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A Comprehensive Chemical Characterization of Leaves of Five Potential Medicinal Plants in Paschim Medinipur District, W. B., India

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Abstract: The physico-chemical and spectroscopic characterization of five selected medicinal plants viz., *Acalypha indica*, *Senna tora*, *Euphorbia hirta*, *Physalis angulata* and *Ziziphus mauritina* are the essence and has been carried out to assess bioactive potential and to establish traditional belief in the light of scientific interpretation. Fresh leaves were collected from Paschim Medinipur district of West Bengal, India, dried and powdered for Proximate analysis, ultimate analysis and compositional analysis, FTIR and GCMS. The study revealed that the highest concentration of Volatile matter (75.452%), Bulk density (0.256 %), Swelling index (1.456 %), Cellulose content (61.727 %), Hemicellulose content (4.372 %) and Tannins (0.635 g/ 100g) was observed to occur in *Acalypha indica* leaf than other experimental plants. In *Physalis angulata* the percentage composition of fixed carbon (15.251%) and extractive content (1.974 %) was higher than others. Ash content (12.327 %), hemicellulose content (4.372 %), and lignin content (5.531 %) were observed to be higher in the *Senna tora* leaf than others. Total carbohydrate concentrations (9.619 gm/100 gm) and nitrogen content (2.050 %) were observed to be higher in *Euphorbia hirta* than others. Total protein (2.321 gm/100 gm), total fat (0.655 gm/100 gm), oxygen content (34.222 %) and Higher heat value (HHV) (16.546 %) were higher in *Ziziphus mauritina* than others. The existence of functional groups and the relevance of the presence of a specific bioactive component were determined using FTIR (Fourier Transform Infrared Spectroscopy) of the leaves of five plant samples. According to GCMS (Gas Chromatography Mass Spectroscopy), five different medicinal plants contain significant bioactive chemicals. By using GC-MS analysis, a total of 10 bioactive compounds (*Acalypha indica*, *Senna tora*) and 8 bioactive compounds (*Ziziphus mauritina*, *Euphorbia hirta*, *Physalis Angulata*) were found in the peaks of methanolic extracts of five potential medicinal plants. The bioactive compounds like Cyclotrisiloxane, Hexamethyl-, Dodecane, 1-Fluoro-, Myo-Inositol, 4-C-Methyl obtained from *Acalypha indica* leaf extract, Palmitic Acid, Phthalic Acid, Cyclopentadecanol obtained from *Senna tora* leaf extract and 4-Tert-Octylphenol, TMS Derivative obtained from *Euphorbia hirta* leaf extract has no records of individual bioactivity as per Dr. Duke's ethnobotanical and pharmaceutical database and are considered as novel bioactive compounds. Isolation of these compounds and successive bioactivity studies should be the thrust area for future researchers. Also, the current study confirms that specific plant leaves are a great source of important phytochemicals and can be used to make herbal formulations, practical medications, and complementary medicines.

Introduction

Many medicinal plants are valued for their bioactive compounds, natural chemicals that can have various physiological effects on the human body. Plant secondary metabolites, which are chemical compounds produced by plants that are not directly involved in their primary metabolic processes like growth and reproduction, play various roles in plants, including defense against herbivores and pathogens, the attraction of pollinators, and responses to environmental stresses (*Maiti* et al., 2010, 2013; Banerjee et al., 2014; Eljounaidi and Lichman, 2020; Acharya et al., 2022). These bioactive

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compounds, such as alkaloids, flavonoids, terpenoids, phenolic compounds, and more, can be found in different parts of the plant, including the leaves.

The secondary metabolites may change in its concentration in various ways. Such as, due to Climate change that, including rising temperatures, increased carbon dioxide (CO_2) levels, and changes in precipitation patterns. Edaphic factors refer to the soil-related factors such as soil composition, pH, moisture content, and nutrient availability. Altitudinal variation, which refers to changes in elevation or altitude above sea level, can significantly impact plant secondary metabolites. As plants grow at different altitudes, they experience variations in environmental factors such as temperature, precipitation, light intensity, atmospheric pressure, and oxygen levels. These changes can influence the production and composition of secondary metabolites (Ma et al., 2020). So, the chemical characterization of medicinal plants is urgently needed today to find actual bioactive phyto-constituent used by indigenous people.

The chemical characterization of leaves from medicinal plants involves identifying and quantifying various chemical compounds present in the leaves. Medicinal plant leaves can contain a wide range of bioactive compounds and understanding their chemical composition is essential for assessing their potential therapeutic properties.

Some common methods and techniques used for the chemical characterization of medicinal plant leaves include: Extraction of Bioactive Compounds using appropriate solvents like ethanol, methanol, water, or a mixture of solvents, Qualitative and Quantitative Analysis through thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and Mass spectrometry is used for the determination of molecular weights and the identification of compounds based on their mass spectra gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) etc.

Paschim Medinipur (West Midnapore), located in the Indian state of West Bengal, is rich in biodiversity and has a long history of traditional herbal medicine practices. The region is home to various indigenous communities that have relied on the abundant flora for their medicinal needs.

In the present investigation, five potential medicinal plants viz., *Acalypha indica*, *Senna tora*, *Euphorbia hirta*, *Physalis angulata* and *Ziziphus mauritiana*, have been collected from Paschim Medinipur district of West Bengal, India and screened for their potential bioactive phytochemicals.

Acalypha indica, commonly known as Indian copperleaf or Indian nettle, belongs to the family Euphorbiaceae. It is a perennial herb that typically grows to a height of 30-80 centimetres. It is native to India and is found in various parts of South Asia, Southeast Asia, and other tropical and subtropical regions. This plant is known for its various traditional and medicinal uses. It has simple, alternate leaves that are typically green, with serrated edges. *Acalypha indica* has a long history of traditional medicinal uses in various cultures. It is believed to have anti-inflammatory, analgesic, and antipyretic (fever-reducing) properties. Traditional medicine has been used to treat conditions like skin disorders, wounds, diarrhea, and as a pain reliever. The plant contains various phytochemicals, including alkaloids, flavonoids, tannins, and terpenoids, which are believed to contribute to its medicinal properties (Mondal et al., 2021; Islam et al., 2019).

Senna tora, commonly known as sickle senna, sicklepod, or coffee senna, is a plant species belonging to the Fabaceae family. It is an annual or short-lived perennial herb that can grow to a height of 30-90 centimeters. It is native to many tropical and subtropical regions, including parts of Asia, Africa, and the Americas. It has pinnately compound leaves with oblong leaflets. *Senna tora* has a history of traditional medicinal uses in various cultures. It is believed to have laxative, purgative, and anthelmintic (worm-expelling) properties. In traditional medicine systems like Ayurveda, the plant has been used to treat constipation, skin diseases, and other ailments. The seeds are often used in traditional remedies. *Senna tora* leaves contain several bioactive compounds, including anthraquinones, flavonoids, and other phytochemicals responsible for its medicinal properties.

Euphorbia hirta, commonly known as asthma weed, garden spurge, or hairy spurge, is a small, herbaceous plant belonging to the Euphorbiaceae family. It is a low-growing, prostrate or erect annual herb typically reaches a height of 20-50 centimeters. It is native to various parts of the world, including tropical and subtropical regions of Asia, Africa, the Americas, and the Pacific Islands. The plant is covered with fine, soft hairs, which give it a somewhat fuzzy appearance.

The leaves are opposite and oblong to lanceolate, with serrated edges. It is used as a remedy for a wide range of ailments, including respiratory conditions like asthma, bronchitis, and coughs (Jain et al., 2021). It is also used for digestive issues, diarrhea, dysentery, and skin conditions like wounds and rashes (Iskandar et al., 2021). The plant contains various phytochemicals, including alkaloids, flavonoids, tannins, and terpenoids, which are believed to contribute to its medicinal properties (Ezikanyi and Linian, 2021; Islam et al., 2019).

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Physalis angulata, commonly known as hairy ground cherry, is a small annual herbaceous plant belonging to the Solanaceae family, is a low-growing plant, typically reaching heights of 30-60 centimeters. This plant is native to tropical regions of the Americas but has become naturalized in various parts of the world due to its adaptability and ability to spread. The leaves are ovate to lanceolate, with serrated edges. In some traditional herbal medicine systems, parts of the plant, including the leaves and fruit, are used for their potential medicinal properties. They are believed to have anti-inflammatory, diuretic, and antimicrobial properties. However, it's essential to exercise caution and consult with a healthcare professional before using any plant for medicinal purposes. The plant contains various chemical compounds such as Alkaloids, Flavonoids, Steroids, Terpenoids, Phenolic Compounds bearing potential medicinal values.

Ziziphus mauritiana, commonly known as Indian jujube, is a small deciduous tree or shrub belonging to the family Rhamnaceae. It is a small to medium-sized shrub or tree that typically reaches heights of 2 to 8 meters. It is native to various parts of Asia, the Middle East, and Africa. This plant is known for its edible fruits and has several other traditional and ecological uses. It has a bushy and often spiny appearance, with glossy green leaves that are ovate to elliptical in shape. In traditional herbal medicine, various parts of the plant have been used to treat ailments such as digestive disorders, coughs, and colds. The fruit is sometimes used to alleviate stomach discomfort. The chemical composition of leaves can vary depending on factors such as the plant's age, environmental conditions, and geographical location. However, some common classes of compounds found in the leaves include Phenolic Compounds, Alkaloids, Triterpenoids, Saponins, Volatile Compounds etc.

Figure 1. ***Acalypha indica***

Figure 2. ***Senna tora***

Figure 3. ***Euphorbia hirta***

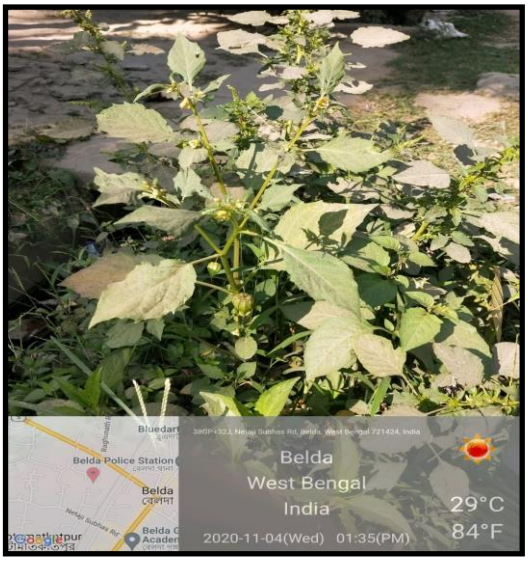

Figure 4. ***Physalis angulata***

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Figure 5. Ziziphus mauritiana

Material and Methods

Study area

Paschim Medinipur, also known as West Midnapore, is a district in West Bengal, India. It is situated in the southwestern part of West Bengal and is known for its diverse geography, which includes forests, rivers, and agricultural land. It shares borders within West Bengal districts of Bankura, Purulia, Jhargram, and Paschim Bardhaman. The latitude of the district is between 21.47^0N – 23.00^0N and the longitude is between 86.40^0E – 87.52^0E. The district also has a coastline along the Bay of Bengal to the south.

Temperature:

Summers that are hot and humid, a monsoon season, and comparatively moderate winters are the characteristics of a tropical climate. Summers (March to June) can be hot, with temperatures often exceeding 30°C (86°F). April and May are typically the hottest months. Winters (December to February) are milder, with temperatures ranging from 15°C to 25°C (59°F to 77°F).

Climate: Paschim Medinipur experiences a monsoon season from June to September, during which it receives significant rainfall. The southwest monsoon brings heavy rains to the region, which are essential for agriculture but can also lead to flooding in some areas. The post-monsoon months of October and November are generally pleasant, with cooler temperatures and less humidity. The district also has some forested areas, which contribute to its climate and ecology.

Collection of sample and authentication

The plant has been collected from Paschim Medinipur district, West Bengal, India, identified and authenticated by a book entitled Bengal Plants by David Prain. After collection, the plant is prepared for herbarium following standard methodology. The herbarium sheet has been deposited at the Department of Botany, Belda College, Paschim Medinipur, West Bengal. Mature fresh leaf samples of five selected medicinal plants have been collected from the study area and kept in different sterilized polythene bags. The sample bags were marked according to the name of the plants. To preserve the chemical integrity of the leaves, carefully dry them using the air drying method and store them in airtight containers in a cool, dark place to prevent degradation. Dried leaf samples were ground and made into a powder with a minimum particle size of 1 mm.

Physical analysis

Proximate Analysis

Proximate composition analysis has been achieved through a standard test method for ash in biomass (ASTM E1755). The volatile matter has been analyzed through a standard test method for volatile matter in the analysis of particulate wood fuels [E 872 – 82 (Reapproved 1998)]. Fixed Carbon was determined through difference.

Bulk density and Swelling index

Bulk density was analyzed through Standard Test Methods for Specific Gravity of Solids (ASTM D 854-92) and the swelling index was calculated through ASTM D4829 – 11.

Compositional analysis

Compositional analysis was achieved through ASTM: American Society for Testing and materials (ASTM International) standards, 2015, Van Soest et al., 1991.

Chemical and spectroscopic analysis

Preparation of Extract: Preparation of extract using a Soxhlet apparatus, an 8-hour extraction was performed in methanol. The extract was then concentrated by evaporation under a rotary vacuum.

Qualitative Phytochemical Studies

Preliminary Phytochemical Screening

Qualitative Phytochemical tests are conducted (Balamurugan et al., 2019; Ogbuagu et al., 2020; Junaid and Patil, 2020; Sahira Banu and Cathrine, 2015; Silva, GO et al., 2017; Singh and Kumar, 2017; Nazril et al., 2016) to identify the presence of active phytochemical components (Alkaloids, Proteins, Carbohydrates, Total Phenolic compound, Tannins, Fat etc.).

The method is performed as described below:

Test for carbohydrates

A. Benedict's test

The Benedict's reagent was added to approximately 0.5 ml of the extracted solution. The mixture was cooked for about 2 minutes in a bath of boiling water. As a result, a red precipitate emerges, indicating that sugar is present.

B. Molisch's test

2 ml of the solution was combined and shaken with 2 drops of the -napthol alcohol solution. Later, 2-3 drops of concentrated H_2SO_4 were dropped into the test tube's side

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wall. The appearance of a violet ring demonstrates the presence of sugar in it detects proteins.

Test for protein

A. Biuret test

To around 2 ml of the extracted solution, a drop of 2% copper sulphate solution was added. Then, 1 ml of 95% ethanol was added. Next, KOH pellets were added. A pink solution formed in the ethanolic layer, suggesting the presence of protein.

B. Xanthoproteic test

Two millilitres of the extracted solution were combined with a few drops of concentrated nitric acid (HNO_3) to produce a yellowish output. A protein is present because of its colour.

Test for fat

A. Saponification test

About 2 ml of the extracted solution was mixed with a small amount of 0.5N alcoholic KOH and a small amount of phenolphthalein. The mixture was heated for around two hours. There are indications that fixed oils or fats are present in the sample when soap is produced or when alkali is partially neutralised.

B. Spot test

An oil stain resulted from applying a small amount of the extract to one filter paper and pressing it with a different filter paper. It is obvious that solidified oil is present there.

Test for alkaloid

A. Wagner's Test (Potassium iodide)

In the test tube holding 500 ml of the extract solution, 1–2 drops of Wagner's reagent (1.27 g I2 + 2 g KI in 100 ml distilled water) were applied along the side. For 30 minutes, the combinations were heated at 60°C. Alkaloids can be detected using Wagner's reagent by looking for a reddish-brown precipitate.

B. Dragendroff's Kraut's test

The extracted solution was mixed with 1-2 drops of Dragendroff's reagent (10 ml stock solution, 20 ml acetic acid and distilled water). A reddish-brown precipitate emerges as a result, indicating the presence of an alkaloid.

Test for phenols

A. Ferric Chloride Test

An appropriate mixture was made with 1 ml of the extracted solution, 1 ml of ethanol, and a few drops of FeCl3. The plant extracts revealed blue, green, purple, and red phenolic chemicals.

B. Ellagic Acid Test

5% glacial acetic acid and then 5% sodium nitrite solution were added to 2 ml of the extract aqueous solution. The presence of a muddy solution or Niger brown precipitation confirmed the presence of phenols.

Tests for tannins

A. 10% NaOH test

0.4 ml of plant extraction solution was added to 4 ml of 10% NaOH solution, which was then violently agitated for a time. The development of an emulsion indicated the existence of hydrolysable tannins.

B. Gelatin test

5 ml of distilled water is used to dilute the plant extract. Later, 1% gelatin solution and 10% sodium chloride were added. Because of the tannin, the precipitate turns white.

Quantitative Phytochemical studies

Estimation of the Value of Carbohydrates

The anthrone technique was used to estimate the quantity of carbohydrates. 4ml of anthrone reagent was added to 1ml of sample. After 8 minutes of incubation in a boiling water bath, the absorbance was measured at 630 nm and compared to a blank for the reagent. The results were represented as mg/gdw sample after the estimate was carried out in triplicate.

Estimation of Value of Protein

Using the Lowry technique, protein content was estimated. Two millilitres of the sample were obtained, five millilitres of newly made alkaline copper reagent were added, and after 10 minutes at room temperature, Folin-Ciocalteau reagent was added. Incubate at room temperature for 30 minutes in the dark after thoroughly mixing. Additionally, a reagent blank was originated. The blue colour that was displayed was read at 660 nm.

Estimation of the Value of Fat

The test samples were dried and ground into powder, and 100 mg of the mixture was added to a conical flask with 30 ml of chloroform and methanol (2/1:v/v) before being transferred. This mixture was well combined before being incubated at room temperature in the dark for the whole extraction process. Then, 20 ml more chloroform and 2 ml of water were added and centrifuged to separate the two layers. All of the lipids (in chloroform) were present in the lowest layer and were gathered in the pre-weighed glass vials. All the water-soluble components were included in the pigmented layer that was separated in methanol. In every test sample, the thick medium layer was removed.

Estimation of value of Alkaloids

Alkaloids were identified using the Harborne (1973) technique. 200 ml of 10% acetic acid in ethanol was added to 5 g of the sample, covered, and left to stand for 4 hours. It was filtered, and the filtrate was then concentrated to a fourth of its original volume in a water

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bath. Drop by drop, concentrated NH4OH was added to the filtrate until the precipitation was finished. After allowing the whole solution to settle, the precipitate was collected, cleaned with weak NH4OH, and then filtered. The alkaloid, which was dried and weighed, is the residual.

Estimation of Value of Total Phenolic compound

The Folin-Ciocalteu reagent technique was modified to measure the total phenolic content. 7% Sodium carbonate solution in Distilled Water was added to 1 ml of plant extract, 5 ml of Folin-Ciocalteu reagent, and 5 ml of plant extract. The mixes were let to stand at room temperature for 30 minutes. Using a UV-visible spectrophotometer, the absorbance at 760 nm was measured in comparison to a blank made up of all the same ingredients as the test sample but without the test extract. Gallic acid solutions in methanol at concentrations of 1.0, 1.5, 3.0, 6.0, 12, and 24 g/ml were used to create the standard calibration curve (R2 = 0.998). Gallic Acid Equivalents (mg of GAE/g dry weight of extract), a standard reference ingredient, were used to represent the total phenolic content in the seed extract.

Estimation of value of Tannins

By using the Siddhuraj and Manian method, the amount of tannins in *H. radicata* was calculated. The extracts were divided into 500 L test tubes, each of which received 500 L of distilled water and 100 mg of polyvinyl polypyrrolidone. For four hours, this mixture subsequently incubated at 4°C. The sample was then centrifuged for five minutes at a speed of 5000 rpm, and 20 L of the supernatant was collected.

This supernatant contains no tannins and just simple phenolics (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). At 725 nm, the supernatant's phenolic content was determined and represented as the amount of free phenolic content based on dry matter. The extract's tannin content was determined using the results mentioned above in the following manner:

Total phenolics (mg GAE/g extract) minus free phenolics (mg GAE/g extract) equals tannins (mg GAE/g extract).

Ultimate analysis

Materials can contain a variety of chemical combinations. To analyze and determine the chemical components of that specific item, the technique known as "Ultimate Analysis" can be used (Sarkar et al., 2022). This procedure is more precise than "Proximate Analysis' method. Following this procedure, a sample of the plant species was analyzed by the method- Solid biofuels - Determination of total carbon, hydrogen, and oxygen content is covered in CEN/TS 15104:2005.

HHV

The Standard Test Method for Ash in Biomass (ASTM E1755) and the Standard Test Method for Volatile Matter in the Analysis of Particulate Wood Fuels [E872 - 82 (Reapproved 1998)] were used to explore Volatile Matter, Ash Content, Fixed Carbon and Higher heat value (MJ/kg).

FTIR

In this process, the sample is scanned through an infrared light which has a radiation of 10,000 to 100cm-^{1}. The radiation is absorbed in some cases and in some cases, it passes through the sample object. The sample molecules convert the absorbed into rotational or vibrational energy. The detector of the resulting signal generally presents a spectrum from 4000 cm-1 to 400cm-^{1}, reflecting a molecular fingerprint of the sample (Bagchi et al., 2021).

Model: Thermo Fisher Scientific Nicolet TM iS10 FTIR Spectrometer, USA

GCMS

Preparation of extract

With the aid of an electric grinder, the plant's leaves were ground into a powder and sieved. Methanolic extract was prepared for further GC-MS research.

GC Programme

Model: GC-MS Model: Perkin Elmer Clarus 680 GC/ 600C MS

Oven: Initial temp 60°C for 1 min, ramp 7°C/min to 200°C, hold 3 min, ramp 10°C/min to 300°C, hold 5 min, Inj Aauto=280°C, **Volume**=0 µL, Split=10:1, **Carrier Gas**=He, Solvent Delay=8.00 min, Transfer Temp=180°C, Source Temp=150°C, **Scan**: 50 to 600Da, **Column** 60.0m x 250µm.

MS Programme

Spectrum analysis has been done using the Version 2.0g of the NIST mass spectral library search programme.

Identification of the phytochemical constituents

The individual mass spectral peak values of the unidentified phytochemical components included in the methanol extract of the stem were compared with the 62,000-pattern database of the National Institute of Science and Technology. The phytochemicals were then identified based on the obtained results after contrasting the unknown peak value and chromatogram from GCMS with the known chromatogram and peak value from the NIST Library database.

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Observation

Physical analysis

Physical analysis involves analysis of proximate composition, bulk density and Swelling Index. The observed data has been represented in tabular form in Table:

Table 1. Observed data on physical analysis

Sample	VM	AC	FC	BD	SI
Acalypha indica	**75.452**	11.555	12.993	**0.256**	**1.456**
Physalis angulata	67.616	12.307	**15.251**	**0.223**	1.428
Senna tora	66.454	**12.327**	15.161	0.227	1.155
Euphorbia hirta	**55.289**	**10.242**	**12.365**	0.236	**1.060**
Ziziphus mauritiana	58.236	11.254	12.444	0.235	1.222

VM=Volatile Matter (%), **AC**=Ash Content (%), **FC**=Fixed Carbon (%), **BD**=Bulk Density (gm/cm3), **SI**=Swelling Index (%). *Red colour indicates Higher Values, Blue colour indicates Lower Values*.

Compositional analysis

The Compositional analysis involves percentage calculation of Cellulose, Hemicellulose, Lignin, and Extractive content. The observed data has been represented in tabular form in Table:

Table 2. Observed data on compositional analysis.

Sample	CELC	HEMC	LC	EC
Acalypha indica	**61.727**	**4.372**	4.531	1.163
Physalis angulata	60.640	3.685	5.147	**1.974**
Senna tora	61.393	**4.372**	**5.531**	1.390
Euphorbia hirta	38.453	**2.327**	4.420	**0.749**
Ziziphus mauritiana	**37.245**	3.254	**4.222**	1.235

CELC=Cellulose Content (%), **HEMC**=Hemicellulose Content (%), **LC**=Lignin Content (%), **EC**=Extractive Content (%). *Red colour indicates Higher Values, Blue colour indicates Lower Values*

Chemical and Spectroscopic Analysis

Qualitative and quantitative phytochemical studies

Preliminary phytochemical studies (Qualitative and quantitative) were performed to assess the presence of bioactive phytoconstituents. Observed data has been represented in Table:

Table 3. Observation on Qualitative phytochemical studies.

Phytochemicals	Results				
	Acalypha Indica	***Physalis Angulata***	***Senna tora***	***Euphorbia hirta***	***Ziziphus Mauritiana***
Carbohydrate	+	+	+	+	+
Protein	+	+	+	+	+
Total Fat	+	-	-	+	-
Alkaloids	+	+	+	+	+
Phenolics	+	+	+	+	+
Tannin	+	+	+	+	+
Saponins	-	-	-	-	-
Terpenoids	-	-	-	-	-

+= Present, -= Absent

Table 4. Observed data on quantitative phytochemical analysis.

Sample	TCH	TPR	TFA	ALKD	PHEL	TANN
Acalypha indica	**3.907**	1.201	0.330	1.132	1839.946	**0.635**
Physalis angulata	8.123	**1.040**	**0.311**	**1.261**	**2907.845**	**0.101**
Senna tora	4.761	1.932	0.591	0.801	**1281.466**	0.458
Euphorbia hirta	**9.619**	1.194	0.642	1.095	2011.451	0.330
Ziziphus mauritiana	9.142	**2.321**	**0.655**	**0.235**	2000.451	0.354

TCH =Total Carbohydrate (gm/100 gm), **TPR** =Total Protein (gm/100 gm), **TFA** =Total Fat (gm/100 gm), **ALKD** =Alkaloids (g% W/V) of dry weight), **PHEL** =Phenolics (mg/100 gm), **TANN** =Tannin (g/100g). *Red colour indicates Higher Values, Blue colour indicates Lower Values*

Ultimate analysis

In Table: the percentage values of C, H, O, N and HHV (Higher Heat Value) has been represented.

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Table 5. Observed data on ultimate analysis

Sample	CC	HC	OC	NC	HHV
Acalypha indica	**39.322**	6.289	32.795	1.158	16.267
Physalis angulata	42.773	6.525	32.411	**0.974**	16.269
Senna tora	**44.656**	**7.289**	30.793	1.117	15.425
Euphorbia hirta	42.600	4.560	**27.320**	**2.050**	**15.088**
Ziziphus mauritiana	43.222	**4.369**	**34.222**	0.987	**16.546**

CC=Carbon Content (%), **HC**=Hydrogen Content (%), **OC**=Oxygen Content (%), **NC**=Nitrogen Content (%), HHV=Higher Heat Value (%) *Red colour indicates Higher Values, Blue colour indicates Lower Values.*

FTIR

The FTIR graph, which is shown in Figures (4.1–4.5), showed the presence of several peaks corresponding to absorbance and wave number. Table (4.5–4.9) shows the positions of these peaks as well as information about their functional groups, chemical classes, and peak details.

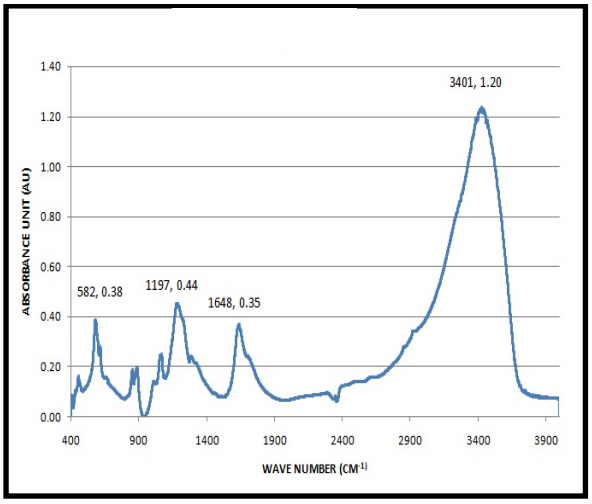

Figure 6. FTIR spectra of *Zizipus mauritiana.*

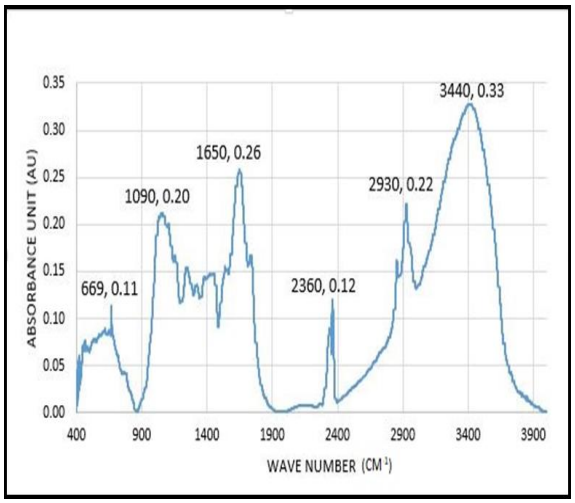

Figure 7. FTIR spectra of *Euphorbia hirta.*

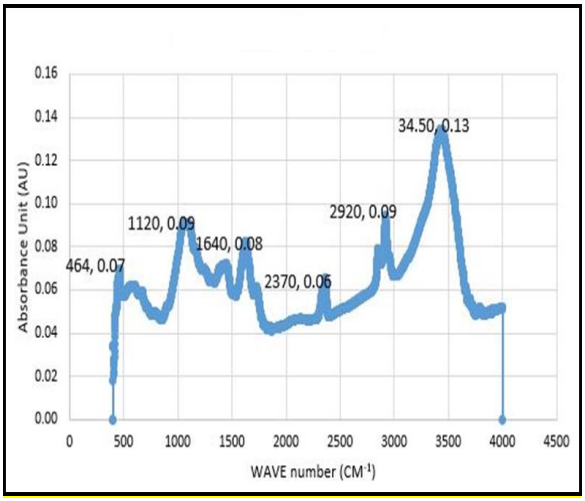

Figure 8. FTIR spectra of *Senna tora*

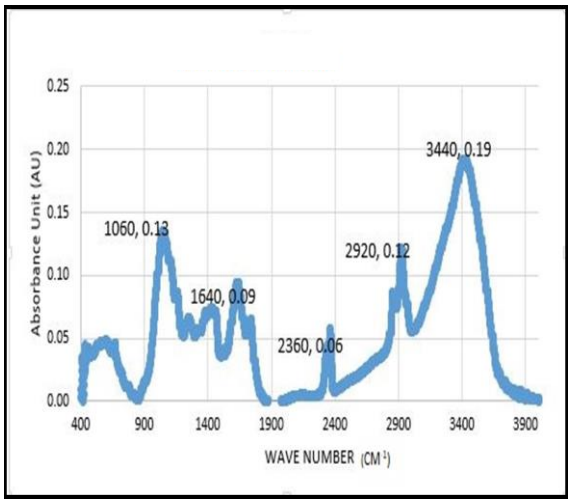

Figure 9. FTIR spectra of *Physalis angulata.*

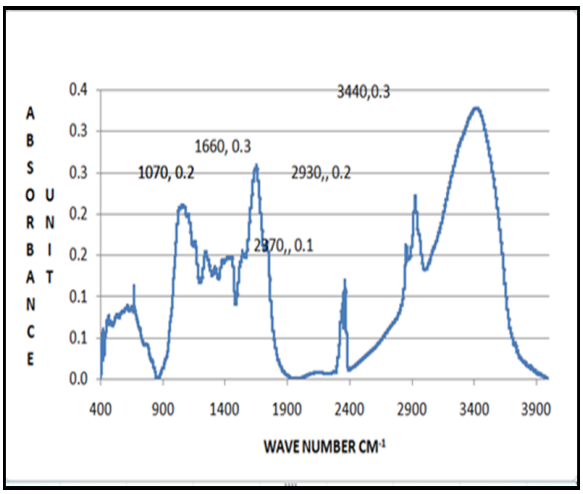

Figure 10. FTIR spectra of *Acalypha indica.*

Figures 6-10. Various peaks corresponding to absorbance and wave number of leaves of 5 different medicinal plant.

Table 6. Peak positions, functional groups, chemical class and peak details of leaves of five potential medicinal plants.

	Peak Position	Range	Group	Class	Peak Details
Ziziphus mauritiana	582	515-690	C-Br stretching	Halo Compound	Strong
	1197	1124-1205	C-O Stretching	Tertiary Alcohol	Strong
	1648	1566-1650	C=C Stretching	Cyclic Alkene	Strong
	3401	3300-3400	N-H Stretching	Aliphatic Primary Amine	Medium
Euphorbia hirta	669	665-730	C=C Bending	Alkene	Strong
	1090	1087-1124	C-O Stretching	Secondary Alcohol	Strong
	2360	2360	O=C=O Stretching	Carbon Dioxide	Strong
	2930	2840-3000	C-H Stretching	Alkene	Medium
	3440	3200-3550	O-H Stretching	Alcohol	Strong, Broad
Senna tora	1060	1050-1085	C-O Stretching	Primary Alcohol	Strong
	1640	1640-1690	C=N Stretching	Imine / Oxime	Strong
	2360	2360	O=C=O Stretching	Carbon Dioxide	Strong
	2920	2840-3000	C-H Stretching	Alkene	Medium
	3440	3200-3550	O-H Stretching	Alcohol	Strong / Broad
Physalis angulata	464	1087-1124	C-O Stretching	Secondary Alcohol	Strong
	1640	1638-1648	C=C Stretching	Alkene	Strong
	2370	2370	O=C=O Stretching	Carbon Dioxide	Strong
	2920	2840-3000	C-H Stretching	Alkane	Medium
	3450	3200-3550	O-H Stretching	Alcohol	Strong, Broad
Acalypha indica	1070	1030-1070	S=O stretching	Sulfoxide	Strong
	1660	1665-1675	C=C stretching	Alkene	Weak
	2370	2349-2370	O=C=O stretching	Carbon Dioxide	Strong
	2930	2840-3000	C-H stretching	Alkane	Medium
	3440	3200-3550	O-H stretching	Alcohol	Strong, broad

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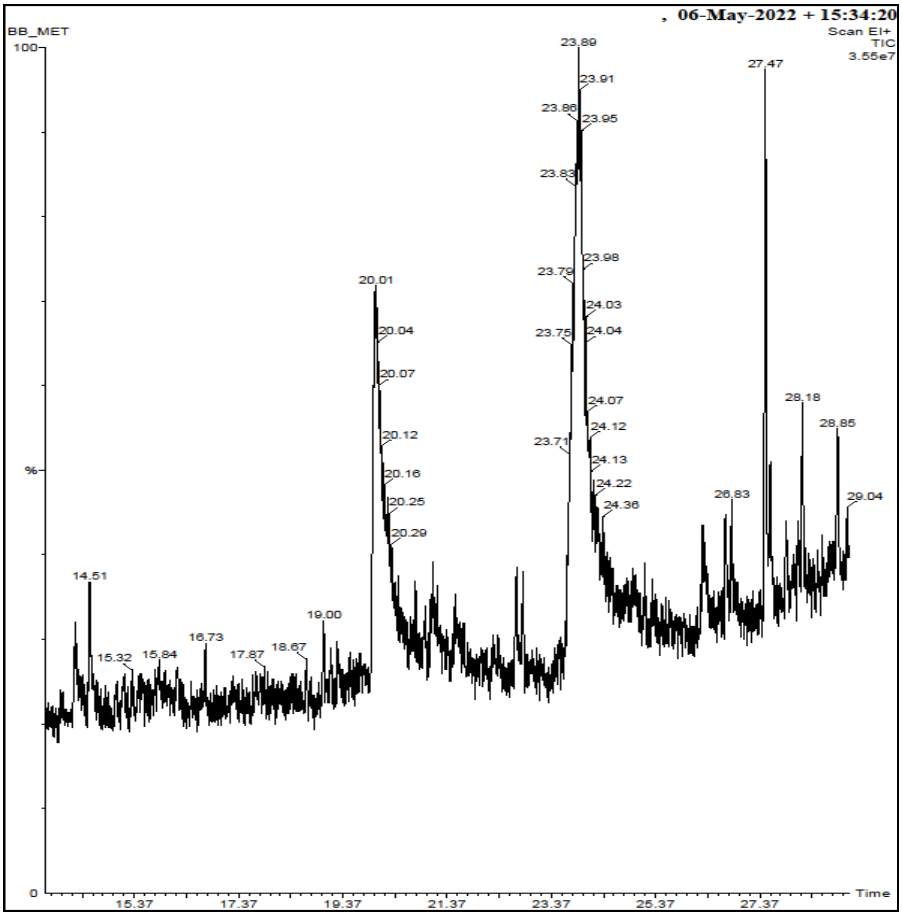

Figure 11. GC-MS Chromatogram of Methanol extract of *Acalypha indica* leaf.

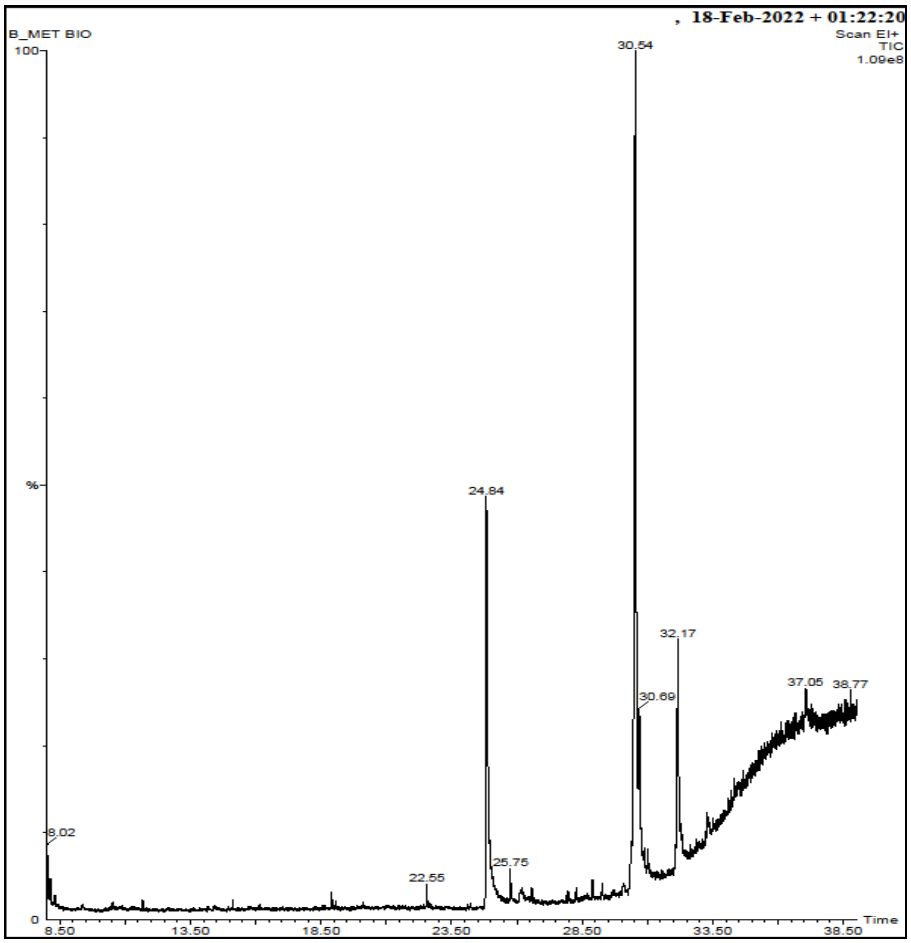

Figure 12. GC-MS Chromatogram of Methanol extract of *Senna tora* leaf.

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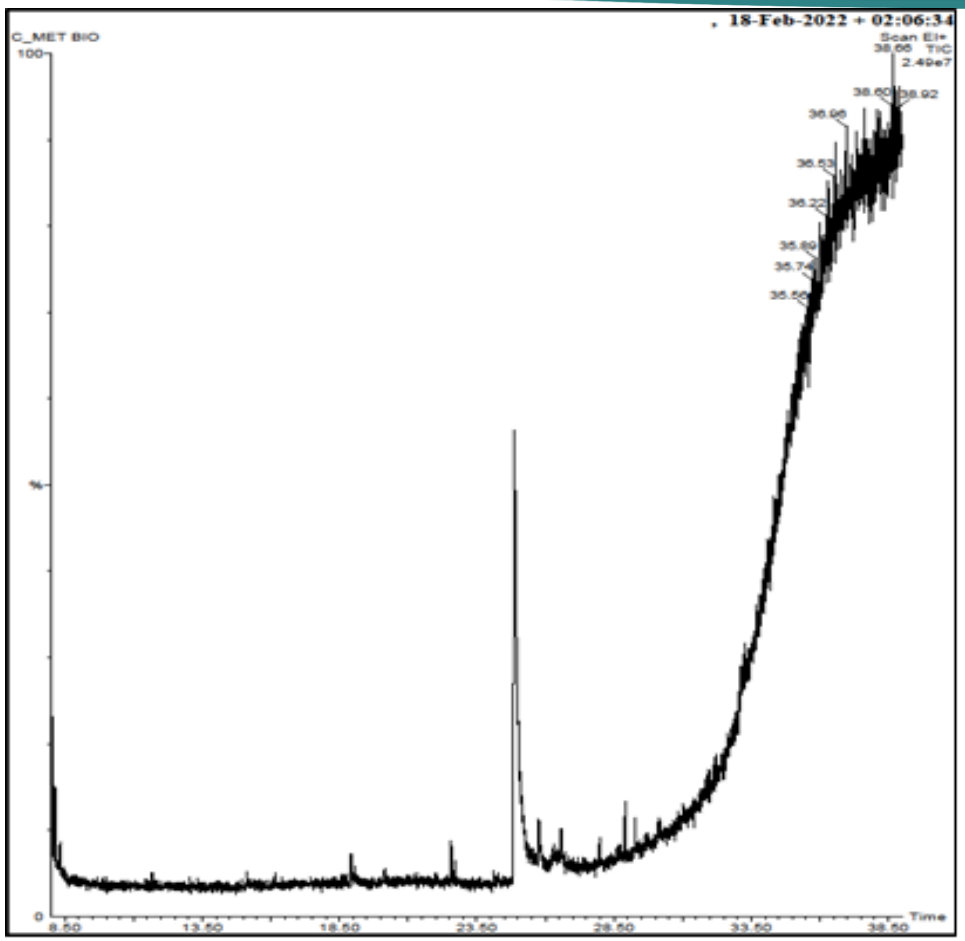

Figure 13. GC-MS Chromatogram of Methanol extract of *Euphorbia hirta* leaf.

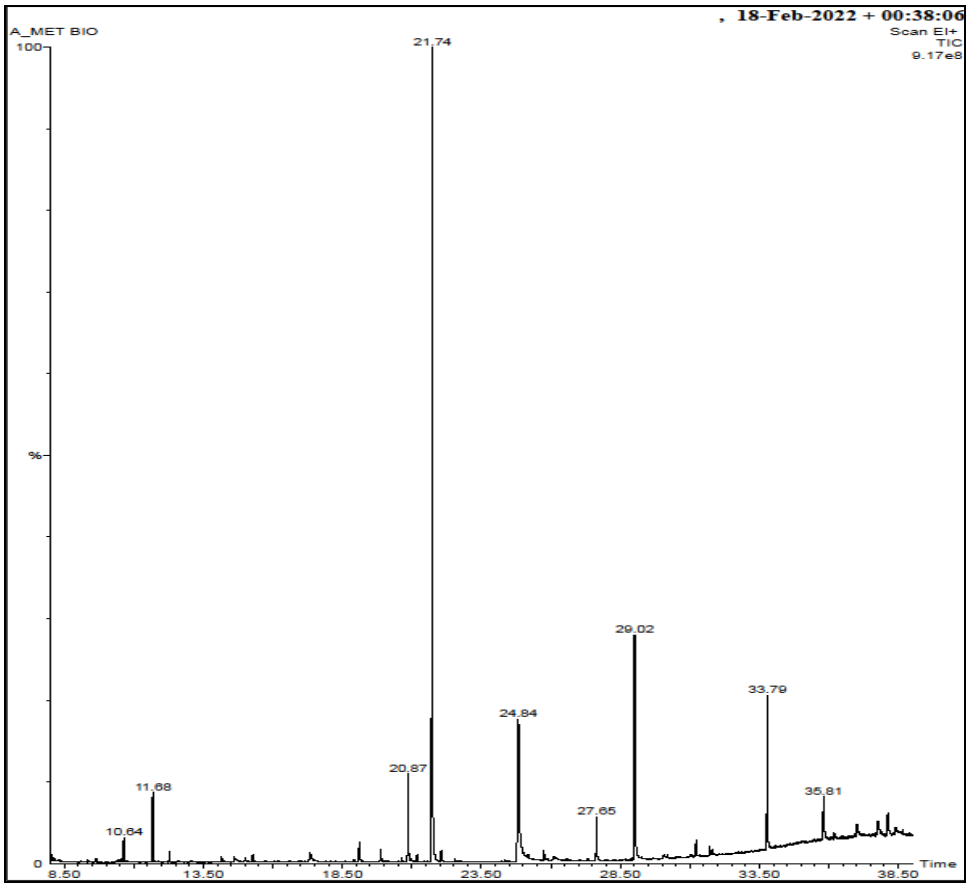

Figure 14. GC-MS Chromatogram of Methanol extract of *Physalis angulata* leaf.

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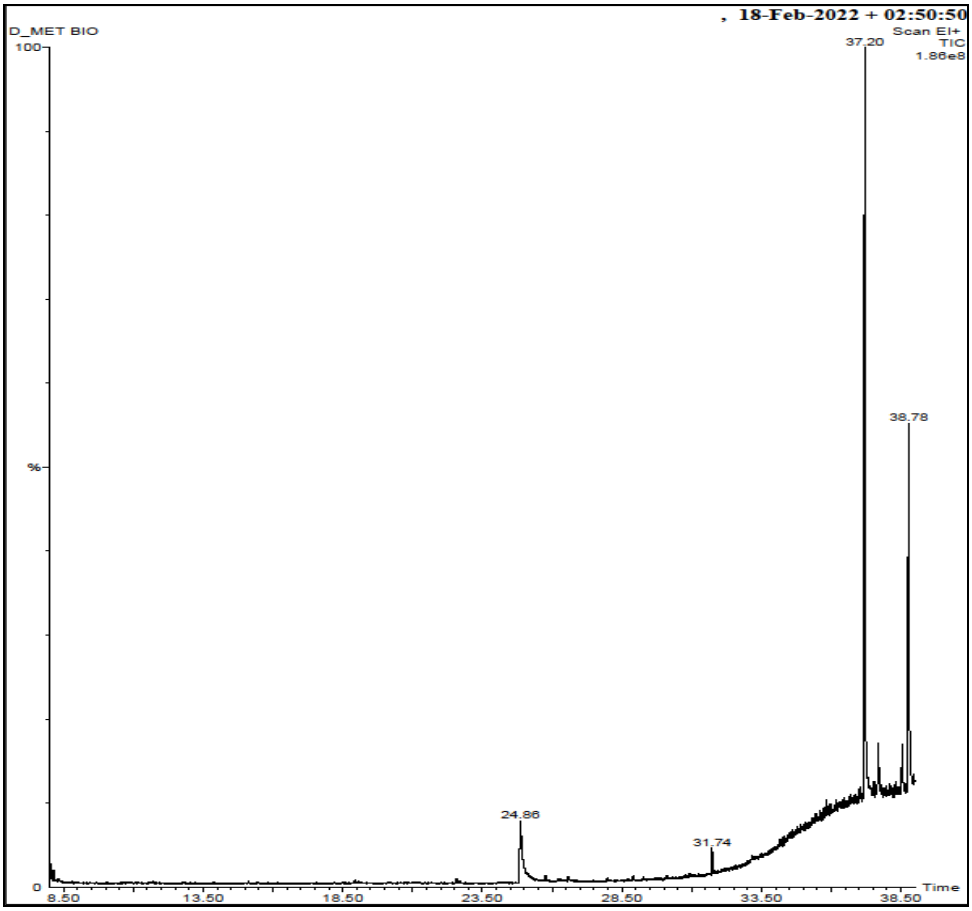

Figure 15. GC-MS Chromatogram of Methanol extract of *Ziziphus mauritiana* leaf.

Table 7. Phyto-components identified with their respective peaks, molecular weight (MW) and retention time (RT) in the methanolic extracts of Various Plants. Source: Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search/list)

	Peak	RT	Compounds	MW (Da)	Bioactivity
Acalypha indica	1	35.883	CYCLOTRISILOXANE, HEXAMETHYL-	222	No record found (Novel)
	2	9.389	CIS-2,4-DIMETHYLTHIANE, S,S-DIOXIDE	162	Anticancer (stomach)
	3	14.509	1-NONADECANAMINE, N,N-DIMETHYL-	311	Antitumor (nasopharynx)
	4	20.011	TRIDECANE, 2,2,4,10,12,12-EXAMETHYL-7-(3,5,5-TRIMETHYLHEXYL)-	164	Arachidonic-Acid-Inhibitor
	5	22.697	DODECANE, 1-FLUORO-	188	No record found (Novel)
	6	23.888	MYO-INOSITOL, 4-C-METHYL	194	No record found (Novel)
	7	27.474	TETRAMETHYL-2-HEXADECEN-1-OL	296	Oligosaccharide Provider
	8	31.371	Z,Z-6,28-HEPTATRIACTONTADIEN-2-ONE	530	Increase Zinc Bioavailibility
	9	37.923	ARSENOUS ACID, TRIS(TRIMETHYLSILYL) ESTER	342	Increase Aromatic Amino Acid Decarboxylase Activity
	10	38.864	ARSENOUS ACID, TRIS(TRIMETHYLSILYL) ESTER	342	Inhibit Production of Uric Acid

DOI: https://doi.org/10.52756/ijerr.2023.v36.002

	Peak	RT	Compounds	MW (Da)	Bioactivity
Senna tora	1	21.1	PALMITIC ACID	256.5	No record found (Novel)
	2	25.7	ALPHA-TOCHOPHEROL	430.72	Antioxidant, Neuroprotective
	3	22.73	PHYTOL OR 3,7,11,15-TETRAMETHYL-2HEXADECEN-1-OL	297	Neuroprotective, Antimicrobial, Antiasthmatic
	4	16.8	LAMINITOL OR 1-METHYL1,2,3,4,5,6CYCLOHEXANEHEXOL	194.2	Anti-malarial
	5	23.01	LINOLENIC ACID	278.43	Antioxidant, Neuroprotective
	6	15.9	TREHALOSE	342.3	Neuroprotective
	7	26.17	PHTHALIC ACID	391	No record found (Novel)
	8	19.395	PHYTOL	297	Neuroprotective, Antimicrobial, Antiasthmatic
	9	23.15	STEARIC ACID	284.4	Neuroprotective, cholesterol-lowering
	10	26	CYCLOPENTADECANOL	226.35	No record found (Novel)

	Peak	RT	Compounds	MW (Da)	Bioactivity
Ziziphus mauritiana	1	24.86	PHENOL, 3-METHOXY-2-METHYL-	138	Methyl-Guanidine-Inhibitor
	2	32.2	PHENOL, 2-(2-HYDROXY-1,2-DIMETHYLCYCLOPENTYL)-, 1-ACETATE, TRANS-	248	Decrease Glutamate Pyruvate Transaminase
	3	38.776	AROMADENDRENE, DEHYDRO	202	Alcohol-Dehydrogenase-Inhibitor
	4	13.87	PENTADECANOIC ACID	242	Antimicrobial, antifungal, Antioxidant
	5	7.26	HEPTADECANE	240	Antioxidant
	6	14.07	1,2-BENZENEDICARBOXYLIC ACID, DIHEXYL ESTER	334	Reduces β-induced neurotoxicity
	7	16.3	HEXADECANOIC ACID, ETHYL ESTER	284	Nematicide, Pesticide, Lubricant, Antiandrogenic
	8	15.9	9-OCTADECENOIC ACID	282	Anemiagenic, anti-alopecic, choleretic, dermatitigenic

	Peak	RT	Compounds	MW (Da)	Bioactivity
Euphorbia hirta	1	6.224	1,2,3- BENZENETRIOL	126	Anticancer (Lung), Antiseptic, Antioxidant, Antidermattic
	2	23.888	MYO-INOSITOL, 4-C-METHYL-	194	Myo-neuro-stimulant
	3	10.85	PHYTOL	296	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
	4	12.53	9- TETRADECENAL, (Z)-	210	Sex pheromone
	5	14.24	13-OCTADECENAL,(Z)-	266	Antimicrobial
	6	27.474	Z,Z-6,28-HEPTATRIACTONTADIEN-2-ONE	530	Increase zinc Bio-availability
	7	38.864	4-TERT-OCTYLPHENOL, TMS DERIVATIVE	278	No record found (Novel)
	8	9.49	HEXADECANOIC ACID, METHYLESTER	270	Antioxidant, Flavor, Hypocholesterolemic

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	Peak	RT	Compounds	MW (Da)	Bioactivity
Physalis Angulata	1	26.15	DODECANOIC ACID	200	Hypocholesterolemic agent
	2	18.9	DECANOIC ACID, METHYL ESTER	186	Antibacterial, Anti-inflammatory
	3	32.48	HEXADECANOIC ACID, METHYL ESTER		Antibacterial, Anti-inflammatory, Anticancer
	4	36.2	PHYTOL	296	Antioxidant, Diuretic and Anti-cancer
	5	35.78	9,12-OCTADECADIENOIC ACID (Z,Z)-,METHYL ESTER	944	Anti-cancer, Anti-inflammatory
	6	36.27	METHYL STEARATE	298	Anti-helminthic, Anti-inflammatory
	7	34.03	N-HEXADECANOIC ACID	256	Anti-bacterial, Anti-fungal
	8	33.4	DIBUTYL PHTHALATE	278	Antibacterial, Anti-inflammatory

Results and Discussion

Physical analysis

The percentage composition of volatile matter was highest in *Acalypha indica* (75.452%) whereas it was lowest in *Euphorbia hirta* (55.289%). The percentage composition of Ash content was highest in *Senna tora* (12.327%), whereas it was lowest in *Euphorbia hirta* (10.242%). The percentage composition of Fixed carbon was highest in *Physalis angulata* (15.251%), whereas it was lowest in *Euphorbia hirta* (12.365%). The percentage composition of Bulk Density was highest in *Acalypha indica* (0.256%), whereas it was lowest in *Physalis angulata* (0.223%). The percentage composition of the Swelling Index was highest in *Acalypha indica* (1.456%), whereas it was lowest in *Euphorbia hirta* (1.222%).

Compositional analysis

The percentage composition of Cellulose Content was highest in *Acalypha indica* (61.727%) whereas it was lowest in *Ziziphus mauritiana* (37.245%). The percentage composition of Hemicellulose Content was highest in *Acalypha indica* (4.372%) and *Senna tora* (4.372%), whereas it was lowest in *Euphorbia hirta* (2.327%). The percentage composition of Lignin Content was highest in *Senna tora* (5.531%), whereas it was lowest in *Ziziphus mauritiana* (4.222%). The percentage composition of Extractive Content was highest in *Physalis angulata* (1.974%), whereas it was lowest in *Euphorbia hirta* (0.749%).

Chemical and Spectroscopic Analysis

The highest concentration of Total Carbohydrate has been observed to occur in *Euphorbia hirta* (9.619 gm/100 gm), whereas it was lowest in *Acalypha indica* (3.907 gm/100 gm). The highest concentration of Total Protein has been observed to occur in *Ziziphus mauritiana* (2.321 gm/100 gm), whereas it was lowest in *Physalis angulata* (1.040 gm/100 gm). The highest concentration of Total Fat has been observed to occur in *Ziziphus mauritiana* (0.655 gm/100 gm), whereas it was lowest in *Physalis angulata* (0.311 gm/100 gm). The highest concentration of Alkaloids has been observed to occur in *Physalis angulata* (1.261 gm/100 gm), whereas it was lowest in *Ziziphus mauritiana* (0.235gm/100 gm). The highest concentration of Phenolics has been observed to occur in *Physalis angulata* (2907.845 gm/100 gm), whereas it was lowest in *Senna tora* (1281.466 gm/100 gm). The highest concentration of Tannin has been observed to occur in *Acalypha indica* (0.635 gm/100 gm), whereas it was lowest in *Physalis angulata* (0.101 gm/100 gm).

The percentage composition of Carbon Content was highest in *Senna tora* (44.656%), whereas it was lowest in *Acalypha indica* (39.322%). The percentage composition of Hydrogen Content was highest in *Senna tora* (7.289%) whereas it was lowest in *Ziziphus mauritiana* (4.369%). The percentage composition of Oxygen Content was highest in *Ziziphus mauritiana* (34.222%), whereas it was lowest in *Euphorbia hirta* (27.320%). The percentage composition of Nitrogen Content was highest in *Euphorbia hirta* (2.050%), whereas it was lowest in *Physalis angulata* (0.974%). The percentage composition of Higher Heat Value was highest in *Ziziphus mauritiana* (1.456%), whereas it was lowest in *Euphorbia hirta* (15.088%).

FTIR (Fourier Transform Infra-red spectroscopy) of the leaf of five plant samples was conducted to determine the presence of functional groups and understand the significance of particular bioactive components. The values of peaks and functional groups were depicted in Figures (6-10) and Table-6, respectively.

The presence of functional groups like Halo Compound, Tertiary Alcohol, Cyclic Alkene and

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Aliphatic Primary Amine in the leaves of *Ziziphus mauritiana* was confirmed by the more intense absorption band occurring at 582, 1197, 1648 and 3401 cm^{-1} corresponding to C-Br stretching C-O Stretching, C=C Stretching and N-H Stretching, bending, vibrations respectively.

The presence of functional groups like Alkene, Secondary Alcohol, Carbon Dioxide, Alkene and Alcohol in the leaves of *Euphorbia hirta* was confirmed by the more intense absorption band occurring at 669, 1090, 2360, 2930 and 3440 cm^{-1} corresponding to C=C Bending, C-O Stretching, O=C=O Stretching, C-H Stretching and O-H Stretching, bending, vibrations respectively.

The presence of functional groups like Primary Alcohol, Imine / Oxime, Carbon Dioxide, Alkene and Alcohol in the leaves of *Senna tora* were confirmed by the more intense absorption band occurring at 1060, 1640, 2360, 2920 and 3440 cm^{-1} corresponding to C-O Stretching C=N Stretching, O=C=O Stretching, C-H Stretching and O-H Stretching, bending, vibrations respectively.

The presence of functional groups like Secondary Alcohol, Alkene, Carbon Dioxide, Alkane and Alcohol in the leaves of *Physalis angulata* were confirmed by the more intense absorption band occurring at 464, 1640, 2370, 2920 and 3450cm^{-1} corresponding to C-O Stretching, C=C Stretching, O=C=O Stretching, C-H Stretching, O-H Stretching, bending, vibrations respectively.

The presence of functional groups like Sulfoxide, Alkene, Carbon Dioxide, Alkane and Alcohol in the leaves of *Acalypha indica* was confirmed by the more intense absorption band occurring at 1070, 1660, 2370, 2930 and 3440 cm^{-1} corresponding to S=O stretching C=C stretching, O=C=O stretching, C-H stretching and O-H stretching, bending, vibrations respectively.

GCMS (Gas Chromatography Mass Spectroscopy) reveals the presence of important bioactive compounds in five different medicinal plants. A total of 10 (*Acalypha indica, Senna tora*), 8 (*Ziziphus mauritiana, Euphorbia hirta, Physalis Angulata*) bioactive compounds were obtained from the peaks of methanolic extracts of five different medicinal plants by GC-MS analysis. The details of the bioactive compounds, their retention time, molecular weight and bioactivity were tabulated (Table 7). Figure (11-15) shows the chromatogram of the compounds detected using GC-MS. The bioactive compounds like Cyclotrisiloxane, Hexamethyl-, Dodecane, 1-Fluoro-, Myo-inositol, 4-C-Methyl obtained from *Acalypha Indica* leaf extract, PALMITIC ACID, PHTHALIC ACID, CYCLOPENTADECANOL obtained from *Senna tora* leaf extract and 4-TERT-OCTYLPHENOL, TMS DERIVATIVE obtained from *Euphorbia hirta* leaf extract has no records of bioactivity as per Dr. Duke's ethnobotanical and pharmaceutical database and are considered as novel bioactive compounds.

Conclusion

Medicinal plants are the 'gift of nature' as they contain important bioactive chemical compounds (Acharya, 2015; Acharya, 2016; Acharya et al., 2021a,b; Acharya et al., 2022; Sarkar and Madhu, 2017; Sarkar et al., 2021; Sarkar et al., 2022; Alam et al., 2022; Antony et al., 2018; Roy et al., 2023; Sarkar et al., 2023). *Acalypha indica, Senna tora, Euphorbia hirta, Physalis angulata, and Ziziphus mauritiana* were chosen as the five medicinal plants for physico-chemical and spectroscopic characterization in order to assess bioactive potential and to support traditional beliefs in the context of scientific interpretation. Fresh leaves from the Paschim Medinipur district of West Bengal, India, were collected, dried, and pulverized for proximate, ultimate, and compositional analyses, as well as FTIR and GCMS. The study found that compared to other experimental plants, *Acalypha indica* leaves had the highest percentage of volatile matter (%), bulk density (%), swelling index (%), cellulose content (%), hemicellulose content (%), and tannins (g/100g). The percentage composition of fixed carbon and extractive content in *Physalis angulata* was higher than that in other species. Ash, hemicellulose, and lignin contents were observed to be higher in the *Senna tora* leaf than others. In comparison to other plants, *Euphorbia hirta* had the greatest quantities of total carbohydrates and nitrogen. *Ziziphus mauritiana* had higher protein, fat, oxygen content, and heat values than other plants. FTIR (Fourier Transform Infrared Spectroscopy) was used to analyse the leaves of five different plant samples to identify the presence of functional groups and the significance of the presence of a particular bioactive component.

GCMS (Gas Chromatography Mass Spectroscopy) has revealed the presence of major bioactive compounds in five different medicinal plants. Five different medicinal plants' methanolic extracts were examined using GC-MS analysis, and a total of 10 bioactive compounds (*Acalypha indica, Senna tora*) and 8 bioactive compounds (*Ziziphus mauritiana, Euphorbia hirta, Physalis Angulata*) were identified.

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Conflict of interest

None

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