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Phytochemical Analysis and Bioactive Components of a Medicinal Plant *Acalypha paniculata* Miq.

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ABSTRACT: In the current study, ethyl acetate and methanol extracts of *Acalypha paniculata* were analyzed using Gas Chromatography and Mass Spectrometry (GC-MS) to identify the bioactive components. The preliminary qualitative phytochemical tests for the presence of carbohydrates, flavonoids, alkaloids, tannins, saponins, phenols, fatty acids, gums and mucilage were carried out according to the standard technique. Using Perkin-Elmer Gas Chromatography-Mass Spectra, the chemical contents of the methanolic and ethyl acetate extract of *Acalypha paniculata* were examined. The result revealed the presence of 7 compounds from ethyl acetate extract and 4 compounds from methanol extract whereas, Ethane, 1,2,2-trichloro-1,1-difluoro, Methane, oxybis[dichloro, Trichloromethane, Nonadecanoic acid, Methane, dichloronitro, L-Gala-L-Ido-octose, Methane, bromodichloro and Methane, oxybis[dichloro (47.214%), 4-amino-3-methoxypyrazolo[3,4-d] pyrimide (22.465%) were major compounds. The compounds identified by GC-MS in ethyl acetate and methanol extract may be medicinally important and possess different pharmaceutical applications. The identified phytocomponents needs further research on toxicological aspects to develop safe drugs.

Keywords: *Acalypha paniculata*, Phytochemistry, Secondary metabolites, GC-MS.

INTRODUCTION

Nature has an abundant amount of herbs that are rich in medicinal value and a number of innovative drugs are separated from plants since 1000 years ago. Different herbs are used as medicine for several diseases throughout the world (Siddiqui *et al.,* 2009). Plants are considered as major source of traditional medicine. Herbal medicine is the oldest form of health care system known to mankind. The medicinal value of the plant lies in several chemical substances that make a specific physiological action on the human body (Iniaghe *et al.,* 2009). Medicinal plants have been worth sources of bioactive compounds that were traditionally used for containing human ailments in the history of human life. Potentiality of medicinal plants worldwide is deeply rooted in the fact that many people, about 70–90%, depend on herbal drugs for primary health care system. Of course, many people particularly from the developing countries use the herbal medicines probably because it is entirely the poor people can afford them. Traditionally, the herbal medicines are taken in various forms such as decoctions, paste and infusion to treat human ailments such as skin fungal infections (Mlozi *et al.,* 2022). *Acalypha paniculata,* categorized under the family Euphorbiaceae scattered in Western Ghats in all districts of shady moist places and it is an erect herb about 1m tall, with minute hairs, leaves simple, alternate, broadly ovate 4-8×2-5cm, apex accuminate, subcordate or base rounded, margins are crenate, serrate, sparsely hispid membranous, basally 3-5 nerved, with 4-7cm long petiole. Flowers monoecious, very minute, male flowers in slender axillary spikes, tepals 4, stamens 8, filament 6-10 cm long, Female flowers in terminal panicle, bracteate, tepals 3-6, ovary 3 lobed, 3 locular, ovules one per locule, style filiform in 3 groups of 3 each. Fruit capsule, 5-2mm across with globose seeds (Bhavadharaniparkavi and Abirami 2020). Extracts of *A. paniculata* was used for treating pimples, stomach ache, kidney problems, hernia, body pain, anaemia and antidote. In India, *Acalypha paniculata* Miq. leaf and young shoots are used as vegetables (Gabrilla and Ameenah 2008).

The extraction of plant components is essential to isolate bioactive compounds which are aimed to understand their role in the treatment of a number of diseases (Muddasir *et al.,* 2018). Numerous phytochemicals have been used in clinical settings by thousands of physicians and tens of millions of people regularly consume them under medical supervision. Large-scale human exposure to natural products may result from using unprocessed plant extracts and medications made from natural chemicals, even by pharmaceutical corporations (Kavitha *et al.,* 2021). Alkaloids, phenols, flavonoids, saponins, essential oils, tannins, carbohydrates, and other chemical compounds with therapeutic qualities are among the biologically active chemical components (phytochemicals) found in

medicinal plants (Adegoke *et al.,* 1968)**.** India has a rich source of plants and many herbs and their products are known to exhibit medicinal properties for several diseases. Phytochemical screening plays an important role in finding new sources of medically and commercially significant substances, such as carbohydrates, alkaloids, phenolic compounds, flavonoids, tannins, saponins etc., in plants support their use in the treatment of wounds and burns in herbal medicine (Pradeep *et al.,* 2013).

Gas Chromatography- Mass Spectrography analysis is a suitable method to examine the quantitative amount of bioactive compounds in plant extracts (Sami 2022). For the investigation of medicinal plants, GC-MS studies have been used more and more. The most frequently employed approach for identification and quantification purposes is Gas Chromatography-Mass Spectroscopy, which is a hyphenated system and a very compatible technique (Vasthi and Karthiga 2018). Therefore, the objective of the current study was to investigate the phytochemical and GC-MS analysis in ethyl acetate and methanol extract of *Acalypha paniculata* Miq.

MATERIALS AND METHODS

The aerial parts of *Acalypha paniculata* Miq. were obtained from Eratti hills of the Burgur range reserve forest, which is located in the North East of Erode district, Tamil Nadu. The local floras (Gamble and Fischer), (Mattew, 1983), and the Botanical Survey of India, Southern region center, Coimbatore, Tamil Nadu, were used to taxonomically identify the plant material. "BSI/SRC/5/23/2019/Tech" is the herbarium number issued by BSI. The Vellalar College for Women (Autonomous), located in Erode, Tamil Nadu, India, 638 012, has a voucher specimen collection.

Preparation of Extracts (Anonymous 1966). The plant powder of *Acalypha paniculata* was extracted using Soxhlet apparatus with petroleum ether (60-80°C), hexane (63-70°C), chloroform (60°C), and alcohol (78°C). Powdered materials were dried in an air oven below 50°C before extracting with the next solvent each time. The extracts were dried over anhydrous sodium sulfate and kept in vials that were sealed in the refrigerator (5-8°C) until analysis. The aqueous extract was then obtained by macerating marc in chloroform water for 24 hours. The extract was concentrated by removing the solvent from it and then drying it out in a water bath.

Phytochemical Analysis

Qualitative phytochemical analysis. Qualitative phytochemical tests were conducted using a variety of extracts, including ethyl acetate and methanol. According to Kokate *et al.* (1995), standard procedures were used for the phytochemical analysis of plant extract to check for the presence of carbohydrates, alkaloids, fatty acids, saponins, tannins, phenols and flavonoids.

Test for proteins

(a) **Biuret test:** 1ml of the extract was mixed with an equivalent volume of a 40% sodium hydroxide solution and two drops of 1% copper sulfate. The presence of proteins is indicated by the emergence of the violet colour.

(b) **Ninhydrin test:** To the extract, 2 drops of freshly made 0.2% ninhydrin reagent were heated. The development of a pink colour shows the presence of proteins.

Test for carbohydrates. 5 ml of Benedict's and Fehling's reagents was added to 2 ml of extract under suitable conditions. The presence of carbohydrates is indicated by the reagents' ability to produce a brick red colour.

Test for alkaloids. A few drops of diluted H_2SO_4 were added to 1 ml of extract, and the filtrate was then individually treated with Dragendorff's, Hager's, Mayer's, and Wagner's reagents. Alkaloids are present when orange brown, yellow cream, pink, and reddish brown precipitates form in reaction to the above mentioned reagents, respectively.

Test for flavonoids. To 1 ml of the plant extract sodium hydroxide solution was added. The appearance of pink or red-colored foam was an indicator of the presence of flavonoids.

Test for Glycosides. Aqueous NaOH solution was added after a little quantity of plant powder had been dissolved in 1 ml of water. Glycosides are present when a yellow colour appears.

Test for fixed oils and fats. Between two filter papers, a drop of concentrated extract was placed and left alone. The presence of oils and fats is shown by the oil stains on the paper.

Test for saponins. The alcoholic and aqueous extracts were dissolved separately in 20 ml of water and shaken in a graduated cylinder for 15 minutes. It was considered a positive reaction when a layer of foam measuring 1 cm thick.

Test for tannins and phenols. A small amount of aqueous and alcoholic extracts were dissolved in water, and 5% FeCl3 was then added. Tannins and phenols are present when a dark bluish black colour is observed.

Test for steroids. Salkowski's test: An equal volume of concentrated sulfuric acid was added after the extract was dissolved in 2 ml of chloroform. Steroids are present when the test tube's upper layer goes red and its lower layer turns yellow with a green fluorescence.

Test for terpenoids. To the extract, 2ml of chloroform and conc. H_2SO_4 was added and the mixture was heated for 2 mints. Terpenoids are present because of the formation of a grey colour.

Test for Coumarin. 10% NaOH was added to 2 ml of extract and shaken for 5 minutes. The presence of coumarin is indicated by the appearance of yellow colour.

Test for gums and mucilages. 25 ml of pure alcohol should be slowly mixed with 10 ml of aqueous extract. Gums and mucilage are present when there is precipitation.

Quantitative phytochemical analysis

Estimation of total alkaloids (Ferguson, 1956). Separately, 100 mg of plant powder was consumed and soaked in alcohol for 24 h. After filtering, the filtrate was extracted in 0.1 N HCl and partitioned in a separating funnel with chloroform. The aqueous layer

was basified with ammonium hydroxide to pH and partitioned with chloroform in a separating funnel after the chloroform layer was rejected. The chloroform layer was evaporated and the resulting material was treated as total alkaloid after the aqueous layer was rejected.

Estimation of total Flavonoid (Shahiladevi and Jegadeesan 2006). Fifty ml of distilled water was added to twenty ml of ethyl acetate extract. After an hour, precipitation was observed. This precipitate was recovered through filtering. The precipitate was also heated slowly for 5 mints, agitated for 15 mints to dissolve it in 100 ml of chloroform, and then filtered while still hot. The insoluble portion of the chloroform that was still on the filter paper was dissolved in ethyl acetate and then crystallized with methanol. The soluble portion of the chloroform was discarded. The residue that was left over was then weighed.

Estimation of total terpenoids (Ferguson, 1956). A hundred milligrams of plant powder was immersed in ethanol for 24 h. The extract was filtered, and using a separating funnel, petroleum ether was separated from the filtrate. The total terpenoids were used to treat the ether extract.

Estimation of total saponin (Nahapetian and Bassiri 1975). 10g of plant powder was dissolved in 100 ml of 20% ethanol to create the suspension. This sample suspension was continuously stirred while being heated in a water bath for 4 hours at 55ºC. This sample was filtered, and the extracted liquid was put in a beaker with a capacity of 200 ml. Re-extraction of the obtained residue was done using 100ml of 20% ethanol. A water bath was used to heat the mixture of extracts until the volume was reduced to 40 ml. The concentrate was poured into a 250 ml separating funnel, agitated quickly, and then 10 ml of diethyl ether was added. While the ether layer was discarded, the aqueous layer was recovered. The cleansing procedure was repeated. N-butanol (30 ml) was added. Two washes of 10 ml of 5% aqueous sodium chloride were performed on the combined n-butanol extracts. In a water bath, the residual solution was warmed. The sample was weighed and dried in the oven following evaporation.

$$\text{Weight of saponins } \% = \frac{\text{Weight of saponins}}{\text{Weight of sample}} \times 100$$

Estimation of Tannin –free total glycoside (Shahiladevi and Jegadeesan). A hundred grams of air dried powder was extracted using ethanol and water (2:1). The tannins found in the resulting aqueous ethanol extracts typically obstruct biological processes. Therefore, this needs to be eliminated by treating with a 5% neutral lead acetate reagent, which precipitates the tannins as lead tannate. The precipitated lead tannate was filtered out after the aqueous ethanol solution was treated with an 8% neutral lead acetate solution. Until no more precipitate is obtained, this process is repeated. The excess unprecipitated lead ions in the solutions were now present in the clear filtrate, and they were eliminated by adding H_2S gas to the solution. Remove the lead ions using this complicated, insoluble black lead sulfide. The filtering of the black precipitate was typically reported until no more black precipitate was produced and the solution had a strong H_2S odour. The normally syrupy solution was concentrated over a water bath that was kept at a temperature of 55°C. This technique was used to get rid of the extra H_2S. From the above results, the tannin contents of the sample were calculated as follows:

Tannin (%) = Total glucosides (%) – Non–tannin glycosides (%).

Statistical analysis. The samples were examined in triplicate for the purpose of quantifying phytochemical and the values were represented as Mean Standard error (S.E).

GC-MS Analysis (Jennings and Shibamoto 1980). Therma GC-Trace Ultra ver. 5.0 was used for the GC-MS analysis of the methanol (A&B) and ethyl acetate (A&B) fractions of *Acalypha paniculata*, and a gas chromatograph interfaced to a mass spectrometer equipped with a thermo MS DS Q II fused a DB35-MS capillary standard non-polar column (30 × 0.25m ID × 0. For GC-MS detection, an injection volume of 1 ml was used (with a split ratio of 10:1), along with an impact mode with an ionization energy of 70 eV Helium as the carrier gas. The temperature of the injector was kept constant at 250°C. The oven temperature was set to 150°C for 2 minutes, then to 220ºC for 5 minutes, then to 260ºC for 10 minutes, and finally to 350ºC for 20 minutes of isothermal cooking. At 70 eV, mass spectra were recorded. 0.5 second scan time with fragment sizes ranging from 50 to 650 Da. The entire running time for the GC-MS was 37.50 mints and the solvent delay ranged from 0 to 4 mints. Comparing each component's average peak area to the total areas allowed us to determine the proportional percentage amount of each component.

RESULT AND DISCUSSION

Acalypha paniculata plant extracts made from ethanol and methanol were subjected to phytochemical analysis to check for the presence of several bioactive components.

The phytochemical screening of ethyl acetate and methanol extract of *Acalypha paniculata* have shown the presence of various medicinally active constituents represented in Table 1. The result of phytochemical analysis obtained from ethyl acetate extract of *Acalypha paniculata* indicated the presence of protein, carbohydrate (Fehling's reagent), alkaloids, flavonoids, fixed oil and fats, tannins and phenols, steroids, terpenoids and coumarin were present, whereas carbohydrate (Benedict's reagent), glycosides, saponin, gums and mucilage were tested absent. These results are consistent with a previous investigation by Satyaprasad *et al.* (2015) in *Acalypha indica* and *Croton bonplandianum.* Methanolic extract showed the presence of protein, carbohydrates, alkaloids, flavonoids, glycosides, fixed oil and fats, saponins, tannins and phenols, steroids, terpenoids and coumarin in *Acalypha paniculata.* Protein, alkaloids, tannins, glycosides, phenols, coumarin and terpenoids were present in methanol extracts of *Jatropha gossypiifolia* (Reetu Dubey *et al.,* 2020), *Euphorbia hirta* (Aska and Kubmarawa 2016) except gums and mucilage.

The quantitative examination of phytoconstituents such as flavonoids, alkaloids, terpenoids, tannins and saponins are shown in Table 2. The highest percentage was represented in flavonoids (0.42±0.12) and in *Euphorbia hirta* lowest percentage of flavonoids was present (Aska *et al.,* 2019). The amount of total flavonoids (0.42±0.12) is found higher followed by total alkaloids (0.30± 0.05). The present investigation showed that tannin content was present in the extract (0.108± 0.08). These observations conform with the previous reports of phytochemical screening in *Euphorbia cuneata* (Awaad *et al.,* 2017) tannin content was lower (9.15 ± 1.21). The number of total terpenoids and total saponins were (0.172±0.10) and (0.120±0.09). According to Aska *et al*. (2019), this is following the most recent studies on *Euphorbia hirta.*

The results of GCMS analysis revealed the presence of seven and four major separated compounds in methanol and ethyl acetate fractions of *Acalypha paniculata.* The compounds are identified based on the Retention Time (RT), Peak area (%), Molecular Weight (MW), Molecular Formula (MF), Nature of compounds and activities. Gas chromatogram of ethyl acetate and methanol extract of *Acalypha paniculata* are shown in Fig. 1 and 2 respectively. The compounds in ethyl acetate fractions with the highest percentage were Trichloromethane, Methane, bromodichloro, L-Gala-L-Ido-octose, Ethane, 1,2,2-trichloro-1,1-difluoro, Methane, oxybis[dichloro, Methane, dichloronitro and Nonadecanoic acid in the proportion of 21.995%, 17.057%, 16.418%, 13.018%, 12.733%, 10.661%, 8.110% respectively (Table 3). Whereas, in the methanol fractions, the compounds with the highest percentage were Methane, oxybis[dichloro, 4-amino-3-methoxypyrazolo[3,4-d] pyrimide, Methane, dichloronitro and Propanenitrile,3-(5-diethylamino-1-methyl-3-pentynyloxy) in the proportion of 47.214, 22.465%, 16.114%, 14.207% respectively (Table 4). Similarly, 4-amino-3-methoxypyrazolo [3,4-d] pyrimidne and propanenitrile, 3-(5-diethylamino-1-methyl-3-pentynyloxy) was the main component present in the ethanolic leaf extract of *Acalypha indica* (Azhagumadhavan, 2021).

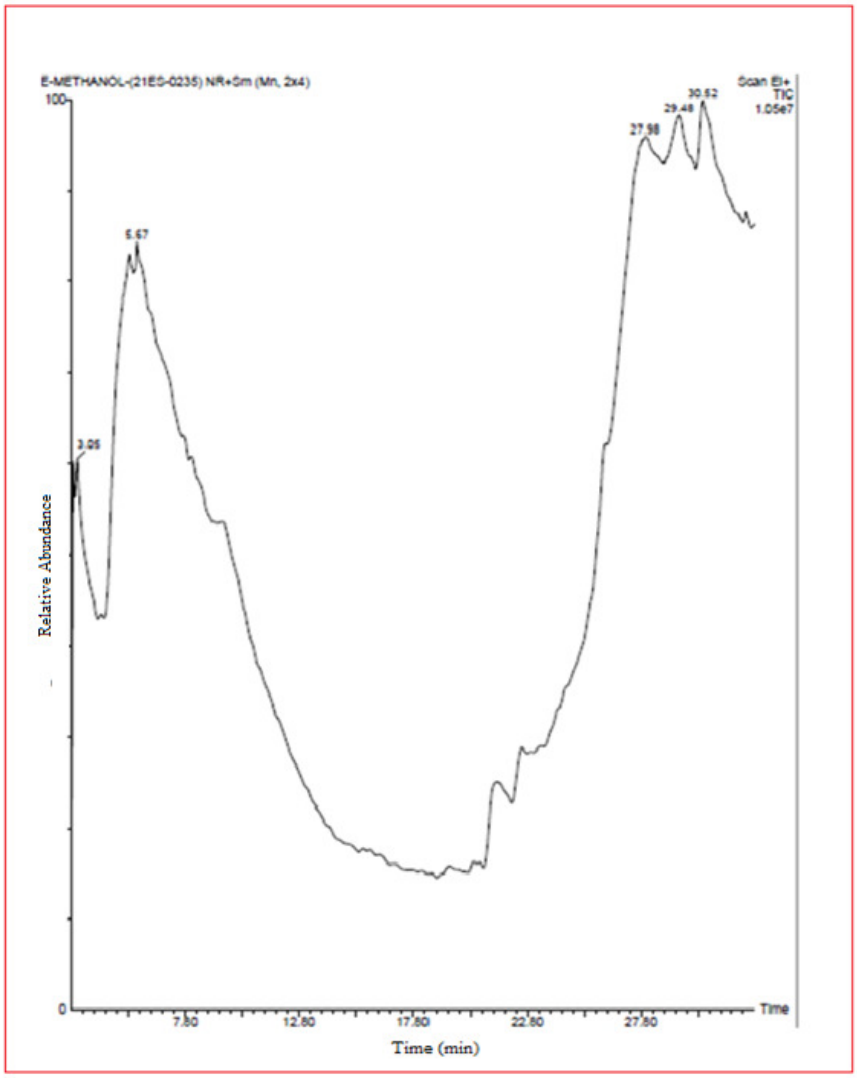


Fig. 1. GC-MS chromatogram of ethyl acetate fraction – EAAS3 of *Acalypha paniculata.*

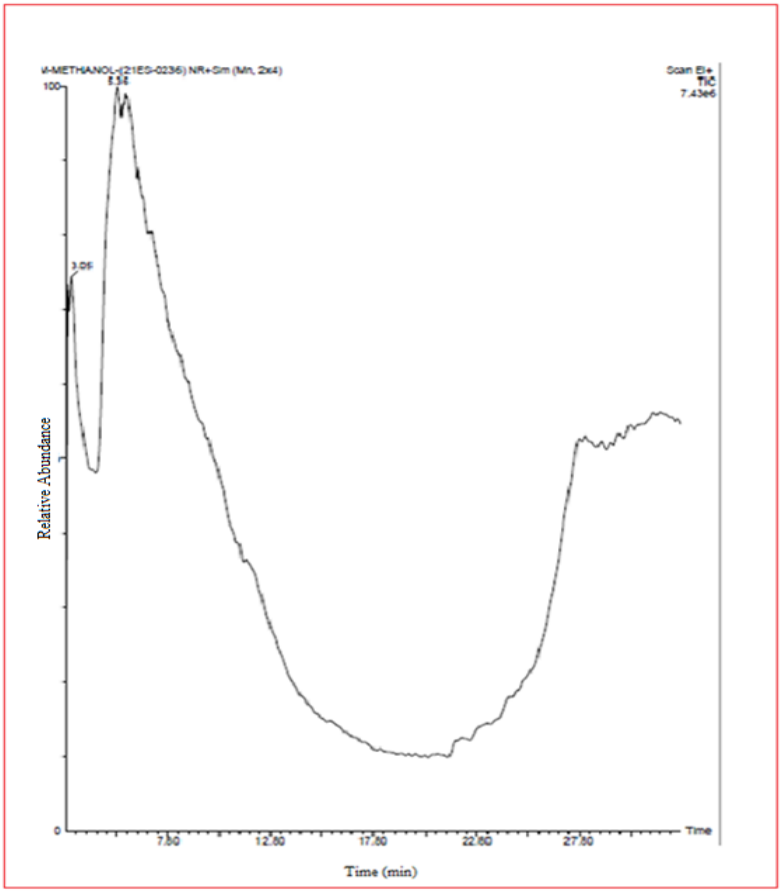


Fig. 2. GC-MS chromatogram of methanol fraction – MEAS3 of *Acalypha paniculate.*

Table 1: Qualitative phytochemical analysis of successive solvent extracts of *Acalypha paniculata* plant powder.

Sr. No.	Phytoconstituents	Extracts	
		Ethyl acetate	Methanol
1.	Protein	+	+
		+	+
2.	Carbohydrates	+	+
		-	+
3.	Alkaloids	+	+
		+	+
		+	+
4.	Flavonoids	+	+
5.	Glycosides	-	+
6.	Fixed oils and fats	+	+
7.	Saponins	-	+
8.	Tannins and phenols	+	+
		+	+
9.	Steroids	+	+
10.	Terpenoids	+	+
11.	Coumarin	+	+
12.	Gums and mucilage	-	-

"+" indicates presence while "-" stands for absence

Table 2: Quantitative phytochemical studies of *Acalypha paniculata* plant powder.

Sr. No.	Phytoconstituents	Values (%)
1.	Alkaloids	0.30± 0.15
2.	Flavonoids	0.42±0.12
3.	Terpenoids	0.172±0.10
4.	Saponins	0.120±0.09
5.	Tannins	0.108±0.08

Values are means of three independent analysis of the extract ± SE (n = 3)

Table 3: GC-MS Profiling of Ethyl acetate fraction – EAAS3 of *Acalypha paniculate.*

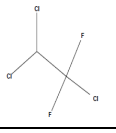
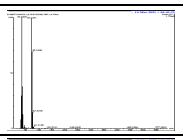
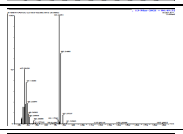
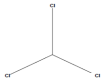
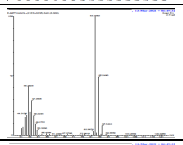
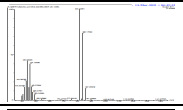
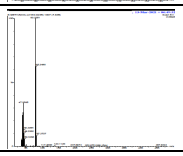
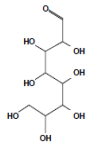
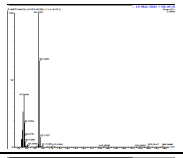
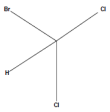
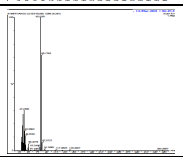
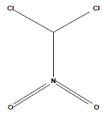
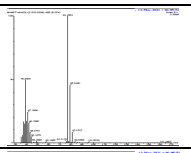
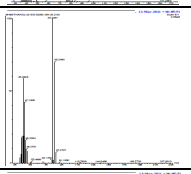
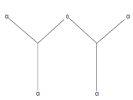
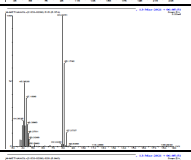
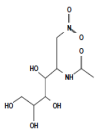
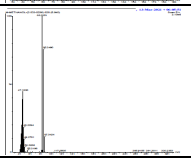
Sr. No.	Name of the compound	Structure	Hit spectrum	Molecular Formula	Molecular Weight	Retention time	Peak Area %
1.	**Ethane, 1,2,2-trichloro-1,1-difluoro**			$C_2HCl_3F_2$	168	4.784	13.018
2.	**Methane, oxybis[dichloro**			$C_2H_2OCl_4$	182	5.660	12.733
3.	**Trichloromethane**			$CHCl_3$	118	6.000	21.995
4.	**Nonadecanoic acid**			$C_{19}H_{38}O_2$	298	6.135	8.110
5.	**Methane, dichloronitro**			CHO_2NCl_2	129	7.835	10.669
6.	**L-gala-l-ido-octose**			$C_8H_{16}O_8$	240	8.371	16.418
7.	**Methane, bromodichloro**			$CHCl_2Br$	162	9.291	17.057

Table 4: GC-MS Profiling of Methanol fraction – MEAS3 of *Acalypha paniculata*

Sr. No.	Name of the compound	Structure	Hit spectrum	Molecular Formula	Molecular Weight	Retention time	Peak Area %
1.	**Methane, dichloronitro**			CHO_2NCl_2	129	5.074	16.114
2.	**4-amino-3-methoxypyrazolo[3,4-d] pyrimidne**			$C_6H_7H_5O$	165	5.219	22.465
3.	**Methane, oxybis[dichloro**			$C_2H_2OCl_4$	182	5.374	47.214
4.	**Propanenitrile,3-(5-diethylamino-1-methyl-3-pentynyloxy)**			$C_{13}H_{22}N_2O$	222	5.940	14.207

Chandra Mohan *et al.,* (2012) studied the GC-MS analysis of *Acalypha indica* leaf and the presence of five compounds like 1H-Pyrrole-2,5-dione,1- ethenyl, 3,8-Nanodiene-2-one, (E), Proline, 3,4- didehydro-, 4-Amino-3-methoxypyrazolo[3,4d] pyrimidine, Propanenitrile, 3-(5-diethylamino-1-methoxy-3-pentynyloxy) were reported. These findings are in agreement to our study. The identified compounds in the ethyl acetate and methanol extract of *Acalypha paniculata* possessed many pharmacological and biological activities (Table 3 and 4).

CONCLUSIONS

The GC-MS analysis of *Acalypha paniculata* determines the number of phytochemicals in their plant extract. Most plant extracts had some biological activities which are used in the field of medicine. These plants were used from ancient times in foods and medicines to treat pimples, stomach aches, kidney problems, hernia, body pain, anaemia and antidote. So that it can be recommended as a plant phytopharmaceutical importance. Further investigations are required to determine the nature and toxicity of substances present in the *Acalypha paniculata*.

FUTURE SCOPE

Generally, the presence of secondary metabolites in plants provides phytoconstituents, which supports the therapeutic effect of future drug preparations.

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Conflict of Interest. None.

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