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A comparative physico-chemical, phytochemical and spectroscopic analysis of two medicinal plants belongs to Euphorbiaceae family: *Acalypha indica* L. and *Euphorbia hirta* L. growing in Paschim Medinipur District, West Bengal, India

Manna De and Lata Sharma*

Department of Botany, DR. CV Raman University, Kota, Bilaspur, C.G., India

E-mail/Orcid Id:

MD, manmade.botany@gmail.com, https://orcid.org/0000-0002-1319-8146; *LS,* lata.sh.bsp@gmail.com, https://orcid.org/0009-0003-1742-9217

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Abstract: Plants belong to Euphorbiaceae family, bearing enormous Medicinal potential due to the presence of various pharmacologically important secondary metabolites. Now a days, with the help of various tools and techniques like FTIR, GCMS researchers are engaged to systematically find out various biologically active phytoconstituents. The present study investigated *Acalypha indica* L. and *Euphorbia hirta* L. leaf to establish the fact. Physico-chemical parameters of the experimental plants have been investigated. The result shows that *Acalypha indica* possesses maximum values on Swelling index, volatile matter, ash contents, fixed carbon, higher heat value, hydrogen content, oxygen content, carbon content, hemicellulose content, lignin content and extractive values. In contrast, the percentage composition of bulk density, carbon content, and nitrogen content is higher in *Euphorbia hirta*. In the present investigation, the FTIR analysis reveals that *Acalypha indica* L. possessed different chemical classes like Sulfoxide, Alkene, Carbon Dioxide, Alkane, Alcohol etc., whereas FTIR analysis reveals the presence Alkene, Secondary Alcohol, Conjugated Alkene, Carbon Dioxide, Alkene, Alcohol in *Euphorbia hirta* L. The methanolic leaf extracts of two different plants have been screened through GC-MS, revealing many important bioactive phytoconstituents. *Acalypha indica* L. possesses Cyclotrisiloxane, Hexamethyl-, cis-2,4-dimethylamine, S, S-dioxide, 1- nonadecanamine, n, n-dimethyl-, tridecane, 2,2,4,10,12,12-hexamethyl-7-(3,5,5-trimethylsilyl)-, dodecane, 1-fluoro-, whereas, *Euphorbia hirta* L. possesses myo- inositol, 4-c-methyl-, z,z-6,28-heptatriactontadien-2-one, phytylpalmitate, hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-, 4-tert-octylphenol, TMS derivative. Both these plants have chemical substances that have proven bioactivity in human welfare. The GC-MS of leaf methanolic extract reveals that the phytochemical cis-2,4-dimethylthiane, S, S-dioxide have Anticancer (stomach) activities, 1-nonadecanamine, N, N-dimethyl bears Antitumor (nasopharynx) activities, myo-inositol, 4-C- methyl bearing Myo-neuro-stimulant activities and Z, Z-6,28-heptatriactontadien-2-one having Increase zinc bioavailability activities. These activities of individual phytoconstituents have been proven through Dr. Duke's Phytochemical and Ethno-botanical Databases: U.S. Department of Agriculture. This information might be helpful to the pharmacologists, chemists, etc., for future novel drug discovery.

Introduction

The family Euphorbiaceae is the fifth largest family of flowering plants and contains about 7,500 species organized into 300 genera, 37 tribes and three subfamilies: Acalyphoideae, Crotonoideae and Euphorbioideae (Jain et al., 2021; Thakur, 2011), including many different vegetative forms, some of which are of great importance. The plants belonging to

***Corresponding Author:** lata.sh.bsp@gmail.com

this family have great medicinal importance as they possess various active principles or secondary metabolites (Islam et al., 2019). This diverse array of the productions of phyto-constituents probably due to their existence all over the world with all kinds of habitats to which they have to adapt, thus producing a variety of chemicals (secondary substances) used for survival/ defence (Eljounaidi and Lichman, 2020). Succulence and the Crassulacean Acid Metabolism (CAM) pathway that characterizes many of its members have been cited as several adaptations that facilitate colonization and survival to achieve this induction. On the other hand, it was observed that a variety of environmental stressors, including temperature, salinity, drought, and others, worked in concert with genetic factors, including gene expression and mutation loads, to cause the synthesis of a wide assortment of secondary substances, which may likely be the cause of the family's medicinal properties (Ma et al., 2020).

Table 1. Plant description

Binomial Name:	***Acalypha indica* L.**	***Euphorbia hirta* L.**
Local Name:	Muktajaschuri.	Dudhia.
Family:	Euphorbiaceae.	Euphorbiaceae.
Habit:	Small herb, rarely sub-shrub.	Herb.
Habitat:	Waste, moist and shady places and river banks	Gardens where it is cultivated as an ornamental plant.
Flower color:	Olive green.	Red.
Flowering season:	December-April	December-February
Parts utilized:	Leaf, stem, flowers, roots and seeds	Leaf, stem, roots and flower

Acalypha indica L., a common annual medicinal herb, is found mostly in moist places, generally backyards of houses throughout the plains of India (Mondal et al., 2021). The presence of nutrition and phytochemicals supports the traditional use of *Acalypha indica* as an alternative treatment for curing certain health conditions (Nazril et al., 2016).

The plant has enormous medicinal potential as an emetic, expectorant, laxative and diuretic. It is useful in bronchitis, pneumonia, asthma and pulmonary tuberculosis (Mohan, 2012). The Leaves of this plant are laxative and anti-parasiticide. They are ground with common salt, quicklime, or lime juice applied externally in scabies (Mohan, 2012). In order to treat ringworm, it has been suggested to use lime juice and leaf paste. Children should avoid drinking leaf juice (Mohan, 2012). An earache remedy is a leaf decoction. Children are given dry leaf powder or a decoction with a small amount of garlic to help them eliminate worms (Mohan, 2012). The plant is used in homoeopathy for severe coughs associated with pulmonary bleeding, hemoptysis, and early phthisis.

Kaempferol, a cyanogenetic glucoside, a base called triacetonamine, and an alkaloid called acalyphine are all present in the plant. Acalyphamide, other amides, 2-methyl anthraquinone, tri-O-methyl ellagic acid, -sitosterol, ß-sitosterol glucoside, stigmasterol, n-octacosanol, quinine, tannin, resin, and essential oil are also present. Acalyphine, which is present in *Acalypha indica*, is used to treat sore gums (Bedon, 1982).

Figure 1. *Acalypha indica* L.

Figure 2. *Euphorbia hirta* L.

Euphorbia hirta L. is astringent and haemostatic; as a poultice applied to abscesses, inflamed glands, ulcers, oedema and phlegmon and also used in affections of childhood, in worms, bowel complaints and cough (Iskandar et al., 2021). The plant has good nutritional and phytochemical values (Ezikanyi and Linian, 2021). The juice is believed to be tonic,

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narcotic, anti-asthmatic and febrifuge, and effective against colic, dysentery, diarrhea, and amoebiasis. Decoction is beneficial for treating chronic bronchial diseases and asthma. This plant's extract sedatives the cardiovascular system and the mucous membranes of the respiratory and genitourinary tracts.

The plant can be used as an analgesic to treat pregnancy pains, colic, rheumatism, severe headaches, and toothaches. This plant contains polyphenols, flavonoids, steroids, tannins, and alkaloids as its primary phytochemicals.

The present study deals with the GCMS analysis of methananolic leaf extracts and individual spectra obtained through chromatogram have been authenticated through NIST database for the presence of the phytoconstituents. For their bioactivities, Individual phytoconstituents have been assessed through Dr. Duke's ethno-botanical and pharmacological database. This information would be helpful to the researchers, planners, policymakers and pharmacologists for future novel drug discovery.

Thus, the study highlights chemical and spectroscopic study with the help of modern tools and techniques like FTIR, GCMS to assess the existence of specific bioactive phyto-constituents of two different medicinal plants belonging to Euphorbiaceae family growing in Paschim Medinipur district, West Bengal, India.

Material and methods:

Plant collection 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. Collected leaves are carefully washed in tap water and then with distilled water to remove dust and soil particles. The leaves are dried in the shade and grind in an electrical grinder and used for extraction.

Preparation of extract

Using a Sohxlet apparatus, an 8-hour extraction was performed in methanol. The extract was then concentrated by evaporation under a rotary vacuum.

Collection site

Plants have been collected from Paschim Medinipur district, West Bengal, India, Latitude and longitude coordinates are 22.4080° N, 87.3811° E. After collection the plant has been carried out to the laboratory for further process.

Physico-chemical studies

Bulk density, Swelling index, HHV and Proximate analysis

Analysts and scientists perform a testing process called Proximate Analysis to calculate the quantity of substances in a material (Sarkar et al., 2022). Through this method, quality controls can be set against various materials to check whether they contain any hazardous chemicals and components that are harmful to human health.

The method involves the assessment of Bulk Density (gm/cm3), Fixed Carbon (%), Higher Heat Value (MJ/kg), Particle Size (mm), Volatile Matter (%) and Ash Content (%). Two different plant species collected from Paschim Medinipur were used as a sample to investigate the same. Through the Protocol: ASTM D4829 – 11, the swelling index was studied Through the technique Standard Test Methods for Specific Gravity of Solids (ASTM D 854-92). The bulk density had been examined.

The Volatile Matter (%), Ash Content (%), Fixed Carbon (%) and Higher Heat Value (MJ/kg) were investigated through the protocol: Standard Test Method for Ash in Biomass (ASTM E1755), Standard Test Method for Volatile Matter in the Analysis of Particulate Wood Fuels [E872 – 82 (Reapproved 1998)], FC was determined through difference respectively.

Ultimate analysis

Various chemical composites can be contained in a material. The method 'Ultimate Analysis' can be performed to analyze and identify the chemical compounds of that particular material (Sarkar et al., 2022).

In comparison to "Proximate Analysis' method, this process is more accurate. The sample of the plant species was put into this process and has been analyzed as follows: CEN/TS 15104:2005, Solid Biofuels – Determination of total carbon, hydrogen and oxygen content.

Compositional analysis

Many components comprise a material. To identify and measure the components of that particular material, Composition Analysis and materials identification processes are used to study them. Through the process, the components can be identified and compared to other similar materials. Here, the plant species' samples are evaluated to determine the presence of cellulose, lignins, hemicellulose and extractive value through the protocol-ASTM: American Society for Testing and Materials (ASTM International) Standards, 2015.

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Phytochemical studies

Preliminary Phytochemical screening

To identify the presence of active phytochemical components (Alkaloids, Proteins, Carbohydrates, Total Phenolic compound, Ascorbic acid, Tannins, Fat etc) Qualitative Phytochemical tests are conducted (Balamurugan et al., 2019; Ogbuagu et al., 2020; Junaid and Patil 2020; Sahira Banu and Cathrine, 2015; Silva et al., 2017; Singh and Kumar 2017). The method is performed as described below:

Test for carbohydrates

A. Benedict's test

To about 0.5 ml of the extracted solution was taken, in which 0.5 ml of Benedict's reagent was added. The mixture was heated for about 2 minutes in a boiling water bath. As a result, a red precipitate comes out, which refers to the presence of sugar in it.

B. Molisch's test

2 drops of alcoholic solution of α-napthol were mixed and shaken up with 2 ml of the extracted solution. Later, concentrated H_2SO_4 was dropped in the side wall of the test tube in a very small quantity of 2-3 drops. A violet ring appears, which proves the availability of sugar in it.

Test for Protein

A. Biuret test

A drop of 2% copper sulphate solution was added to around 2 ml of extracted solution. 1ml of 95% ethanol was added after that. KOH pellets were included next. In the ethanolic layer, a pink-coloured solution formed, indicating protein's presence.

B. Xanthoproteic test

A few drops of concentrated Nitric Acid (HNO3) was mixed with 2 ml of extracted solution, resulting in a yellow-coloured output. The colour indicates the presence of protein.

Test for Fat

A. Saponification test

A little bit of 0.5N alcoholic KOH and a little bit of phenolphthalein were added to around 2 ml of the extracted solution. About two hours were spent heating the mixture. The production of soap or the partial neutralisation of alkali are signs that fixed oils or fats are present in the sample.

B. Spot test

A small amount of the extract was putted on a filter paper and pressed with another filter paper, producing an oil stain. It is evident that fixed oil is available in it.

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 (Potassium bismuth iodide)

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 $FeCl_3$. 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

4 ml of 10% NaOH solution and around 0.4 ml of plant extraction solution were combined and then shaken vigorously for a while. Emulsion formation suggested the presence of hydrolysable tannins

B. Gelatin test

Plant extract is diluted in 5 ml of distilled water. Later, 10% sodium chloride and 1% gelatin solution were added. As tannin is present, the precipitate turns white in colour.

FTIR analysis

Another analytical tool is "Fourier Transform Infrared Spectroscopy" which is popular as FTIR analysis method. Basically, it is used to perform chemical identification of the components of any organic, inorganic or polymeric materials. In this process, the sample is scanned through an infrared light with a radiation of 10,000 to 100 cm^{-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 presents a spectrum generally from 4000 cm^{-1} to 400$cm^{-1,}$ which reflects a molecular fingerprint of the sample. Each and every chemical or molecule form will generate a unique and inimitable spectral fingerprint.

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Figure 3. Thermo Fisher Scientific NicoletTM iS10 FTIR Spectrometer, USA

Figure 4. GC-MS Model: Perkin Elmer Clarus 680 GC/ 600C MS

GCMS Analysis

GC-MS stands for Gas Chromatography-Mass Spectroscopy, an important tool to analyze the compounds forming a plant species. The combination of Gas Chromatography and Mass Spectroscopy is applied in the technique to perform the testing process.

Preparation of extract

The leaves of the plant have been powdered with the help of an electric grinder. Sieved and prepared methanolic extract for further GC-MS study.

GC Programme

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

NIST mass spectral library search program (Version 2.0g) has been used for spectrum analysis.

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.

Results and discussions

Results on Physico-chemical Contents

Physico-chemical parameters of the experimental plants have been investigated. The result shows that *Acalypha indica* possesses maximum values on Swelling index, volatile matter, ash contents, fixed carbon, higher heat value, hydrogen content, oxygen content, carbon content, hemicellulose content, lignin content and extractive values. In contrast, the percentage composition of bulk density, carbon content, and nitrogen content is higher in *Euphorbia hirta*.

Conclusion

Medicinal Plants derived novel phyto-constituents are now an appealing objective for the researchers and chemists as these bioactive chemical compounds are safer, reliable and accessible to the common man also (Sarkar et al., 2022; Acharya et al., 2021a; Acharya et al., 2021b; Acharya, 2016; Acharya, 2015; Sarkar et al., 2021; Alam et al., 2022; Sarkar and Madhu, 2017). One of the promising medicinal plants, *Acalypha indica* L., is growing in the Paschim Medinipur district of West Bengal, India. It has been found to contain many significant bioactive phytoconstituents.

The methanolic leaf extracts of two different plants have been screened through GC-MS, revealing many important bioactive phyto-constituents. *Acalypha indica* L. possesses Cyclotrisiloxane, Hexamethyl-, cis-2,4-dimethylamine, S,S-dioxide, 1- nonadecanamine, n, n-dimethyl-, tridecane, 2,2,4,10,12,12-hexamethyl-7-(3,5,5-trimethylhexyl)-, dodecane, 1-fluoro-, whereas, *Euphorbia hirta* L. possesses myo- inositol, 4-c-methyl-, z,z-6,28-heptatriactontadien-2-one, phytylpalmitate, hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-, 4-tert-octylphenol, tms derivative. Both these plants have chemical substances that have proven bioactivity in human welfare.

The GC-MS of leaf methanolic extract reveals that the phytochemical cis-2,4-dimethylamine, s, s-dioxide have Anticancer (stomach) activities, 1-nonadecanamine, N, N-dimethyl bears Antitumor (nasopharynx) activities, myo-inositol, 4-C- methyl bearing Myo-neuro-stimulant

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activities and Z, Z-6,28-heptatriactontadien-2-one having Increase zinc bioavailability activities. These activities of individual phytoconstituents have been

Table 2. Results on Physico chemical studies of *Acalypha indica and Euphorbia hirta*

Sample	BD	SI	VM	AC	FC	HHV	CC
Acalypha indica	0.26	1.456	75.452	11.555	12.993	16.267	39.322
Euphorbia hirta	0.236	1.06	55.289	10.242	12.365	15.088	42.6

Table 3. Results on Physico chemical studies of *Acalypha indica* and *Euphorbia hirta*

Sample	HC	OC	NC	CC	HEMC	LC	EC
Acalypha indica	6.289	32.795	1.158	61.727	4.372	4.531	1.163
Euphorbia hirta	4.56	27.32	2.05	38.453	2.327	4.42	0.749

BD=Bulk Density (gm/cm^3), SI=Swelling Index (%), VM=Volatile Matter (%), AC=Ash Content (%), FC=Fixed Carbon (%), HHV=Higher Heat Value (MJ/kg), CC=Carbon Content (%)
HC=Hydrogen Content (%), OC=Oxygen Content (%), NC=Nitrogen Content (%), CC=Cellulose Content (%), HEMC=Hemicellulose Content (%), LC=Lignin Content (%), EC=Extractive Content (%)

Table 4. Results on Phytochemical studies of *Acalypha indica and Euphorbia hirta*

Phytochemicals	Results	
	Acalypha indica	***Euphorbia hirta***
Carbohydrate	+	+
Protein	+	+
Total Fat	+	+
Alkaloids	+	+
Phenolics	+	+
Tannin	+	+

Results on FTIR analysis of both the plants

FTIR:

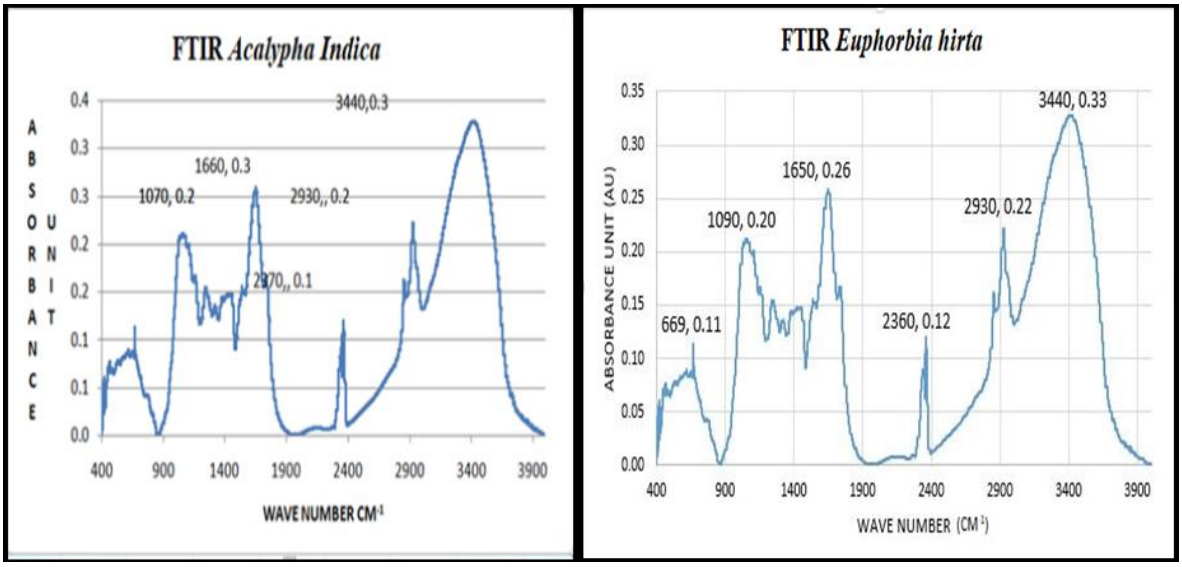

Figure 5. Various peaks correspond to absorbance and wave number of leaf of *Acalypha indica* and *Euphorbia hirta*

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Table 5. Peak position, functional group, chemical class and peak details of *Acalypha indica* and *Euphorbia hirta*

Plant specimen	Peak position	Range	Group	Class	Peak details
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
Euphorbia hirta	669	665-730	C=C Bending	Alkene	Strong
	1090	1087-1124	C-O Stretching	Secondary Alcohol	Strong
	1650	1600-1650	C=C Stretching	Conjugated Alkene	Medium
	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

Results on GCMS analysis of both plants

GCMS Chromatogram

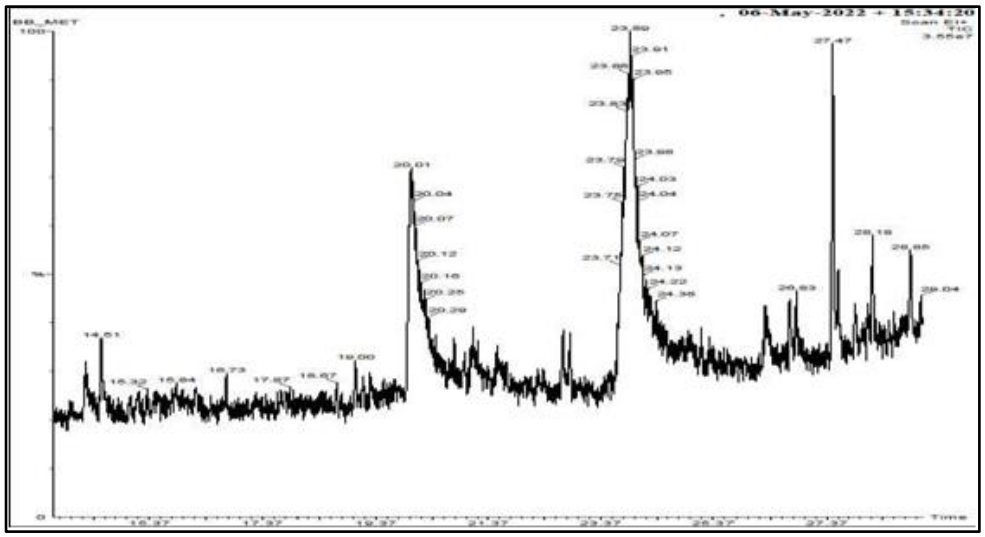

Figure 6. Chromatogram obtained from GCMS of leaf extract of *Acalypha indica* L. growing in Paschim Medinipur district, West Bengal, India

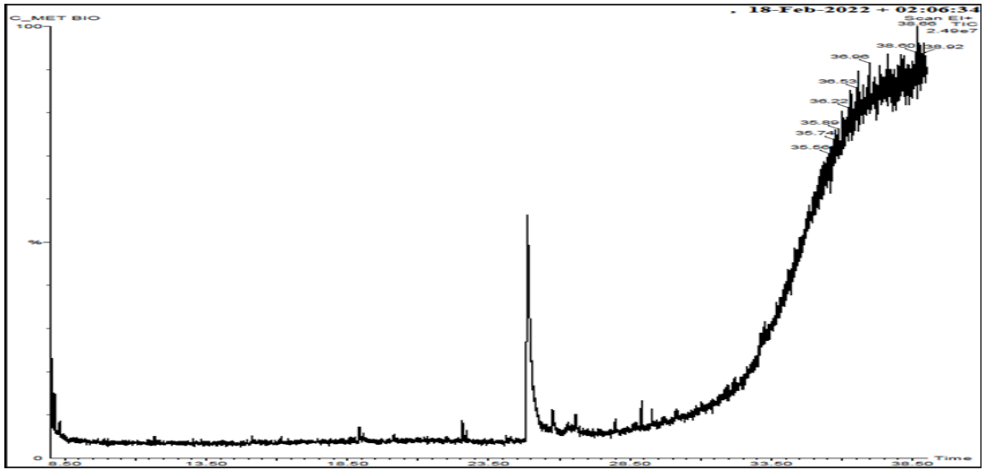

Figure 7. Chromatogram obtained from GCMS of leaf extract of *Euphorbia hirta* growing in Paschim Medinipur district, West Bengal, India

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Table 6. Individual Phyto-constituents, their retention time, molecular weight obtained from GCMS of leaf extract of *Acalypha indica* L. growing in Paschim Medinipur district, West Bengal, India

No.	*RT*	*Name of Compound*	*MW*	*Bioactivity*
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)-	394	No record found (Novel)
5	22.697	Dodecane, 1-fluoro-	188	No record found (Novel)

Source of Bioactivity: USDA: Dr. Duke's Phytochemical and Ethno-botanical Databases: U.S. department of agriculture. **RT**=Retention time, **MW**=Molecular Weight

Table 7. Individual phyto-constituents, their retention time, molecular weight obtained from GCMS of leaf extract of *Euphorbia hirta* L. growing in Paschim Medinipur district, West Bengal, India

No.	*RT*	*Name of Compound*	*MW*	*Bioactivity*
1	23.888	Myo-inositol, 4-C-methyl-	194	Myo-neuro-stimulant
2	27.474	Z,Z-6,28-heptatriactontadien-2-one	530	Increase zinc Bio-availability
3	31.371	Phytyl palmitate	534	No record found (Novel)
4	37.923	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethyl-	430	No record found (Novel)
5	38.864	4-tert-octylphenol, tms derivative	278	No record found (Novel)

Source of Bioactivity: USDA: Dr. Duke's Phytochemical and Ethno-botanical Databases: U.S. department of agriculture. **RT**=Retention time, **MW**=Molecular Weight

proven through Dr. Duke's Phytochemical and Ethno-botanical Databases: U.S. Department of Agriculture. This information might be helpful to the pharmacologists, chemists, etc., for future novel drug discovery.

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Conflict of interest

None

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