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

THESIS

Submitted for partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

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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.

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FTIR graph 1 Bougainvillea spectabilis

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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 anti-inflammatory, antioxidant, antidiabetic anticancer, antimicrobial, and antiviral activities. Flavonoids, phenols, alkaloids, tannins, terpenoids, steroids, and saponins contributes 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 rely on traditional herbal medicine for primary healthcare. Herbal medicines are important because they provide natural alternatives for disease management and play a significant role in pharmaceutical research and drug discovery. 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 used for treating several health problems. Its therapeutic importance is mainly attributed to the presence of bioactive compounds like flavonoids, glycosides, alkaloids, phenolics, tannins 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.

Secondary metabolites of plants are separated into different sets created on their chemical construction. 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.

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 facts gathered from numerous review and research articles. The thesis emphases on the estimation of secondary metabolites in two different medicinal plants. The thesis also contains information about their therapeutic mechanism in disease control.

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

Chapter 2 is the review of literature section which covers 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, 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]

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 are 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

The most distinctive and structurally distinctive phytochemicals of B. spectabilis are betalains, a class of water-soluble, nitrogen-containing pigments found solely in plants of the order Caryophyllales (including Nyctaginaceae). Betalains are classified into two groups, i.e., yellow-orange betaxanthins (e.g., indicaxanthin and vulgaxanthin) and red-violet betacyanins (e.g., betanin and isobetanin). The structural basis is the betalamic acid chromophore, which offers remarkable 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

Flavonoids have been isolated from different parts of B. spectabilis. HPLC analysis proved the presence of quercetin, kaempferol and their glycosidic derivatives (3-O-glucoside of quercetin and kaempferol-3-O-rutinoside) in leaf and bract extracts [11]. The finding of rutin (quercetin-3-O-rutinoside) has also been done. These substances are important contributors to the anti-inflammatory and antioxidant properties of the plant. (16)

2.2.3 Alkaloids and Pinitol

B. spectabilis was found to contain a few alkaloids including bougainvillin and a trace quantity of chemicals of the indole type (Jawad et al., 2012). A derivative of inositol, pinitol, is of pharmaceutical interest as it has been proven to possess insulin-mimetic and antidiabetic

properties [15]. Pinitol has been identified in extracts of B. spectabilis leaves and stems and is one of the main substances that are assumed to be responsible for the hypoglycemic action.

2.2.4 Tannins, Saponins, and Phenolic Acids

Qualitative and quantitative phytochemical screening methods always contain hydrolyzable tannins, saponins, terpenoids and steroids in Bougainvillea spectabilis extracts. It has been shown that the phenolic acid gallic acid is present in bract extracts and has potent antioxidant and hepatoprotective properties [3]. The total phenolic content of leaf methanol extracts ranged from 18.4 to 62.7 mg gallic acid equivalent (GAE)/g dry weight depending on the extraction solvent and plant section [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).

The polar fraction is much richer in rosmarinic acid (RA), an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (3,4-DHPL) with 6–34 mg/g dry weight, in T. vulgaris compared to the other Lamiaceae species [18]. The leaf surface wax contains two non-volatile triterpenoids: ursolic acid and oleanolic acid. HPLC and LC-MS are reliable methods for finding flavones, luteolin and luteolin 7-O-glucoside, apigenin, naringenin and eriodictyol. Minor components are tannin, saponins and flavanones.

2.4 Phytochemical profile of *Thymus vulgaris*

2.4.1 Essential Oil: Thymol and Carvacrol

The dried plant contains 0.8-2.6% of the essential oil after steam distillation of aerial portions of Thymus vulgaris. The main constituents of the oil that are always discovered by GC-MS analysis are thymol (2-isopropyl-5-methylphenol) and carvacrol (5-isopropyl-2-methylphenol) in 30-80% of the total oil composition depending on the chemotype and geographical origin [17]. Other monoterpenes present are p-cymene (5-30%) , γ-terpinene (2-10%) , linalool and borneol. Thymol is the most studied bio-active constituent of thyme and accounts for most of the antibacterial and antifungal activity of the herb.

2.4.2 Polyphenolic Acids

Thymus vulgaris contains an exceptional amount of hydroxycinnamic acids. Rosmarinic acid (RA) (6–34 mg/g dry weight) is the main phenolic acid present in the leaf extracts and is largely responsible for the plant's anti-inflammatory and antioxidant properties [18]. Other substances 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

Thymus vulgaris includes diterpenoids such as oleanolic acid, ursolic acid, in addition to the volatile monoterpenes, which were discovered to have anti-inflammatory and hepatoprotective activities. Ursolic acid is a pentacyclic triterpenoid with antitumour action that binds to the NF-κB pathway. Thyme also contains flavanones, tannins & saponins in small quantities.

2.5 *Nyctanthes arbor-tristis*

72 *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]

64 Chemical investigations of the leaves, flowers, stem bark and seeds of N. arbor-tristis have led to the isolation of a diverse array of secondary metabolites. The seeds as well as flowers contain the highest concentration of pharmacologically significant chemicals. These are iridoid glycosides notably nyctanthoside A, B and C which are esterified iridoids generated 5 from 8-epiloganic acid and have been isolated and characterized by NMR and mass 5 spectrometry [21] The major flavonoids identified in the leaves by HPLC-UV and LC-MS/MS fragmentation pattern are the flavonoids astragalin and nicotiflorin [22]. The leaves are a major source of the sugar alcohol, mannitol, which is of biosynthetical importance. The orange corollar tube contains nyctanthin (crocetin di-β-D-gentiobioside) which is a carotenoid derived pigment, comparable in structure to the active ingredients of saffron. Tannins, oleanolic acid, ursolic acid, β-sitosterol and phenolic glycosides, such as syringin 3 and verbascoside have also been reported [7], [8]. The primary volatile components of the leaf essential oil were identified by GC-MS as linalool, geraniol and β-ionone.

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]. 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 both the flowers and leaves of N. arbor-tristis include various flavonoids such as quercetin, astragalin, nicotiflorin and rutin [24]. The plant has been reported to have excessive antioxidant and anti-inflammatory properties of the aforesaid flavonoids (Nair et al., 2020). The Folin–Ciocalteu technique demonstrated the presence of 24.3–58.7 mg GAE/g dry weight total phenolics in the methanol leaves. The extremely efficient liquid chromatography (HPLC) of the leaf extracts demonstrated the presence of caffeic acid, p-coumaric acid and chlorogenic acid that are the basis for an antioxidant mode of action as in other Oleaceae . The major volatile elements of the flower essential oil characterized by GC-MS are geraniol, linalool and beta-ionone.

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. is a long-lasting evergreen aromatic shrub of the Lamiaceae family native to the Mediterranean basin and currently utilized all over 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. It is used for rheumatic pain and as a brain tonic (muqawi-e-dimagh) in the South Asian Unani Tibb medical system.

Being among the best plant sources of phenolic diterpene antioxidants is R. officinalis. The abietane-type diterpenes, carnosic acid and its oxidation product carnosol, are the most prevalent lipophilic antioxidants in leaf extracts. These compounds are specific to the Lamiaceae subfamily Nepetoideae and give Rosmarinus officinalis its exceptional capacity to stabilize food products through oxidative reactions [6]. It, belonging to the family Lamiaceae, is rather unique. The main components of its essential oil are 1,8-cineole (eucalyptol; 15–55%), camphor (10–25%), α-pinene (10–25%) and camphene, according to GC-MS analysis. Ursolic acid and oleanolic acid are the triterpenoids that always constitute the non-volatile fraction.

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 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

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). 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).

2.8.3 *Rosmarinic Acid, Flavonoids, and Other Polyphenols*

One out of the several water-soluble polyphenols present in rosemary plants, such as Thymus vulgaris, is rosmarinic acid, which is present in the plant at quantities of 0.5–2.5% of the dry leaf mass. In addition to the flavonoids luteolin, apigenin, diosmin, hesperidin, genkwanin, and nepitrin, as well as the diterpenoid acids rosmaridiphenol and rosmanol, S. rosmarinus shares the same biosynthesis, structure (an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid), and antioxidant mechanisms as thyme (Pintore et al., 2002). Significant levels of ursolic acid and oleanolic acid, two pentacyclic triterpenoids shared with Thymus vulgaris, are also responsible for rosemary's anti-inflammatory and anti-cancer qualities.

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.

These are small organic molecules, 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 [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). 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 major 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 (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.

All four plants examined contain different types of terpenoids. Monoterpenes are volatile essential oil fractions in T. vulgaris and R. officinalis. They include thymol, carvacrol, p-cymene, γ-terpinene, 1,8-cineole, α-pinene and camphor. The most abundant antioxidants in Rosmarinus officinalis specific to the Lamiaceae are carnosic acid and carnosol (C_{20}), which belong to the abietane-type diterpenes. All four plants contain the drug allopurinol and pentacyclic triterpenoids (C_{30}), such as ursolic acid and oleanolic acid, which have anti-inflammatory and anti-cancer effects. The iridoids from N. arbor-tristis (arbortristoside A–C) are monoterpenoid glycosides which are produced by geraniol as a critical step, then cyclised and oxidized 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).

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 antibacterial and analgesic activity seen in crude plant extracts is caused by alkaloids, which have a variety of forms and are significant leads in the development of pharmaceuticals.

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. Tyrosine is converted to the ring-opening product betalamic acid by DOPA-dioxygenase during betalain biosynthesis. This product then interacts non-enzymatically with amino acids and amines to form betaxanthins and with cyclo-DOPA glucoside to produce betacyanins [12]. Interestingly, this mechanism is absent from Caryophyllales, which likewise lack the anthocyanin biosynthesis pathway. Additionally, as no plant is known to generate both, the betalain system is a stand-alone solution to the pigmentation issue.

2.9.4 Flavonoids

These are a class of phenylpropanoids in which two phenyl rings (A and B) are joined by a three-carbon bridge (C-ring) usually containing an oxygen atom to give the C–structure. The enzyme chalcone synthase (CHS) catalyzes the condensation of one molecule of p-coumaroyl-CoA (from the phenylpropanoid pathway) with one molecule of malonyl-CoA (from the acetate-malonate pathway) to form naringenin chalcone, a universal flavonoid precursor. Chalcone isomerase (CHI) catalyzes the conversion to naringenin, a flavanone, which gives rise to the major classes of flavonoids: flavones by flavone synthase activity, dihydroflavonols and flavonols by flavanone 3-hydroxylase activity, and anthocyanidins by dihydroflavonol 4-reductase activity [27].
The pharmacological importance is hydroxylation of the B-ring and nature of substituents at specific positions. Quercetin (3,3',4',5,7-pentahydroxy) as glycosides in B. spectabilis and N. arbor-tristis possesses antioxidant, anti-inflammatory and antidiabetic properties because of the presence of catechol B-ring (radical scavenging), C2=C3 double bond (planar geometry that allows π-stacking interactions with protein binding sites) and 3-OH group (metal chelation). Luteolin (3′,4′,5,7-tetrahydroxy), the most common flavones in T. vulgaris and R. officinalis, has strong anti-inflammatory effects by suppressing the STAT3 and NF-kB pathways.Luteolin (3',4',5,7-tetrahydroxy) is the major flavone of T. vulgaris and R. officinalis and differs from quercetin in the absence of the 3-hydroxyl, but the presence of the catechol B-ring and the 5,7-hydroxylation pattern of the A-ring. Both compounds have potent anti-inflammatory effects by inhibiting the STAT3 and NF-κB pathways. Kaempferol (4',5,7-trihydroxy), present in B. spectabilis and N. arbor-tristis, has one hydroxyl in the B-ring instead of the catechol system and somewhat less radical scavenging ability, but is still an antiproliferative and anticancer substance. The main flavonoid constituent of N. arbor-tristis

is astragalin (kaempferol-3-O-glucoside), which has been demonstrated to possess potent anti-inflammatory and antiparasitic activities in experimental systems [22].

2.10 Extraction Method for Plants Secondary Metabolites

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 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. 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 total phenolic content (TPC) was determined using the Folin-Ciocalteu assay (absorbance at 765 nm, calibrated with gallic acid), the total flavonoid content (TFC) using the aluminium chloride colorimetric method (absorbance at 415–510 nm, calibrated with quercetin or rutin) and the total betalain content (betacyanins at 535 nm, betaxanthins at 480 nm). In B. spectabilis, bract methanol extracts showed high absorption peak at 535 nm which is corresponding to the absorption of betacyanins (betanin: λmax 536 nm) and another peak was observed at 480 nm for betaxanthins [14]. The UV absorption of the extract was observed in the region of 240–280 nm (Band II, A-ring) and 350–380 nm (Band I, B-ring) and is attributed to flavonoids. The rosmarinic acid has a UV absorption at 328 nm, typical for extracts of T. vulgaris, and hence it can be readily quantified. The total phenolic content of the leaf methanol extracts of R. officinalis has been found to be 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 leaf ethanol extracts of N. arbor-tristis was in the range of 18–45 mg GAE/g and TFC was in the range of 12–28 mg QE/g depending on the solvent used for extraction and the plant part (Rani et al., 2012).

2.11.2 Fourier-Transform Infrared Spectroscopy

The FTIR spectroscopy provides a molecular fingerprint of the plant extracts since it depends upon the characteristic absorption bands associated with the vibrational modes of the functional groups. The presence of large O-H stretching bands in the 3200-3600 cm-1 area seen in all four plants in their extracts indicates the existence of phenolic hydroxyl groups. The C=O carbonyl stretches appear at ~1690-1730 cm-1 for the carboxylic acid groups of phenolic acids (rosmarinic, gallic, carnosic), the ester carbonyl of carnosic at ~1720 cm-1 and the lactone carbonyl of carnosol at 1745 cm-1. The existence of aromatic C=C ring stretching vibrations in the area of 1,500-1,600 cm-1 indicates the existence of flavonoid and phenylpropanoid frameworks in all the four plants.
FTIR analysis of the bract extracts showed characteristic peaks at 3420 cm-1 (O-H stretch), 1626 cm-1 (C=N stretch of betalamic acid chromophore 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 good diagnostic feature for the betalain compounds and is found at approximately 1620-1640 cm-1. The essential oil-depleted extracts of T. vulgaris exhibited 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), characteristic of a rosmarinic acid-rich profile (Petersen & Simmonds, 2003). The extracts of R. officinalis contain unique FTIR spectra with a wide O-H hydrogen bond at 3,450 cm-1, C=O (carboxylic acid) at 1,718 cm-1 and characteristic conjugated diene system at 1,590-1,620 cm-1. FTIR spectra of the extracts of leaves of N. arbor-tristis showed 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) [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 among 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 stimulates the Nrf2/ARE signalling pathway, leading to increased effect 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

T. vulgaris shows its bactericidal effect on both gram-positive and gram-negative bacteria with MIC values ranging from 0.05 to 2.0 mg/mL [31] by disturbing the bacterial cytoplasmic membrane by intercalation into the phospholipid bilayer, dissipating the proton motive force and causing ion leakage. Essential oil of R. officinalis, 1,8-Cineole and α-pinene has similar effects on the fungal ergosterol membranes and demonstrate synergistic anti-fungal activity against C. albicans. B. spectabilis tannins and gallic acid precipitate bacterial surface proteins that are involved in the formation of the cell wall [3]. Iridoid glycosides are especially the arbortristoside compounds of N. arbor-tristis, which have exhibited antifungal and antiparasitic efficacy, probably due to their inhibition of 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 ro

smarinic acid, which disrupts the activity of PTP1B and enhances the endogenous insulin signalling pathway (Kim et al., 2010). The useful 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)

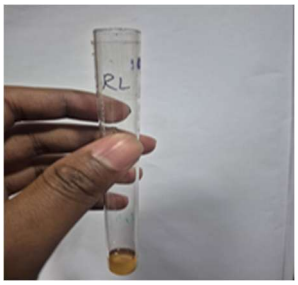
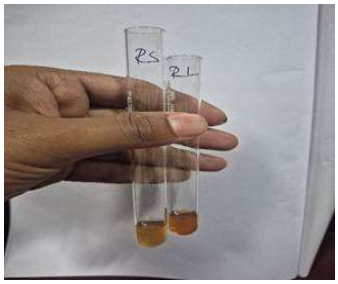
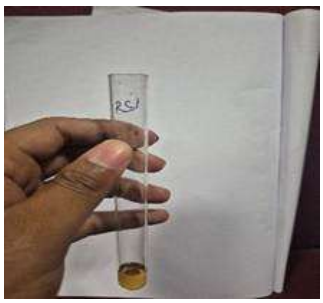
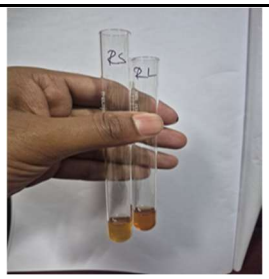
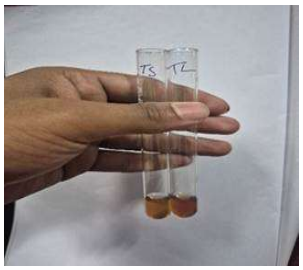
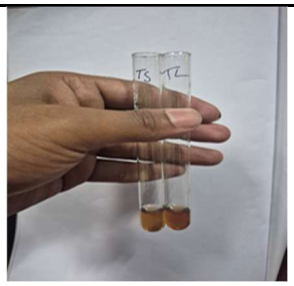
Fats and oils were detected in *Thymus vulgaris* leaf, *Bougainvillea spectabilis* leaf, flower and *Nyctanthes arbor-tristis* stem, because of the change of colour of the solution to blue, while absent in *Rosmarinus officinalis* and most other extracts.

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		

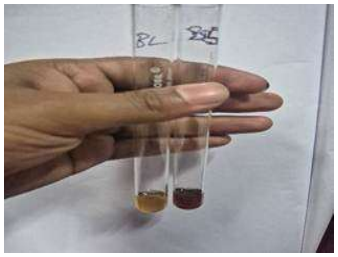
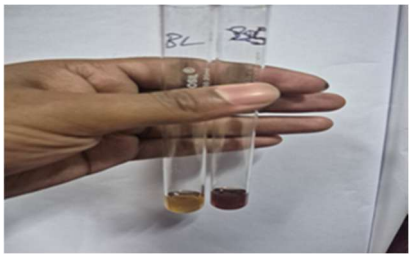
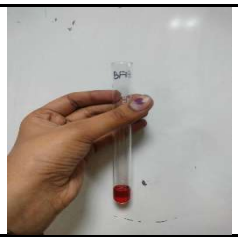
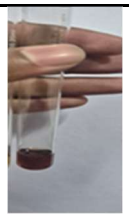
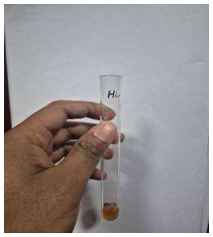
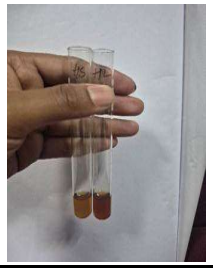
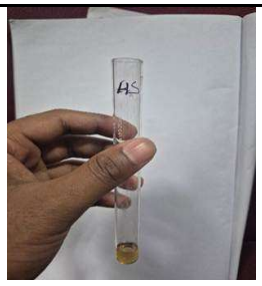
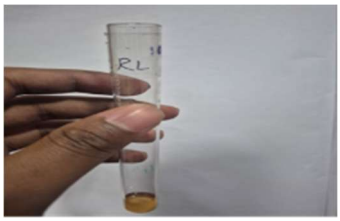
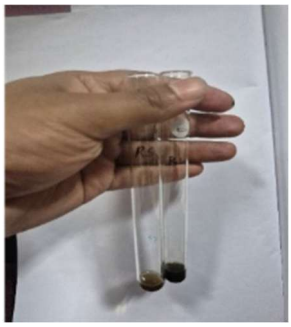
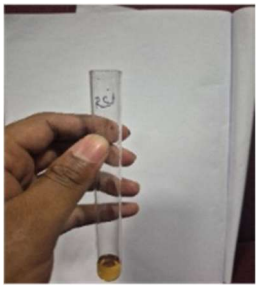
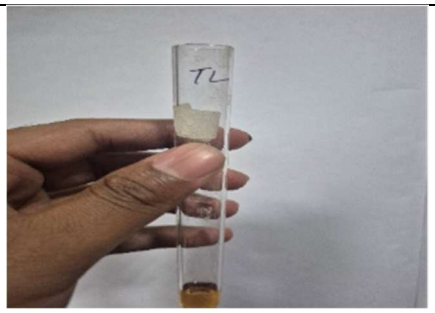
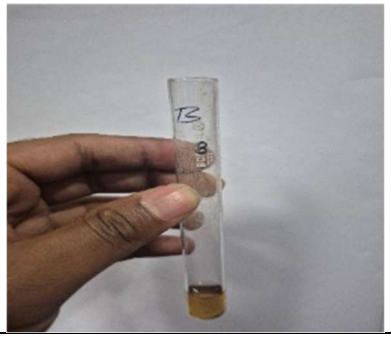
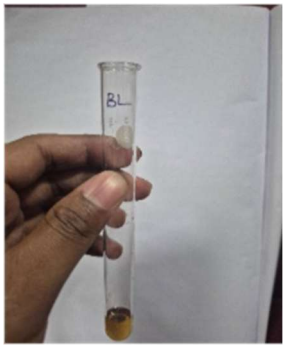
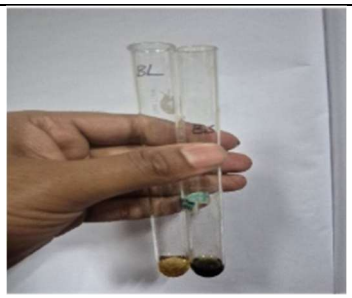
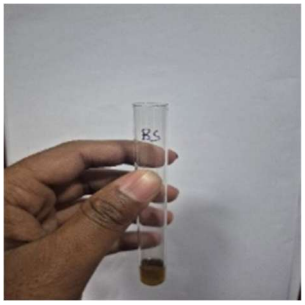
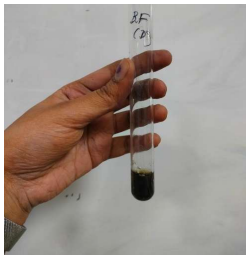
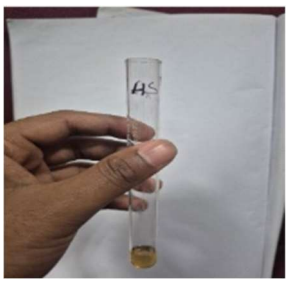
Bougainvillea	leaf		
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

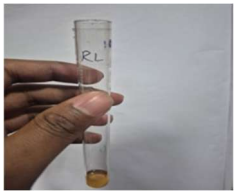
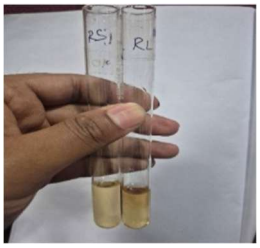
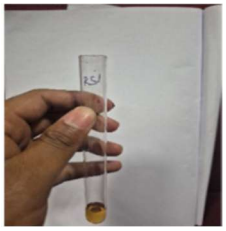
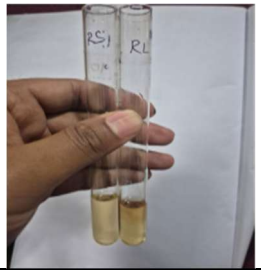
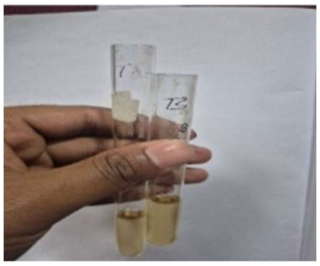
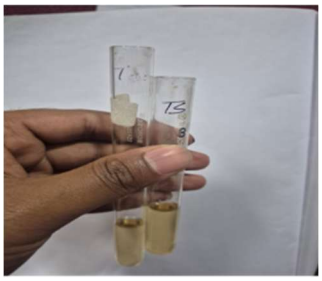
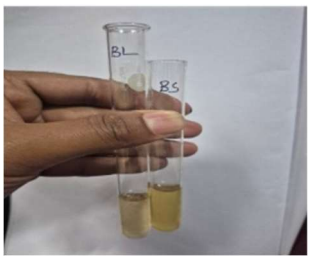
Figure 1: Alkaloid test dried plant

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		

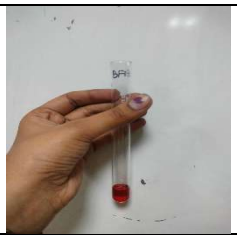
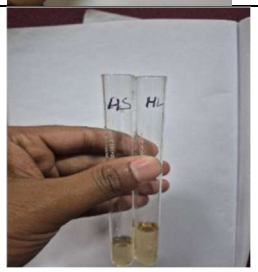
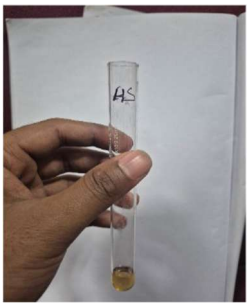
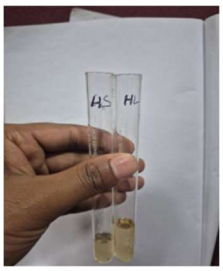
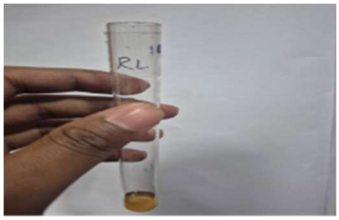
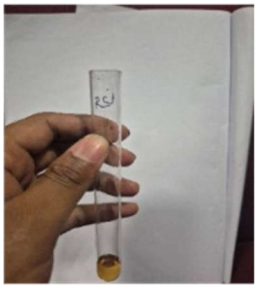
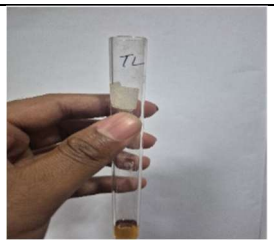
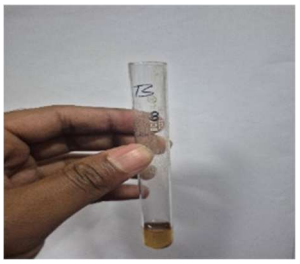
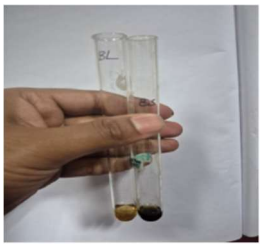
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

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		

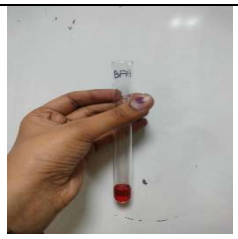
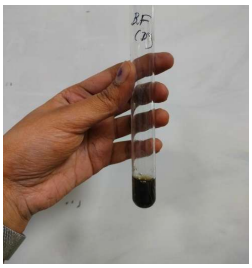
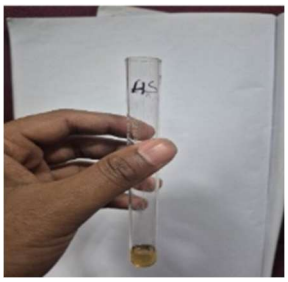
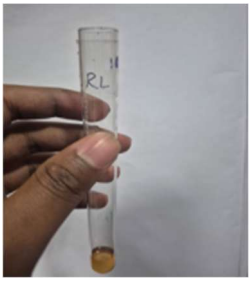
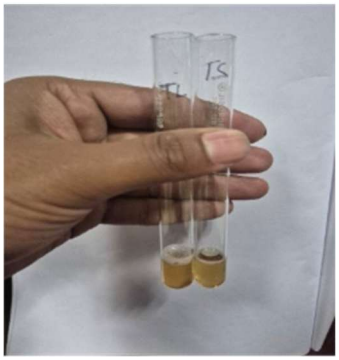
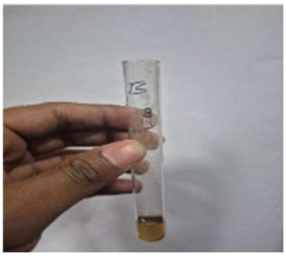
			
Harsingar	Leaf		
Harsingar	Stem		

Figure 3: Tannins test dried plant

e. saponins

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

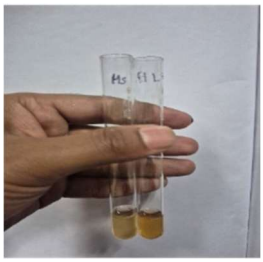
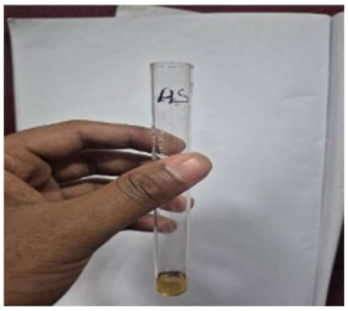
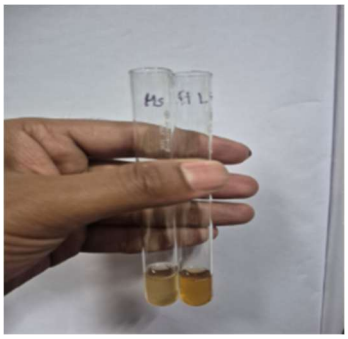
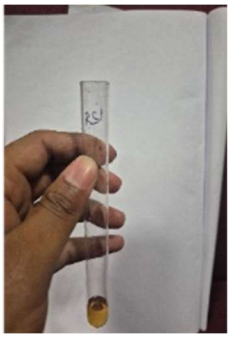
Bougainvillea	leaf		
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

Figure 4: Saponins test dried plant

f. Terpenoids

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

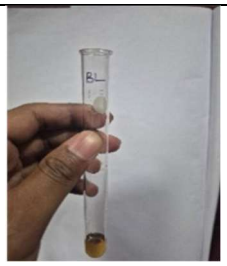
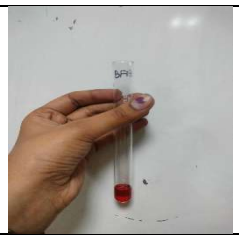
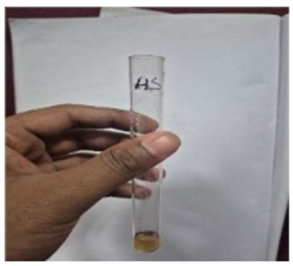
Bougainvillea	leaf		
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

Figure 5: Terpenoids test dried plant

g. Quinones

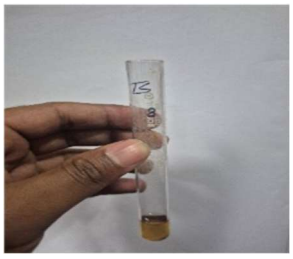
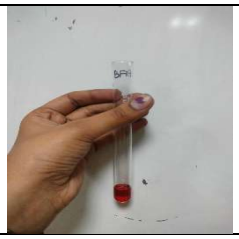
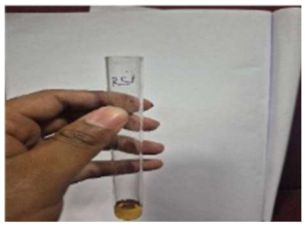
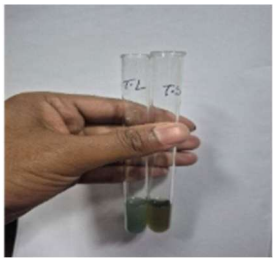
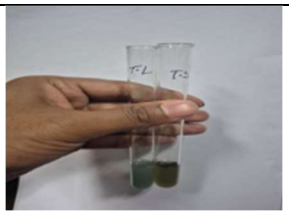
Plant name	Part	Before	After
Rosemary	stem		
Thyme	stem		
Bougainvillea	stem		
Bougainvillea	flower		

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		

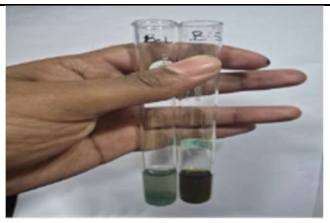
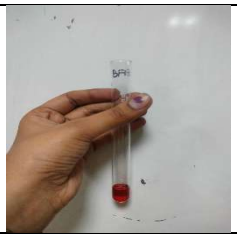
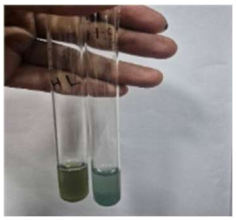
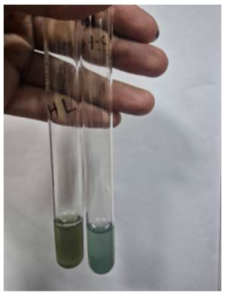
Bougainvillea	stem		
Bougainvillea	flower		
Harsingar	Leaf		
Harsingar	Stem		

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

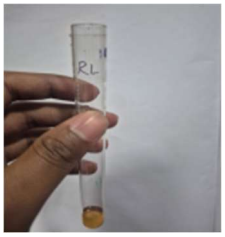
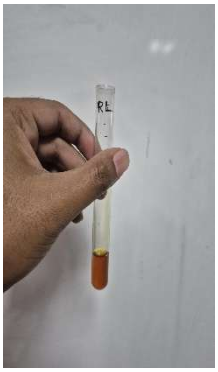
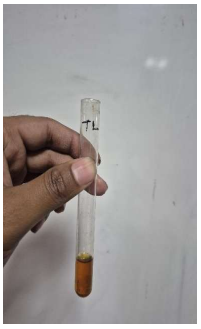
Plant Name	Before	After
Rosemary		
Thyme		
Bougainvillea (leaf)		
Bougainvillea (flower)		

Figure 8: Alkaloid test fresh plant

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

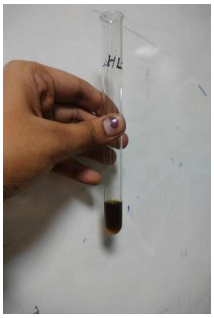
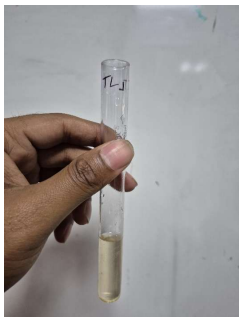
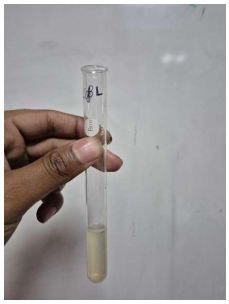
Harsingar		

Figure 9: Phenols test fresh plant

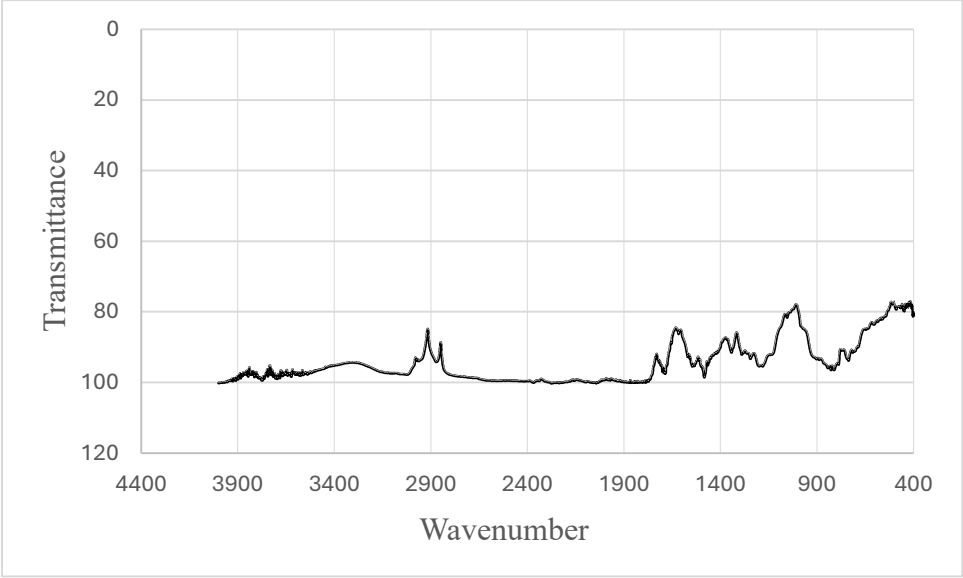
Plant Name	Before	After
Rosemary		
Thyme		
Bougainvillea (leaf)		
Bougainvillea (flower)		

Harsingar		

Figure 10: Flavonoid test fresh plant

4.3 FTIR Results

4.3.1 *Bougainvillea spectabilis*



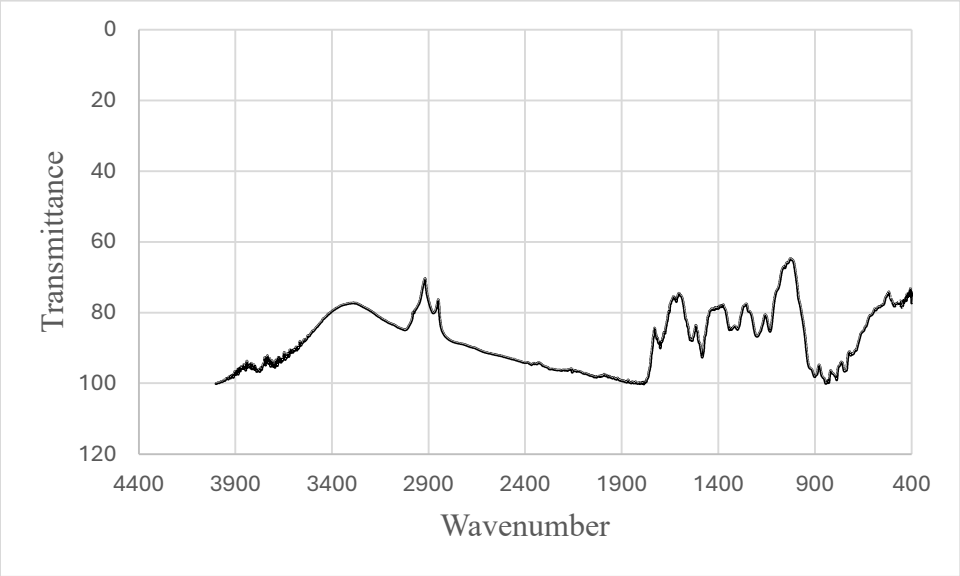
FTIR graph 1 Bougainvillea spectabilis

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

Peak Position (cm^{-1})	Probable Functional Group	Possible Compound Type / Interpretation
~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*



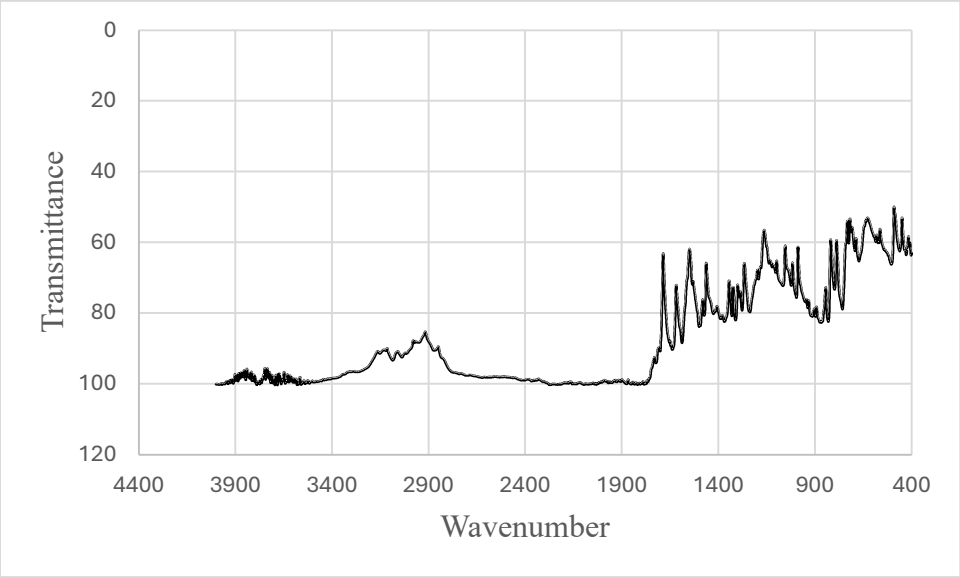
FTIR graph 2 Thymus vulgaris

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

Peak (cm^{-1})	Functional Group	Possible Phytochemical/Interpretation
~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*

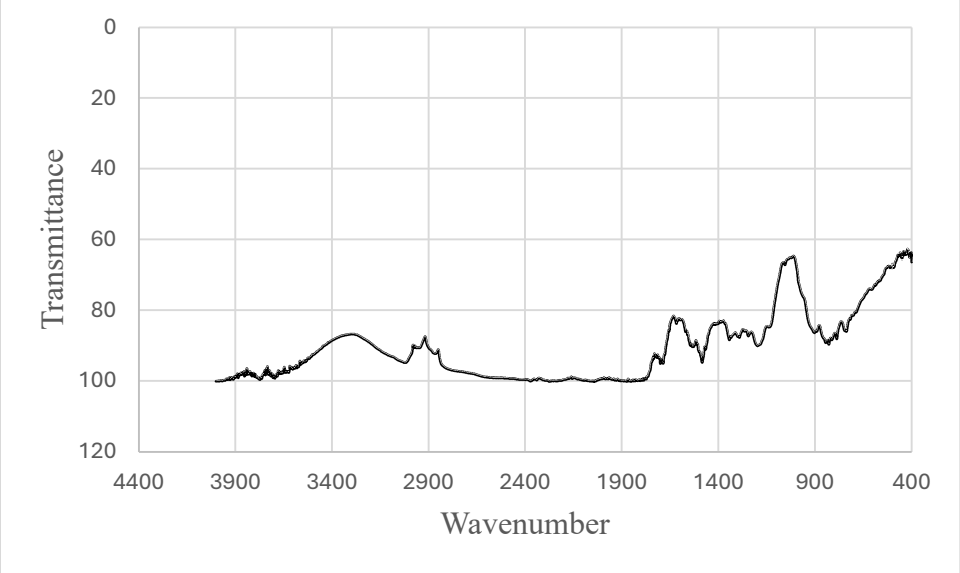


FTIR graph 3 Rosmarinus officinalis

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

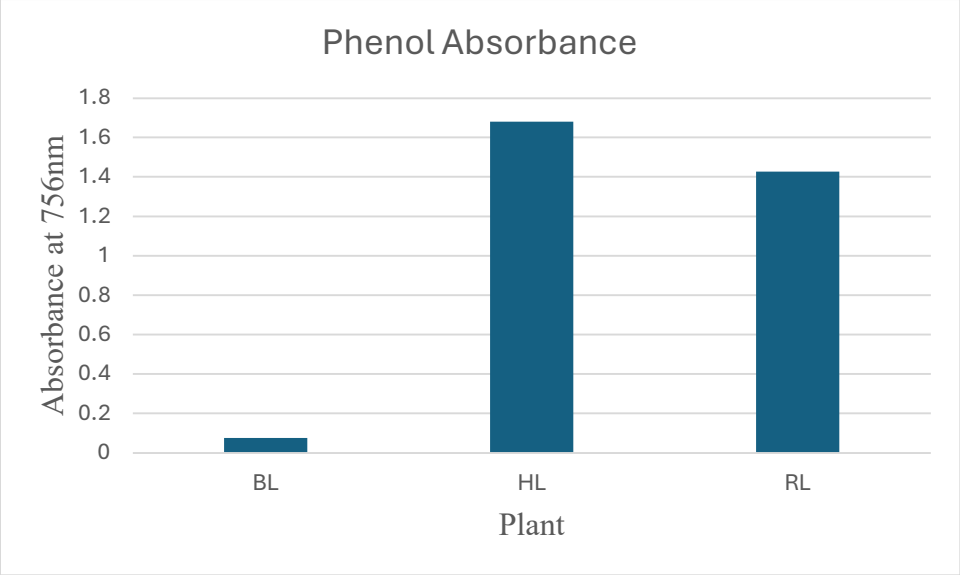
Peak (cm^{-1})	Functional Group	Possible Phytochemical/Interpretation
~3296	O–H stretching	Phenols, flavonoids, alcohols
~2918	C–H stretching	Terpenoids, lipids, alkanes
~1632	C=C stretching / Amide	Aromatic compounds, proteins
~1374	C–H bending	Alkanes and methyl groups
~1273	C–N stretching	Alkaloids,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

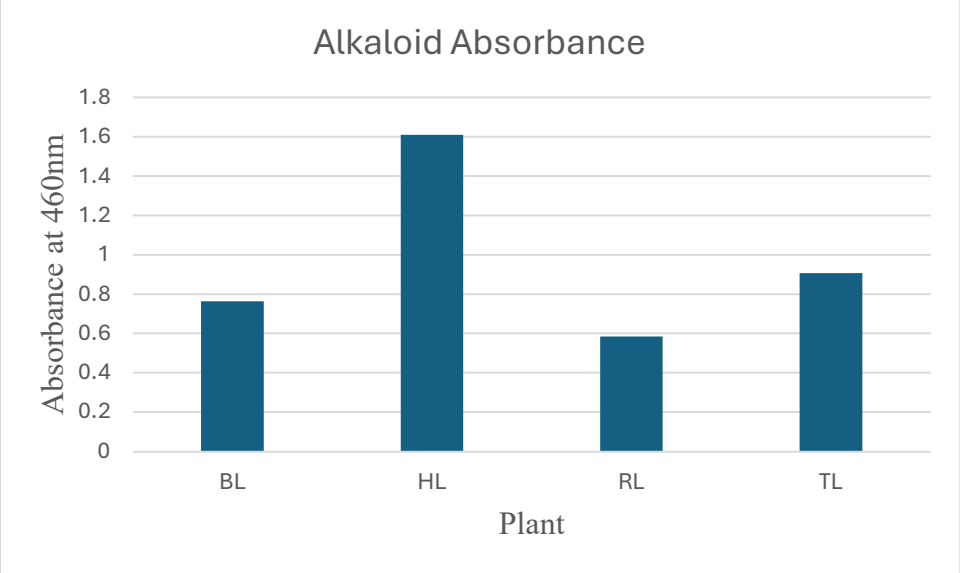
4.4 Spectroscopy analysis result and discussion

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 work used qualitative phytochemical screening, FTIR spectroscopy, and UV–visible spectrophotometric analysis to evaluate the phytochemical content of medicinal plants, including Rosmarinus officinalis, Thymus vulgaris, Bougainvillea spectabilis, and Nyctanthes arbor-tristis. Alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, quinones, lipids, and oils were among the secondary metabolites found in the fresh and dried extracts, according to qualitative analysis. 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 occurrence 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 study using qualitative analysis, FTIR spectroscopy and UV–visible spectrophotometric estimate successfully determined the phytochemical richness of the selected medicinal plants. The findings verified that both fresh and dried extracts included a number of significant secondary metabolites, including alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, quinones, lipids, and oils. UV–visible spectroscopy revealed differences in the levels of phenolics and alkaloids in the plant extracts, and FTIR analysis confirmed the presence of functional groups associated with these bioactives. Nyctanthes arbor-tristis and Thymus vulgaris showed higher therapeutic potential and a comparatively higher amount of phytochemicals than other samples tested. The study results confirmed the traditional application of these plants in the medical and health care sector and also showed

their potential for use in pharmaceutical, nutraceutical and herbal remedies. In the future, the creation of new therapeutic medicines may benefit from the separation, purification, and biological activity assessment of the individual chemicals from these medicinal plants.