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Phytochemical Screening and GC-MS Analysis of Acetone Leaf Extract of *Acalypha indica* (Linn.)

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Abstract: *Acalypha indica distributed in the southern part of India particularly in Tamilnadu has potential medicinal properties and it is used as diuretic, anthelmintic and for respiratory problems such as bronchitis, asthma and pneumonia. Phytochemical screening of leaves extract revealed the presence of alkaloids, tannins, steroids, saponins, terpenoids, flavanoids, cardiac glycosides and phenolic compounds. The aim of this study was to screen the phytochemicals present in the leaf of Acalypha indica and further analysis of the components present in it by GC-MS analysis. The leaves were sequentially extracted based on the polarity viz., petroleum ether, chloroform, ethyl acetate, acetone and methanol. The acetone extract showed the presence of all phytoconstituents studied. The GC-MS analysis of the acetone extract revealed the presence of five major compounds.*

Keywords: *preliminary phytochemical analysis, GC-MS acetone leaf extract.*

1. INTRODUCTION

Acalypha indica it is a common annual herb, found mostly in the backyards of houses and waste places throughout the plains of India. Plants are emetic, expectorant, laxative and diuretic; useful in bronchitis, pneumonia, asthma and pulmonary tuberculosis. Leaves are laxative and antiparasiticide; ground with common salt or quicklime or lime juice applied externally in scabies. Leaf paste with lime juice is prescribed for ringworm; leaf juice is emetic for children. A decoction of the leaves is given in earache. Powder of the dry leaves is given to children to expell worms; also given in the form of decoction with little garlic. In homoepathy, the plant is used in severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis. The plant contains kaempferol, a cyanogenetic glucoside, a base, triacetonamine and an alkaloid, acalyphine. It also contains the amide, acalyphamide and some other amides, 2-methylanthraquinone, tri-O-methyl ellagic acid and γ-sitosterol, ß-sitosterol glucoside, stigmasterol, n-octacosanol, quinine, tannin, resin and essential oil [6]. The plant is traditionally used as an expectorant against asthma and pneumonia, and also as an emetic, emmenagogue and anthelminthic [12]. *Acalypha indica* contains acalyphine which is used in the treatment of sore gums [2]. The plant is reported to have a post-coital antifertility effect [12], anti-venom properties [1], wound healing effects [15], antioxidant activities [11], anti-inflammatory effects [8], acaricidal effects [13], diuretic effects [5] and anti bacterial activities [7]. Till now, the investigation of phytocomponents by GC-MS has not been done on *Acalypha indica*. In the present study, the acetone leaf extract of *Acalypha indica* were evaluated for GC-MS analysis.

2. MATERIAL AND METHODS

Fresh and healthy *Acalypha indica* leaves were collected from Annamalai University campus Annamalai nagar, Cuddalore district, Tamilnadu. The plant was taxonomically identified by using flora of Madras presidency. Herbarium was deposited in Department of Botany, Annamalai University, (Voucher specimen No: AUBOT ≠ 249). In the laboratory, the leaves were washed 2-3 times with running fresh water followed by rinsing with distilled water, were shade dried at room temperature then pulverized into powder and stored in air tight container till further use. 100 g of leaves of *Acalypha indica* were subjected to successive extraction with different solvents in increasing polarity *viz*. petroleum ether, chloroform, ethyl acetate, acetone and methanol using soxhlet apparatus for phytochemical screening as per the method given by [10] the solvents were evaporated through

rotary vacuum flash reduced pressure and stored in desiccator at 4 ºC. The acetone extracts was used for GC-MS analysis.

2.1. Gc-Ms Analysis

Acetone extract of *Acalypha indica* leaves was analyzed with the help of GC-MS analyzer (GC Clarius 500 Perkin Elmer). On Elite-1 column the data was generated. The carrier gas helium (99.999%) was used at flow rate of 1 ml per min in split mode (10: 1). 8 µl of acetone sample was injected to column at 250 ºC injector temperature. Temperature of oven starts at 80 ºC and hold for 2 min and then it was raised at rate of 10 ºC per min to 200 ºC without holding. Holding was allowed for 9 min at 280 ºC at program rate of 5 ºC per min. Temperature of ion source was maintained at 200 ºC. The injector temperature was set at 230 ºC and detector temperature was set at 260 ºC. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode from 45 to 450 Da atomic mass units. A 0.5 seconds of scan interval and fragments from 45 to 450 Da was maintained. Total running time was 40 minutes.

2.1.1. Identification of Components

Identification was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the version (2005), software, Turbomas 5.2. This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extracting these compounds.

3. RESULT AND DISCUSSION

3.1. Phytochemical Screening

In the present study, the phytochemical screening was studied with petroleum ether, chloroform, ethyl acetate, acetone and methanol extract of the leaves of *Acalypha indica*. The results revealed, acetone leaf extracts of *Acalypha indica* recorded the presence of alkaloids, terpenoids, steroids, flavonoids, phenolic compounds, tannins and saponins followed by other extracts (Table.1) Phytochemical constituents such as tannins, flavonoids and several other aromatic compounds or secondary metabolites of plants serve as defense mechanism against predation by many microorganisms. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, cardiac glycosides, phenolic compounds, saponins and steroids [3]. The presence of alkaloids, saponins, flavonoids, phenolic compounds, tannins, steroids and terpenoids in the leaf extract are very important and are used in analgesic, anti plasmodic and bactericidal activities [14]. Thus the preliminary screening test may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Table1. *Preliminary Qualitative Phytochemical analysis of leaves extracts of Acalypha indica L.*

S. No	Phytoconstituents	Petroleum ether	Chloroform	Ethyl acetate	Acetone	Methanol
1	Alkaloids	+	+	-	+	-
2	Cardiac glycosides	-	-	-	-	-
3	Terpenoids	-	+	+	+	+
4	Steroids	+	+	+	+	+
5	Flavonoids	-	+	+	+	+
6	Phenolic compounds	-	-	+	+	+
7	Tannins	-	-	-	+	-
8	Saponins	-	-	+	+	+

(+) = Positive (present); (-) =Negative (absent)

3.1.1. Gc – Ms Analysis

The compounds present in the acetone leaf extract of *Acalypha indica* were identified by GC-MS analysis are presented in Figure -1. The active principle, Molecular Weight (MW), Concentration (%), Molecular Formula (MF), and Retention Time (RT) is presented in Table 2. More than five major compounds were identified in the extract being 1,3-Dioxolane, 4- Ethyl-5-Octyl-2,2-Bis (Trifluoromethyl)-, Trans- (28.924 %), Clotrisiloxane, Hexamathyl- (15.508 %), Trimethyl [4-(1,1,3,3, Tetramethylbutyl) phenox] Silane (8.543 %), Phenol,24 BIS(1,1-Dimethylethyl) (3.232%) and 2-Methyl-3(3-Methyl-But-2 Enyl)-2-(4-Methul-Pent-3-Enyl)-Oxetane (2.961%) respectively along with other minor constituents. The identified compounds in the leaf of acetone extract of *Acalypha indica* possessed many biological properties. Among the identified phytochemicals, 2 hydroxy-4-methoxy benzaldehyde a phenolic compound have the property of antioxidant and antifungal activity [9]. Ethanolic extract of *Acalypha indica* was subjected to GC-MS analysis for identification of phyto constituents are screened, and most of the constituents are 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)-compounds [4]. Thus, this type of GS-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

Table2. *Compounds identified in acetone leaf extract of Acalypha indica*

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
1	12.872	Phenol,24 BIS(1,1-Dimethylethyl)	$C_{14}H_{22}O$	206	3.232
2	18.000	2-Methyl-3(3-Methyl-But-2 Enyl)-2-(4-Methul-Pent-3-Enyl)-Oxetane	$C_{15}H_{26}O$	222	2.961
3	26.518	Trimethyl[4-(1,1,3,3, Tetramethylbutyl) phenox] Silane	$C_{17}H_{30}OSi$	278	8.543
4	26.808	Clotrisiloxane, Hexamathyl-	$C_6H_{18}O_5Si_4$	282	15.508
5	28.544	1,3-Dioxolane, 4- Ethyl-5-Octyl-2,2-Bis (Trifluoromethyl)-, Trans-	$C_{15}H_{24}O_2F_6$	350	28.924

4. CONCLUSION

From the present study it is concluded that the maximum extraction of phytochemicals was observed in acetone extract than petroleum ether, chloroform, ethyl acetate and methanol. More over Gas Chromatography and Mass spectrometry analyses showed the existence of various compounds with variable chemical structures which suggests the contribution of these compounds on pharmacological activities. Hence, the leaves of *Acalypha indica* may be utilized for the development of traditional medicines and further investigation is needed to elute novel active compounds which may create the new way to treat many incurable diseases.

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VIT Qualitative Report

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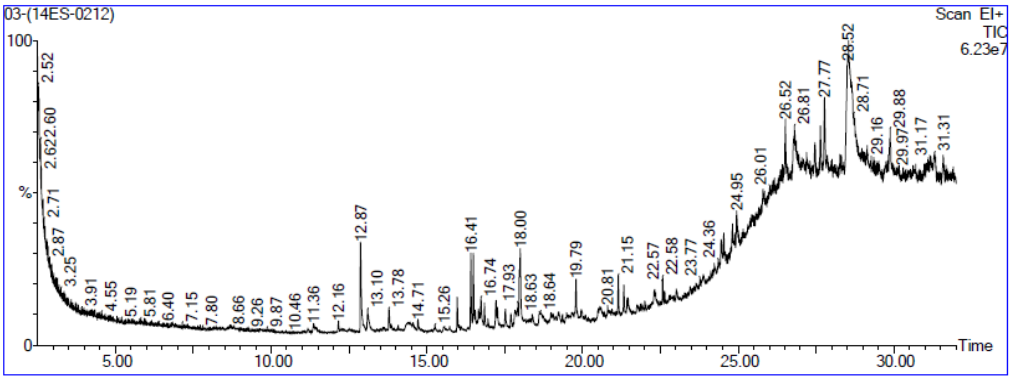

Fig1. *GC-MS analysis results of Aclypha indica L. with acetone leaf extracts*