

## GC-MS Analysis and Antimicrobial Activity of *Acalypha indica* Linn.

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In this study ethanolic extract of leaves of *Acalypha indica* L. (Family: *Euphorbiaceae*) was tested for antimicrobial activity by disc diffusion method. It was found that the ethanolic extract of the leaves showed antimicrobial activity against both gram (+) and gram (-) bacteria. Maximal antibacterial activity was seen against *Streptococcus faecalis* while maximal antifungal activity was against *Candida albicans*. Diameter of zone of inhibition exhibited by *Acalypha indica* ethanolic extract against tested organisms ranged between 9-15 (in mm). GC-MS analysis of same extract revealed presence of di and triterpenes like squalene, phytol and also ethyl ester of octanoic acid all known for their antimicrobial activity. *Acalypha indica*. L is thus a potential medicinal plant for therapeutic use.

**Key Words:** *Acalypha indica*, Antimicrobial activity, Maximum zone of inhibition, Medicinal plant, Therapeutic use.

### INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The more important of these bioactive constituents of plants are alkaloids, tannins, flavanoids and phenolic compounds<sup>1</sup>. Plants are an important component of the healthcare system. There are about 45,000 plant species in India with concentrated hot spots in the regions of Eastern Himalayas, Western Ghats and Andaman, Nicobar islands. The officially documented plants with medicinal potential are 3000 but traditional practitioners use more than 6000 plants. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world<sup>2</sup>. Approximately 20 % of these plants in the world have been subjected to pharmacological or biological tests<sup>3</sup>. The systemic screening of antimicrobial plant extracts represents a continuing effort to find new compounds with the potential to act against multicellular pathogenic bacteria and fungi.

A knowledge of the chemical constituents of plants such as *Acalypha indica* L. is desirable not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources of such economic materials such as tannins, oils, gums, precursors for the synthesis of complex chemical substances,

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*etc.* In addition the knowledge of the chemical constituents of medicinal plants like *Acalypha indica* L. would further be valuable in the discovering of the actual value of folklore remedies.

*Acalypha indica* L. is an irritant to the gastro-intestinal mucous membrane. It is used as a substitute for ipecacuanha. It also acts as an expectorant and in larger doses as an emetic. This drug has also been used as a laxative and anthelmintic<sup>4</sup>. *Acalypha indica* L. has been used as an emetic, expectorant, bronchitis, asthma, pneumonia, scabies in Bangladesh. In India, this plant finds use as an acrid expectorant, purgative, emetic, gastro-intestinal, skin disease, constipation, ulcers, bronchitis, vitiated and otalgia<sup>5</sup>. Since the plant is gaining importance in indigenous medicine and in allopathy for curing various ailments, the research with this plant is most suitable to evaluate its efficacy. Hence present study has been attempted to analyze the chemical constituents of *Acalypha indica* L. and to carry out antimicrobial studies.

### EXPERIMENTAL

The leaves of *Acalypha indica* L. were collected from the grounds and open space areas in the village of Avoor, Pudukottai district and used for preparation of ethanolic extract. The leaves of *Acalypha indica* L. were shade dried and powdered with the help of a blender 20 g of powdered sample was soaked in 50 mL absolute alcohol over night. The soaked sample was filtered using Whatman-41 filter paper along with 2 g sodium sulphate to remove the traces of moisture residue in the filtrate. The filtrate was concentrated by bubbling nitrogen gas into the solution, 1  $\mu$ L of the filtrate was then injected into the GC-MS equipment and the sample was analyzed.

The antimicrobial activity was determined using filter paper disc diffusion method<sup>6</sup> by measuring zone of inhibition in mm and comparing with standard drugs Ciproflaxin (2  $\mu$ g/disc for bacteria) and fluconozale (10  $\mu$ g/disc for fungi).

### RESULTS AND DISCUSSION

**Antimicrobial activity:** Ethanolic extract of *Acalypha indica* L. leaves showed antimicrobial activity against the tested organisms in the order of *S. faecalis* (15 mm), *B. subtilis* and *Klebsiella* (13 mm), *E. coli* (12 mm), *P. aeruginosa* (10 mm) and *S. aureus* (9 mm). In case of fungi, antimicrobial activity against tested organisms was in the order of *C. albicans* (10 mm) and *A. niger* (9 mm). Maximal antibacterial activity was seen against *Streptococcus faecalis* while maximal antifungal activity was against *Candida albicans* (Table-1).

**GC-MS studies:** GC-MS studies were carried out on the alcoholic extract of the leaves of *Acalypha indica* L. The various phytocomponents found in the leaves of *Acalypha indica* (alcoholic extract) are listed out in Table-2.

The peak at RT 28.89 with a peak area of 100 % is phytol. This compound is a diterpene with a m.f. of C<sub>20</sub>H<sub>40</sub>O and molecular weight (m.w.) of 296. Phytol is found to show antimicrobial, anticancer and antiinflammatory activities.

TABLE-1  
ANTIMICROBIAL ACTIVITY OF ETHANOLIC  
EXTRACT OF *Acalypha indica* L. LEAVES

Name of microorganism	Diameter of zone of inhibition (mm)	
	Sample	Standard
<i>Staphylococcus aureus</i>	9	19
<i>Streptococcus faecalis</i>	15	22
<i>Bacillus subtilis</i>	13	17
<i>Escherchia coli</i>	12	14
<i>Pseudomonas aeruginosa</i>	10	23
<i>Kliebsiella aerogenes</i>	13	18
<i>Candida albicans</i>	10	13
<i>Aspergillus niger</i>	9	12

TABLE-2  
PHYTOCOMPONENTS IDENTIFIED IN THE ALCOHOLIC  
EXTRACT OF *Acalypha indica* LEAVES [GC-MS STUDIES]

No.	RT	Name of the compound	Formula	m.w.	Peak area (%)
1	2.97	1,1-Diethoxy butane	C <sub>8</sub> H <sub>18</sub> O <sub>2</sub>	146	0.69
2	4.08	1,1-Diethoxy pentane	C <sub>9</sub> H <sub>20</sub> O <sub>2</sub>	160	0.61
3	5.97	1,1,3-Triethoxy propane	C <sub>9</sub> H <sub>20</sub> O <sub>3</sub>	176	0.66
4	6.37	1,1-Diethoxy hexane	C <sub>10</sub> H <sub>22</sub> O <sub>2</sub>	174	0.17
5	7.60	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	2.32
6	8.57	Ethyl pentanate	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	172	0.11
7	13.35	Ethyl decanate	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	200	0.12
8	14.05	Trifluoromethyl <i>t</i> -butyl disulfide	C <sub>5</sub> H <sub>9</sub> S <sub>2</sub> F <sub>3</sub>	190	4.19
9	19.26	4-C-methyl-myo-inositol	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194	10.94
10	25.67	Didodecyl phthalate	C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>	502	0.63
11	25.96	<i>n</i> -Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	8.40
12	28.89	Phytol	C <sub>20</sub> H <sub>40</sub> O	296	23.41
13	34.79	1-Monolinoleoylglycerol trimethylsilyl ether	C <sub>27</sub> H <sub>54</sub> O <sub>4</sub> Si <sub>2</sub>	498	1.06
14	39.45	Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	7.05
15	41.81	Squalene	C <sub>30</sub> H <sub>50</sub>	410	39.65

The peak at RT 41.81 with a peak area of 25 % corresponds to the compound called squalene. Squalene has the m.f. C<sub>30</sub>H<sub>50</sub>. It has a m.w. of 410. Squalene has a number of activities such as antibacterial, antioxidant, antitumour and cancer preventive. It is also shown to be an immunostimulant, chemo preventive, lipoxygenase-inhibitor, pesticide and diuretic.

Corresponding to the peak at RT 41.81 and peak area 35 % is vitamin E of  $\alpha$ -tocopherol. The molecular formula and molecular weight of this compound is C<sub>29</sub>H<sub>50</sub>O<sub>2</sub> and 430, respectively. This vitamin is known to be antiaging antidiabetic, antiinflammatory, antidermatic, antileukemic and anticancer in its activity. It also is hepatoprotective, hypocholesterolemic, antiulcerogenic, antispasmodic and antibronchitic.

*n*-Hexadecanoic acid is found to be responsible for the peak at RT 25.96 with a peak area 28 %. This fatty acid has the m.f.  $C_{16}H_{32}O_2$ . It has a m.w. of 256. This compound has various activities like antioxidant, hypocholesterolemic, nematocidal, pesticide, lubricant and inhibitory activity such as 5- $\alpha$  reductase inhibition.

The polyhydroxy aromatic compound 4-C-methyl-myo-inositol is found to be the cause of the peak at RT 19.26 with a peak area of 12 %. This compound has the m.f.  $C_{17}H_{14}O_6$  and m.w. of 194. It is mainly hypoglycemic in its activity (Table-2). The peak at RT 8.57 with a peak area of 0.11 % is the ethyl ester of octanoic acid. This compound has the m.f. of  $C_{10}H_{20}O_2$ . Molecular weight of this compound is 172. It is a fatty acid with antimicrobial activity. The peak at RT 14.05 with a peak area 4 % is trifluoromethyl *t*-butyl disulphide. Molecular formula and molecular weight of this compound is  $C_5H_9F_3S_2$  and 190, respectively. It is a sulphur compound having hypoglycemic activity.

### Conclusion

Spectral data from GC-MS studies (Fig. 1) show that the major components are the diterpene, 2-*trans*-phytol (peak area -100 %), vitamin E or  $\alpha$ -tocopherol (peak area 35 %), squalene-a known lipoxygenase inhibitor<sup>7</sup> as well as a antibacterial compound (peak area 25 %). The other components are identified as 1,1-diethoxy butane, pentane, 1,1,3-triethoxy propane, 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran, ethyl esters of octanoic and decanoic acids, respectively, trifluoromethyl *t*-butyl disulfide, 4-C-methyl-myo-inositol, didodecyl phthalate, *n*-hexadecanoic acid and 3,7,11,15-tetranethyl-2-hexadecen-1-ol. Moreover it was found that the ethanolic extract of the leaves of *Acalypha indica* L. was effective against both gram positive, gram negative bacteria as well as fungi.

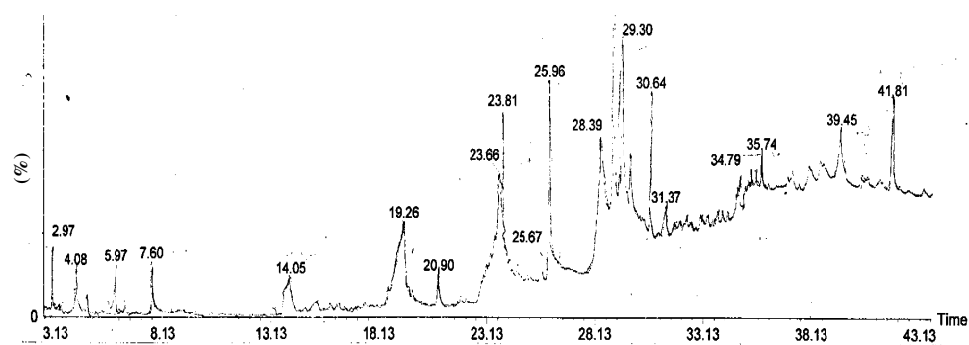


Fig. 1. *Acalypha indica* Alcoholic ext medicinal plant analysis 82.

It therefore can be concluded that antimicrobial activity of *Acalypha indica* L. against bacteria and fungi shows its medicinal value and supports the widespread use of the plant as a local remedy for a variety of ailments ranging from ulcers to bronchitis.

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