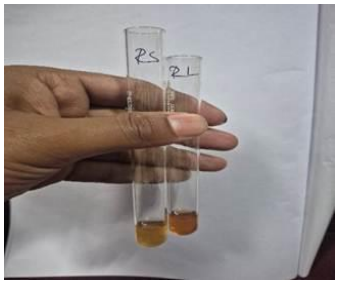
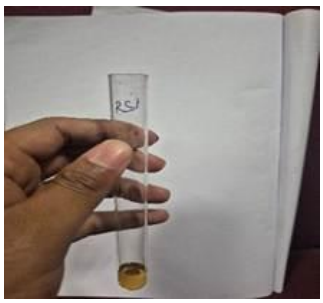
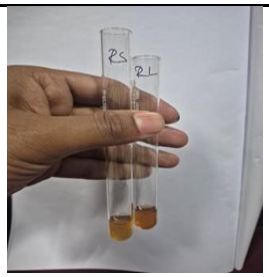
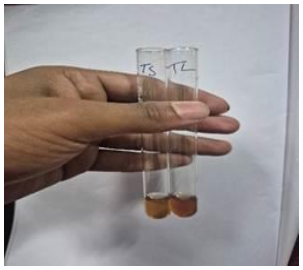
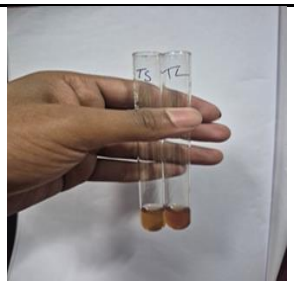
Fats and oils were detected in *Thymus vulgaris* leaf, *Bougainvillea spectabilis* leaf, flower and *Nyctanthes arbor-tristis* stem, because of the change of colour of the solution to blue, while absent in *Rosmarinus officinalis* and most other extracts.

Plant name	Part used	Alkaloids	Phenols	Flavonoids	Tannins	Saponins	Terpenoids	Quinones	Fats and Oils
Rosmarinus officinalis	Leaf	+	+	++	++	++	+	-	-
Rosmarinus officinalis	Stem	-	-	+	++	+	+	+	-
Thymus vulgaris	Leaf	++	-	++	+	+	++	-	+
Thymus vulgaris	Stem	+	+	+	++	-	+	++	-
Bougainvillea spectabilis	Leaf	-	-	+	-	+	++	-	+
Bougainvillea spectabilis	Stem	++	+	-	++	+	+	++	-
Bougainvillea spectabilis	Flower	+	+	-	+	+	+	+	+
Nyctanthes arbor-tristis	Leaf	+	+	++	++	-	+	-	-
Nyctanthes arbor-tristis	Stem	-	+	+	+	-	-	-	+

Table 1 Results Qualitative Analysis of dried plant extract

4.1.1 Picture of the result

a. Alkaloids

Plant name	Part	Before	After
Rosemary	Leaf		
Rosemary	stem		
Thyme	leaf		
Thyme	stem		

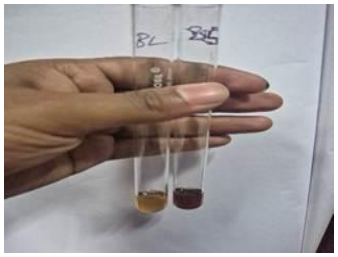
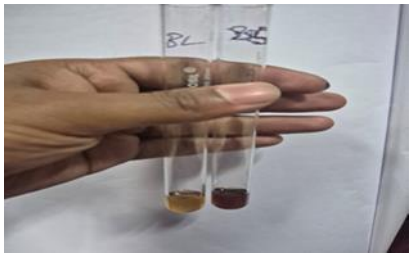
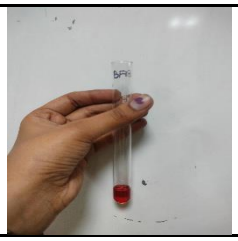
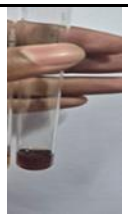
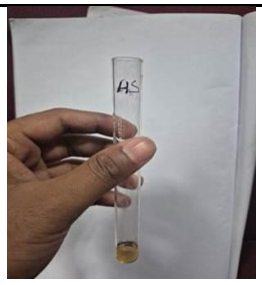
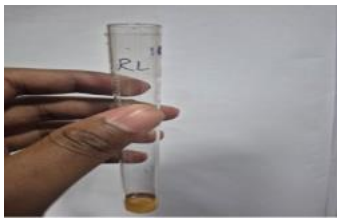
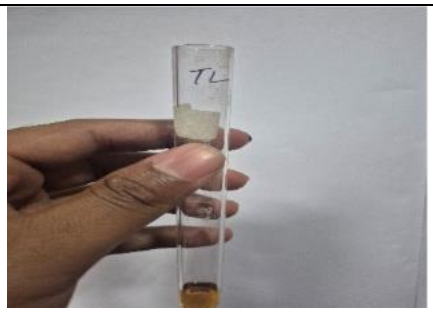
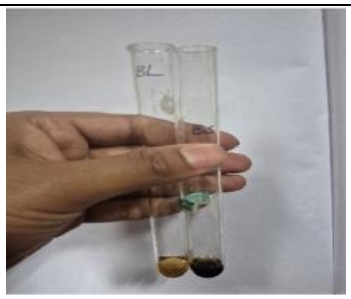
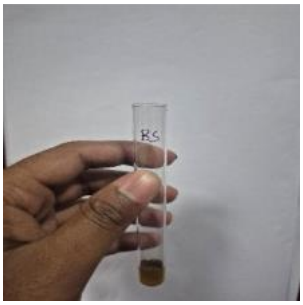
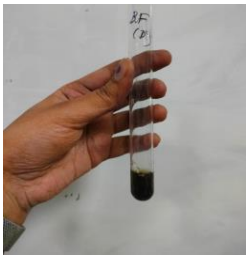
Bougainvillea	leaf		
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

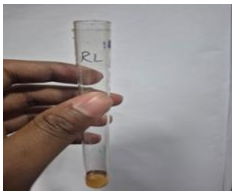
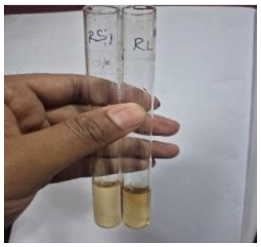
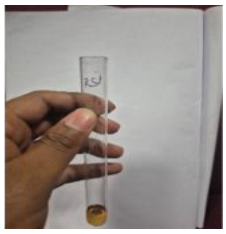
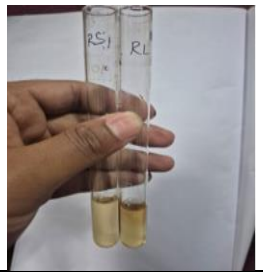
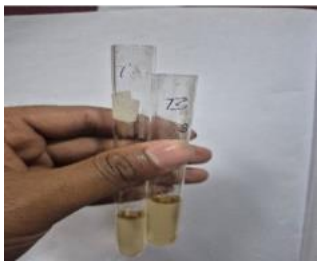
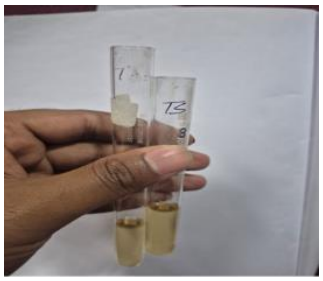
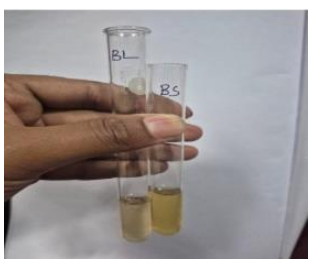
Figure 1: Alkaloid test dried plant

b. Phenols

Plant name	Part	Before	After (phenols)
Rosemary	Leaf		
Rosemary	stem		
Thyme	leaf		
Thyme	Stem		

Bougainvillea	leaf		
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

c. Flavonoids

Plant name	Part	Before	After
Rosemary	Leaf		
Rosemary	stem		
Thyme	leaf		
Thyme	stem		
Bougainvillea	leaf		

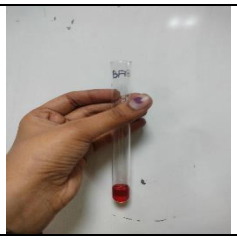
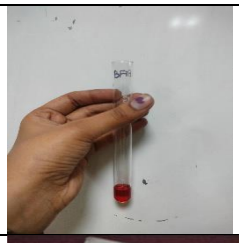
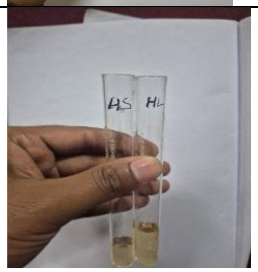
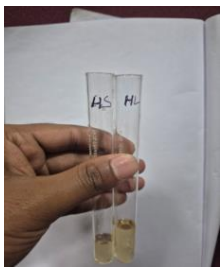
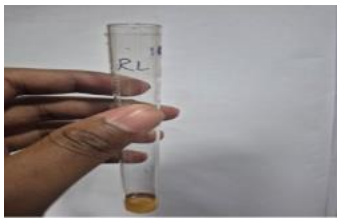
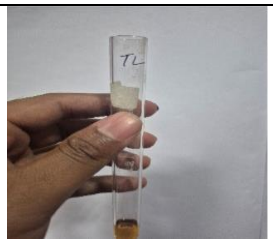
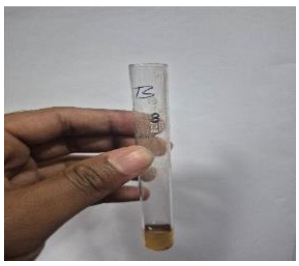
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

Figure 2: Flavonoid test dried plant

d. Tannins

Plant name	Part	Before	After
Rosemary	Leaf		
Rosemary	stem		
Thyme	leaf		
Thyme	stem		
Bougainvillea	leaf		

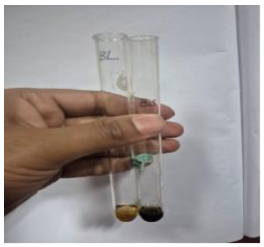
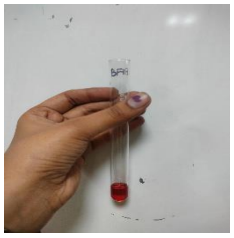
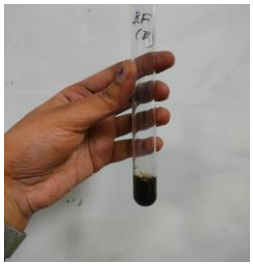
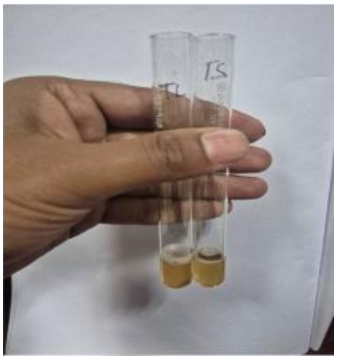
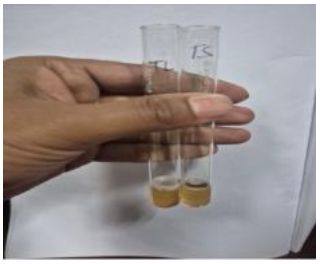
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

Figure 3: Tannins test dried plant

e. saponins

Plant name	Part	Before	After
Rosemary	Leaf		
Rosemary	stem		
Thyme	leaf		
Thyme	stem		

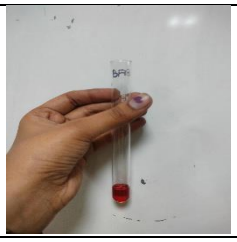
Bougainvillea	leaf		
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

Figure 4: Saponins test dried plant

f. Terpenoids

Plant name	Part	Before	After (Terpenoids)
Rosemary	Leaf		
Rosemary	stem		
Thyme	leaf		
Thyme	stem		

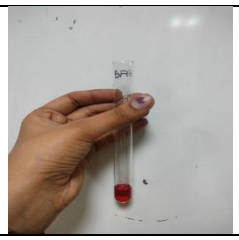
Bougainvillea	leaf		
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

Figure 5: Terpenoids test dried plant

g. Quinones

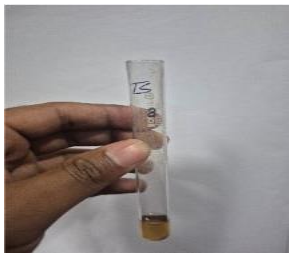
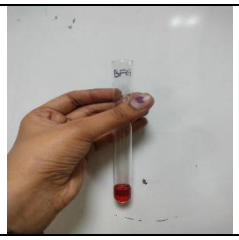
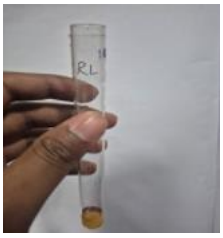
Plant name	Part	Before	After
Rosemary	stem		
Thyme	stem		
Bougainvillea	stem		
Bougainvillea	flower		

Figure 6: Quinones test dried plant

h. Fats and oil

Plant name	Part	Before	After (Fats and oil)
Rosemary	Leaf		
Rosemary	stem		
Thyme	leaf		
Thyme	stem		
Bougainvillea	leaf		

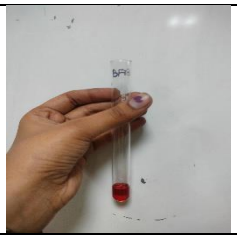
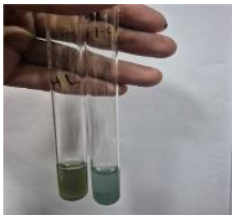
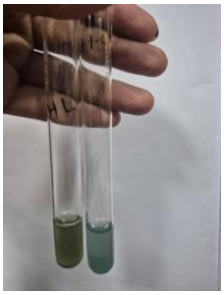
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

Figure 7: Fats and oils test dried plant

4.2 Qualitative Analysis of Fresh extract

a. Test for alkaloids

Thymus vulgaris leaves show highest presence of alkaloids. Leaves of *Rosmarinus officinalis*, *Thymus vulgaris*, *Nyctanthes arbor-tristis*, *Bougainvillea spectabilis* and the flower also indicate positive results.

b. Test for phenols

Except *Bougainvillea spectabilis* leaves, every other sample indicates the presence of phenols.

c. Test for flavonoids

A strong presence of flavonoids is seen in *Rosmarinus officinalis*, *Thymus vulgaris*, and *Nyctanthes arbor-tristis*. Whereas leaves of *Bougainvillea spectabilis* show positive results, the flower shows an absence of flavonoids.

d. Test for tannins

All of them have given positive results, whereas *Bougainvillea spectabilis* flower has shown the strongest presence of tannins.

e. Test for saponins

Persistent frothing was observed in *Rosmarinus officinalis* leaf, *Thymus vulgaris* leaf, *Bougainvillea spectabilis* leaf and the flower, *Nyctanthes arbor-tristis* leaf.

f. Test for terpenoids

Leaves of *Thymus vulgaris* and *Bougainvillea spectabilis* have shown strongest presence of terpenoids. And *Rosmarinus officinalis*, *Nyctanthes arbor-tristis*, *Bougainvillea spectabilis* flower also shows positive results.

g. Test for quinones

Except *Rosmarinus officinalis* leaves, all others show positive results for quinones.

h. Test for fats and oils (glycerine)

All the solution shows the blue colour change means presence of glycerine.

Plant name	Part used	Alkaloids	Phenols	Flavonoids	Tannins	Saponins	Terpenoids	Quinones	Fats and Oils
Rosmarinus officinalis	Leaf	+	+	++	+	++	+	-	+
Thymus vulgaris	Leaf	++	++	++	+	+	++	+	+
Bougainvillea spectabilis	Leaf	+	-	+	+	+	++	+	+
Bougainvillea spectabilis	Flower	+	+	-	++	+	+	+	+
Nyctanthes arbor-tristis	Leaf	+	+	++	+	++	+	+	+

Table 2 Results Qualitative Analysis of fresh plant extract

4.2.1 Pictures of the result

a. Alkaloids

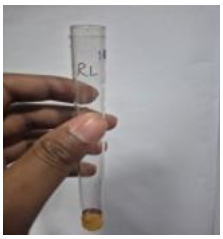
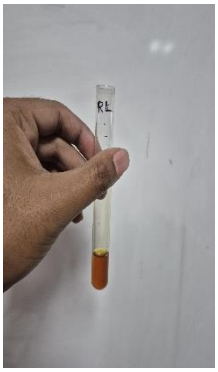
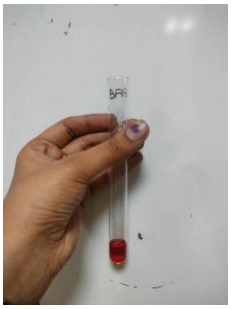
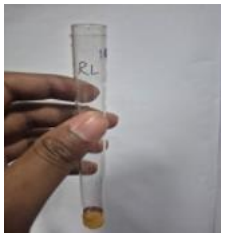
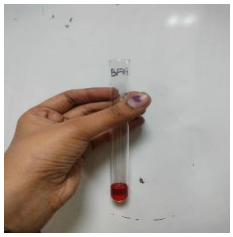
Plant Name	Before	After
Rosemary		
Thyme		
Bougainvillea (leaf)		
Bougainvillea (flower)		

Figure 8: Alkaloid test fresh plant

Plant Name	Before	After
Rosemary		
Thyme		
Bougainvillea (leaf)		
Bougainvillea (flower)		

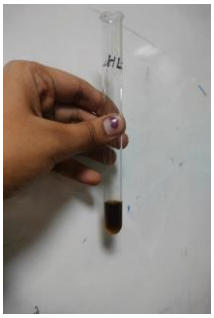
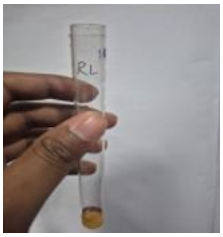
Harsingar		

Figure 9: Phenols test fresh plant

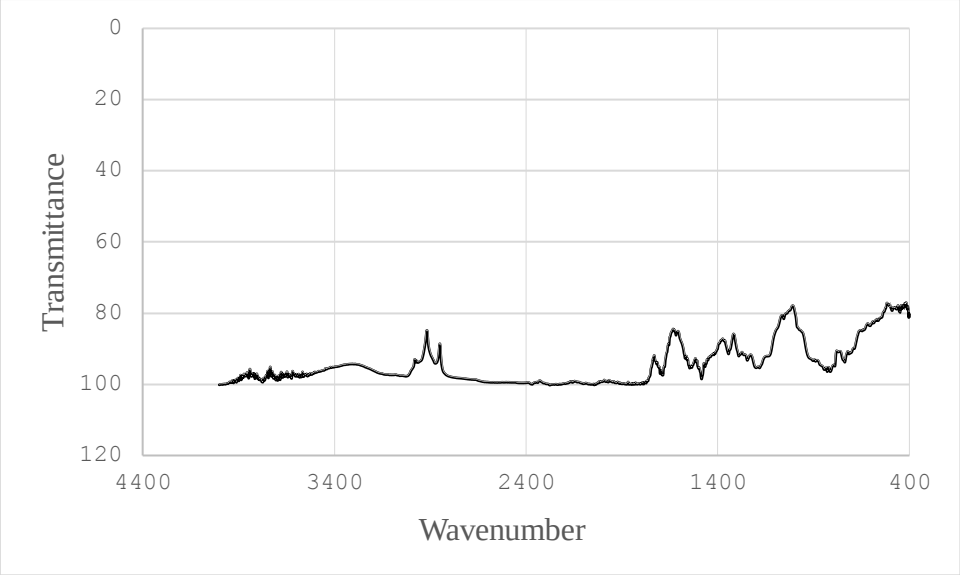
Plant Name	Before	After
Rosemary		
Thyme		
Bougainvillea (leaf)		
Bougainvillea (flower)		

Harsingar		

Figure 10: Flavonoid test fresh plant

4.3 FTIR Results

4.3.1 *Bougainvillea spectabilis*

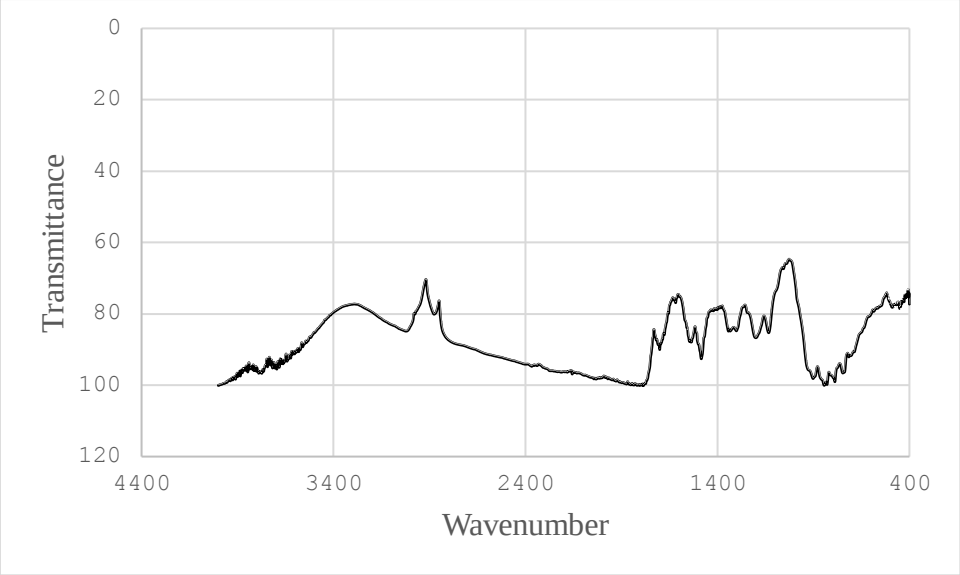


FTIR graph 1 Bougainvillea spectabilis

Peak Position (cm^{-1})	Probable Functional Group	Possible Compound Type / Interpretation
~3306 cm^{-1}	O–H stretching	Alcohols, phenols, flavonoids, polyphenols
~2916 cm^{-1}	C–H stretching	Alkanes, lipids, terpenoids
~2849 cm^{-1}	C–H symmetric stretching	Fatty acids and hydrocarbons
~1731 cm^{-1}	C=O stretching	Esters, aldehydes, ketones, carboxylic acids
~1632 cm^{-1}	C=C stretching / amide band	Aromatic compounds, proteins, flavonoids
~1516 cm^{-1}	Aromatic ring vibrations	Phenolic compounds and flavonoids
~1374 cm^{-1}	C–H bending	Alkanes and methyl groups
~1317 cm^{-1}	C–N stretching	Amines and alkaloids
~1008 cm^{-1}	C–O stretching	Alcohols, polysaccharides, carbohydrates
~779 cm^{-1}	Aromatic C–H bending	Aromatic compounds

Table 3 Interpretation of possible compound in Bougainvillea spectabilis by FTIR graph

4.3.2 *Thymus vulgaris*

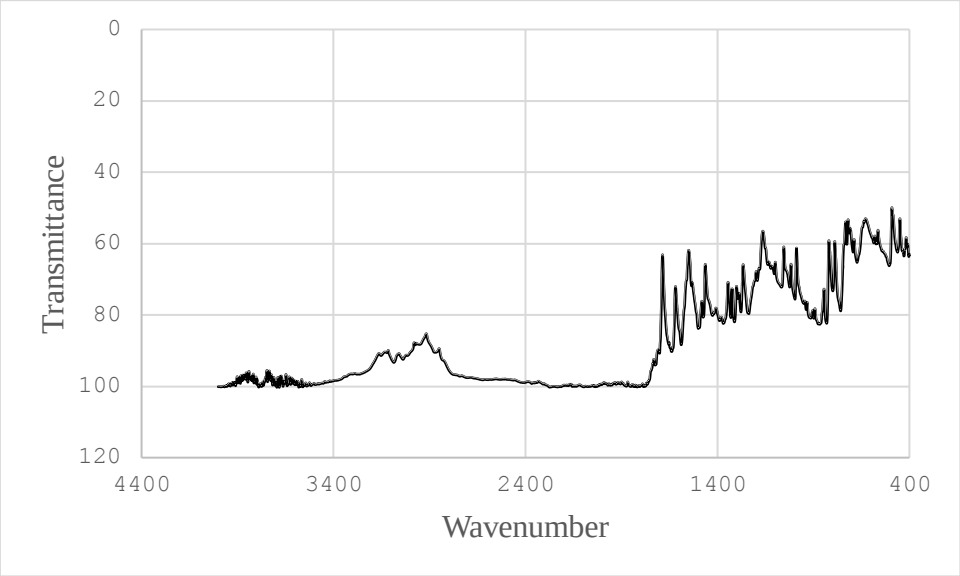


FTIR graph 2 Thymus vulgaris

Peak (cm^{-1})	Functional Group	Possible Phytochemical/Interpretation
~3289	O–H stretching	Phenols, flavonoids
~2919	C–H stretching	Terpenoids, lipids
~1731	C=O stretching	Carbonyl compounds
~1605	C=C stretching	Aromatic compounds
~1517	Aromatic ring vibration	Phenolic compounds
~1375	C–H bending	Alkanes
~1257	C–N stretching	Amines, alkaloids
~1158	C–O stretching	Alcohols, ethers
~1027	C–O stretching	Carbohydrates
~518	Halogen/low-frequency vibration	Secondary metabolites

Table 4 Interpretation of possible compound in Thymus vulgaris by FTIR graph

4.3.3 *Rosmarinus officinalis*

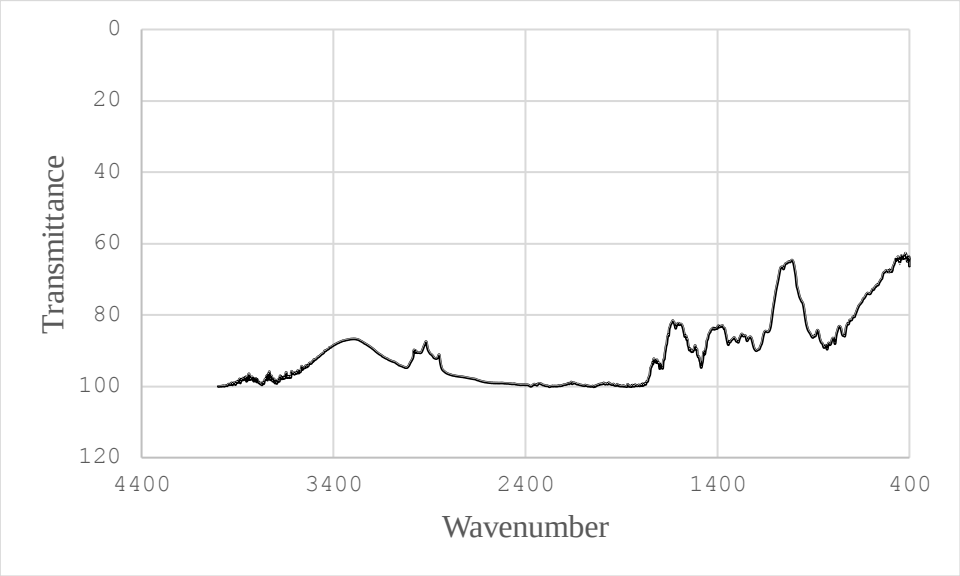


FTIR graph 3 Rosmarinus officinalis

Peak (cm^{-1})	Functional Group	Possible Phytochemical/Interpretation
~2917	C–H stretching	Terpenoids, essential oils
~1686	C=O stretching	Carbonyl compounds, aldehydes
~1618	C=C stretching	Aromatic compounds, flavonoids
~1550	Aromatic ring vibration	Phenolic compounds
~1463	C–H bending	Alkanes
~1266	C–N stretching	Amines, alkaloids
~1163	C–O stretching	Alcohols, ethers
~988	C–O stretching	Carbohydrates
~820	Aromatic C–H bending	Aromatic compounds
~789	Aromatic ring vibration	Phenolic compounds

Table 5 Interpretation of possible compound in Rosmarinus officinalis by FTIR graph

4.3.4 *Nyctanthes arbor-tristis*



FTIR graph 4 Rosmarinus officinalis

Peak (cm^{-1})	Functional Group	Possible Phytochemical/Interpretation
~3296	O–H stretching	Phenols, flavonoids, alcohols
~2918	C–H stretching	Terpenoids, lipids, alkanes
~1632	C=C stretching / Amide	Aromatic compounds, proteins
~1374	C–H bending	Alkanes and methyl groups
~1273	C–N stretching	Alkaloids, amines
~1012	C–O stretching	Carbohydrates, polysaccharides
~765	Aromatic C–H bending	Aromatic compounds

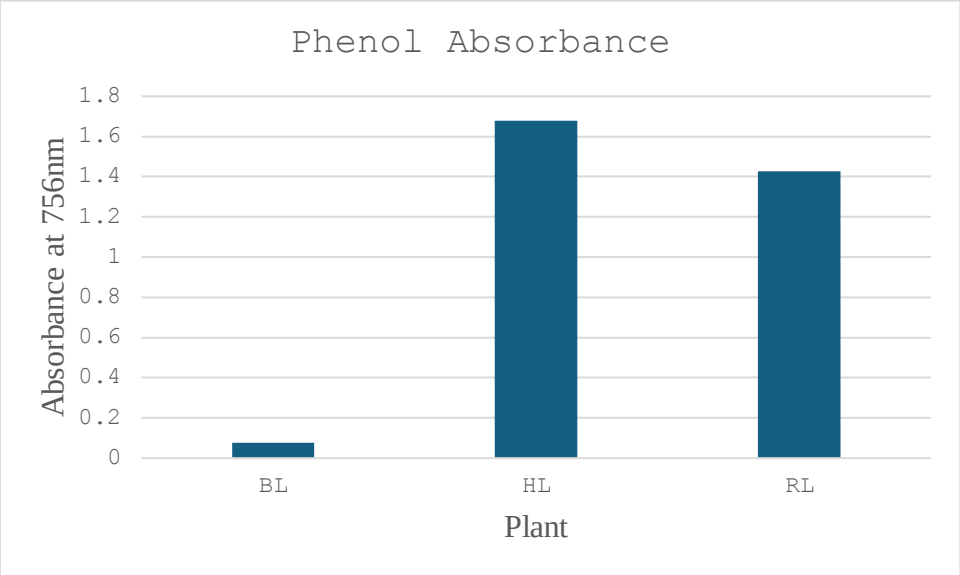
Table 6 Interpretation of possible compound in Nyctanthes arbor-tristis by FTIR graph

4.4 Spectroscopy analysis result and discussion

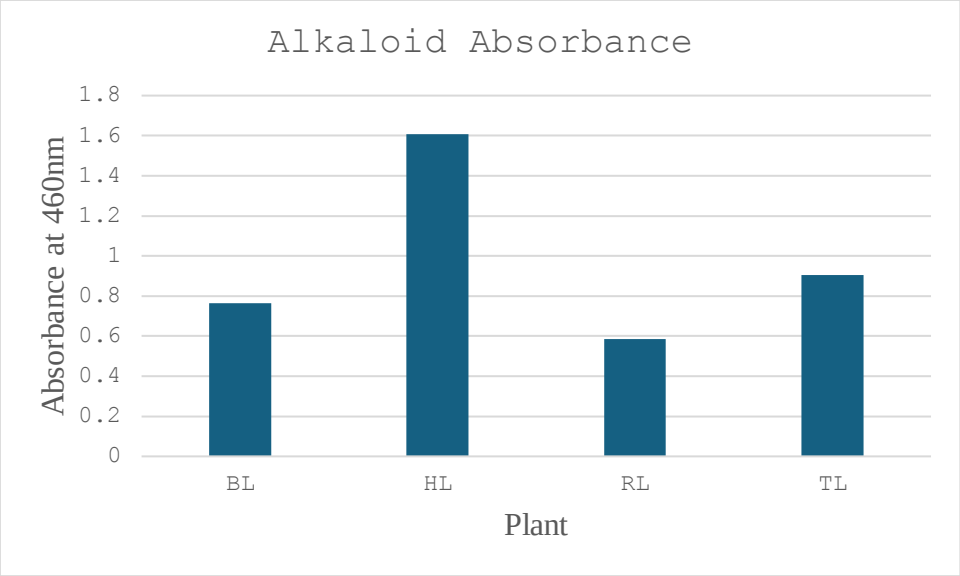
UV-Visible spectrophotometry was used for quantitative estimation of phenolics and alkaloids of various plant extracts. Absorbance of phenolic compounds was measured at 756 nm, and

alkaloid content was estimated at 460 nm. The absorbance values from Bougainvillea leaves (BL), Harsingar leaves (HL), Rosemary leaves (RL) and Thyme leaves (TL) are shown in graph.

The results showed that there was variation in phytochemical content of the tested plant extracts. Harsingar leaves (HL) showed comparatively higher absorbance value for both phenolic and alkaloid content which indicates that there is high concentration of bioactive constituents in the leaves. The leaves of rosemary (RL) also had good phenolic absorbance, while Bougainvillea leaves (BL) had comparatively low phenolic absorbance.



Graph absorbance for phenol



Graph absorbance for Alkaloid

CHAPTER 5: DISCUSSION

This study was performed to assess the phytochemical composition of medicinal plants such as Rosmarinus officinalis, Thymus vulgaris, Bougainvillea spectabilis and Nyctanthes arbor-tristis by qualitative phytochemical screening, FTIR spectroscopy, and UV–Visible spectrophotometric analysis. Qualitative analysis of the fresh extracts and dried extracts showed the presence of various secondary metabolites including alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, quinones, fats and oils. The distribution of phytochemicals also varied between different plant species and plant parts, which suggests that the concentration of phytochemicals depends significantly on plant species and plant part and extraction method. Thymus vulgaris leaf and Bougainvillea spectabilis stem demonstrated strong positive result with alkaloids whereas Rosmarinus officinalis leaf and Nyctanthes arbor-tristis leaf had high level of flavonoids and tannins. The presence of terpenoids in almost all the extracts indicated the ubiquitous presence of biologically active volatiles in these medicinal plants.

Qualitative screening of fresh extracts also revealed high phytochemical content. The flower of Bougainvillea spectabilis had the highest content of tannins while the fresh leaves of Thymus vulgaris had the highest content of alkaloids and terpenoids. Majority of fresh extracts gave positive results for phenols, flavonoids and quinones, suggesting that fresh plant material might have higher active metabolites than dried samples. The high occurrence of tannins and flavonoids in most of the plants indicates a high antioxidant activity, whereas the presence of alkaloids and terpenoids gives an indication of their potential for antimicrobial, anti-inflammatory and therapeutic properties.

The FTIR spectroscopy also confirmed presence of various bioactive functional groups in the plant extracts. O–H stretching vibration around 3289–3306 cm^{-1} was identified in Bougainvillea spectabilis, Thymus vulgaris and Nyctanthes arbor-tristis. The C-H stretching vibrations of terpenoid groups and alkanes had peaks in the range 2916-2919 cm-1, while strong peaks around 1731 cm-1 indicated the presence of carbonyl groups (such as aldehyde, ketone and ester) in the molecule. The presence of phenolic and flavonoid compounds was also confirmed by the presence of aromatic C=C stretching and aromatic ring vibrations observed near 1600–1517 cm^{-1}. The peaks corresponding to C–N and C–O stretching confirmed the presence of alkaloids, carbohydrates, ethers and polysaccharides. The FTIR profiles of the four plants showed that the extracts are rich in various chemicals, which confirmed the qualitative phytochemical screening results.

Therefore, the UV–Visible spectrophotometric analysis was carried out to determine the quantitative estimation of the phenolic and alkaloid content of the plant extracts. The absorbance recorded at 756 nm for phenols and 460 nm for alkaloids showed considerable differences between the samples tested. The highest absorbance values were observed for the leaves of harsingar for both phenolic and alkaloid content indicating high concentration of bioactive compound. Rosemary leaves also exhibited good absorbance whereas Bougainvillea leaves indicated low amount of phenols. If there are any differences in absorbance, then that means there are variations in the concentration of phytochemicals among different plant species. The antioxidant, antimicrobial and therapeutic potential of the plants are related to their higher phenolic and alkaloid contents. So, the spectrophotometric results also corroborate the results of the phytochemical screening analysis revealing that among the tested plants, Nyctanthes arbor-tristis and Thymus vulgaris might have relatively high medicinal value.

CONCLUSION

The present research successfully established the phytochemical richness of the selected medicinal plants by qualitative analysis, FTIR spectroscopy and UV–Visible spectrophotometric estimation. The results confirmed the presence of several important secondary metabolites such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, quinones, fats and oils in both fresh and dried extracts. FTIR analysis confirmed the presence of functional groups related to these bioactives, and UV–Visible spectroscopy showed differences between the plant extracts in terms of the number of phenolics and alkaloids. Nyctanthes arbor-tristis and Thymus vulgaris were comparatively rich in phytochemical content and have a higher medicinal value when compared with the other samples tested. The results of this study validated the traditional application of these plants in medical and health care industry and showed their potential use in pharmaceutical, nutraceutical and herbal medicines. The isolation, purification and biological activity evaluation of the individual compounds from these medicinal plants can be useful in the development of novel therapeutic agents in future.

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Title: A Review on Role of Plant Secondary Metabolites in Suppression of Disease-Associated Protein

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Date of conference: 9-10 may, 2026.


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