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Chemical investigation of aerial parts of *Acalypha fruticosa* forssk

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ABSTRACT

Acalypha fruticosa Forssk. [Family Euphorbiaceae] commonly known as 'Chinnichedi' and 'Birch-leaved Acalypha' is a strong smelling bushy shrub traditionally used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites. In the present study the aerial parts of Acalypha fruticosa were analysed for phytochemicals and minerals. Qualitative analysis of phytochemicals of the various extracts of the aerial parts of Acalypha fruticosa indicated the presence of triterpenoids, steroids, saponins, tannins, phenols, flavonoids, alkaloids, anthraquinones and sugars. Quantitative estimation of phytoconstituents in the powdered samples of Acalypha fruticosa showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and steroids. 1, 2- Benzenedicarboxylic acid diisooctyl ester, n-Hexadecanoic acid, 9, 12-octadecadienoic acid [z, z], α-D-glucopyranoside and eicosyltrichlorosilane were identified by Gas Chromatogram-Mass spectrometry [GC-MS] analysis of the extracts. Potassium, sodium, calcium, magnesium, sulphur, zinc, copper, iron, manganese, boron and molybdenum were estimated using atomic absorption spectrophotometer. Phytochemicals and minerals analysed in the present study may account for the medicinal properties of Acalypha fruticosa.

Keywords: *Acalypha fruticosa*, phytochemicals, minerals, GC-MS, atomic absorption spectrophotometer

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INTRODUCTION

Acalypha fruticosa Forssk. [Family Euphorbiaceae] commonly known as 'Chinnichedi' and 'Birch-leaved Acalypha' is a strong smelling and bushy shrub. *Acalypha fruticosa* is used to treat dyspepsia, stomachache, skin diseases, wounds and poisonous bites. [1-7]. In Yemen, leaf and stem have been used to treat skin diseases, malaria and wound [8]. In Tanzania, it is used to treat fungal infections and a leaf decoction is drunk to treat epilepsy. A leaf infusion is taken to treat stomach problems and swellings of the body. Leaf maceration is used in eye infections. Leaf sap is used as nose drops to treat cough and chest problems. Leaf paste is applied to scabies and

sores. Stems ground in water are applied to wounds of animals [9]. Several pharmacological studies have revealed its antidiarrhoeal [10], antioxidant, anti-inflammatory [11], anticancer [12], antiplasmodial [13], wound healing [14] and cytotoxic properties [15]. However there are no reports on the detailed chemical investigation of this potent medicinal plant. The present study was undertaken to analyze the phytochemicals and minerals present in the aerial parts of *Acalypha fruticosa*.

MATERIALS AND METHODS

Plant material

Aerial parts of *Acalypha fruticosa* were collected from Courtallam hills, Western Ghats of South India, Tamil Nadu. The plant was identified by Dr. V. Chelladurai, Research officer (Botany), Survey of Medicinal and Aromatic Plants Unit–Siddha, CCRAS, Palayamkottai, Tirunelveli District, Tamil Nadu, India. A voucher specimen (MSU-38) has been kept in the Herbarium of the Department of Pharmaceutical Chemistry, Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

Preparation of powder and extract

The aerial parts were shade-dried and pulverized to powder in a mechanical grinder. The powder (1kg) was successively extracted with various solvents such as petroleum ether (40°-60°C), chloroform, ethanol and water. The extracts were concentrated under reduced pressure in a rotary evaporator (Buchi, USA). The powder and extracts of the plant were used for phytochemical studies.

Qualitative phytochemical analysis

The qualitative phytochemical tests for steroids, reducing sugars, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins, tannins and anthraquinones were carried out on the concentrated extracts using the standard procedures to identify the constituents as described by Brinda *et al*. [16].

Quantitative estimation of phytoconstituents

Quantitative estimation of phytoconstituents like alkaloids [17], flavonoids [18], tannins and phenols [19], saponins [20] and steroids [17] were carried out in the powdered samples of *Acalypha fruticosa.*

Isolation and characterization of chemical compounds by GC-MS analysis

The fraction of the extract of petroleum ether (40°-60° C) and the ethanolic extract of *Acalypha fruticosa* extract were subjected to Gas Chromatogram- Mass spectrometry (GC-MS) analysis.

GC-MS analysis of the extracts was carried out on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30mm x 0.25mm ID x 1 µMdf, composed of 100 % Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99. 999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 µl was employed (split ratio of 10:1); injector temperature 250 ^{o}C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5 °C / min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Interpretation on mass spectra of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST). The mass spectrum of the

unknown component was compared with that of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Estimation of mineral elements

The amount of potassium, sodium, calcium, magnesium, sulphur, zinc, copper, iron, manganese, boron and molybdenum in the powdered sample was quantitatively estimated using atomic absorption spectrophotometer (Solaar AA series, Atomic Absorption Spectrophotometer).

RESULTS AND DISCUSSION

The qualitative analysis of the phytochemicals of the various extracts of the aerial parts of *Acalypha fruticosa* indicated the presence of triterpenoids, steroids, saponins, tannins, phenols, alkaloids, flavonoids, anthraquinones and sugars in *Acalypha fruticosa* (Table 1). Quantitative estimation of phytoconstituents present in the powdered samples of *Acalypha fruticosa* showed that flavonoids were present in high amount when compared to alkaloids, tannins, phenols and steroids (Table 2). These phytoconstituents are known to show medicinal activity [21]. Soladoye *et al*. [22] reported the presence of alkaloids, tannins, saponins, and cardenolides in *A. fimbriata, A. hispida, A. ornata, A. racemosa* and *A. wilkesiana.* The presence of terpenoids and flavonoids in *A. fruticosa* is confirmed with the reports of Mothana *et a*l. [12].

GC-MS chromatogram of the ethanolic extract of *A. fruticosa* (Figure-1) showed three peaks indicating the presence of three compounds. The chemical compounds in the ethanolic extract of *Acalypha fruticosa* are presented in Table 3, with their retention time (RT), molecular formula, molecular weight and peak area (%). The compounds identified were 1, 2-Benzenedicarboxylic acid diisooctyl ester, n-Hexadecanoic acid and 9, 12-Octadecadienoic acid [z, z]. GC-MS chromatogram of the fraction of the extract of petroleum ether (40°-60°C) showed 8 peaks (Figure-2), of which, two peaks (peak-6 and peak-8) were prominent. When the mass spectra of these two peaks/compounds were compared with those of the compiled data for known compounds, peak with Retention time 12.29 was found to be identical with

α-D-glucopyranoside and the peak with Retention time 13.85 was identified as Eicosyltrichlorosilane. Presence of the anti-oxidant compounds like n-Hexadecanoic acid and 9, 12-Octadecadienoic acid [23] may possibly play a role in curing skin diseases.

Table 4 shows the results of quantitative estimation of minerals in the dried powder of *Acalypha fruticosa*. The concentration of macro elements (K, Na, Ca, Mg and S) ranged from 0.01% to 4.23% and that of the microelements (Zn, Cu, Fe, Mn, Bo and Mo) ranged from 0.02 ppm to 87.62 ppm. Of the macro elements analyzed, calcium was present in high amount followed by magnesium and potassium. Among the minor elements, iron and manganese were present in higher concentrations. Minerals are essential for the normal functioning of muscles, heart, nerves and in the maintenance of body fluid composition. Therapeutic role of certain medicinal plant materials has been correlated with the presence of specific elements in their composition. Pereira and Felcman [24] analyzed the concentration of five minerals, *viz*. silicon, manganese, iron, copper and zinc in sixteen medicinal plants which were used in wound healing to study their possible role in the healing processes. Mineral composition of *Acalypha wilkesiana* was investigated by Ikewuchi and Ikewuchi [25]. Topical zinc-containing treatments, have improved healing of wounds [26]. Magnesium is a cofactor for many enzymatic reactions including collagen synthesis. Copper is a cofactor in protein synthesis and is essential for wound healing. Iron is required for hydroxylations of proline and lysine, both the amino acids are essential for

collagen synthesis [27]. The presence of magnesium, copper, iron, manganese and zinc in *A. fruticosa* may be responsible for its wound healing activity.

CONCLUSION

In the present study the result on the analysis of phytochemicals showed the presence of bioactive compounds. 1, 2-Benzenedicarboxylic acid diisooctyl ester, n-Hexadecanoic acid, 9, 12-Octadecadienoic acid (z, z), α-D-glucopyranoside and Eicosyltrichlorosilane were identified by Gas Chromatogram-Mass spectrometry (GC-MS) analysis of the extracts. Substantial amount of macroelements and microelements were present in the aerial parts of ***Acalypha fruticosa.***

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Table 1: Qualitative phytochemical analysis of the extracts of *Acalypha fruticosa*

		Extracts			
Sl.No.	**Phytochemicals**	**Petroleum ether (40-60°C)**	**Chloroform**	**Ethanol**	**Water**
1	Triterpenoids	_	_	+	+
2	Steroids	-	_	+	+
3	Flavonoids	_	-	+	+
4	Phenols	_	_	+	+
5	Saponins	+	+	+	+
6	Anthraquinones	_	_	_	+
7	Alkaloids	_	+	+	_
8	Tannins	_	_	+	+
9	Sugars	+	+	_	+

Table 2: Quantitative analysis of phytoconstituents in the powder of aerial parts of *Acalypha fruticosa*

Sl.No	**Name of the phytoconstituents**	**Amount (mg/100g)**
1	Alkaloids	0.36
2	Flavonoids	1.19
3	Tannins	0.56
4	Phenols	0.30
5	Saponins	0.06
6	Steroids	0.05

Table 3: Chemical constituents of the ethanolic extract of *Acalypha fruticosa*[GC-MS]

Compound number	Retention Time	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	16.16	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.45
2	18.74	9,12-Octadecadienoic acid (z,z)	$C_{18}H_{32}O_2$	280	3.33
3	24.70	1,2-Benzene dicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	91.23

Table 4: Estimation of minerals in the aerial parts of *Acalypha fruticosa*

S.No	Name of the minerals	Quantity
1	Potassium	2.87%
2	Sodium	0.01%
3	Calcium	4.23%
4	Magnesium	3.16%
5	Sulphur	0.59%
6	Zinc	7.65ppm
7	Copper	0.46 ppm
8	Iron	87.62 ppm
9	Manganese	59.16 ppm
10	Boron	0.92 ppm
11	Molybdenum	0.02 ppm

Figure 1. GC-MS Chromatogram of the ethanolic extract of aerial parts of *Acalypha fruticosa*

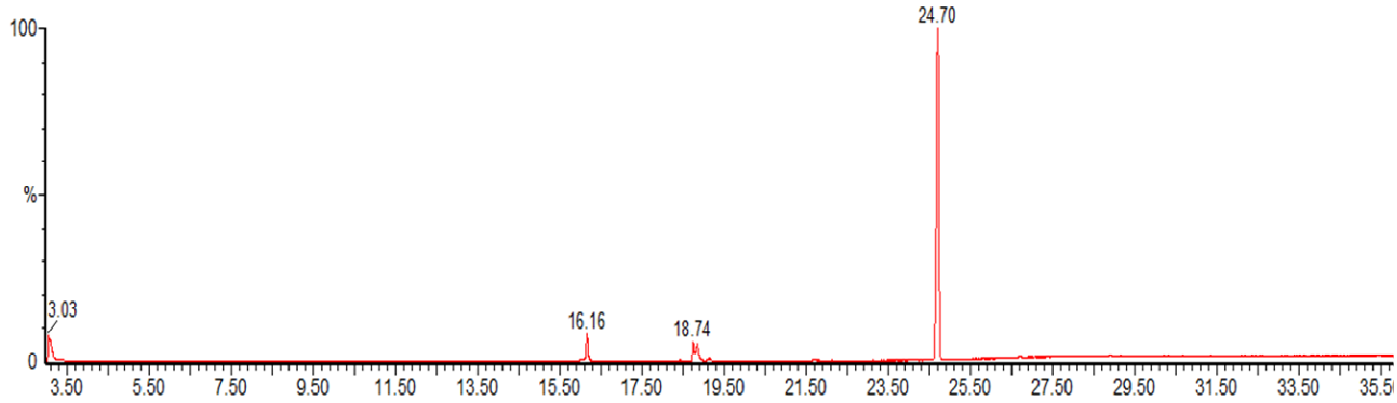
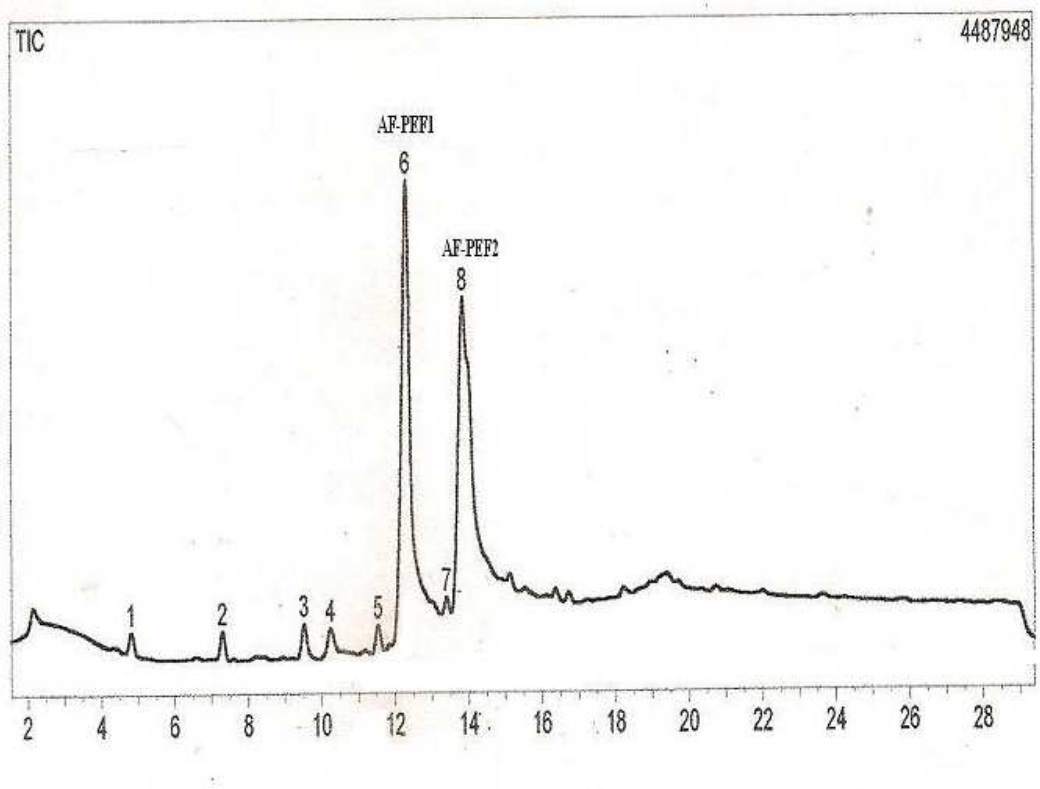


Figure 2. GC-MS Chromatogram of the fraction of petroleum ether extract of *Acalypha fruticosa*



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