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Identification of New Bioactive Compounds from Fruit of *Abutilon indicum* through GCMS Analysis

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ABSTRACT: ***Abutilon indicum*** **(L.) commonly known as Kaghi in Hindi and Atibala in Sanskrit is an important medicinal plant of family Malvaceae. It is used as source of drug since ancient period and mentioned as Bhav Prakash and Raj-Nighantu in Ayurveda, Unani and Siddha system of medicines. The plant is used in the treatment of piles, gonorrhea, diabeties, ulcer, urinary disorders and many more health complications. In present study, the ethanolic extract of *A. indicum* fruit was subjected to Gas chromatography Mass spectroscopy (GCMS) analysis and thirteen new compounds have been identified. Some of these identified compounds are reported to possess significant medicinal applications. The present study led to the identification of medicinally important compounds from the fruit of *A. indicum* which could be used as lead compound for the manufacture of herbal drug.**

Keywords: *Abutilon indicum*, Bioactive compounds, GCMS

INTRODUCTION

During the last few decades, there has been growing interest in the use of medicinal plants due to their easy availability, therapeutic potential, least side effects and minimum cost. Phytoconstituents isolated from medicinal plants are playing pivotal role in development of novel compounds, which might be useful in drug discovery. Various plants available in the nature are still unexplored for their medicinal potential. Merely a small percentage of plants have been subjected to phytochemical investigations, and the fractions submitted to pharmacological screening is very low. Gas chromatography mass spectrometry (GCMS) has been emerged as key technological platform for secondary metabolite isolation in both plant and non-plant species (Fernie *et al.* 2004; Kell *et al.* 2005).

Abutilon indicum is a small shrub of the family Malvaceae native to tropical and subtropical regions. The plant is widely known for its various medicinal properties. The fruit is used in piles, gonorrhea and cough treatment (Samy *et al.* 2008; Ignacimuthu *et al.* 2008). Fruit decoction mixed with ammonium chloride is given orally to treat hemorrhagic septicemia (Ali *et al.* 1999). The bark has been recommended as febrifuge, anthelmintic, alexeteric (Singh *et al.* 2002). Roots are used to treat fever, chest infection, gonorrhea, haematuria, strangury, leprosy, dry cough, bronchitis, gout, polyuria and uterine hemorrhagic discharge (Giri *et al.* 2009). The leaves are used to cure ulcer, inflammation, rheumatism, syphilis of penis, piles, uterus displacement, inflammation of bladder, catarrhal bilious diarrhea, bronchitis, gonorrhea, fever and to relieve leg pains (Dhanalakshmi *et al.* 1990; Jain *et al.* 2005; Kaladhar 2012). *A. indicum* extract possess analgesic (Bagi *et al.* 1984; Almeida *et al.* 2001), antidiarroheal (Chandrashekhar *et al.* 2004) hepatoprotective (Porchezhian and Ansari 2005), hypoglycemic (Seetharam *et al.* 2002), immunomodulatory (Dixit *et al.* 1978; Singh *et al.* 2008), larvicidal (Rahuman *et al.* 2008), and wound healing activity (Roshan *et al.* 2008).

The present study was aimed to investigate the presence of various biomolecules in *A. indicum* by first preparing the ethanolic extract of fruit and separation and identification of the compounds by GCMS analysis.

MATERIALS AND METHODS

A. Collection of Plant material

Fruits of *A. indicum* were collected from Phoolpur, Allahabad. Taxonomical description and identification of plant was done at Botany Department, University of Allahabad and Voucher specimen was kept in the Duthie herbarium of the Department.

B. Preparation of extract

For preparation of extract, fruits of the plant were thoroughly washed under running tap water. Plant material was dried for 8 hr at 60°C in oven, and then powdered in mechanical grinder. The powdered sample (10 g) was extracted by 250 ml of ethanol by using a Soxhlet extractor for 48 h at a temperature not exceeding the boiling point of the solvent (Lin *et al.* 1999).

C. Gas chromatography mass spectroscopy (GCMS) analysis

Gas chromatography mass spectroscopy analysis was carried out by Trace-1300 GC copupled to TSQ 8000 triple quadrupole MS detector. The TGSMS column with dimentions of 30m × 0.25mm × 0.25um was used for the analysis. 1.0 µL of clear extract was injected into GC-MS with a oven programming of 50ºC for 5min to 280ºC for 10min at the rate of 15 ºC/min. The injector temperature and MS transfer line temperature was maintained at 250ºC and 280ºC respectively. The carrier gas used in the analysis was helium which had the flow rate of 1ml/min. The compounds were identified by matching with NIST- MS library.

RESULTS AND DISCUSSION

GC-MS analysis of the ethanol extract of *A. indicum* fruit led to the identification of thirteen compounds (Table 1). These compounds were identified through mass spectrometry attached with GC. The identified compounds with their retention time (RT), molecular formula, molecular weight and concentration (peak area %) are presented in Table 1. The GC-MS chromatogram of identified compounds with their chemical structures is depicted in Fig. 1-5. The GC-MS spectrum confirmed the presence of 13 compounds with the retention time; 5.65, 6.44, 24.31, 30.41, 30.58, 30.64. Three compounds 2-Pentanone, 4-hydroxy-4-methyl-, 2-Hexanol, 2-methyl and 2-Pentanol, 2, 3-dimethyl- were identified at retention time 5.65 (Fig. 2). Among these isolated compounds 2-Pentanone, 4-hydroxy-4-methyl- had highest probability (70.72 %), followed by 2-Hexanol,2-methyl (18.51 %) and 2-Pentanol,2,3-dimethyl- (3.59 %), thus 2-Pentanone, 4-hydroxy-4-methyl- seems to be most stable compound. Benzene, 1, 3-dimethyl (m-xylene), p-xylene and o-xylene were identified at retention time 6.44 with highest probability (34.35 %) of Benzene, 1,3-dimethyl, followed by p-xylene (23.57 %) and o-xylene (19.91 %) (Fig. 3). It appears from the result that Benzene, 1,3-dimethyl (m-xylene) is the more stable compound than p-xylene and o-xylene. Three compounds c-sitosterol, a-sitosterol and Cholest-5-en-3-ol, 4, 4-dimethyl-(3a)- were identified at retention time 24.31 with highest probability of c-sitosterol (88.05 %) followed by a-sitosterol (4.35 %) and Cholest-5-en-3-ol,4,4-dimethyl-(3a)- (0.87 %) (Fig. 4). It is evident from the result that c-sitosterol is the most stable compound. Three compounds Lupeol, Lup-20(29)-en-3-ol, acetate, (3a)- and 9,19-Cyclo-9a-lanostane-3a,25-diol were found at retention time 30.41 30.58 and 31.10 with higher probability of lupeol (79.47 %), followed by Lup-20(29)-en-3-ol, acetate, (3a)- (3.46 %) and 9,19-Cyclo-9a-lanostane-3a,25-diol (2.37 %), thus lupeol appears to be most stable among these three. The compound lupeol, Lup-20(29)-en-3-ol, acetate, (3a)- and Taraxasterol were identified at retention time 30.64. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of those compounds which can be identified from the data library. Some of these isolated compounds are reported to possess significant medicinal applications, which are presented in Table1. The present study helps to predict the formula and structure of 13 biomolecules. Further investigation may lead to isolation of bio-active compounds, their structural elucidation and screening of pharmacological activity which will be helpful in drug development.

This is the first report for the identification of these compounds from *A. indicum* extract. Only two compounds gamma-sitosterol and lupeol has been identified from hexane extract of *A. indicum* leaves through GCMS analysis (Shanthi *et al.* 2011). Hussain *et al.* (2014) has done quantitative estimation of lupeol from *A. indicum* extract.

Table 1: Bioactivity of isolated compounds.

S.N.	R T	Name of compound	Molecular formula	Molecular weight	Probability	Bioactivity
1	5.65	2-Pentanone,4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116	70.72	Antimicrobial
2	5.65	2-Hexanol,2-methyl	$C_7H_{16}O$	116	18.51	-
3	5.65	2-Pentanol,2,3-dimethyl-	$C_7H_{16}O$	116	3.59	-
4	6.44	m-xylene	C_8H_{10}	106	34.35	-
5	6.44	p-xylene	C_8H_{10}	106	23.57	Antipsoriatic, Antimicrobial, Antioxidant, Antifungal
6	6.44	o-xylene	C_8H_{10}	106	19.91	Antimicrobial, Antioxidant, Antifungal
7	24.21	c-Sitosterol	$C_{29}H_{50}O$	414	86.81	-
8	24.21	a-Sitosterol	$C_{29}H_{50}O$	414	4.55	Antimicrobial, Anticancer, Antiarthritic, Diuretic,Anti-asthma, Anti-inflammatory
9	24.21	Cholest-5-en-3-ol, 4,4-dimethyl-,(3a)-	$C_{29}H_{50}O$	386	0.97	Prevents the risk of infraction and brain hemorrhage
10	30.41	Lupeol	$C_{30}H_{50}O$	426	76.74	Antimicrobial, Anti-inflammatory Antitumor, Antimalarial, Anti-hyperglycemic, Antitumor
11	30.41	Lup-20(29)-en-3-ol,acetate,(3a)-	$C_{32}H_{52}O_2$	468	3.02	-
12	30.41	9,19-Cyclo-9a-lanostane-3a,25-diol	$C_{30}H_{52}O_2$	444	2.90	-
13	30.64	Taraxasterol	$C_{30}H_{50}O$	426	2.55	Chemoprotective, Anti-inflammatory, Anti- tumor, Anti- ulcer.

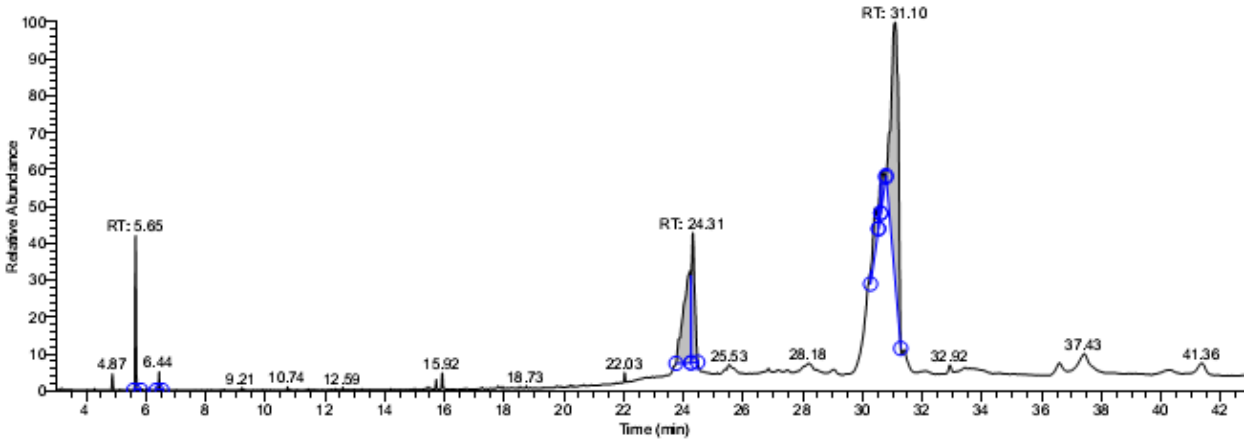

Fig.1. GCMS chromatogram of ethanol extract of *Abutilon indicum* fruit extract.

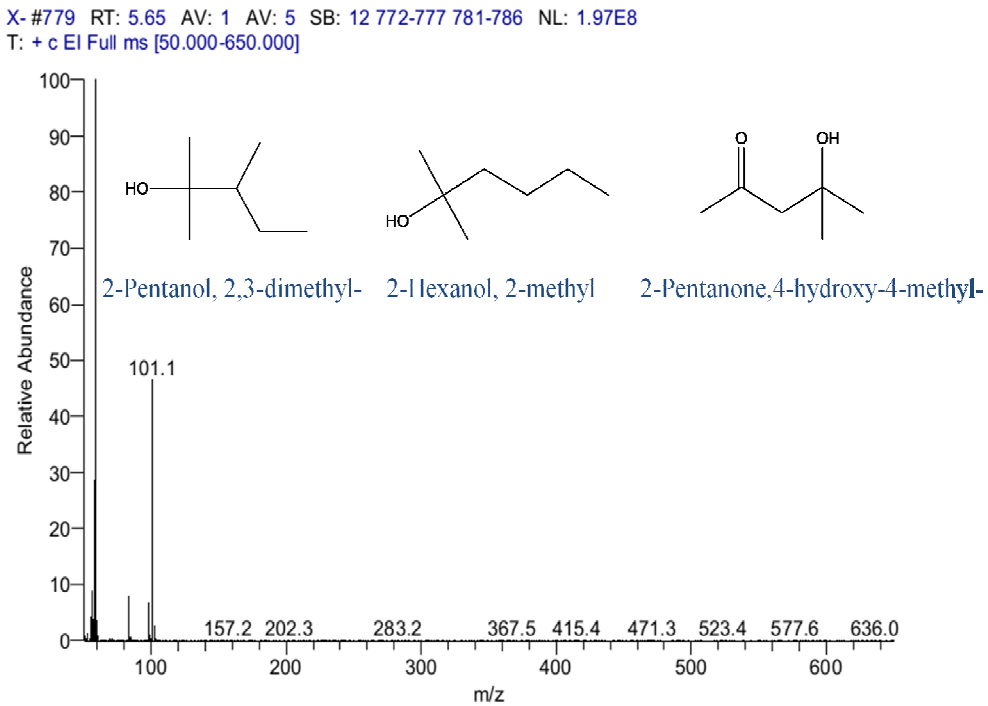

Fig. 2. Mass spectrum and structure of compounds identified at retention time 5.65.

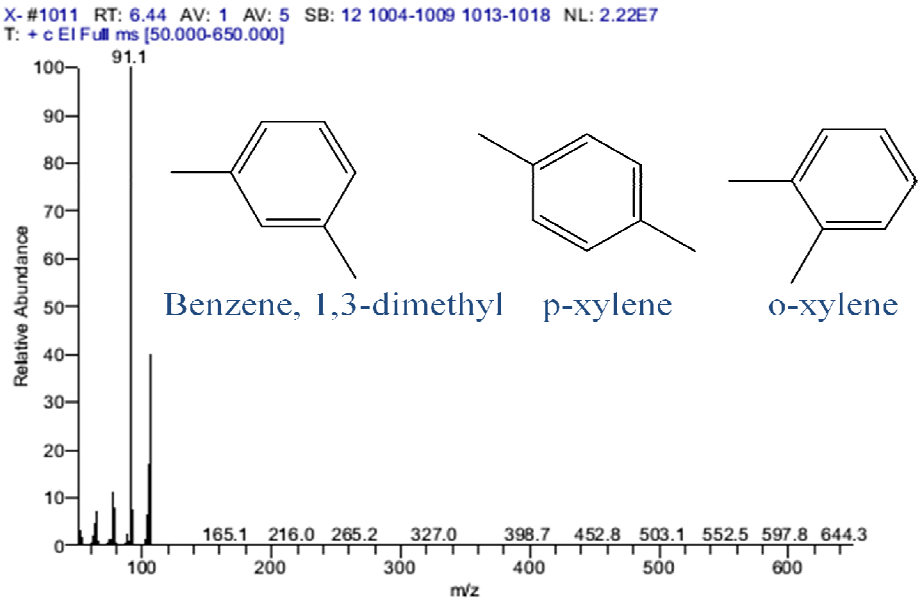

Fig. 3. Mass spectrum and structure of compounds identified at retention time 6.44.

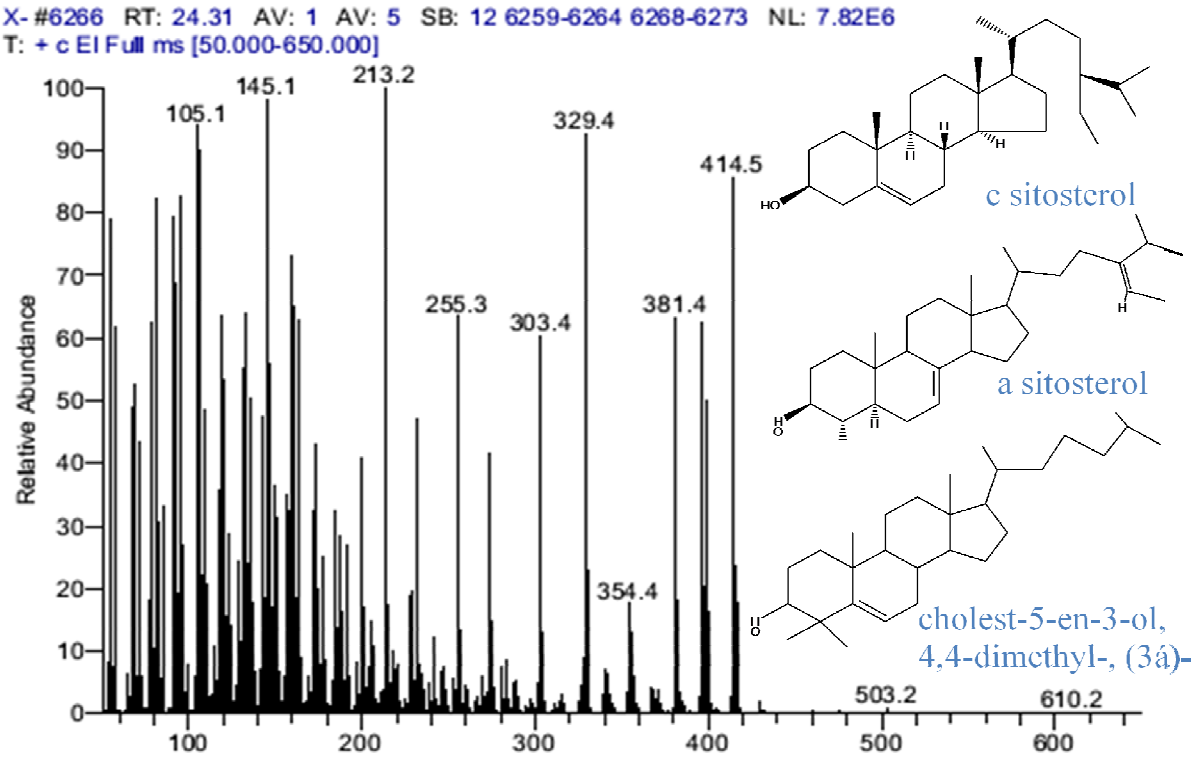

Fig. 4. Mass spectrum and structure of compounds identified at retention time 24.31.

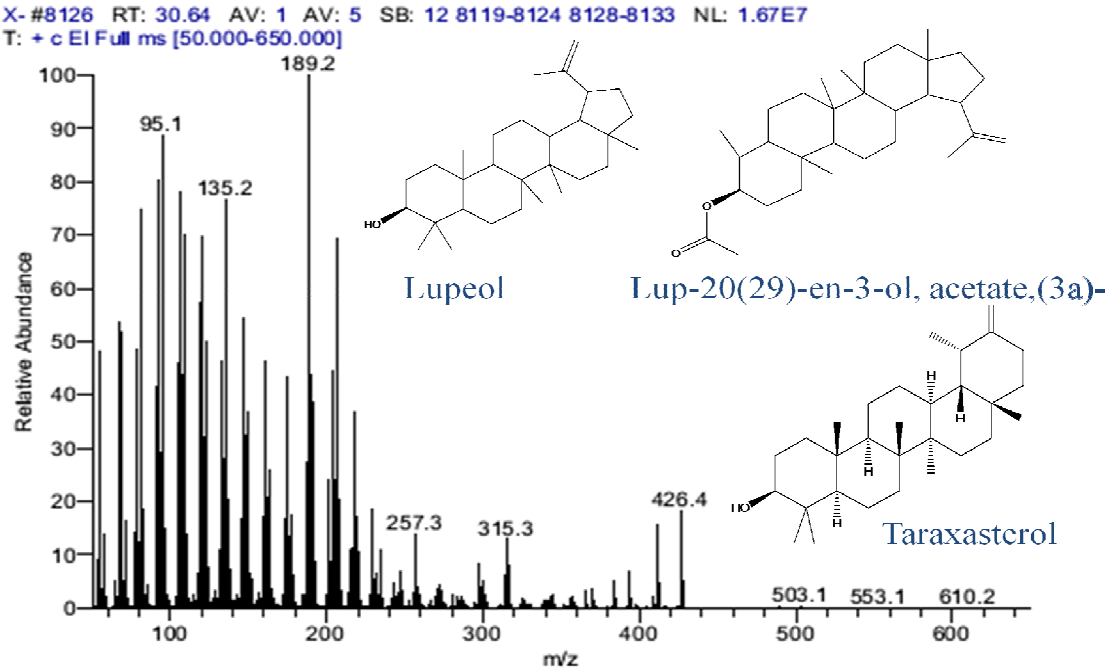

Fig. 5. Mass spectrum and structure of compounds identified at retention time 30.64.

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