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**GC-MS, FTIR AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF  
*ABUTILON INDICUM***

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**ABSTRACT**

The present study states the phytochemical investigation and antimicrobial activity of crude methanol and chloroform extracts of the leaves of *Abutilon indicum* (Malvaceae). Preliminary phytochemical studies revealed the presence of alkaloids, flavonoids, steroids, cardiac glycosides, saponins, phenols, terpenoid and tannins in the methanolic extract. The gas chromatography-mass spectrometry (GC-MS) analysis and FTIR was performed. The extracts showed inhibitory activity against clinical isolates of the gram negative bacteria such as *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Shigella flexneri*. The results showed that the methanol extracts was more potent than the chloroform extracts.

**Key Words:** *Abutilon indicum*, Antibacterial activity, Phytochemical screening.

**INTRODUCTION**

In recent years usage of commercial antimicrobial drugs against human pathogenic microorganisms increased extensively. Effective antimicrobials have been developed over the past years, several reports development of antibiotic resistance of human pathogens to available antibiotics (Martino *et al.*, 2002). Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance, search for plant resources has been increased for their potential antimicrobial activity (Hammer *et al.*, 1999). The new compounds from plant sources are not based on the obtainable synthetic antimicrobial agents, and also phytochemicals from plants have different structures from microbial derived antibiotics and atypical modes of action (Fabricants *et al.*, 2001) antibiotic resistance can be much reduced.

Knowledge of the phytochemical constituents is very essential to facilitate search of the actual effectiveness

of the plant in medicine. Bioactive compounds are normally accumulated as a secondary metabolite in all plant cells but their concentration varies according to the plant parts, season, climate, and growth phase. Leaf is one of the important edible parts and easy to extract, and people are generally preferred it for the therapeutic purpose.

*Abutilon indicum* belongs to the family Malvaceae and distribute in all parts of tropical and sub tropical region of India. All parts of the plant have been recognized to have medicinal properties. The plant is commonly called as Thutti. The traditional method of medicine, the plant used as anthelmintic, anti-inflammatory and is useful in urinary and uterine discharges, piles and lumbago (Porchezian *et al.*, 2000), jaundice, ulcer and leprosy. *A. indicum* leaves are used in the treatment of toothache, lumbago, piles, anti-fertility and liver disorders (Anyensu *et al.*, 1978). Root and bark are used as aphrodisiac, antidiabetic (Lakshmayya *et al.*, 2003), nervine tonic, and diuretic. The plant extracts and their products for antimicrobial activity have shown that a potential source of novel antibiotic prototypes of higher plants (Afolayan *et al.*, 2003). The crude plant extracts for screening series is potentially more successful

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in initial steps than the pure compounds (Kasamota *et al.*, 1995).

Plant source is still mostly unexplored and merely a small percentage of them has been subjected to phytochemical investigations, and the fractions submitted to pharmacological screening is very low. The present investigation was undertaken to evaluate the phytochemical constituents using FT-IR and GC-MS and antibacterial activity of leaf extract *A. indicum*.

## MATERIALS AND METHODS

### Collection of plant materials

*Abutilon indicum* wild plants grown on the road side were collected in Batlagundu, Dindigul district of Tamil Nadu. The plant species was identified with the help of Prof. Rajangam, Horticulture Research Station, Kodaikanal. The leaves were rinsed in distilled water, dried and powdered.

### Preparation of extract

Fifty grams of the powdered plant material were extracted with 250 ml of the solvents namely methanol and chloroform in conical flasks. Then, the samples were incubated at room temperature for 24 hrs. The extracts were filtered using Whatmann No.1 filter paper. The filtrates were then concentrated under vacuum at 40°C and the extracts stored at -18°C.

### Phytochemical analysis

The leaves extract was analyzed for the presence of phytochemical components for the presence of Alkaloids, Flavonoids, Steroids, Cardiac glycosides, Saponins, Phenols, Terpenoid, Tannins, Anthroquinone, Carbohydrates were tested by using standard procedures (Sofowora, 1993).

### Antibacterial screening

Antimicrobial activity of the leaf extracts of *Abutilon indicum* was tested using the discs diffusion method (Doughari *et al.*, 2008). Nutrient agar plates were prepared and inoculated test organisms namely *S. typhi*, *P. aeruginosa*, *E. coli*, *P. mirabilis*, *K. pneumoniae* and *S. flexneri* by a spread plate method. The sterilized filter paper disc of 5 mm diameter (Whatmann's No. 1 filter paper) was used and the leaf extracts of various concentrations of 25 µl, 50 µl, 75 µl, and 100 µl were added to each disc. The sterile impregnated disc with plant extracts were dried and placed on the agar surface with forceps and pressed gently down to ensure complete contact of the disc on the agar surface. All the plates were incubated at 37°C for 24 hours. The antimicrobial activity of plant extract was assessed by the presence or absence of inhibition zone and the diameter of the zone were measured.

### GC-MS analysis

A required quantity of powder was weighted and extracted using methanol. The flask was incubated for 24 hours and extract was filtered. The extract evaporated to dryness by using a rotary evaporator. The final residue thus obtained was then subjected to GC-MS analysis. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were determined.

### FT-IR analysis

For FT-IR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 minutes and filtered through Whatmann No.1 filter paper by using a high pressure vacuum pump. The dried residues were characterized by IR and their functional groups were recorded.

## RESULTS AND DISCUSSION

In this study, results of phytochemical analysis of *A. indicum* leaves extracts showed the presence of carbohydrates, alkaloids, flavonoids, steroids, cardiac glycosides, saponins, phenols, terpenoid and tannins in methanolic extract (Table 1). Steroids, saponins, terpenoid and tannins, carbohydrate was absent in the chloroform extract. Anthroquinone was absent both methanol and chloroform leaf extract. According to Dhanalaksmi *et al.* (1990) stated that the presence of flavonoids and triterpenoids was presented in the ethanolic extract of *A. indicum*. Phytochemicals or secondary metabolites usually occur in complex mixtures that differ among plant organs and stages of development (Wink, 2004). Understanding of the phytochemical constituents is important for exploration of the authentic effectiveness of the plant.

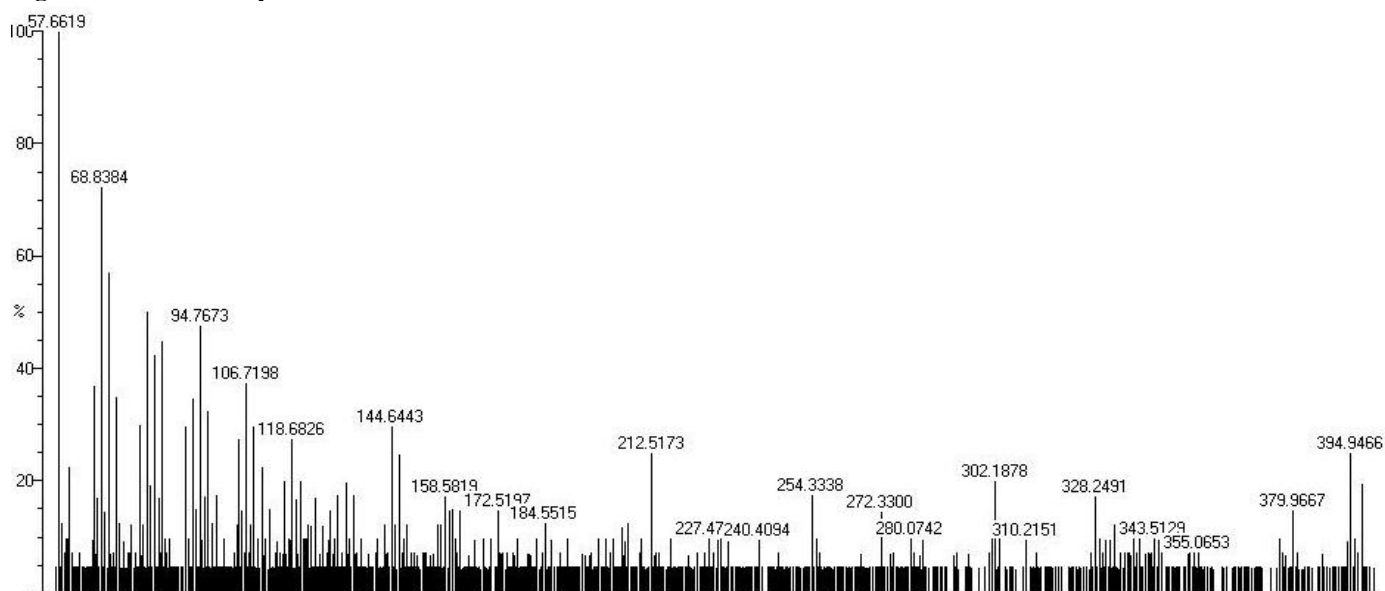
The results pertaining to GC-MS analysis leads to the identification of the number of compounds from the GC fractions of the methanol extract of *A. indicum* (Fig.1). In this study, the presence of six different compounds namely Dasycarpidan1 was found as major component followed by acetate (ester) (C<sub>20</sub>H<sub>47</sub>O), Phytol (C<sub>20</sub>H<sub>40</sub>O), Hexadecanoic acid 1,2 Ethanyl Ester (C<sub>35</sub>H<sub>68</sub>O<sub>5</sub>), Geranylisovalerte (C<sub>15</sub>H<sub>26</sub>O<sub>2</sub>), Heptadecane A Hexyl (C<sub>23</sub>H<sub>48</sub>), Octadecane-2 Hexadocyloxy (Ethox) (C<sub>25</sub>H<sub>28</sub>O<sub>4</sub>) was presented in Table 2. Flavonoids, Terpenes, Amino acids, Aldehyde, Hydrocarbon, Ketone, Fatty acids and esters were reported in *A. indicum* (Ramasubramaniraja, 2011).

The FT-IR spectrum was used to identify the functional group of the active components. The outcome of FT-IR functional groups were represented in Table 3. The FT-IR spectrum profile was illustrated in the Figure 2. The FT-IR spectrum confirmed the presence of Alkanes (CH<sub>3</sub>), Alkanes (CH<sub>2</sub>), Ketone-6-ring, Alikane butyl, Sulfate, Tri substituted Alkanes and S.Halides.

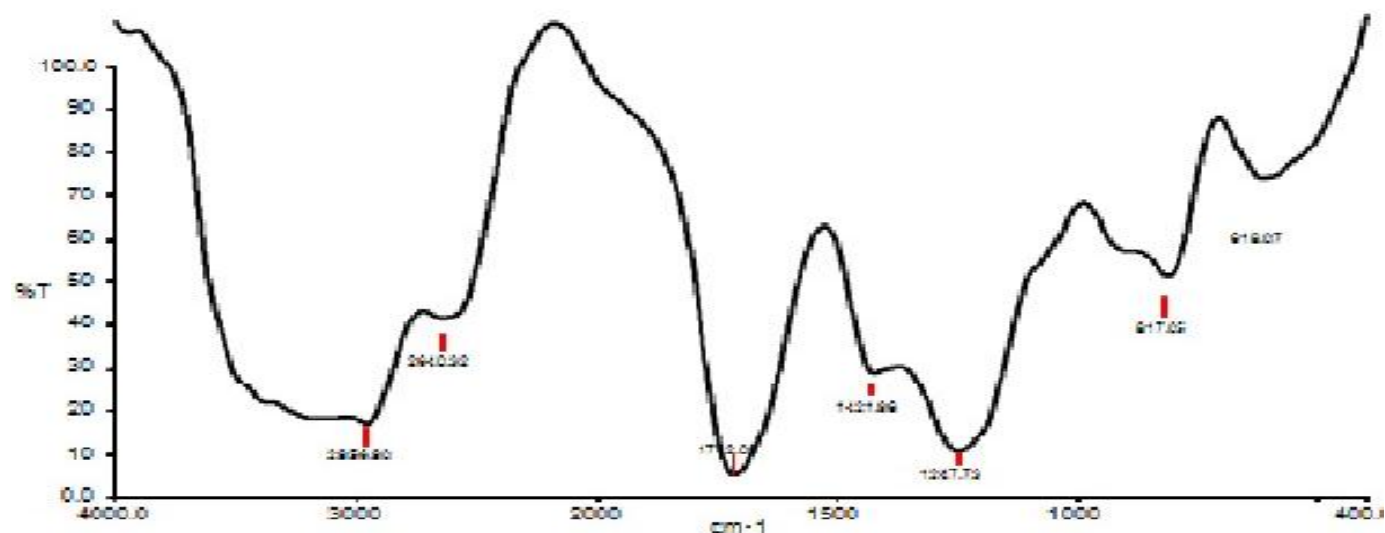
The effect of inhibitions (Table 4) however showed that the antibacterial activity of the extracts increased with increase in concentration. For instance the activity of the extracts at the concentration of 100 $\mu$ l in methanol *P. mirabilis* showed zone of inhibition high 29.3mm followed by *E. coli* (28mm), *K. pneumoniae* and *P. aeruginosa* showed 25.6mm, *S. flexneri* (22.1mm) and *S. typhi* (19.6mm). The chloroform extract showed a degree of growth inhibitions less compared to methanol extract. The maximum inhibition showed at 100  $\mu$ l was *E. coli* (8.9mm) followed by *K. pneumoniae* (6.2mm). The minimum activity by *S. typhi* (1.5mm). The activities of some phyto-components with compound nature of flavonoids, palmitic acid (hexadecanoic acid, ethyl ester

and n-hexadecaonoic acid), unsaturated fatty acid and octadecatrienoic acid might cause as antimicrobial (Kumar *et al.*, 2010). All the test organisms were susceptible to the plant methanol extracts with various degree of sensitivity. Flavonoids from different plants have been reported for their antibacterial activities. Microorganism acquired resistant against the commercial drugs by the production of enzymes, resistant plasmids, alteration of metabolic pathway in the pathogens. Natural compounds of their extract provide unrestricted for the development of new drugs due to the availability of chemical diversity. The plant secondary metabolites are promising sources of preventive agents in the pathogenesis and competent of microbial diseases.

**Figure1. GC-MS analysis of Methanol extract of *A. indicum***



**Figure 2. FTIR Spectrum of Methanolic extract of *A. indicum***



**Table 1. Phytochemical analysis of *A. indicum* leaf extracts**

Phytochemical constituents	Methanol	Chloroform
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	-
Cardiac glycosides	+	+
Saponins	+	-
Phenols	+	+
Terpenoid	+	-
Tannins	+	-
Anthroquinone	-	-
Carbohydrates	+	-

**Table 2. GC-MS analysis of Methanolic extract of *A. indicum***

Name	Retention Time	Molecular Weight	Molecular Formula
Dasycarpidan-1,Methanol Acetate	29.28	296.53	C <sub>20</sub> H <sub>47</sub> O
Phytol	11.4	296.627	C <sub>20</sub> H <sub>40</sub> O
Hexadecanoic acid, 1,(hydroxymethyl 1,2-ethanodyl	19.04	568.91	C <sub>35</sub> H <sub>68</sub> O <sub>5</sub>
Geranyl Isovalerte	21.65	238.37	C <sub>15</sub> H <sub>26</sub> O <sub>2</sub>
Heptadecane, $\alpha$ Hexyl	23.19	324.627	C <sub>23</sub> H <sub>48</sub>
Octadecane 1-2,hexadocyloxy	29.28	351.556	C <sub>25</sub> H <sub>28</sub> O <sub>4</sub>

**Table 3. FT-IR peak values and Functional groups of Methanol extracts of *A. indicum***

Frequency	Wave Length (cm <sup>-1</sup> )	Intensity	Compounds	Stretching
2956.9	3.36-3.39	M	Alkanes(CH <sub>3</sub> )	C-H Str
2640.32	3.40-3.43	M	Alkanes(CH <sub>2</sub> )	C-H Str
1712.09	5.83-5.88	S	Ketone-6-ring	C=O Str
1247.73	7.97-8.33	s,d	Alkane butyl	SkeletalStr
1421.96	-	S	Sulfate	S=O Str
817.05	11.90-12.66	S	Tri substituted Alkanes	C-H Def
618.07	12.50-16.67	S	S.Halides	C- Cl str

**Table 4. Antibacterial activity of Leaves of *A. indicum* using Disc diffusion method**

Test organisms	Methanol Extract				Chloroform Extract			
	25 $\mu$ l	50 $\mu$ l	75 $\mu$ l	100 $\mu$ l	25 $\mu$ l	50 $\mu$ l	75 $\mu$ l	100 $\mu$ l
<i>E. coli</i>	12.3	20	21	28	6.4	7.9	8.4	8.9
<i>K. pneumoniae</i>	12	9.3	12.6	25.6	4	5.2	5.6	6.2
<i>P. aeruginosa</i>	10.2	13.3	23.3	25.3	4	4.8	5.3	6
<i>P. mirabilis</i>	8.3	15.6	23.3	29.3	2	3.9	4.4	5.1
<i>S. flexneri</i>	9	21	23	22.1	1	3	3.1	3.8
<i>S. typhi</i>	10.3	13.6	15.6	19.6	0	1	1.4	1.5

## CONCLUSION

*A. indicum* have been subjected to preliminary screening of phytoconstituents using GCMS and FTIR. The presence of functional groups of compounds was as Alkanes, Ketone, Sulfate, and Sulphate Halides. The study concluded that the methanolic extracts exhibited significant

antibacterial activities in a concentration dependant manner. This study revealed the presence of six phytochemical compounds. Therefore, the plant extracts can be used for the treatment of infections caused by the strains of the test bacterial organisms.

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