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Antimicrobial, Phytochemical and GC-MS Analysis of *Abutilon indicum* (Linn.) Sweet Parts used as Ayurvedic Drug for Painful Urination as Per *Mādhav Cikitsā*

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Abstract : Painful urination is a suffering of human population from incontinence of urine caused mostly due to unhygienic internal and surrounding conditions. It is also commonly known as dysuria. This problem was existing since ancient time. To which, *Āyurveda*, the oldest science of healthy living describes treatment through Sanskrit Medical compositions/treatises/ literature under the term as '*Mūtrakṛcchra'*and enlisted different herbal formulations for the treatment. *Mādhav Cikitsā*is one of the *Āyurvedic* compendia written by *AcāryaMādhava*(Ca 7-8th CE) that mainly deals with treatment part of diseases in similar line to his *MādhavaNidānam*. The present study was an attempt to investigate the antimicrobial activity and phytochemical analysis of plant part extracts of the sampled out medicinal herb *Abutilon indicum*(Linn.) Sweet (Malvaceae), which has been mentioned in *MādhavCikitsā*for *Mūtrakṛcchra* and to rationalize the findings with the treatment of the disease. The antimicrobial activity test experiments conducted with the whole plant extract responded positive to establish the plant's antibiotic nature. The qualitative phytochemical tests confirm that the plant extract (made in different solvents) contains alkaloids, flavonoids, steroids, glycosides, saponins, phenols, terpenoid and tannins. The Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanolic extract of whole plant further validates the qualitative phytochemical data. The compounds identified are Oleic acid, 10-Undecen-1 al, 2-methyl, (Z)6, (Z)9-Pentadecadien-1-Ol, Phytol, 17-Octadecyonic Acid, n-Hexadecanoic acid, Oxirane, tetradecyl, 3,7,11,15-Tetramethyl-2-hexadecane-1-ol. These compounds are having proven anti-microbial, anti- inflammatory, diuretic and anti-oxidant, etc. properties. Hence, use of *Abutilon indicum* for the treatment of *Mūtrakṛcchra*by *Mādhava* establishes its merit.
Keywords : Painful urination,*Āyurveda, Mūtrakṛcchra*, *Acārya Mādhava,* antimicrobial activity, phytochemical analysis, *Mādhav Cikitsā,* GC-MS analysis.

Introduction:

Painful urination is a common problem affecting people from neonates to geriatric age group worldwide due to urinary tract infections (UTI), commonly known as dysuria[1]. It is estimated that about 150 million UTIs occur yearly world-wide[2]. More than 80% UTI cases are caused by single bacterial species *Escherichia coli*[3]. The main factor predisposing to genitourinary tract infection has been attributed to poor personal hygiene and culture habit imposition[4]. The modern treatment is medication of antibiotics. However, UTI condition has been a health problem of people since ancient time too, where different symptoms and treatment for this problem

have been enumerated in ancient medical texts of India. *Āyurveda* is an extensively practiced system of traditional medicine. It is mainly based on medicinal plants for the treatment of many diseases[5]. It is believed that about 80% of the world population is still dependanton traditional medicines[6,7].*Āyurveda* explains the condition of painful urination or incontinence of urine as *Mūtrakṛcchra*[7]. *Āyurveda* says that it happens due to imbalance in vata, *pītta* and *kaphadośas* and there are 8 types of *Mūtrakṛcchra* (*Vātīk, Paitīk, Ślaiśmīk, Sānnīpatīk, Śalya, Puriṣaj, Śukraj* and *Aśmarij*) as per the symptoms[8]. *AcaryaMādhav* was one of the Ayurvedic practitioners of the middle age (Ca7th-8thCE) and his text *MādhavCikitsā* can be said to be one of the earliest texts written on *Cikitsā*of different aliments. *Mādhav* wrote the *Cikitsā*text based on the already established knowledge from *CarakaSamhitā, SuśrutSamhitā*and *Aṣṭāṅgahṛdayam* compendia in an user friendly compositions. Though *Mādhav Cikitsā* did not receive as much popularity as his *MādhavNīdana,*but it can be said to be a milestone in *Āyurvedic* history which initiated a new tradition of *Cikitsā* grantha[9]. *Mādhav* described the *Cikitsā* (treatment) for painful urination in 30th chapter under the name of *Mūtrakṛcchra Cikitsā* in his book *Mādhav Cikitsā*[10]. Various medicinal herbs with different combinations and preparations are described in this text which are meant to provide relief from the specified disorders.

Abutilon indicum (Linn.) Sweet (Syn. *Abutilon indicum* G. Don.) belonging to family Malvaceaeis known as Kākhi, Kākhīyā, Kaṅgahiin Vernacular terms. In Sanskrit it is narratedas *Kaṅgahi, Ṛṣyaprōktā, Kaṅkatika, Atībalā*[7, 11]. From ancient times, *Abutilon indicum* has been used as *Āyurvedic* medicine with greater benefits[11]. *AcaryaMādhav*has mentioned use of this herb,(*Balā*) in his text *MādhavCikitsā* under the topic *MūtrakṛcchraCikitsā* that is for the treatment of dysuria. Other traditional literature also describes that this*Abutilon indicum* plant could be used inurinary and uterine discharges, piles, lumbago, jaundice, ulcer and leprosy. It has also anthelmintic and anti-inflammatory action[12].*Abutilon indicum*(Linn.) Sweet is a perennial shrub, softly tomentose and up to 3 m in height. It grows in wild dry climate[11, 13].

Botanical Identification of *Abutilon indicum* (Linn.) Sweet (*Kangahi, Ṛṣyaprōktā, Kaṅkatika, Atībalā* [14, 15])–

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Malvales
Family	Malvaceae
Genus	*Abutilon*
Species	*indicum*
Authority	Linnaeus and Sweet

Biological active compounds (alkaloids, tannins, saponins, flavonoids, Sterols, Glycosides etc.) from natural source have always been of great interest to scientists working on diseases. These compounds are responsible for qualifying medicinal properties of plants. Biochemical compounds produced by plants are having pharmacological and toxicological effects[16]. Knowledge of the phytochemical constituents has been expanding with new research to relate the actual effectiveness of the plant as medicine[17]. A number of medicinal herbs at the same time also prevent microbial growth in the body for their antimicrobial properties.The present investigation is an attempt to revalidate the use of *Abutilon indicum* (Linn.) Sweet for its medicinal value based on antimicrobial property and phytochemical constituents for the treatment of dysuria.

Materials and Methods:

Collection of plant material and identification

Abutilon indicum (Linn.) Sweet plants(Local/Marathi name- Cakrabheṇdi, Petāri, Mudrā[18]) grown on road sides were collected from Latur district of Maharashtra. The plant was identified and authenticated by Botanical Survey of India, Western Regional Centre, Pune(Specimen No. BSI/WRC/IDEN.CER./2016/390).

Preparation of plant extracts

The fresh plant materials of *Abutilon indicum* (Linn.) Sweet were washed under running tap water, air dried in shade and then pounded into fine powder (Root, Stem, Leaves and Whole plant separately) using an electric blender. Each powdered content was stored in airtight bottles until required for further analysis[15, 19, 20]. Dried powder of different parts (Root, Stem, Leaves and whole plant) of *Abutilon indicum* (Linn.) Sweet were subjected to extraction in Soxhlet extractor using different solvents like Petroleum ether, Benzene, Chloroform, Methanol, Ethanol and Water. The extracts were filtered and concentrated under reduced pressure to obtain extracts as solid residues[20, 21].

Antibacterial activity

Pure culture of *Escherichia coli*was obtained from Department of Microbiology, Maharashtra Institute of Medical Sciences and Research (MIMSR) Latur, Maharashtra, India, and culture was maintained at 4°C. Antimicrobial activity of methanolic extracts of Root, Stem, Leaf and Whole plant was determined by agar well diffusion method. Microorganism was seeded by pouring 24 hr enriched culture in to nutrient agar plates and after solidification of media, agar surface was bored by using sterilized gel borer to make wells (7 mm diameter). 30 µl of methanolic extract was poured in to each well. Standard antibiotics (Ampicillin, Penicillin and Ciprofloxacin) solutions (500µg/ml, 50µg/ml,30µg/ml) were also poured in to separate wells of *E. coli* infected plates. The control plate well was filledin with only methanol of 30 µl. Plates were incubated at 37°C for 48 hours. Triplicate plates were maintained for observation of results[21,22].

Phytochemical analysis

Qualitative analysis-

Standard qualitative phytochemical identification tests[19,23] for different parts of plant extracts were performed,that istest for Alkaloids, Tannins, Flavonoids, Proteins, Carbohydrates, Sterols, Glycosides, Phenols, Saponins, Terpenoids, Gum and mucilage as shown below in Table No.1.

Table No. 1 - Phytochemical analysis tests

Name of the test	Type of plant part	extract solution
For Alkaloids i) Mayer's test ii) Wagner's test iii) Hager's test iv) Tannic acid test	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Tannins i) Ferric chloridetest ii) Gelatin test iii) Lead acetate test iv) Potassium dichromate test	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Flavonoids i) Test with NaOH ii) Test with H_2SO_4 iii) Shidona test	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Proteins i) Biuret test ii) Ninhydrintest iii) Xanthoproteictest	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Carbohydrate i) Benedict's test ii) Molisch'stest	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Sterols	Leaf, stem, root and	Pet ether, chloroform, benzene, ethanol,

i) Libermann-Burchard Reagent test ii) Salkowski'stest	whole plant	methanol and water
For Glycosides i) Keller- Killanitest ii) Baljettest iii) Salkowskitest	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Phenols i) Ferric Chloride test	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Saponins i) Foam test	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Terpenoids i) Chloroform test	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water
For Gums and Mucilages i) Test with 95% alcohol ii) Molisch's test	Leaf, stem, root and whole plant	Pet ether, chloroform, benzene, ethanol, methanol and water

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

EI- MS Spectrum was scanned at 70 eV with instrument details as follows:

Model of MS: Joel, Model: Accu Time of Flight Analyzer (TOF) GCV, Specification: Mass range of 10-2000 amu and resolution of 6000.

Make of GC: Agilent 7890, Detector: Flame Ionization Detector (FID), Run Time: 30 min.

GC-MS analysis was performed by splitless injection (spilt 20:80-8-200-5M-8-260-10M-10-280-HP5-ETOH) of 1.0 µL of the sample in methanol on a Hewlett Packard 6890 (USA) gas chromatograph fitted with a cross-linked 5% phenyl methyl Siloxane HP-5 MS capillary column (30 m x 0. 25 mm x 0. 26 µm coating thickness), coupled with a mass detector.

The GC-MS operating conditions were as follow:

The initial column temperature was 35 °C with a hold time of 3 minutes. The temperature was programmed to increase by 4K/min with a final temperature of 230°C. In a typical process, 1µl of the sample was injected into the port and immediately vapourised and moved down the column with helium as the carrier gas. After the separation in the column, the components were identified and further analysed by FID and the individual components were identified by NIST MS 2.0 structural library[24].

Results:

i) **Antibacterial activity**

The inhibitory effect of bacterial growth i.e. against *E.coli*, (which causes the urinary tract infection) was observed for the methanolic extracts of root, stem, leaf and whole plant of *Abutilon indicum*(Table No. 2, 3 and Fig. 1). Higher antibacterial activity which was 5.5 mm zone of inhibition was shown by whole plant extract followed by root zone of inhibition of 3.7 mm in size, stem and leaf zone of inhibition as 1.25 mm and 0.88 mm respectively (Table No.2). On the other hand,the zone of inhibition by chemical antibiotic drugs has been observed differently. The zone of inhibition was 7.25 mm by Ciprofloxacin only and there was no zone of inhibition by Ampicillin and Penicillin against *E.coli* (Table No.3). The results were observed from 3 sets of experiments (Fig. 1).

Table No. 2 – Antibacterial activity of plant

Sr. No.	Methanolic extract of plant parts	Zone of Inhibition (mm)
1	Leaf	0.88 +/- 0.13
2	Stem	1.25 +/- 0.1
3	Root	3.7 +/- 0.11
4	Whole plant	5.5 +/-0.3

Table No. 3 – Antibacterial activity of antibiotics

Sr. No.	Antibiotic solutions	Zone of Inhibition (mm)
1	Ampicillin	0
2	Penicillin	0
3	Ciprofloxacin	7.25 /-0.2

Fig. 1 – Antibacterial activity

ii) **Phytochemical analysis**

The results of qualitative phytochemical analysis of *Abutilon indicum* (Linn.) Sweet extracts of Leaf, Stem, Root and Whole plant in different solvents showed the presence of Alkaloids, Tannins, Flavonoids, Protins, Carbohydrates, Sterols, Glycosides, Phenols, Saponins, Terpenoids, Gum and mucilage (Table 4). Tannins, Flavonoids, Proteins, Sterols, Phenols, Saponins, Gum and mucilage were absent in the Petroleum ether, Benzene and chloroform extract tests. But majority of the phytochemicals were found in methanolic, ethanolic and aqueous (water) extracts of all parts.

Table No. 4 – Qualitative Phytochemical analysis tests (L-Leaf, S- Stem, R- Root, W- Whole plant)

Sr no	Phytochemical	Test	Petroleum ether				Benzene				Chloroform				Methanol				Ethanol				Water			
			L	S	R	W	L	S	R	W	L	S	R	W	L	S	R	W	L	S	R	W	L	S	R	W
1	Alkaloids	Mayer's test	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+
		Wagner's test	+	+	+	+	-	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
		Tannic acid test	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+

		Hyger's test	+	+	+	+	-	+	+	+	-	-	-	-	++	+	+	+	+	+	++	+	+	+	+	+
2	**Tannins**	**Ferric chloridetest**	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+
		Gelatin test	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	+	+	-	+	+	+
		Lead acetate test	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Potassium dichroma-te test	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
3	**Flavonoids**	**Test with NaOH**	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
		Test with H_2SO_4	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	-	-	-	+	+	+	+
		Shidona test	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+
4	**Proteins**	**Biuret test**	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	+	-	+	+	+
		Ninhydrin test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Xantho pro-teictest	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+	-	-	-	-
5	**Carbohydrate**	**Bendict's test**	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
		Molisch'stest	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
6	**Sterols**	**Liberman-Burchord test**	-	-	-	-	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	-	+	+	+
		Salkowski test	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
7	**Glycosides**	**Keller-Killani test**	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
		Baljet test	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
		Salkowski test	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
8	**Phenols**	**Ferric chloridetest**	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	+	+	+	+	+
9	**Saponins**	**Foam test**	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	+
10	**Terpenoids**	**Chloroform**	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+

		test																							
11	**Gum &mucilage**	**Test with alcohol**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	+
		Molisch's test	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+

iii) **GCMS Analysis**

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 *Abutilon indicum* (Linn.) Sweet. In this study, presence of 08 different compounds namely, Oleic acid ($C_{18}H_{34}O_2$) followed by 10-Undecen-1 al, 2- methyl ($C_{12}H_{22}O$), (Z)6, (Z)9-Pentadecadien-1-Ol ($C_{15}H_{28}O$), Phytol ($C_{20}H_{40}O$), 17-Octadecyonic Acid ($C_{18}H_{32}O_2$), n-Hexadecanoic acid ($C_{16}H_{32}O_2$), Oxirane, tetradecyl ($C_{16}H_{32}O$), 3,7,11,15-Tetramethyl-2-hexadecane-1-ol ($C_{20}H_{40}O$) were found as major compounds (Fig No. 3-10). Fig.2 represents the total GC-MS spectra of the methanolic extract of whole plant, while Fig. 3 to 10 show expanded pictures of peaks of Fig. 2 representing individual mass spectrum of identified compounds.

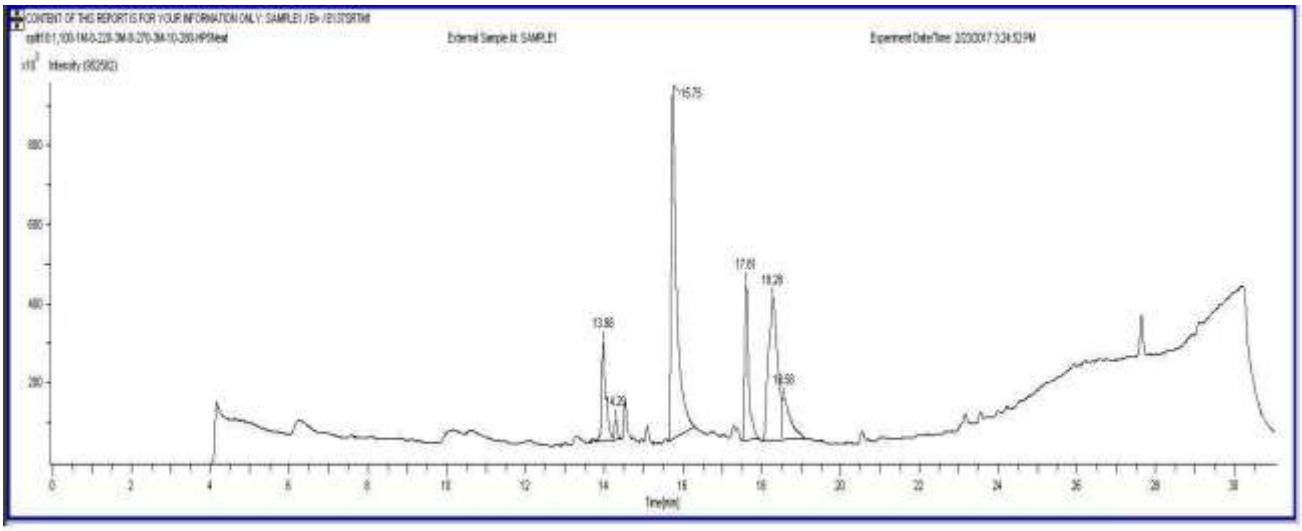

Fig. 2- GC-MS analysis of methanolic extract of *Abutilon indicum* (Linn.) Sweet whole plant

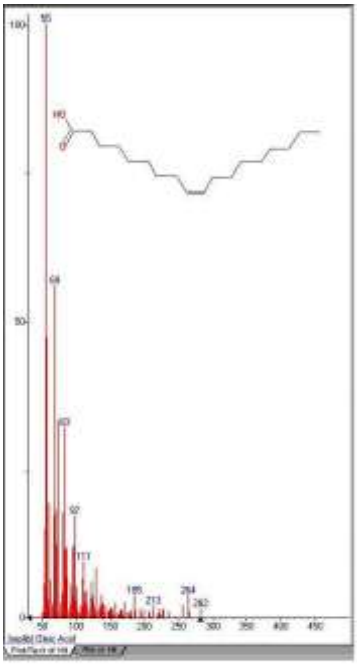

Fig. 3 - Mass spectrum of Oleic acid ($C_{18}H_{34}O_2$) – MW- 282

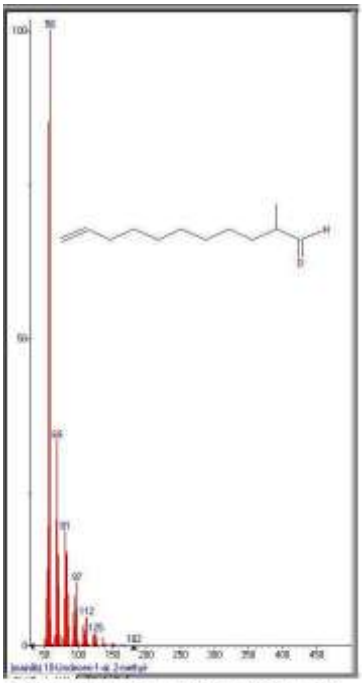

Fig 4. - Mass spectrum of 10-Undecen-1 al, 2-methyl ($C_{12}H_{22}O$)- MW-182

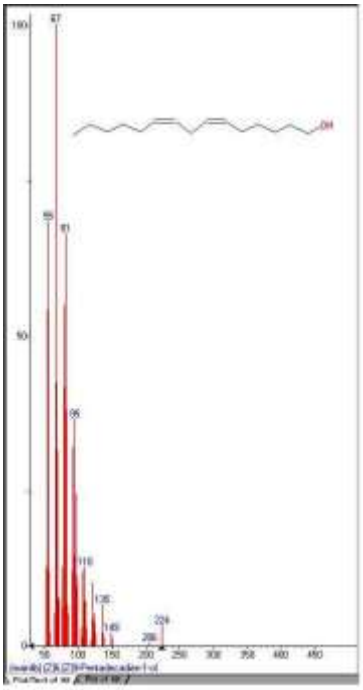

Fig. 5 - Mass spectrum of (Z)6, (Z)9-Pentadecadien-1-Ol (C15H28O)- MW-224

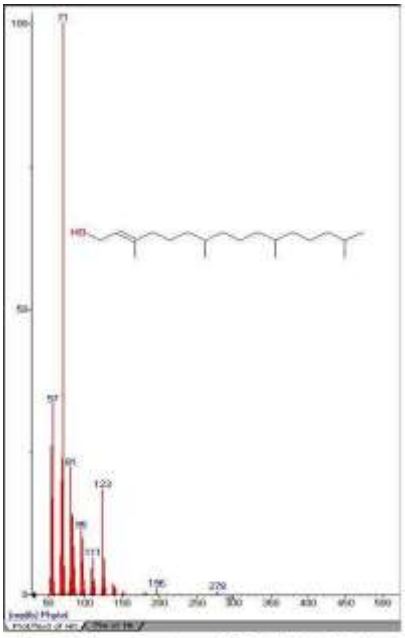

Fig. 6 - Mass spectrum of Phytol ($C_{20}H_{40}O$) – MW-296

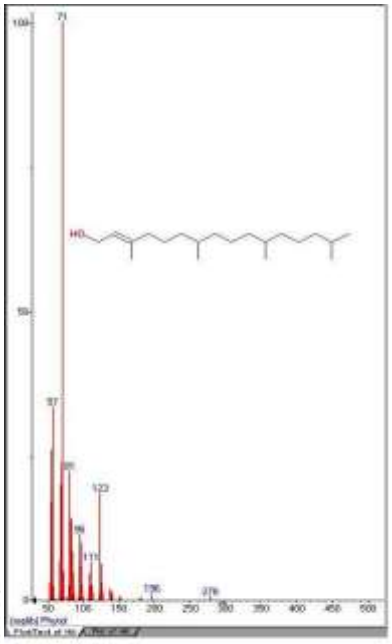

Fig. 7 - Mass spectrum of 17-Octadecyonic Acid ($C_{18}H_{32}O_2$)- MW- 280

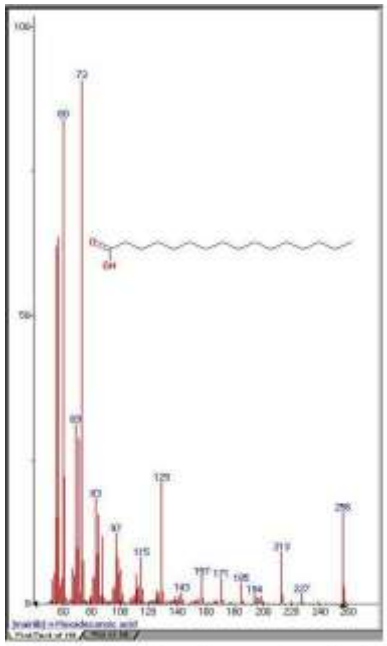

Fig. 8 - Mass spectrum of n-Hexadecanoic acid ($C_{16}H_{32}O_2$)-MW-256

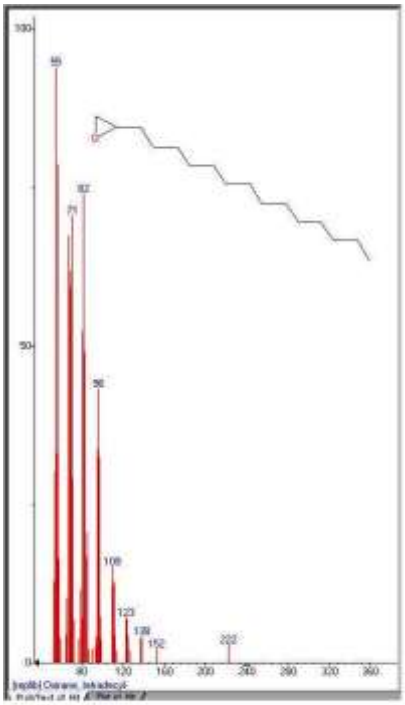

Fig. 9 - Mass spectrum of Oxirane, tetradecyl ($C_{16}H_{32}O$) –MW-240

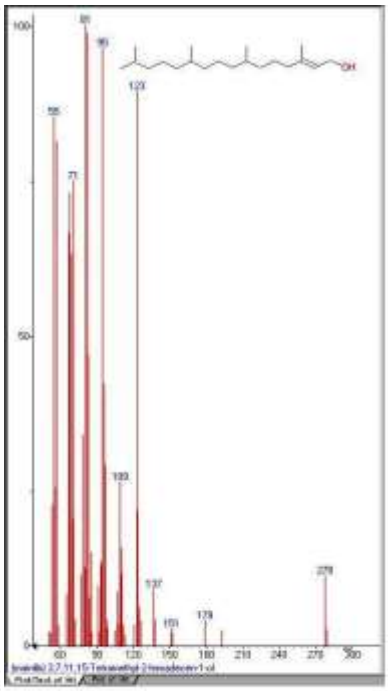

Fig. 10 - Mass spectrum of 3, 7, 11, 15-Tetramethyl-2-hexadecane-1-ol ($C_{20}H_{40}O$)-MW- 296

Discusssion:

In present study methanolic extracts of*Abutilon indicum* plant parts shown decent inhibitory activity against *E.coli*, which is the most infectious bacteria found in urinary tract infections. However, it was observed that*E. coli*has shown resistance against antibiotics like ampicillin andpenicillin, but itis sensitive to ciprofloxacin antibiotics.*E. coli* could be multidrug resistantat certain circumstances[25,26]. The whole plant methanolic extract of *Abutilon indicum*although shows greater inhibition against *E.coli* than other plant parts,this observationverifies its antibacterial nature in the treatment of urinary infections as reported earlier[27,28].

The plant parts of *Abutilon indicum* subjected to qualitative phytochemical determinations in different solvents showed distinguished confirmatory tests, whichare normally considered as diagnostic index.Most of the phytochemicals were found in polar solvents that are methanol, ethanol and water,showing presence of Alkaloids, Tannins, Flavonoids, Protins, Carbohydrates, Sterols, Glycosides, Phenols, Saponins, Terpenoids, Gum and mucilage. It is observed that the methanolic extracts responded for more phytochemicals in the confirmatory tests.Several studies confirmed that total phenolic compounds depend on organic solvent polarity and methanol is one of the most suitable solvents for the extraction of total phenolic compounds from plants[29]. Water and methanol have high dielectric constants and dipole moments as polar protic solvents. Moreover, the use of methanol makes the process of evaporation easier when compared to water[30].The selection of appropriate solvent for preparation of plant extracts is thus an art and experience apart from scientific values. The GC-MS analysis of whole plant methanolic extract reveals about 8 different compounds.These compounds have been described for different medicinal activities as shown in Table No. 5.

found in GC-MS analysis

Sr No.	Compound name	RT	Molecular formula	Molecular weight	Medicinal Activity
1	Oleic acid	23.25	$C_{18}H_{34}O_2$	282	Anti-inflammatory, Flavor,Insectifuge, Antiandrogenic, Cancer preventive, Dermatigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic[31, 32]
2	10-Undecen-1 al, 2- methyl	18.58	$C_{12}H_{22}O$	182	Anti-microbial, Ant- inflammatory activity[33]

3	(Z)6, (Z)9-Pentadecadien-1-Ol	18.3	$C_{15}H_{28}O$	224	Anti-oxidant, Anti-microbial activity[32, 34]
4	Phytol	17.61	$C_{20}H_{40}O$	296	Hypocholesterolemic, Anti-microbial, Anti-cancer, Cancer preventive, Diuretic Anti-inflammatory[31, 32, 34]
5	17-Octadecyonic Acid	17.29	$C_{18}H_{32}O_2$	280	Anti-hypertensive [35]
6	n-Hexadecanoic acid	15.74	$C_{16}H_{32}O_2$	256	Lubricant, Anti-androgenic flavor, Hypocholesterolemic flavor, Hemolytic, Anti-oxidant, Pesticide, 5-alpha reductase inhibitor [31, 36]
7	Oxirane, tetradecyl	14.54	$C_{16}H_{32}O$	240	No activity reported [31]
8	3,7,11,15-Tetramethyl-2-hexadecane-1-ol	13.97	$C_{20}H_{40}O$	296	Anti-microbial and Anti-inflammatory activity[36, 37]

These activities of the compounds do help in curing urinary disorders like *Mūtrakṛcchra* (painful urination) or urinary infections. To know the effectiveness of the plant against a disease/disorder/ailment, it is important to understand the phytochemical constituents of plants since there is relevance of these chemicals in controlling the cell activities[38].

Study of active biochemical compounds reveals the medicinal secrets of the plants which are enlisted in the ancient *Āyurvedic*texts. Study of these secondary metabolic chemicals of *Abutilon indicum* (Linn.) Sweet shrub proves and strengthens the knowledge of pharmaceutical property of this plant described in the *MādhavaCikitsā*. Hence, use of *Abutilon indicum* for the treatment of *Mūtrakṛcchra*by *Mādhava* establishes its merit.

Multiple uses of plants for medicinal purposes have necessitated their large scale collection as raw material andsupply to the pharmaceutical industries leading to over exploitation and disappearing of the wild populations of such plants. Pharmaceutical industries can directly produce these active biochemical compounds commercially in large amount by using the plant tissue culture technique which will be helpful for conservation of herbal diversity[39].

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