# Navneeta Bharadvaja

## 12 (1) (1)

### Document Details

Submission ID
trn:oid:::27535:140526792

Submission Date
May 26, 2026, 11:46 AM GMT+0

Download Date
May 26, 2026, 11:51 AM GMT+0

File Name
12 (1) (1).docx

File Size
3.7 MB

42 Pages

9,267 Words

58,252 Characters

# 7% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

## Match Groups

**64** Not Cited or Quoted 7%
Matches with neither in-text citation nor quotation marks

**0** Missing Quotations 0%
Matches that are still very similar to source material

**0** Missing Citation 0%
Matches that have quotation marks, but no in-text citation

**0** Cited and Quoted 0%
Matches with in-text citation present, but no quotation marks

## Top Sources

4% Internet sources
3% Publications
6% Submitted works (Student Papers)

## Integrity Flags

**0 Integrity Flags for Review**

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

## Match Groups

- **64** Not Cited or Quoted 7%
  Matches with neither in-text citation nor quotation marks
- **0** Missing Quotations 0%
  Matches that are still very similar to source material
- **0** Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- **0** Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

## Top Sources

- 4% Internet sources
- 3% Publications
- 6% Submitted works (Student Papers)

## Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

| | | |
|---|---|---|
| 1 | Internet | |
| bsj.com.bt | | <1% |
| 2 | Internet | |
| www.mdpi.com | | <1% |
| 3 | Internet | |
| www.spectroanalysis.com | | <1% |
| 4 | Student papers | |
| Krea University on 2026-05-23 | | <1% |
| 5 | Publication | |
| Mahendra Rai, Chistiane M. Feitosa. "Eco-Friendly Biobased Products Used in Micr... | | <1% |
| 6 | Student papers | |
| Higher Education Commission Pakistan on 2011-01-04 | | <1% |
| 7 | Student papers | |
| Vellore Institute of Technology on 2026-05-05 | | <1% |
| 8 | Internet | |
| spiral.imperial.ac.uk | | <1% |
| 9 | Student papers | |
| Higher Education Commission Pakistan on 2025-08-23 | | <1% |
| 10 | Internet | |
| etd.aau.edu.et | | <1% |

| | | |
|---|---|---|
| 11 | Student papers | universititeknologimara on 2024-06-22 <1% |
| 12 | Student papers | Coventry University on 2026-03-17 <1% |
| 13 | Student papers | Higher Education Commission Pakistan on 2025-08-13 <1% |
| 14 | Student papers | B.S.Abdur Rahman Crescent Institute of Science & Technology on 2025-04-22 <1% |
| 15 | Internet | ijsrst.com <1% |
| 16 | Student papers | University of Witwatersrand on 2025-10-28 <1% |
| 17 | Internet | www.sryahwapublications.com <1% |
| 18 | Student papers | Abu Dhabi University - BB Ultra on 2025-10-19 <1% |
| 19 | Student papers | Higher Education Commission Pakistan on 2013-07-10 <1% |
| 20 | Internet | research.nottingham.edu.cn <1% |
| 21 | Student papers | University of West London on 2025-05-26 <1% |
| 22 | Internet | atrium.lib.uoguelph.ca <1% |
| 23 | Student papers | University of Chichester on 2013-07-05 <1% |
| 24 | Publication | Hasana Logita Haile. "Natural insecticides and phytochemical analysis of gaggass... <1% |

25 Student papers
Higher Education Commission Pakistan on 2016-09-06 <1%

26 Student papers
Sri Lanka Institute of Information Technology on 2025-04-20 <1%

27 Student papers
Delhi Technological University on 2026-05-25 <1%

28 Internet
baadalsg.inflibnet.ac.in <1%

29 Internet
www.draymanabdelhameed.com <1%

30 Student papers
9715 on 2015-04-14 <1%

31 Student papers
Higher Education Commission Pakistan on 2015-10-07 <1%

32 Student papers
Nilai University College on 2018-03-08 <1%

33 Publication
T. Pullaiah. "Phytochemistry and Pharmacology of Medicinal Plants", Apple Acade... <1%

34 Student papers
University of Wales Institute, Cardiff on 2025-12-06 <1%

35 Student papers
Vellore Institute of Technology on 2026-05-01 <1%

36 Internet
ir.avinuty.ac.in <1%

37 Internet
journals.innovareacademics.in <1%

38 Internet
link.springer.com <1%

39 Internet

pure.manchester.ac.uk <1%

40 Internet

repository.ju.edu.et <1%

41 Student papers

Higher Education Commission Pakistan on 2015-11-03 <1%

42 Student papers

Our Lady of Fatima University on 2025-10-01 <1%

43 Publication

Kiran Zahid, Syed Muhammad Faheem Ahmad, Maqsood Ahmed, Farah Khan. "An... <1%

# Comparative Analysis of Phytochemicals in Medicinal Plants using UV-Visible and FTIR

**A Thesis Submitted**

**in Partial Fulfillment of the Requirements for the Degree of**

## MASTER OF SCIENCE IN BIOTECHNOLOGY

**By**

**CHETNA RANI &AKASNHA**

**(Roll No. 24/MSCBIO/53)**

**(Roll No. 24/MSCBIO/26)**

**Under the Supervision of**

**DR. NAVNEETA BHARADVAJA**

**Associate Professor, Department of Biotechnology**
**Delhi Technological University**

**To the**

**Department of Biotechnology**

**DELHI TECHNOLOGICAL UNIVERSITY**

**(Formerly Delhi College of Engineering)**

**Shahbad Daulatpur, Main Bawana Road, Delhi-110042**

**May, 2026**

29

# LIST OF FIGURES



22

22

# LIST OF TABLES

# TABLE OF CONTENTS

# TITLE: - Comparative Analysis of Phytochemicals in Medicinal Plants using UV-Visible and FTIR


## ABSTRACT: -

The objective of this research was to analyze the phytochemical constituents and medicinal values of certain medicinal plants through qualitative, quantitative, and spectroscopic analysis. The aqueous extracts of selected medicinal plants that is *Euphorbia milii, Matricaria chamomilla*, *Parthenium hysterophorus* and *Stevia rebaudiana*, were obtained, and certain phytochemical constituents were determined qualitatively. Qualitative analysis indicated the existence of alkaloids, phenols, tannins, flavonoids, saponins, terpenes, sterols, and quinones in the plant extracts. Quantitative analysis emphasized variations in the concentration of phytochemicals in the selected plants. Further, UV–Visible spectroscopy was performed for identification and estimation of phytochemicals. FTIR spectroscopy was performed to determine functional groups in bioactive compounds. The results emphasize the significance of medicinal plants as natural sources of phytochemicals with antioxidative and therapeutic effects.


## INTRODUCTION

Phytochemicals are natural derived compounds occurring in medicinal plants, specifically in plant parts like leaves,roots,stems, bark, flowers, seeds, etc., having a specific mechanism and protective effects.[1][2]Numerous bioactive chemicals in plants affect different physiological functions in humans; hence, medicinal properties in plants can be attributed mainly to phytochemicals.[3] Metabolites in plants are divided mainly into two categories: primary metabolites and secondary metabolites. Primary metabolites, such as sugars, amino acids, lipids, proteins, and chlorophyll, are vital in normal growth and development processes, while SMs include alkaloids, flavonoids, tannins, terpenoids, saponins, phenols, essential oils, and cardiac glycosides.[4]

These secondary metabolites display various biological functions such as antioxidant, antiapoptotic, antiaging, anticarcinogenic, anti-inflammatory, antiatherosclerosis, cardiovascular protecting, antiangiogenic, and antiproliferative properties.[5] As a result of their medicinal properties, phytochemical screening techniques have been adopted widely as a means of detecting and identifying bioactive compounds in plants, which could also form the

foundation for drug development in the future. Phytochemical qualitative screening can be done to determine different kinds of biologically active compound.[6]

Phenolics, alkaloids, terpenes, tannins, and saponins are common secondary metabolites found in plants with important pharmacological applications.[8] Plants with significant amounts of secondary metabolites are important sources of natural medicinal substances and can be used in the development of drugs.[9]Alkaloids are nitrogenous compounds known for their numerous applications in modern medicine due to their high therapeutic potential.[7] These nitrogenous metabolites are important for antimicrobial, analgesic, and anti-inflammatory actions.[8] Some of the phytochemicals including alkaloids and polyphenols have proved effective in providing antimicrobial, anticancer, and anti-inflammatory actions.[7]

Phenolic metabolites provide important contributions towards the antioxidant potential due to their hydrogen donating action against harmful free radicals.[8] Polyphenols are important phytochemicals known for their antioxidant capacity due to their ability to protect against oxidation.[7]Flavonoids and phenolic components are important metabolites in protecting against free radicals by neutralizing their harmful effects. Therapeutic importance of phenolic compounds can be associated to their ability to protect against oxidative stress-related diseases.[10]Tannins are polyphenolic phytochemicals known for their wide-spectrum antibacterial activity against pathogenic microorganisms. Antibacterial properties of tannins can be attributed to inhibition of enzymes, interference with cellular processes, and prevention of nutrient transport.[11]

Natural antioxidants in the form of polyphenols from plants have drawn substantial attention in modern medicine.[11]Saponins are glycosides occurring in medicinal plants. Antioxidant activity of saponins from medicinal plants has been attributed to their radical scavenging and membrane protection activity.[12]

*Euphorbia milii* is a valuable medicinal plant whose use for skin inflammation treatment, because of the presence of diterpenoids, triterpenoids, flavonoids, and macrocyclic lactones. Some biological activities observed in the extracts obtained from *Euphorbia milii* include antioxidative, antimicrobial, anti-inflammatory, anticancer, antiparasitic, and other health benefits.[14]*Matricaria chamomilla* one of the earliest used medicinal plants in treating inflammatory diseases, gastrointestinal issues, and skin infections. Bioactive components found in chamomile include flavonoids, terpenoids, coumarins, and essential oils, giving this medicinal plant excellent antioxidative, anti-inflammatory, antimicrobial, wound-healing, and mild sedative activities.[15]

*Parthenium hysterophorus*, having important phenolic compounds, flavonoids, and sesquiterpene lactones, contributing to the pharmacological significance of this medicinal plant. *Parthenium hysterophorus* has shown excellent antioxidative and anti-inflammatory activities; traditionally, this plant was used to treat skin problems, fever, inflammations, and infectious diseases.[16]*Stevia rebaudiana* is a popular because of the presence of intensely sweet steviol glycosides along with stevioside, rebaudioside, flavonoids, and phenols.[17]

Traditionally, *Stevia rebaudiana* was used for managing diabetes, high blood pressure, and metabolic problems; antioxidants and other pharmacological properties of this medicinal plant are due to the phenols and natural glycosides, which are potent free radical scavengers.[13], [18]The process of extraction and spectroscopic characterization of phytochemicals through analytical methods such as UV–Visible and FTIR spectroscopy .[13]

One popular technique in the studies of medicinal plants is aqueous extraction, which is safe, cheap, and eco-friendly concerning the extraction of polar active phytochemicals.[19]Qualitative phytochemical profiling is done to check the existence of certain secondary metabolites in medicinal plants, namely, alkaloids, phenols, tannins, and saponins. The quantification of phytochemicals is an important procedure in determining the content of medicinal substances in plants. UV-Vis spectroscopy is a fast and efficient method for the identification of phytochemicals.[20]

FTIR spectroscopy is an indispensable method for the determination of functional groups and the analysis of the structure of biologically active phenolics and flavonoids.[13], [22]Phytochemical evaluation and spectroscopic characterization can provide scientific substantiation for the pharmacological properties of medicinal plants.[20]

## 1.2 Relevance of the project

Bioactive secondary metabolites are regarded as important sources with various pharmaceutical properties. Alkaloids, phenolic, tannins, and saponins are among the phytochemical compounds that possess antioxidant, antimicrobial, anti-inflammatory and medicinal properties. Natural plant-based products have gained immense popularity in recent times as alternative treatment methods, since they pose less risk compared to synthetic drugs. UV-Visible and FTIR spectroscopy are commonly used in identifying plant extracts through phytochemical and functional group analyses.

## 1.3 Objective of the study

1. Conduct qualitative phytochemical analysis for detection of phytochemical constituents.

2. Phytochemical profiling of plant extracts using UV-Vis Spectroscopy.

3. Determination of functional groups of plant extracts using FTIR spectroscopy.

The current study may provide scientific information regarding phytochemistry of medicinal plants and their medicinal relevance. This could also be useful in promoting phytochemicals of plant origin in future pharmaceutical applications.

## 1.4 Medicinal Plants Selected for the Study

1.*Euphorbia milli*

2.*Matricaria chamomilla*

3.*Parthenium hysterophorus*

4.*Stevia rebaudiana*

# Chapter 2: Review of Literature

Medicinal plants have gained considerable scientific interest in recent years because their phytochemical composition is rich, and also because they have a broad range of therapeutic uses. From these plants, secondary metabolites appear to have high therapeutic potential like antioxidant , antimicrobial , anti-inflammatory and even anticancer activities. In the next section some medicinally relevant plants are mentioned that have been studied for their phytochemical potential in general terms.

## *2.1 Euphorbia milli*

### *2.1.1* Botanical Description

*Euphorbia milii* is a scandent succulent, ornamental shrub because its thick fleshy brownish stems carry sharp spines.[23] It has alternate obovate leaves, and it forms brightly coloured bracts around flowers that are relatively inconspicuous. [24] Native to Madagascar, *E. milii* is well adapted for dry and semi dry conditions, and it contains toxic milky latex in every part of the plant and produces triangular-ovoid capsules with greyish brown seeds, and it flower throughout the year.[23]

### 2.1.2 Phytochemical Constituents

Phytochemical investigations of *Euphorbia milii* have showed that diverse secondary metabolites are present like triterpenoids, flavonoids, alkaloids, diterpenoid and saponins which are notable for medicinal value [24][23]In fact, among these constituents the diterpenoids class seems to be the dominant group of phytochemicals and it is linked with different biological actions of the plant [14][25] UHPLC-MS profiling along with phytochemical screening studies, also supported that coumarins, cardiac glycosides and terpenoid compounds can be found in various extracts of E. milii. [24] [26].

### 2.1.3 Medicinal Importance

Euphorbia milii possesses significant medicinal value because it contains a diverse phytochemical composition and also exhibits diverse pharmacological action [24] In tradition medicine, the plant has often been used for skin infections, inflammatory problems, and wound related conditions with people relying on it as part of home remedies [28] Many studies report that extracts and latex from E. milii can act as antioxidants, as well as antimicrobials and anti-inflammatory agents, mostly because it contains various secondary metabolites [24], [28]The overall pharmacological impact is often linked with tannins, flavonoids, saponins, and phenolic constituents which are connected with tissue repair and therapeutic potential [25]

### 2.1.4 Anti-Microbial Activity

In antimicrobial assays it came out that E. milii extracts exhibited inhibitory action against Staphylococcus aureus, Escherichia coli and several fungal pathogens[24], [27] The plant's antibacterial and antifungal properties is because of the presence of flavonoids, tannins and other phytochemical components [27]

### 2.1.5 Anti-Oxidant Activity

The methanolic and floral extracts from E. milii exhibited significant DPPH radical scavenging action, which points to a strong antioxidant potential[23], [29], [30] . The total phenolic content was very high, together with the flavonoids and saponins, these constituents significantly contribute to antioxidant and the cytotoxic characteristics of plant. [31]Also, the antioxidant effects of E. milii have been associated with phenolic compounds, these exhibit free radical scavenging , and they can participate in metal ion binding too, thereby supporting the observed antioxidant potential [30] Additionally, the phenolic compounds and flavonoids found in Euphorbia species play a major role in anti-inflammatory mechanisms, mostly via free radical scavenging activity [25]

### 2.1.6 Toxicological and Phytochemical Investigations

The milky latex of Euphorbia milii has diterpenoid constituents with both pharmacological and irritant traits and upon excessive exposure it can lead to skin irritation and inflammatory reactions, due to the presence of toxic compounds [14], [25] Even though it is toxic at higher doses, E. milii still shows a noticeable medicinal value, partly due to its diverse phytochemical makeup and therapeutic usefulness [28]. Overall, these phytochemical screening and extraction reports highlight that Euphorbia milii is scientifically significant as a medicinal plant with both Therapeutic effects, and toxicological relevance [26], [27], [28], [32]

x

## *2.2 Matricaria chamomilla L.*

### 2.2.1 Introduction and botanical description

*Matricaria chamomilla L.* often referred to as German chamomile and spread broadly across Europe and Western Asia. [33] Out of all the parts, the flowers are usually treated as the main medicinal part, and they're known for being particularly rich in essential oils as well as in other bioactive phytochemical components. [15] Because it contains pharmacologically active constituents, chamomile gets used in traditional medicine, in cosmetic products and in the more general herbal market.[33]

### 2.2.2 Phytochemical Constituents

Major bioactive constituents that have been reported in chamomile include apigenin, quercetin, luteolin, and chamazulene , and they seem to really help explain a lot of the plant's medicinal , plus pharmacological properties.[34] The essential oil from chamomile is especially abundant in sesquiterpenes and other terpenoid compounds like chamazulene, which are linked with anti-inflammatory effects and a kind of gentle calming activity.[33]Several studies that used methanolic and ethanolic extracts of M. chamomilla have shown a high phytochemical level, along with notable biological activity , mostly because phenols and flavonoids are extracted very efficiently.[34]

### 2.2.3 Anti-Inflammatory and Sedative Activity

Matricaria chamomilla is widely described to have strong anti-inflammatory effects, and it's mostly linked to flavonoids along with terpenoid related constituents that are found in the flower extracts and essential oils [33]. In other words, those components show up in the extracts, not just one part. Some bioactive molecules like chamazulene are reported to hinder inflammatory signals and also help reduce swelling in tissues, so the overall therapeutic impact of chamomile preparations becomes more reliable, and also more consistent [15].Chamomile contains sedative properties and in traditional usage it has been used for anxiety, nervousness and even sleep related troubles [33]. Not only that, people often describe it as a calming herb even if the reasons are biochemical. One particular flavonoid, apigenin, that is found in chamomile has been said to affect benzodiazepine receptors within the brain, which can lead to anxiolytic and sleep-inducing outcomes [35].A number of pharmacological investigations have also shown that chamomile extracts can act as an antispasmodic agent and a muscle relaxing remedy, which may be useful when dealing with gastrointestinal spasms.[15]

### 2.2.4 Gastroprotective Uses

Matricaria chamomilla has been used for a long time to help with different gastro intestinal problems, like indigestion, gastric irritation, and intestinal spasms, mainly because it works in an antispasmodic way and it feels calming.[15]A number of pharmacological investigations have described gastroprotective and anti-ulcer effects from chamomile extracts. This could be linked with blocking inflammatory signaling and lowering the inflammation on the gastric

lining.[35] Chamomile products are often chosen for reducing belly discomfort and digestive spasms since their flavonoids and essential oil components show muscle relaxing actions.[15]

### 2.2.5 Phytochemical and Spectroscopic Studies

UV–Visible spectrophotometric work was used to detect high phenolic and flavonoid levels. FTIR spectroscopic characterization of chamomile extracts indicated functional groups such as hydroxyl and carbonyl group and this gives a spectral fingerprinting effect and also instrumental validation of those pharmacologically relevant phytochemicals.[36]

### 2.2.6 Conclusion

*Matricaria chamomilla* is often treated as a major medicinal herb, mainly because it has a long traditional background, and also because it shows a wide range of pharmacological capabilities that have been confirmed through scientific studies. [33] The secondary metabolites help explain why it is therapeutically valuable for pharmaceutical, cosmetic, and herbal applications.[34]In addition, phytochemical screening, together with UV–Visible and FTIR spectroscopic examinations, has given useful information about how the biologically active constituents in chamomile extracts can be characterized and also validated.[36]

## *2.3 Parthenium hysterophorus L.*

### 2.3.1 Introduction and botanical description

*Parthenium hysterophorus L.* usually called as carrot grass, congress grass and bitter weed. Morphologically, *P. hysterophorus* shows an erect branched stem, leaves that are finely divided, small white flowers, and well-developed root system, which helps it to survive under wide range of environmental conditions Even though it is generally treated as a noxious invasive weed, several segments of the plant—leaves, flowers, and also whole plant extracts—have slowly gained attention for phytochemical and pharmacological studies.[38]

### 2.3.2 Phytochemical Constituents

Phytochemical investigations of *Parthenium hysterophorus* , have shown a range of secondary metabolites , like alkaloids, phenolic substances, tannins, saponin, flavonoids, terpenoids , and glycosides.[38]This plant seems especially abundant in sesquiterpene lactones such as parthenin, hysterin , and ambrosin, and these are widely viewed as central biologically active components that link with a number of pharmacological as well as toxicological activities.[39]When researchers used methanolic as well as ethanolic extracts of P. hysterophorus , the results showing strong phytochemical richness, suggesting that polar solvents work quite well for extract out phenols, flavonoids and various other compounds that are useful in medicine. [40]Organ specific phytochemical profiling has also indicated that chlorogenic acid, caffeic acid, and other phenolic constituents are not uniformly spread.[43]

### 2.3.3 Antimicrobial Activity

In various in vitro studies, methanolic and ethanolic extracts of Parthenium hysterophorus have shown antibacterial, as well as antifungal, effects against different pathogenic microorganisms. [38] For leaf extracts and flower extracts, the antimicrobial potential seems to be mostly linked with phenolic substances, tannins, flavonoids, and terpenoid related constituents, although the exact contribution may vary between reports.[39] Many of the phytochemicals found in *P. hysterophorus* are described to slow down microbial development via actions like cell membrane disruption, protein denaturation, and also by disturbing essential metabolic pathways. [38] Because it contains biologically active secondary metabolites, *Parthenium hysterophorus* has drawn interest as a plausible reservoir of medicinal compounds, with antimicrobial value.[39]

### 2.3.4 Antioxidant Activity

*Parthenium hysterophorus* is shown to have antioxidant phytochemicals, mostly phenolic compounds along with flavonoids, and this is linked with its ability for free-radical scavenging.[40] Studies that used DPPH together with FRAP assays suggest that P. hysterophorus shows antioxidant activity, as well as ability for lowering oxidative stress. [41]A number of investigations also found a correlation between total phenolic content and antioxidant potential.

### 2.3.5 Allergy and toxicological effects

*Parthenium hysterophorus* is very often treated as a harmful invasive weed, and it can cause uncomfortable consequences in humans and animals too. This occurs because the plant carries allergenic compounds along with other biologically active phytochemicals, so it can set off toxic reactions. [37]

Also, plant extracts of Parthenium hysterophorus have been shown to cause cytotoxic and irritant effects. That suggests they might provoke inflammatory and oxidative stress related cellular harm. Even with that toxicological weight and the allergenic profile, researchers still keep looking at it pharmacologically. The reason is its varied bioactive constituents and the possible medicinal uses.[40]

### 2.3.6 Phytochemical and Spectroscopic Studies

From qualitative phytochemical screening of Parthenium hysterophorus extracts , it was found that there are alkaloids , phenols , tannins, saponins, along with other biologically active secondary metabolites in several parts of the plant .[42]Then quantitative phytochemical assessments , and spectrophotometric investigations, showed a clear distinction in phenolic as well as flavonoid levels across leaves, flowers and other organs of P. hysterophorus .[41]In addition , UV–Visible spectrophotometric analysis has been used extensively , not only for estimation but also for characterization of phenolic compounds within methanolic and ethanolic extracts of the plant.[42]Moreover, FTIR characterization work indicated key functional moieties like O–H, C=O, C–O, and N–H , so the results enabled spectral fingerprinting and also acted as an instrumental validation for the phytochemicals that are

present in Parthenium hysterophorus extracts.[41]Overall, phytochemical and spectroscopic observations have offered meaningful scientific support , about the medicinal relevance and the biological effects linked to the bioactive constituents in *Parthenium hysterophorus*.[42]

### 2.3.7 Conclusion

Parthenium hysterophorus is considered a phytochemical significant invasive weed, mainly because it contains a range of biologically active secondary metabolites with pharmacological and toxicological impacts.[37]This plant holds multiple medicinally relevant constituents, including phenols, flavonoids, and sesquiterpene lactones, and these molecules help drive its antimicrobial, antioxidant, anti-inflammatory, and other types of biological effects.

Even if it is slightly allergenic and toxic, *Parthenium hysterophorus* still attracts substantial scientific attention, partly due to its possible therapeutic uses and its pharmacological relevance. Phytochemical exploration together with spectroscopic characterization approaches like UV–Visible and FTIR analyses, are useful for detecting, describing, and confirming bioactive components present in Parthenium hysterophorus. Overall, the scientific references that are available indicate that Parthenium hysterophorus work as a promising source of biologically active phytochemical substances, for upcoming pharmaceutical and medicinal investigations.[39]

## *2.4 Stevia rebaudiana*

### 2.4.1 Introduction and Botanical Description

*Stevia rebaudiana* Bertoni, which is part of the family Asteraceae, is usually called stevia, sweet leaf, and sugar leaf. It is used as a natural low-calorie sweetener.[44] *S. rebaudiana* is a perennial herbaceous plant. It has a branched stem, leaves that are sessile and oppositely arranged and small white flowers. The root system is well developed, and it seems adjusted for warm climatic conditions.[45] In most phytochemical and pharmacological studies, the leaves and leaf extracts are the plant parts most often used. The main reason is that they contain high levels of sweet diterpene glycosides, along with other bioactive metabolites.[43]Because it supports nutraceutical, pharmaceutical, and food industry uses, Stevia rebaudiana is scientifically important herb It is identified to be an economically important medicinal plant, with potential therapeutic effects.[45]

### 2.4.2 Phytochemical Constituents

*Stevia rebaudiana* has a broad set of biologically active secondary metabolites like phenolic compounds, flavonoids, tannins, saponins, alkaloids, terpenoids, and glycosides, and these together support its medicinal role, [45]. In particular, the plant is rich in sweet diterpene glycosides, such as stevioside, rebaudioside A, dulcoside, and steviol, which are often treated as the key bioactive components behind both the sweetness effect, and its therapeutic benefits. [46]Some phytochemical research also described chlorogenic acid and several other phenolic constituents in stevia, because these are linked with profound antioxidant potential and pharmacological effects in stevia leaf extracts. [47] Generally, the occurrence and level of phytochemicals tend to be higher in the leaves so, that part becomes the main focus for

phytochemical and pharmacological studies. [45]Many publications have shown that the medicinal action of *Stevia rebaudiana* as antidiabetic effects are closely associated to its varied phytochemical composition [47]

### 2.4.3 Antidiabetic, and Hypoglycemic Activity

*Stevia rebaudiana* has shown some real antidiabetic potential, mostly because it can reduce blood glucose and also help in glucose metabolism, suggested by variety of experimental and clinical studies [48]The hypoglycemic effect of stevia is mainly linked with steviol glycosides like stevioside and rebaudioside A, and these are considered for diabetes treatment and management.[47]Several investigations have also claimed that stevioside can act in an insulinotropic way, hence it boosts insulin release from pancreatic β-cells that supports glycemic control. [45]People are consuming stevia as a natural low-calorie sugar alternative, not just out of preference, but because it gives sweetness without raising blood glucose.[48]Overall, pharmacological findings indicates that the antidiabetic activity of *S. rebaudiana* control the insulin signaling routes, better glucose usage, and also lowering the oxidative stress that commonly leads to hyperglycemia.[47]

### 2.4.4 Therapeutic and Pharmacological Importance

*Stevia rebaudiana* is known for strong antihypertensive activity, and supports cardiovascular health via vasodilatory actions and a more consistent reduction in blood pressure. [43] Pharmacological work suggests that S. rebaudiana can act as a hepatoprotective and cardioprotective agent, mainly because it decreases oxidative stress and helps protect tissues from metabolic damage. [45] Because of these therapeutic roles, along with its low caloric value, stevia is now widely used across nutraceutical, pharmaceutical, and food domains, as a natural sweetener, and more or less as a health-promoting substitutes. [49]More recently, researchers have been focusing on stevia in functional foods, herbal formulations, and medicinal preparations, since it carries diverse bioactive phytochemicals, and it also shows broad pharmacological properties.[44]

### 2.4.5 Phytochemical and Spectroscopic studies

Some qualitative alongside quantitative phytochemical analysis on *Stevia rebaudiana* have basically confirmed that alkaloids, phenols, tannins, saponins.[45]In many cases UV-Visible spectrophotometry has been used, for measuring and also characterizing phenolic substances and stevia glycosides found within stevia extracts. When FTIR was applied for *S. rebaudiana e*xtract characterization it showed functional groups like hydroxyl,carbonyl and nitryl groups. This evidence validated that various phytochemical constituents are present.[48] Moreover, spectral fingerprinting via UV and FTIR methods has made a big difference for phytochemical profiling for the analytical identification of stevia leaf metabolites and sweet diterpene glycosides.[44] So overall, phytochemical and spectroscopic studies are useful for the medical and pharmacological relevance of those bioactive molecules which are present in *Stevia rebaudiana* [45]

### 2.4.6 Conclusion

*Stevia rebaudiana* is widely seen as a medicinally and commercially relevant plant, mainly because it has a relatively rich phytochemical composition and it is used often as a natural low-calorie sweetener, across the pharmaceutical, nutraceutical, and food industries.[43] Steviol glycosides like stevioside and rebaudioside A are the ones that are responsible for most of the therapeutic effects, and the sweetening impact, that people associate with stevia.[46] A lot of pharmacological investigations have pointed out that *S. rebaudiana* shows antidiabetic and antioxidant potential, so, it becomes an interesting option for handling metabolic imbalance and oxidative stress related disorders.[47]

## 2.5 Introduction to Phytochemicals

Phytochemicals are naturally found bioactive compounds made by plants. In most cases, primary metabolites help with growth and development, while secondary metabolites seem more about ecological adaptation and medicinal use. [50]Secondary metabolites like alkaloids, flavonoids, terpenoids, glycosides and phenolic compounds have a key role in plant defense mechanisms. They help plants fight pathogens, insects, and environmental stress conditions [51] A lot of medicinal plants owe their pharmacological potential, including antioxidant, antimicrobial, anti-inflammatory, and therapeutic effects, to biologically active secondary metabolites that are already present. [50] Because herbal medicine and natural drug discovery keep getting more attention, scientific interest in phytochemicals has also grown. This is mostly due to their therapeutic value and their pharmaceutical relevance [51]

### 2.5.1 Alkaloids

Alkaloids are nitrogen containing metabolites that tend to have strong physiological as well as pharmacological effects, they show up in many medicinal plants [52]. In plants, the production of alkaloids usually starts from amino acids like lysine, tyrosine, tryptophan, and ornithine. This kind of process goes through enzyme mediated metabolic pathways [53]. Medicinal plants seem to make alkaloids for a defensive chemistry, almost like a shield against trouble. They function as protective agents against microbial threat, herbivores, and also environmental pressure [52].

Some therapeutically valuable alkaloid examples are morphine, quinine, atropine, nicotine, and berberine. These compounds are linked with analgesic, antimalarial, antimicrobial and anticancer actions. The antimicrobial activity that we see with alkaloids is often connected to things like blocking nucleic acid synthesis, breaking microbial cell membranes, and interfering with protein formation [54]. Because alkaloids have such a broad biological and pharmacological spectrum, they've become pretty important in today' modern medicine, natural medicine, and drug discovery research too [52].

### 2.5.2 Phenolic Compounds and Polyphenols

Phenolic compounds are this diverse set of plant secondary metabolites, grouped broadly into simple phenols as well as polyphenols. [55] Polyphenolic compounds, like flavonoids, phenolic acids and tannins, are often described as showing strong antioxidant effects.,[56] which helps keep things from spiralling. When you assess antioxidant activity, phenolic compounds are really important for scavenging reactive oxygen species, and they also support the prevention of oxidative damage to cellular biomolecules. [55] At the same time, simple phenols and polyphenols contribute a lot to oxidative stress reduction, mainly by interrupting the free radical chain reactions, and by stabilizing reactive intermediates. [57] Because of that, phenolic antioxidants are often treated as important therapeutic phytochemicals, since they show protective effects against oxidative stress related conditions and degenerative diseases.[56]

### 2.5.3 Tannins

Tannins are polyphenolic compounds, more or less broadly grouped as hydrolysable tannins and condensed tannins..[11], [58] A key feature about tannins, is that they can precipitate proteins via strong hydrogen bonding interactions. [60] Also, tannins show antimicrobial effects on different pathogenic microorganisms, mainly because they disturb microbial enzymes, interfere with membrane proteins, and limit substrate availability.[58] Their antioxidant potential is generally connected to the polyphenolic framework, it lets them scavenge free radicals effectively and also lowers oxidative stress.[59]Because of these antioxidant , antimicrobial, and therapeutic roles, tannins are seen as important bioactive parts in medicinal plants and in traditional herbal medicine. [60]

### 2.5.4. Saponins

Saponins are glycosidic secondary metabolites made up of a hydrophobic aglycone part that is linked to hydrophilic sugar moieties, and that linkage is what gives them their typical surfactant like nature and surface-active behaviour.[61] Because they are amphiphilic in a way, saponins show a strong foaming ability in aqueous solutions and in terms of biological activities, saponins can act as antimicrobial, antifungal, antioxidant, and also immunomodulatory agents. [62]One key reason they show antifungal activity is that they can bind with, or at least interact with membrane sterols in fungal cells, which then leads to higher membrane permeability, and finally to cellular injury. Their anti-inflammatory importance is also tied to blocking inflammatory mediators, and in turn lowering tissue inflammation in different disease related scenarios.

### 2.5.5 Flavonoids

Flavonoids show strong antioxidant activity, mainly because they donate hydrogen atoms or electrons to neutralize those ROS.[65] The antioxidant mechanisms for flavonoids tend to link to metal ion chelation, free radical scavenging, and the hydroxyl groups helping stabilize reactive intermediates, thus minimizing oxidative damage.[63]Flavonoids also have characteristic UV absorption properties, because the conjugated aromatic structures are responsible for this characteristic and they commonly absorb in the UV-A and UV-B regions.[70] A number of flavonoids show important pharmacological effects, for example anti-inflammatory, antimicrobial, anticancer, cardioprotective, and hepatoprotective actions.[64]

### 2.5.6 Terpenoids

Terpenoids are usually broken down into monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes, basically depending on how many isoprene units are present.[67] A lot of terpenoids show up as major parts of essential oils ,and they help form the typical aroma, flavour and that of volatile nature found in medicinal as well as aromatic plants.[68] Volatile terpenoid compounds are also important in ecology ,they take part in plant protection, and help defend against microbial pathogens or environmental stress conditions.[69] Several terpenoids have antimicrobial activity ,they can disturb microbial cell membranes interfere with enzyme systems, and they also limit microbial growth.[68]. In medicine, terpenoids are important because they bring a range of pharmacological effects ,like antioxidant, anticancer, antiviral, antimicrobial, and anti-inflammatory actions.[69]

#### 2.5.6.1 Biosynthesis of Terpenoids

Terpenoid production mostly goes by the mevalonate pathway and the methylerythritol phosphate pathway both of which are present in plants.[71] Isopentenyl pyrophosphate together with dimethylallyl pyrophosphate work like universal precursor molecules, for making many terpenoid groups.[70] After that, sequential linking of isoprene units (or isoprene pieces) is catalysed by prenyltransferase enzymes and this step gradually builds mono-, sesqui-, di-, and triterpenoid skeletons.[71] Then hydroxylases , cyclases and terpene synthase enzymes convert the terpenoid intermediates even more, so that a wider range of structurally different, biologically active terpenoids can be formed.[67]

### 2.5.7 Glycosides

Glycosides are bioactive compounds built from a sugar portion, called glycone, which is attached to a non-sugar component known as aglycone by glycosidic bond, and this connection is showing their biological properties as well as their solubility.[69]In general, the pharmacological effects of glycosides depend mostly on what the aglycone part is made of , while the sugar component tends to influence absorption, movement through tissues, and overall bioavailability.[70] Cardiac glycosides are steroidal glycosides , they show a strong action on heart muscle basically by blocking $Na^+/K^+$-ATPase activity and they remain therapeutically relevant in some cardiovascular disorders.[69] Steviol glycosides , for instance stevioside and rebaudioside A, are sweet diterpene glycosides obtained from *Stevia rebaudiana* and they are commonly used as natural low calorie sweeteners hence antidiabetic as well as antioxidant, and antihypertensive effects. Glycosides play an important role in medicine since their effects are wide ranging, including cardiotonic, antimicrobial, and anticancer activity as well as other therapeutic uses that show up in pharmaceutical research.[70] Many medicinal plants produce glycosides as important secondary metabolites, and those metabolites help support the pharmacological and therapeutic features.[69]The growing scientific curiosity

about glycosides is mostly tied to their broad bioactivity profile, the fact they could be applied in herbal medicine and also in drug development programs.[72]

### 2.5.8 Sterols and Phytosterols

Plant sterols, usually called phytosterols, are similar in shape to cholesterol, and they mainly include β-sitosterol, stigmasterol and campesterol. Phytosterols have a tetracyclic cyclopentanoperhydrophenanthrene core, but there are small differences in side chain substitution , and also where the double bond is located, these little variations are responsible for structural diversity[73] Sterols are needed to keep plant cell membranes stable , and also they help with permeability and fluidity, basically they control membrane arrangement and cell integrity. Sitosterol and stigmasterol are among the main membrane-associated phytosterols, and they help a lot in stabilizing lipid bilayers and in supporting membrane related physiological roles.[74] Phytosterols are also being considered more often in therapy, mostly because of their cholesterol-lowering action and because they could be used in nutraceutical and pharmaceutical industries.[73] Sterol glycosyltransferases are involved in turning free sterols into sterol glycosides, these glycosides take part in membrane organization , and also help with stress adaptation in plants.[76]

#### 2.5.8.1 Biosynthesis of Sterols

In plants, sterol biosynthesis mostly goes through the mevalonate pathway, where acetyl-CoA gets converted into isoprenoid intermediates, and then later on it leads toward squalene and cycloartenol. Cycloartenol is the main starting point for making different phytosterols and several enzymatic changes then produce structurally varied sterol compounds in plants.[77]The bio synthesis and buildup of phytosterols are crucial for plant growth, the steadiness of membranes, and their ability to adjust to stressful environmental conditions.[74]

## 2.6 Extraction and Isolation of Phytochemicals

### 2.6.1 Principles of Phytochemical extractions

In phytochemical extraction, the main idea is really solvent–solute interaction, where bioactive substances into a suitable solvent, based on how they’re chemically characterized and on the solubility features. [78] Solvent polarity matters, but it depends upon the extraction efficiency factor, because polar solvents usually draw out hydrophilic phytochemicals, whereas non-polar solvents do much better with lipophilic components. [81]

### 2.6.3 Solvents used in phytochemical extraction

Extraction solvents is a crucial step in phytochemical studies, because solvent traits end up shaping both the recovery and the actual makeup of those bioactive compounds pulled out from medicinal plants. [78] In real practice, methanol, ethanol, water-based systems, and hydroalcoholic mixtures are very frequently used for phytochemical extraction because they

can dissolve a wide spectrum of secondary metabolites across varied polarities. [80] Solvent polarity matters a lot for the phytochemical yield, and in general polar solvents tend to be better for phenols, flavonoids, and glycosides extraction. [81] Also every extraction solvent has its own set of advantages and little limitations tied to extraction efficiency, toxicity, selectivity, and even how stable the phytochemical constituents remain over time. [79]

#### 2.6.3.1 Methanolic Extraction

Methanolic extraction is often used in medicinal plant research, since methanol seems to work quite well for pulling out phenolics, flavonoids, and other polar phytochemicals.[80] Methanolic extracts, are then used a lot for antioxidant assays, mostly because of their fairly strong ability to scavenge radicals, and also due to the fact that they usually have a rich, or high, set of phenolic constituents.[83]

#### 2.6.3.2 Ethanolic Extraction

Ethanolic extraction is seen as a safer solvent because the toxicity levels are lower and it works with both pharmaceutical, and nutraceutical workflows.[79] Ethanol efficiently extract a range of polar bioactive compounds, like phenols, flavonoids, and glycosides from medicinal plants. [78]

#### 2.6.3.3 Water Based Extraction

Water based extraction has been used for quite a while in herbal medicine preparation because water is an accessible and not toxic type of extraction media [83] In practice, aqueous solvents can work really well when you need to pull-out water-soluble phytochemicals like carbohydrates, glycosides and a few phenolic compounds.[80]

### 2.6.5 Importance of Extraction Methods in Phytochemical Research

In antimicrobial and antioxidant work, choosing the extraction techniques is important, because the extraction efficiency decides how much of the bioactive secondary metabolites are recovered. Those metabolites are related to therapeutic effects.[80]Standardized extraction procedures also matter a lot for UV–Visible and FTIR spectroscopic work, because they improve reproducibility, support consistent phytochemical profiling, and help with validation of herbal extracts.[81] Efficient extraction and concentration method helps a lot with the identification, separation, and characterization of medicinally valuable phytochemicals from plant material. Finally, choosing a best extraction procedure helps a lot with the identification, separation, and characterization of medicinally valuable phytochemicals from plant material.[83]

## 2.7 Phytochemical Screening Techniques

Qualitative phytochemical screening is a first step analytical approach, it's used to check whether major bioactive secondary metabolites are present in medicinal plant extracts.[85]Usually, standard phytochemical tests are determine for the identification of alkaloids, phenols, tannins, and flavonoids. This is based on typical colour changes, and

precipitate formation reaction mixtures.[86] In addition, preliminary screening methods are also used to detect saponins, glycosides, terpenoids, and sterols, these are important phytoconstituents, that are linked to therapeutic activities.[87]

For alkaloids, detection is often done using Dragendorff's, Mayer's, and Wagner's reagents, meanwhile ferric chloride and lead acetate tests are widely used for phenols and tannins.[85]For flavonoids the Shinoda test is a common choice, for saponins the frothing test is applied, and for sterols and terpenoids people often rely on the Liebermann–Burchard test. Together these are major qualitative methods in phytochemical investigations.[86] Overall, preliminary phytochemical analysis matters a lot in medicinal plant research because it helps with the recognition of bioactive compounds, and it also supports further pharmacological, and spectroscopic studies.[87]

## 2.8 UV-Visible Spectroscopy

### 2.8.1 Introduction to UV–Vis Spectroscopy

This technique is built around how molecules absorb ultraviolet and visible radiation, which then leads to electronic excitation of chemical substances.[22], [88]This method is commonly used in phytochemical analysis , for identification and quantitative estimation of bioactive compounds that are found inside medicinal plant extracts.[89]When UV light interacts with molecules it excites electrons from lower energy levels to higher energy levels and this produces characteristic absorption spectra.[90]

### 2.8.2 Principle of UV–Visible Spectroscopy

Molecules absorb ultraviolet or visible radiation at certain wavelengths, these match with electronic transitions inside the molecule.[88] For electronic excitation, molecules undergo transitions like $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, plus $n \rightarrow \pi^*$ , and it depends on the bonding type and the functional groups involved.[90] Chromophores are basically the light-absorbing units that are responsible for the characteristic absorption patterns, while auxochromes are the ones that alter absorption intensity and sometimes shift the wavelength a bit.[88] The absorption spectra obtained during UV analysis indicates about the molecular structure and whether phytochemical constituents are present.[89]

### 2.8.3 Beer–Lambert Law

It states that absorbance of light by a solution is directly related to the concentration of the absorbing compound, as well as the path length of the light.[89]

$A=\varepsilon cl$ here, A means absorbance, $\varepsilon$ stands for molar absorptivity, c is the concentration, and l represents the path length.[90]

The Beer–Lambert law is the base for quantitative estimating of phytochemicals, and also to figure out the concentration of plant metabolites, present using UV spectroscopy. Quantitative

UV spectroscopic analysis is widely used for the estimation of phenols, flavonoids, and other bioactive phytochemicals, in medicinal plant extracts.[91]

### 2.8.4 Instrumentation of UV–Visible Spectroscopy

In general, the UV–Visible spectrophotometer has a few essential parts which consist of light source, monochromator, sample holder, detector, and the recording system. For light sources, tungsten lamps are usually applied for visible region, and deuterium lamps are used for ultraviolet region, during UV spectroscopic analysis. Then the monochromator selects particular wavelengths, while the cuvette or sample holder holds the sample solution so the absorbance can be measured.[90]Finally the detector transforms the transmitted light into electrical signals, which are then processed and finally shown as absorption spectra by the recording system.[91]

## 2.9 FTIR Spectroscopy

### 2.9.1 Introduction and Principle of FTIR Spectroscopy

This method depends on the absorption of infrared radiation by molecules, and this causes vibrational excitation in chemical bonds found inside a compound. [92] When infrared light interacts with bond systems, it gives rise to a recognizable absorption pattern and those patterns are often useful for understanding the chemical composition of phytoconstituents. [93]FTIR spectroscopy tends to be widely applied in phytochemical investigations, mostly because it supports fast identification and also the characterization of functional group motifs in medicinal plant extract.[94]

### 2.9.2 Functional Group Identification and Interpretation of FTIR Spectra

FTIR spectroscopy makes it easier to analyze functional groups by examining characteristic absorption peak, which show up due to stretching and bending movements of molecules. [92] Among the functional groups that are commonly noticed in medicinal plant extracts are O–H, C=O, C–O, and N–H and each one show up within its own typical absorption zone in the infrared range. [95] For interpreting FTIR spectra, researchers usually match the absorption peaks to phytochemical components like phenols, flavonoids, glycosides, and proteins. [93]Overall, the distinctive FTIR absorption bands give useful insight on structural traits, and also on the chemical character of those bioactive plant metabolites. [94]

### 2.9.3 Applications of FTIR Spectroscopy in Medicinal Plant Analysis

FTIR spectroscopy is extensively used in the analysis of medicinal plant extracts, mostly for identifying and characterizing bioactive phytochemicals.[94] This method works well for spotting phenols, flavonoids and glycosides, by looking at the absorption peaks that come from specific functional groups.[92] Spectral fingerprinting using FTIR gives a singular chemical profile for plant extracts and helps in phytochemical characterization and also in authentication studies.[93] In addition FTIR analysis is useful in quality control and standardization of herbal

drugs, because it makes it easier to check consistency and purity in the medicinal plant preparations.[92]

# Chapter 3: - METHODOLOGY

## 3.1 EXTRACTION OF SUNDRIED LEAVES

### 3.1.1 Preparation of Plant Extract

Leaves from four chosen medicinal plants were collected from the nursery of Delhi Technological University, Delhi and then used for phytochemical analysis. The collected leaves were washed well with distilled water, so that dust and other impurities could be removed, and after that the plant samples were placed for sun drying for about two days, under natural sunlight, until the moisture was reduced enough. Once they were fully dried, 5 g of the leaves were weighed and grind into a fine powder, using a grinder.

For making the aqueous extract, 1 g of the dried powdered plant material was put into a conical flask with 100 ml of distilled water, and then boiled for about 3 minutes, mainly so the secondary metabolites can come out more easily. After this heating step, the flask was sealed with aluminium foil and kept without touching until the mixture cooled down to room temperature, or something close to that. The extract was then centrifuged at 4000 rpm, at 4°C for 10 minutes. Next, the upper supernatant was collected carefully and kept safely, so it can be used later for both qualitative and quantitative estimation of phytochemicals including alkaloids, phenols, tannins, saponins, flavonoids, quinones, terpenoids and sterols.


**Fig 1: - Figure shows steps for the extraction of phytochemicals**

## 3.2 EXTRACTION OF FRESH LEAVES

### 3.2.1 Preparation of Plant Extract

Fresh leaves from four medicinal plants were collected from the nursery area of Delhi Technological University, Delhi for phytochemical screening. The collected samples were rinsed properly with distilled water, in order to remove the dust and other surface contaminants that were hanging around. Then about 5 g of fresh leaves was weighed, and later crushed in a mortar and pestle until it turned into a thick, semi solid paste, although it was not completely uniform, slightly rough here and there.

For the extract preparation, roughly 1 g of the crushed plant material was transferred into a conical flask containing 100 ml of distilled water. The flask was kept undisturbed for about 3-4 minutes, so that the bioactive constituents could seep out into the solvent. Once a visible colour change showed up in the solution, the extract was centrifuged at 4000 rpm at 4 °C for 10 minutes. After that, the supernatant was separated carefully and kept aside, it would be used later for qualitative as well as quantitative evaluation of secondary metabolites including alkaloids, phenols tannins, saponins, flavonoids, quinones, terpenoids and sterols.

## 3.3Qualitative Estimation of Secondary Metabolites

For the qualitative phytochemical screening of the prepared plant extracts, we did the detection of some secondary metabolites like alkaloids saponins, phenols, tannins, flavonoids, terpenoids, sterols and quinones. We used the usual standard biochemical tests. The appearance of a characteristic color shifts, or the formation of a precipitate, generally pointed toward the presence of certain phytoconstituents.

### 3.3.1 Alkaloids detection (Wagner's Test)

**Preparation:** Wagner's reagent was made by dissolving 1.27 g iodine and 2 g potassium iodide in 100 ml of distilled water, and stirred until it looked uniform.

**Procedure:** Take 2 ml of the plant extract, then add only a few drops of Wagner's reagent to it.

**Observation:** A reddish-brown precipitate appeared; this was taken as evidence of alkaloids.

### 3.3.2 Saponins detection (Foam test)

**Procedure:** 2 ml plant extract was shaken vigorously for around 30 seconds, then it was left to stand quietly for a while.

**Observation:** Stable froth formation indicated the presence of saponins.

### 3.3.3 Phenols detection (Ferric chloride test)

**Preparation:** A 5% $FeCl_3$ solution + 5 g ferric chloride in 100 ml distilled water.

**Procedure:** Add 1 ml of plant extract, then treat it with a few drops of the 5% $FeCl_3$ solution.

**Observation:** Black coloration was considered confirmation for phenols.

### 3.3.4 Tannins detection

**Preparation:** A 0.5% $FeCl_3$ solution was prepared by dissolving 0.5 g ferric chloride in 100 ml distilled water.

**Procedure:** Mix 2 ml of plant extract with a few drops of the 0.5% $FeCl_3$ solution.

**Observation:** Blue-black colour, was taken as the indication that tannins were present.

### 3.3.5 Flavonoids detection (Alkaline reagent test)

**Preparation:** A 2% NaOH solution was prepared by dissolving 2 g sodium hydroxide in 100 ml distilled water.

**Procedure:** 1 ml plant extract was combined with 2 ml of 2% NaOH solution, after that a few drops of diluted $H_2SO_4$ was added.

**Observation:** Yellow colour that turned colourless was taken as flavonoids positive.

### 3.3.6 Test for Terpenoids (Salkowski Test)

**Procedure:** 2 ml plant extract was mixed with 2 ml chloroform and heated gently.

After evaporation, 2 ml concentrated $H_2SO_4$ was added and heated for 2 minutes.

**Observation:** Black coloration indicated the presence of terpenoids.

### 3.3.7 Test for Sterols

**Procedure:** 1 ml plant extract was mixed with 10 ml chloroform followed by concentrated $H_2SO_4$.

**Observation:** Formation of reddish-brown ring indicated the presence of sterols.

### 3.3.8 Test for Quinones

**Preparation:** Dilute NaOH solution was prepared in distilled water.

**Procedure:** 1ml plant extract was treated with dilute NaOH solution.

**Observation:** Blue-green or red coloration confirmed the presence of quinones.

## 3.5 Quantitative Estimation of Total Phenolic Content by UV–Visible Spectrophotometry

The Folin–Ciocalteu colorimetric method was used, and then the UV–Vis spectrophotometer was involved for the measurement.

### Principle

The phenolic compounds which are in the plant extract react with the Folin–Ciocalteu reagent when the medium is alkaline. This reaction forms a blue coloured complex, and the stronger the colour, the more phenolics are present in the sample. The absorbance was recorded at 765 nm using a UV–Visible spectrophotometer.

### Reagents Required

. Folin–Ciocalteu reagent (1:10 dilution)

. $Na_2CO_3$ solution

. Plant extract

. Distilled water

### Procedure

1 mg of plant extract was weighed, then dissolved with diluted Folin–Ciocalteu reagent (1:10). About 5 ml of the prepared FC reagent solution was added into a flask that already had the plant extract in it. The reaction mixture was left undisturbed for 3–4 minutes.

After the incubation period, 4 ml of $Na_2CO_3$ solution was added into the mixture, and mixed properly. Then the flask was kept in dark conditions for 30 minutes to allow the colour to develop.

### Observation

The appearance of a bluish coloration suggested that the phenolic complex had formed in the reaction mixture.

### Absorbance Measurement

The absorbance of the prepared sample was measured at 765 nm against a suitable blank solution.

### Result Interpretation

If the absorbance readings were higher, it meant a greater phenolic content in the plant extract was present.

## 3.6 Quantitative Estimation of Total Alkaloid Amount by UV–Visible Spectrophotometry

To measure the total alkaloid amount in the plant sample, an ethanolic extraction was done, then the extract was analysed by UV–Visible spectrophotometry

### Principle

In the plant, alkaloids (the alkaloidal compounds) were first extracted with 70% ethanol solution. These compounds absorb light at 460 nm and absorbance was taken for the analysis.

## Reagents Required

. 70% Ethanol

. Dried plant sample

. Distilled water

## Procedure

A small piece of dried plant material, 0.1 g, was weighed and then stirred with about 10 ml of a 70% ethanol solution. It was kept undisturbed, for roughly 30–40 minutes, just to give enough time for the alkaloids to be leached out into the plant solution.

Afterward, the extract was filtered in order to get a clear liquid, and only then the spectrophotometric reading was carried out.

## Observation

If a colored ethanolic extract appeared, it implied that alkaloidal constituents were indeed extracted from the plant sample.

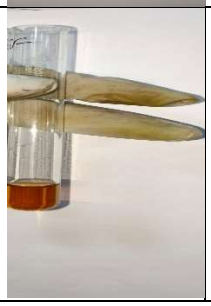
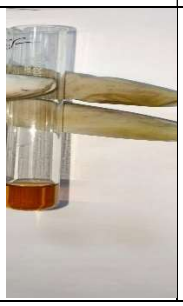
## Absorbance Measurement

6 The absorbance of the prepared ethanolic extract was recorded at 460 nm wavelength, with a suitable blank for comparison.

## Result Interpretation

In general, absorbance being higher meant the plant extract contained more alkaloids, so greater absorbance values corresponded to a higher alkaloid content.

# CHAPTER 4: -OBSERVATION AND RESULTS: -

| PHYTOCHEMICALS | FRESH LEAVES | FRESH FLOWER | SUN DRIED LEAVES | SUN DRIED FLOWER | RESULT |
|---|---|---|---|---|---|
| ALKALOID |  |  |  |  | Red -brown ppt indicate presence of alkaloid |
| FLAVONOID |  |  |  |  | Yellow colour does not change to colourless |
| PHENOL |  |  |  |  | Black colour indicates presence of phenols |
| TERPENOID |  |  |  |  | No greyish colour shows absence of terpenoids |

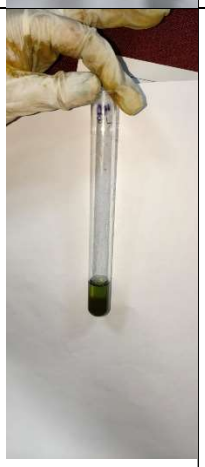
| | | | | | |
|---|---|---|---|---|---|
| TANNINS |  |  |  |  | Blue black colouration indicates tannins are present |
| STEROLS |  |  |  |  | Light bluish colouration shows presence of sterols |
| QUINONES |  |  |  |  | Reddish colouration shows indication of quinones in fresh but not in dried sample |
| SAPONIN |  |  |  |  | Froth formation indicate presence of saponin in fresh flower |

**TABLE 1: - PHYTOCHEMICAL ANALYSIS OF *Euphorbia milli***

xxx

| PHYTOCHEMICALS | FRESH LEAVES | FRESH FLOWER | SUN DRIED LEAVES | SUN DRIED FLOWER | RESULTS |
|---|---|---|---|---|---|
| ALKALOID |  |  |  |  | Reddish brown ppt indicates presence of alkaloid |
| FLAVONOID |  |  |  |  | Yellow colour becomes colourless which shows presence of flavonoid |
| PHENOL |  |  |  |  | No black colouration shows absence of phenols |
| TERPENOID |  |  |  |  | Greyish colouration shows presence of terpenoid in flower but not in leaves |

| | | | | | |
|---|---|---|---|---|---|
| TANNINS |  |  |  |  | No blue-black colour formation shows indication tannins are absent |
| STEROLS |  |  |  |  | Bluish colouration shows presence of phenols |
| QUINONES |  |  |  |  | Reddish colour formation shows presence of quinones in fresh flower |
| SAPONIN |  |  |  |  | No froth formation indicates absence of saponin |

**TABLE 2: - PHYTOCHEMICAL ANALYSIS OF *Matricaria chamomilla***

| PHYTOCHEMICALS | FRESH LEAVES | SUN DRIED LEAVES | RESULTS |
|---|---|---|---|
| ALKALOID |  |  | No reddish-brown precipitate indicates absence of alkaloid |
| FLAVONOID |  |  | Yellow colour does not turn into colourless which indicates absence of flavonoid |
| PHENOL |  |  | Black colouration shows indicates of phenols |
| TERPENOID |  |  | No greyish colour formation indicates absence of terpenoids |

| | | | |
|---|---|---|---|
| TANNINS |  |  | Blue black colouration shows presence of tannins |
| STEROLS |  |  | No bluish colouration which shows absence of sterols |
| QUINONES |  |  | Reddish colouration indicates presence of quinones |
| SAPONIN |  |  | Froth formation indicates presence of saponin |

**TABLE 3: -PHYTOCHEMICAL ANALYSIS OF *Parthenium hysterophorus***

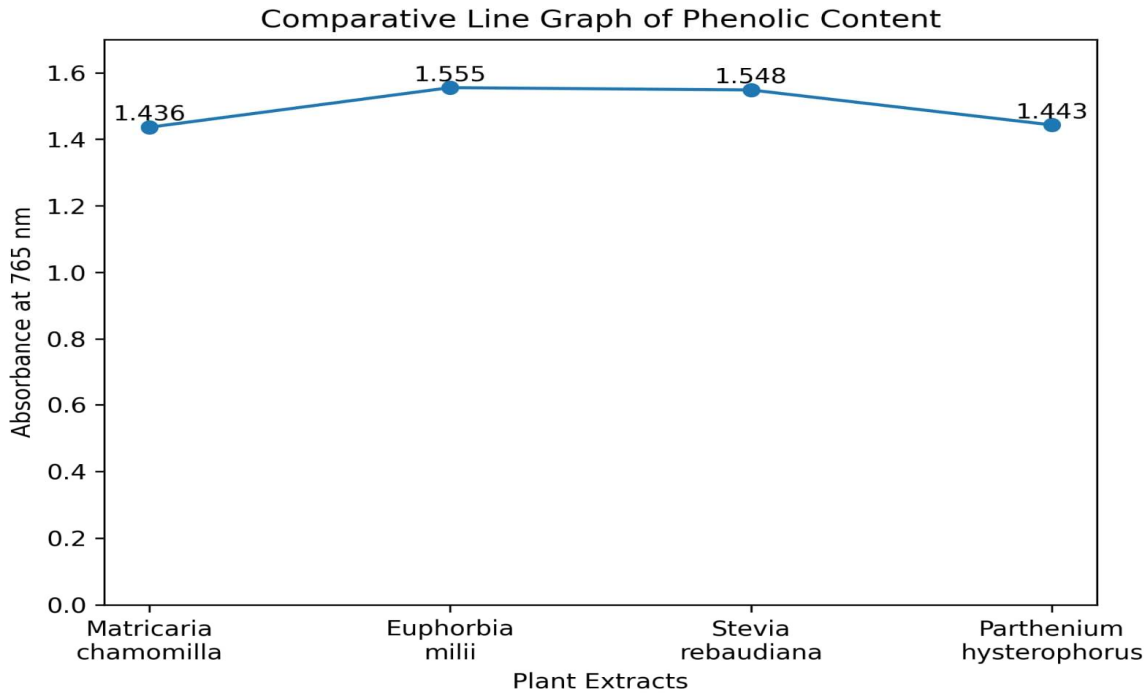
| PHYTOCHEMICALS | FRESH LEAVES | SUN DRIED LEAVES | RESULTS |
|---|---|---|---|
| ALKALOID |  |  | Light reddish brown ppt which indicates absence of alkaloid |
| FLAVONOID |  |  | Yellow colour gets colourless which indicates presence of flavonoid |
| PHENOL |  |  | Black colour formation indicates presence of phenols |
| TERPENOID |  |  | Black colour shows indicates presence of terpenoid |

| | | | |
|---|---|---|---|
| TANNINS |  |  | Blue black colouration indicates presence of tannins |
| STEROLS |  |  | Dark bluish colour observed which indicates presence of sterols |
| QUINONES |  |  | Reddish colour formation which indicates presence of quinones in fresh sample |
| SAPONIN |  |  | Froth formation indicates presence of saponin |

**TABLE: -4 PHYTOCHEMICAL ANALYSIS OF *Stevia rebaudiana***

## 4.2 QUANTITATIVE ANALYSIS: -

### a. UV – VISIBLE SPECT ROPHOTOMETER ANALYSIS FOR PHENOLIC CONTENT




| Sample Code | Plant Name | Absorbance at 765 nm |
|---|---|---|
| CL | *Matricaria chamomilla* | 1.436 |
| EL | *Euphorbia milii* | 1.555 |
| STE | *Stevia rebaudiana* | 1.548 |
| CG | *Parthenium hysterophorus* | 1.443 |

## Interpretation of Graph

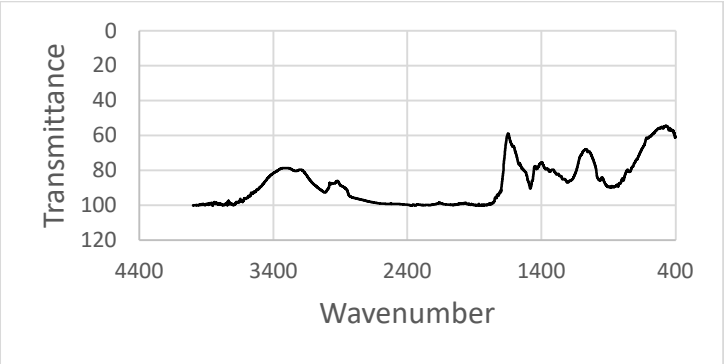
The graph showed an increase in absorbance values corresponding to the phenolic content present in different plant extracts at 765 nm. Among the selected medicinal plants, *Euphorbia milii* exhibited the highest absorbance value (1.555), indicating maximum phenolic content, whereas *Matricaria chamomilla* showed the lowest absorbance value (1.436). The results confirmed the presence of phenolic compounds in all four plant extracts.

5

XXXV

## b. QUANTITATIVE ANALYSIS OF FTIR

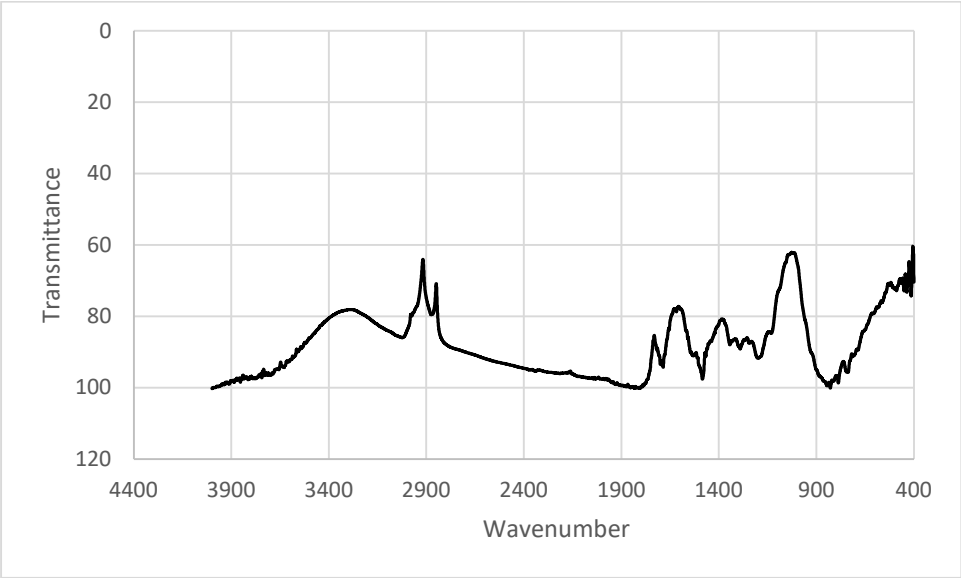
### 1. Chamomile (*Matricaria chamomilla)*




| **Peak Region ($cm^{-1}$)** | Functional Group | Possible Phytochemical Indication |
|---|---|---|
| 3400–3300 $cm^{-1}$ | O–H stretching vibration | Presence of alcohols and phenolic compounds indicating phenols and flavonoids |
| 2950–2850 $cm^{-1}$ | C–H stretching of alkanes | Indicates aliphatic compounds and terpenoid structures |
| 1700–1600 $cm^{-1}$ | C=O stretching vibration | Suggests carbonyl compounds such as ketones, aldehydes and flavonoids |
| 1600–1500 $cm^{-1}$ | C=C aromatic stretching | Indicates aromatic rings commonly present in phenols and flavonoids |
| 1450–1370 $cm^{-1}$ | C–H bending vibration | Presence of methyl and methylene groups |
| 1250–1050 $cm^{-1}$ | C–O stretching vibration | Indicates alcohols, ethers and phenolic compounds |
| 1000–800 $cm^{-1}$ | C–H bending of aromatic compounds | Suggests aromatic phytoconstituents |

Table 1: - Interpretation of possible compound in Chamomile by FTIR graph

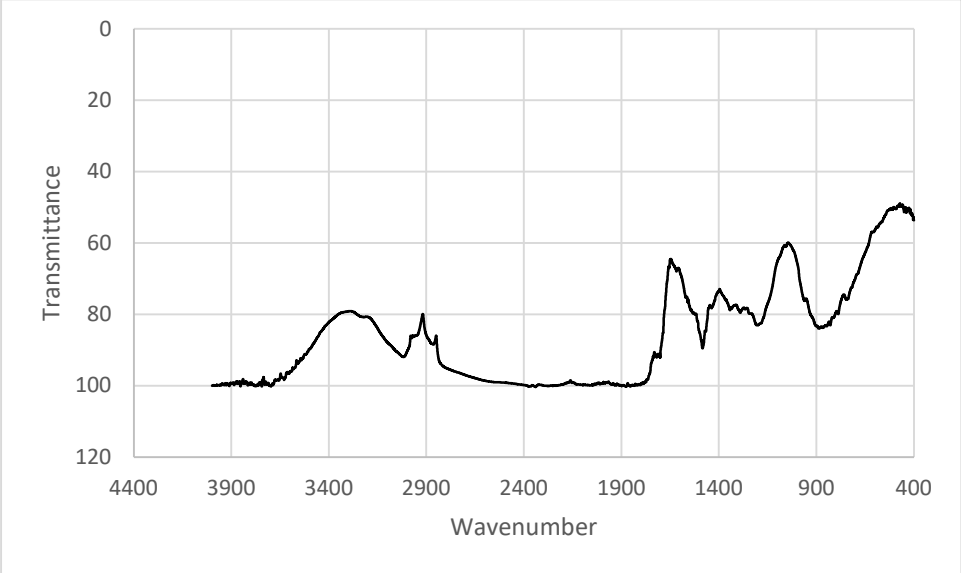
## 2. *Euphorbia milli*




| Peak Region ($cm^{-1}$) | Probable Functional Group | Interpretation |
|---|---|---|
| 3400–3300 | O–H stretching | Indicates presence of alcohols or phenolic compounds due to broad hydroxyl absorption peak. |
| 2950–2850 | C–H stretching | Represents aliphatic C–H bonds of alkanes, suggesting hydrocarbons or terpenoid constituents. |
| 1650–1550 | C=C / N–H bending | May indicate aromatic compounds, amide groups, or alkaloidal constituents. |
| 1450–1380 | $CH_2$ and $CH_3$ bending | Suggests methyl and methylene groups commonly present in plant secondary metabolites. |
| 1250-1050 | C–O stretching | Indicates alcohols, ethers, esters, or glycosidic linkages present in phytochemicals. |
| 950-700 | Aromatic C–H bending | Suggests aromatic ring structures and phenolic compounds. |

Table 2: - Interpretation of possible compounds in *Euphorbia milli* by FTIR

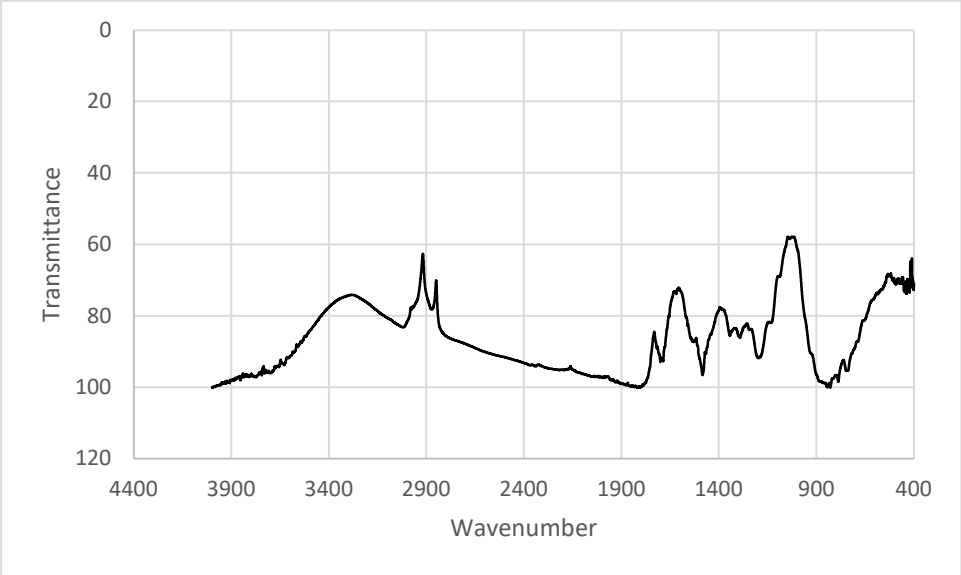
## 3. *Parthenium hysterophorus*




| Peak Region ($cm^{-1}$) | Probable Functional Group | Interpretation |
|---|---|---|
| 3400–3300 | O–H stretching | Broad absorption peak indicating presence of alcohols and phenolic compounds. |
| 2950–2850 | C–H stretching | Suggests aliphatic hydrocarbons and terpenoid constituents. |
| 1650–1550 | C=C / N–H bending | Indicates aromatic compounds, alkaloids, or amide-containing phytochemicals. |
| 1450–1380 | $CH_2$ and $CH_3$ bending | Represents methyl and methylene groups present in organic compounds. |
| 1250-1050 | C–O stretching | Suggests alcohols, ethers, esters, and glycosidic compounds. |
| 950-700 | Aromatic C–H bending | Indicates aromatic ring structures and phenolic derivatives. |

Table 3: - Interpretation of possible compounds in *Parthenium hysterophorus* by FTIR

## 4. *Stevia rebaudiana*




| Peak Region ($cm^{-1}$) | Probable Functional Group | Interpretation |
|---|---|---|
| 3400–3300 | O–H stretching vibration | Broad peak indicating presence of hydroxyl groups associated with phenols and alcohols. |
| 2950–2850 | C–H stretching | Indicates aliphatic hydrocarbons and terpenoid constituents. |
| 1650–1550 | C=O stretching / C=C stretching | Suggests carbonyl compounds, aromatic rings, or conjugated phytochemicals. |
| 1450–1380 | $CH_2$ and $CH_3$ bending | Represents methyl and methylene groups present in plant metabolites. |
| 1250-1050 | C–O stretching vibration | Indicates alcohols, ethers, esters, and glycosidic compounds. |
| 950-700 | Aromatic C–H bending | Suggests aromatic and phenolic ring structures. |

Table 4: - Interpretation of possible compounds in *Stevia rebaudiana*

# CHAPTER 5: DISCUSSION: -

The present study successfully demonstrates quantitative and qualitative phytochemical analysis of 4 important medicinal plants, namely *Euphorbia milii, Matricaria chamomilla, Parthenium hysterophorus,* and *Stevia rebaudiana* by using aqueous extracts and advanced analytical techniques including UV-Vis and FTIR spectroscopic analysis. The phytochemical screening of the medicinal plants has identified the presence of various important phytochemical constituents including alkaloids, phenols, tannins, flavonoids, saponins, terpenoids, sterols, and quinones.

The quantitative assessment has confirmed the variation in phytochemical constituents among the selected plants, highlighting their unique phytochemical composition and medicinal importance. In addition, UV-visible spectroscopy is considered a very useful tool in the identification and quantification of bioactive compound of medicinal plants. Moreover, FTIR spectroscopic analysis of the samples has indicated the presence of functional groups like hydroxyl and carbonyl groups belonging to phenols, flavonoids, glycosides, and other phytoconstituents.

It has been found that those medicinal plants having high content of phenol and flavonoids showed comparatively higher antioxidant activity. Hence, the study can be regarded scientifically as an important source for the validation of the medicinal importance of the selected plants. It can also be concluded that aqueous extraction is a suitable and economic technique for the phytochemical analysis.