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Phytochemical and Antimicrobial screening of *Datura* species against various clinical isolates

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ABSTRACT

The investigation was carried out to determine the possible bioactive components of the leaves and fruits of *Datura stramonium* and *D. metel* by Antimicrobial activity and GC-MS. The chemical compositions of the methanol extract of the *D. stramonium and D. metel* were investigated using Hewlett-Packard Gas chromatography–Mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the Wiley and National Institute of Standards and Technology (NIST) library or with the published mass spectra. Maximum activity was observed in methanolic extracts of fruits of *D. metel* against mycoplasma at 200mg/ml and minimum against *Pseudomonas chrysogenum* at 50mg/ml.

Introduction

Antimicrobial therapy was developed in the first half of the last century .The development of antimicrobial resistance in many bacterial and fungal organism, however, constitutes one of the most serious problems to control infectious diseases. Phytomedicines comes handy in fight against microbial resistance (Osuala *et al*, 2005) and attention has been paid to exploit plant product as novel chemotherapeutants against bacterial and various fungal infection (Mishra and Dubey, 1994). The genus *Datura* belongs to Family Solanaceae. *Datura stramonium* originated in North America, and was first described by Linnaeus in 1753.Commonly it is known as Thornapple. The genus was derived from "datura", an ancient Sanskrit word for the plant. Stramonium is originally from Greek, strychnos (nightshade) and manikos (mad).

All parts of *D.stramonium* plants contain dangerous levels of the tropane alkaloids atropine, hyoscyamine and scopolamine. There is a high risk of fatal overdose amongst uninformed users, and many hospitalizations occur amongst recreational users who ingest the plant for its psychoactive effects (Preissel, 2002).When the plant is younger, the ratio of scopolamine to atropine is approximately 3:1; after flowering, this ratio is reversed, with the amount of scopolamine continuing to decrease as the plant gets older (Nellis, 1997). *D. stramonium* seed contains about 0.1 mg of atropine, and the approximate fatal dose for adult humans is >10 mg atropine or >2–4 mg scopolamine (Arnett, 1995). Exposure of the foetus to *D. stramonium* when a mother use it for asthma, will cause a continuous release of acetylcholine, this can cause damage to foetus (Pretorius and Marx, 2006).

D. metel grows in the wild in all the warmer parts of the world, and is cultivated worldwide for its chemical and ornamental properties. *D.metel* contains tropane alkaloids and are used as sedative, anti-spasmodic and mydriatic agents (Nuhu, 2002). The xanthine oxidase inhibitory activity was assayed for *D. metel* which is traditionally used for the treatment of gout (Umamaheswari*et al*, 2007). Hyoscyamine and scopolamine are the two commercially important anesthetic, antispasmodiac and anticholinergic drugs, also the two most important alkaloids produced in roots and then translocated to the aerial parts of *D. metel* plants (Zhang *et al*, 2004). *D. metel* seed powder possessed blood glucose lowering effect (Murthy *et al*, 2004)

D. metel used for the treatment epilepsy, hysteria, insanity, heart diseases, and for fever of catarrh diarrhea and skin diseases. Scopolamine used in modern medicine for motion sickness, as a bronchodilator, asthma relief and many cold medicine to dry out mucous membranes (Mandal and Shah, 2013).

Materials and Methods

The fresh leaves and fruits of both plants were collected from Botanical garden department of Botany, university of Rajasthan Jaipur. The leaves (1 kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (940 g). The powdered plant sample was packed into a Soxhlet apparatus (5L) and extracted exhaustively with 2L methanol for 72 hours on water bath. The methanol extract was concentrated using a rotary evaporator at 45°C and left on the laboratory bench for 2 days to obtain a dark-brown liquid, further the compounds were separated by column chromatography. Finally isolated compounds were characterized by GC-MS.

Gas Chromatography Mass Spectroscopy (GC-MS)

The extract and the standard samples were analyzed by GC-MS of Hewlett-Packard 6890/5973 operating at 1000 eV ionization energy, equipped with a HP-5. Capillary column (phenyl methyl siloxane, 25 m×0.25 mm i.d) with Helium (He) was used as the carrier gas with split ratio 1:5. Oven temperature was 100 °C (3 min) to 280 °C at 1 to 40 °C/min; detector temperature, 250 to 280°C; carrier gas, Helium (0.9 ml/min).

Retention indices were determined by using retention times of samples that were injected under the same chromatographic conditions. The components of the standard and plant samples were identified by comparison of their mass spectra and retention time with those given in literature and by comparison

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with the mass spectra of the Wiley library or with the published mass spectra. **Table: 7-8**

Antimicrobial Activity

Antimicrobial activity of *D. stramonium* and *D. metel* was studied with their methanolic extracts. Four bacterial and fungal strains were selected for the primary screening.

Microorganisms Used

Clinical laboratory bacterial isolates viz.*Staphylococcus epidermis, Mycoplasma* and fungal isolates viz.*Alternaria solani* and *Penicillium chrysogenum* were collected from the stock cultures of Microbiology Laboratory, SMS Medical College Jaipur, India.

Preparation of Extract

The crude methanol extract was obtained by macerating 30 g of dried plant powder in 100% methanol and kept on a rotary shaker for 24 h. The extract was filtered, centrifuged at 5000 g for 15 min and was dried under reduced pressure. The extract was stored at 4 ºC in airtight bottles.

Culture and Maintenance of Bacteria

Pure cultures of *S. epidermis, Mycoplasma, A.solani and P.chrysogenum* obtained from SMS Medical College, Jaipur, India was used as indicator organisms. These bacteria were grown in Nutrient agar medium (prepared by autoclaving 8% Nutrient agar of Difeco-Laboratories, Detroit, USA, in distilled water at 15 lbs psi for 25-30 min) and incubating at 37°C for 48 h. Each bacterial culture was further maintained on the same medium after every 48 h of transferring. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude methanol extract was studied against gram positive and gram negative bacterial strains by the agar well diffusion method (Perez *et al*, 1990). Mueller Hinton Agar No. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% dimethylsulphoxide (DMSO) at the concentrations of 5 mg/ml. The Mueller Hinton agar was melted and cooled to 48 – 50 ºC and a standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37^{o}C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotics, streptomycin. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed three times to minimize the error and the mean values are presented.

Determination of Antifungal Assay

Antifungal activity of the experimental plant was investigated by agar well diffusion method (Bonjar *et al*, 2005). The yeasts and saprophytic fungi were subcultured on to Sabouraud's Dextrose Agar (SDA; Merck, Germany) and respectively incubated at 37 °C for 24 h and 25 °C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 ml of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37 °C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in cm). All experiments were made in triplicate and means were calculated.

Results

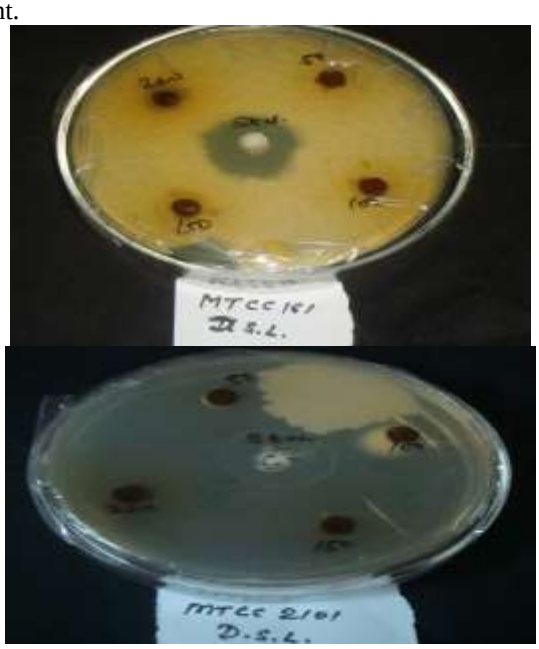
Physico-chemical screening of various metabolites (carbohydrates, proteins flavonoids and alkaloids) of *D. stramonium and D. metel* parts (leaves and fruits) were analysed (Table- 1 and 2). On sequential extraction, all the plant parts and callus tissue exhibited a similar response for all the major groups of metabolites tested. It showed that the biosynthetic potentialities for all types of metabolites in general are retained in their *in vitro* grown tissue culture system also. Among the different solvents used for sequentially extraction, the total extractability was maximum in alcohol, while in C_6H_6 and $CHCl_3$ it was minimum both *in vivo* and *in vitro*.

The methanol extracts of the *D. stramonium* leaves showed potent antibacterial activity against *Mycoplasma* at 200 mg/ml (IZ 2.6 cm) and minimum activity against *S. epidermis* at 50 mg/ml (IZ 0.8 cm).Against fungal strains *P.chrysogenum* showed maximum activity at 200mg/ml (IZ 1.1 cm) and *A. solani* was found to be resistant.

The methanol extracts of the *D. stramonium* fruits showed maximum activity against bacterial strain *Mycoplasma* at 200 mg/ml (IZ 2.4 cm) and minimum activity against *S. epidermis* at 50 mg/ml (IZ 0.9 cm).Against fungal strain *P. chrysogenum* showed maximum activity at 200 mg/ml (IZ 1.0 cm) minimum activity 50 mg/ml (IZ 0.2 cm).

The methanol extracts of the *D. metel* leaves showed potent antibacterial activity against *Mycoplasma* and *S. epidermis* at 200 mg/ml (IZ 2.1 cm) and minimum activity against *S. epidermis* at 50 mg/ml(IZ 0.6 cm).Against fungal strains *P. chrysogenum* showed maximum activity at 200mg/ml (IZ 1.8 cm) and minimum activity against *A. solani* 50mg/ml (0.8 cm).

The methanol extracts of the *D.metel* fruits showed maximum activity against bacterial strain *Mycoplasma* at 200 mg/ml (IZ 2.7 cm) and minimum activity against *S. epidermis* at 50 mg/ml (IZ 0.8 cm).Against fungal strain *P. chrysogenum* showed maximum activity at 200 mg/ml (IZ 1.2 cm)and *A. solani* resistant.



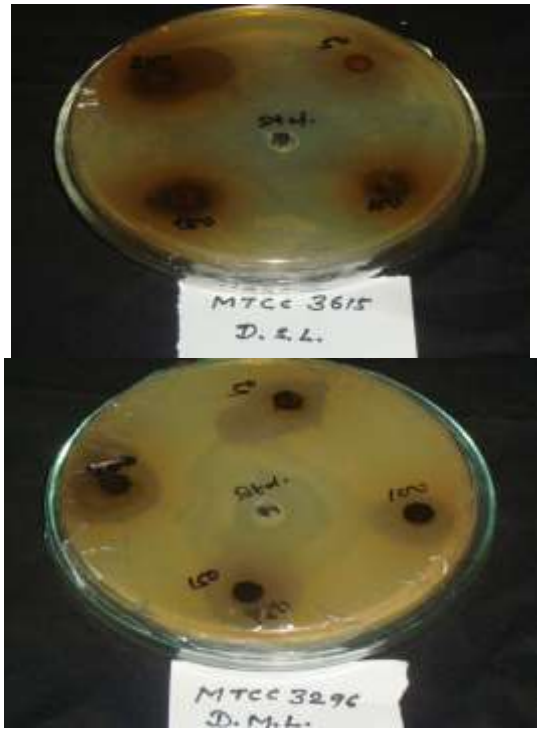


Fig 1: Antimicrobial activity methanol extract of *D. stramonium*leaves.

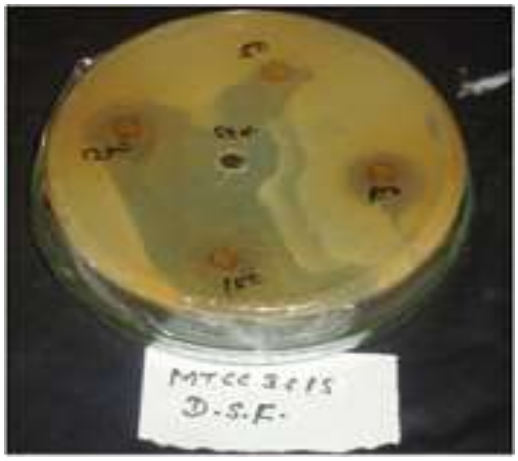


Fig 2: Antimicrobial activity methanol extract of *D. stramonium* fruits.

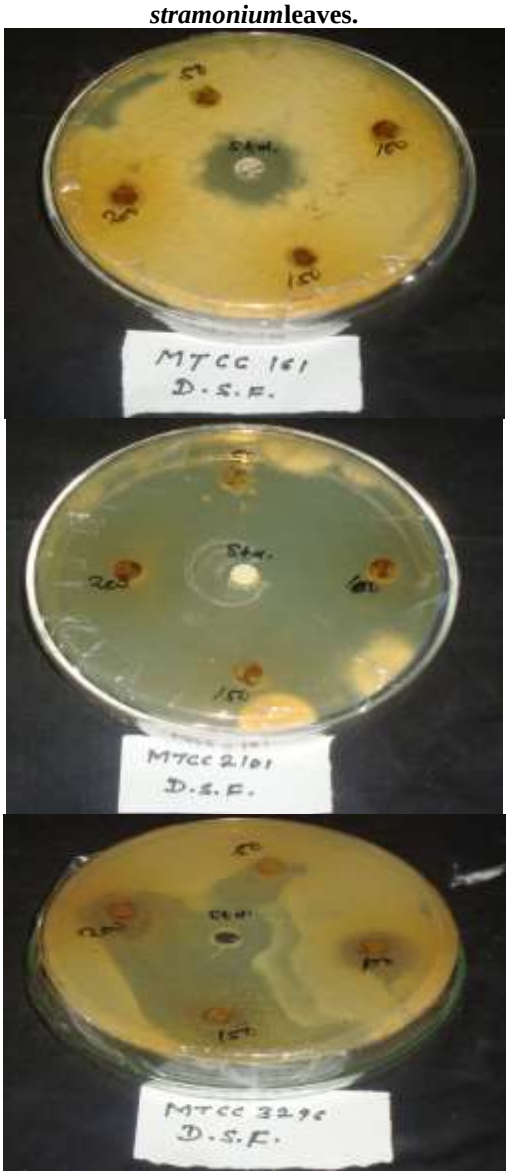


Fig 3: Antimicrobial activity methanol extract of *D. metel* leaves

Fig 4: Antimicrobial activity methanol extract of *D. metel* fruits.

GC-MS Results:-

The GC-MS studied showed that the retention time and peaks of the isolated extracts. identified compounds in plant sample shown in (Table and Fig: 7-8).

Discussion

Plants synthesize variety of phytochemicals as part of their normal metabolic activity. In 2010 a survey of 1000 plants was done out of which, 156 clinical trials for evaluation of their pharmacological activities and therapeutic applications gave encouraging results (Cravotto *et al*, 2010).

Tropane alkaloid biosynthesis in *D. stramonium* mainly takes place in the roots (Conklin, 1976). From the site of synthesis the compounds are translocated to upper parts of the plant. Within cells, the alkaloids most likely occur in the form of crystals in the vacuoles (Verzar-Petri, 1973).

Bouzidi *et al*, 2011 reported that tropane alkaloid profile differs between species. Around thirty different tropane alkaloids have been found in species such as *D. ceratocaula*, *D. inoxia*, *D. stramonium* var. *stramonium*, *D. stramonium* var. *tatula* and *D. stramonium* var. *godronii* (Berkov and Zayed, 2004; Berkov *et al*, 2006). These alkaloids seem to occur in all tissues of the plant, except the capsules and the woody portions of the roots and stems of some *Datura* species, but the largest diversity in constituents is generally found in the roots (Zielinska-Sowicka and Szepczynska, 1972). The stems contain a lower number of tropane alkaloids and even less alkaloids are found in seeds, leaves and flowers (Siddiqui *et al*, 1988; Lounasmaa and Tamminen, 1993; Vitale *et al*, 1995, Philipov and Berkov, 2002). However, for the main tropane alkaloids, hyoscyamine and scopolamine, levels are higher in flowers and leaves than in the root (Witte *et al*, 1987) and seeds contain substantial amounts of these alkaloids (Miraldi *et al*, 2001). Relative amounts of various tropane alkaloids in different tissues (roots, stem, leaves, flowers and seeds) have been reported for *D. ceratocaula* (Berkov, 2003), *D. stramonium* var. *stramonium*, *D. stramonium* var. *tatuala* and *D.stramonium* var. *godronii* (Berkov *et al*, 2006).

Eftekhar *et al*, 2005 reported that the methanol extracts of aerial parts of *D. stramonium* showed the bactericidal activity against Gram-positive bacteria in a dose-dependent manner. However, little or no antibacterial activity was found against *Escherichia coli* and *Pseudomonas aeruginosa*.

Mdee *et al*, 2009 reported antifungal activity of acetone extracts of *D. stramonium* against several fungi including *Penicilliu mexpansum, Aspergillus niger, Aspergillus parasiticus, Colletotrichum gloeosporioides, Fusarium oxysporum, Trichoderma harzianum, Phytophthora nicotiana, Pythium ultimum* and *Rhizoctonia solani*. The MIC of *D. stramonium* extracts ranges from 1.25 to 2.5 mg/mL. The fungicidal effects of the extracts indicate the potential of *D. stramonium* seeds as a natural source of antifungal Agent.

Okwu and Igara, 2009 reported a new antibacterial agent 5`,7`dimethyl 6`– hydroxy3`, phenyl 3 a - amine b - ynesitosterol in *D. metel.*The Compound displayed antibacterial activity against *S.aureus, Pseudomonas aeruginosa, Proteus mirabis, S.typhi, Bacillus subtilis* and *Klebsiella pneumonia* but could not inhibit *Escherichia coli*.

Panda and Bandyopadhyay, 2013 reported chemical information from GC-MS studies of methanolic leaf extract of *Andrographis peniculata* and *D. metel* and their antimicrobial activity against *Pseudomonas aeruginosa* (PB112) Strain. Twelve compounds were identified by GC-MS Investigation of *D. metel*.

Table 1: Physico- chemical evaluation of *Datura stramoinum* L. from leaves and fruits

Parameter		Organic Solvents Used				
		Pet. Ether	Benzene	Chloroform	Alcohol	Water
Physical Appearance	Plant Parts	Yellow Green Sticky	Bright Red Oily	Yellowish Orange Oily	Red Brown Sticky	Brown Dusty Viscous
Carbohydrate	Leaves	-	+	++	++	++
	Fruits	+	++	+	+++	+++
Proteins	Leaves	++	-	+	++	++
	Fruits	+	++	+	++	+
Flavonoids	Leaves	+	++	+++	++	+++
	Fruits	++	++	+++	+	++
Alkaloids	Leaves	++	+ +	+	+ +	+
	Fruits	+	+ +	+	+ ++	-

- absent; + trace amount;++ moderate amount; +++significant amount

Table 2: Physico- chemical evaluation of *Datura metel* L. from leaves and fruits

Parameter		Organic Solvents Used				
		Pet. Ether	Benzene	Chloroform	Alcohol	Water
Physical Appearance	Plant Parts	Yellow Green Sticky	Bright Red Oily	Yellowish Orange Oily	Red Brown Sticky	Brown Dusty Viscous
Carbohydrate	Leaves	-	+	++	++	++
	Fruits	+	++	+	+++	+++
Proteins	Leaves	++	-	+	++	++
	Fruits	+	++	+	++	+
Flavonoids	Leaves	+	++	+++	++	+++
	Fruits	++	++	+++	+	++
Alkaloids	Leaves	++	+ +	+	+ +	+
	Fruits	++	+ +	+	+ ++	-

- absent; + trace amount;++ moderate amount; +++significant amount

Table 3: Antimicrobial activity of *D.stramonium* L .leaves methanol extract.

Organisms	standard	Concentration (mg/ml)			
		50	100	150	200
S. epidermis	2.0 cm	0.8 cm (0.40)	1.4 cm (0.7)	1.5 cm (0.75)	1.8 cm (0.90)
Mycoplasma	2.8 cm	1.9 cm (0.67)	2.1 cm (0.75)	2.3 cm (0.82)	2.6 cm (0.92)
P.chrysogenum	2.0 cm	0.3 cm (.15)	0.4 cm (0.20)	0.6 cm (.30)	1.1 cm (0.55)
A.solani	2.9 cm	R	R	R	R

IZ= zone of inhibition (in cm.)
AI = zone of inhibition of plant/ zone of inhibition of standards. Values are denoted in bracket
R: Resistant

Table 4: Antimicrobial activity of *D.stramonium* L. fruits methanolic extract

Organisms	standard	Concentration (mg/ml)			
		50	100	150	200
S. epidermis	2.1 cm	0.9 cm (0.42)	1.0 cm (0.48)	1.5 cm (0.71)	1.9 cm (0.90)
Mycoplasma	2.9 cm	1.1 cm (0.37)	1.7 cm (0.59)	2.1 cm (0.72)	2.4 cm (0.82)
P.chrysogenum	2.0 cm	0.2 cm (0.1)	0.3 cm (0.15)	0.4 cm (0.20)	1.0 cm (0.5)
A.solani	1.7 cm	0.3 cm (0.18)	0.4 cm (0.23)	0.6 cm (0.35)	0.8 cm (0.47)

IZ= zone of inhibition (in cm.)
AI = zone of inhibition of plant/ zone of inhibition of standards. Values are denoted in bracket
R: Resistant

Table 5: Antimicrobial activity of *D. metel* L. leaves methanol extract

Organisms	standard	Concentration (mg/ml)			
		50	100	150	200
S. epidermis	2.2 cm	**0.6 cm** (0.27)	**1.3 cm** (0.59)	**1.5 cm** (0.68)	**2.1 cm** (0.95)
Mycoplasma	2.4 cm	**1.4 cm** (0.58)	**1.5 cm** (0.62)	**1.7 cm** (0.70)	**2.1 cm** (0.87)
P. chrysogenum	2.9 cm	**1.0 cm** (0.34)	**1.3 cm** (0.44)	**1.5 cm** (0.51)	**1.8 cm** (0.62)
A.solani	1.8 cm	**0.8 cm** (0.44)	**1.3 cm** (0.72)	**1.5 cm** (0.83)	**1.6 cm** (0.88)

IZ= zone of inhibition(in cm.)
AI = zone of inhibition of plant/ zone of inhibition of standards. Values are denoted in bracket
R: Resistant

Table 6: Antimicrobial activity of *Datura metel* L. fruits methanol extract

Organisms	standard	Concentration (mg/ml)			
		50	100	150	200
S. epidermis	2.1 cm	**0.8 cm** (0.38)	**1.2 cm** (0.57)	**1.4 cm** (0.66)	**1.7 cm** (0.80)
Mycoplasma	3.0 cm	**1.2 cm** (0.4)	**2.3 cm** (0.76)	**2.5 cm** (0.83)	**2.7 cm** (0.90)
P.chrysogenum	4.1 cm	**0.2 cm** (0.04)	**0.4 cm** (0.09)	**0.5 cm** (0.12)	**1.2 cm** (0.29)
A.solani	0.6 cm	R	R	R	R

IZ= zone of inhibition (in cm.)
AI = zone of inhibition of plant/ zone of inhibition of standards. Values are denoted in bracket
R: Resistant

Table 7: Compounds identified in leaves of *Datura stramonium* L. by GC-MS

Peak	Retention time	Area%	Name of compound
1	10.959	2.51	2-Pentanone, 5-hydroxy-
2	11.059	0.42	2-Hexanone, 4-hydroxy-3-propyl
3	11.558	0.38	1,7-Dimethyl-4-(1-methylethyl)cyclodecane
4	12.160	3.50	3-Hexadecene, (Z)-
5	12.255	2.15	Tetradecane
6	13.007	1.66	Cyclohexane, octyl
7	13.540	1.50	Pentadecane
8	13.847	0.74	Phenol, 3,5-bis(1,1-dimethylethyl)-
9	13.931	0.67	Cyclohexane, 1,2,4,5-tetraethyl-
10	14.249	0.79	Cyclohexane, 1,2,4,5-tetraethyl-
11	14.633	0.63	Cyclohexane, 1,1'-(1-methylpropylidene)bis-
12	14.840	11.13	1-Pentadecene
13	14.928	3.90	Pentadecene
14	15.849	0.98	Selinan
15	15.963	2.16	n-Nonylcyclohexane
16	16.118	2.18	8-Pentadecanone
17	16.468	0.42	Pentadecane
18	16.665	2.42	4-(4-hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one
19	17.856	12.23	1-heptadecene
20	17.927	2.96	Octadecane
21	18.106	4.19	Loliolide
22	18.891	1.81	Dodecylcyclohexane
23	18.992	7.36	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester
24	19.746	2.52	beta.-selinenol
25	20.066	1.42	dibutyl phthalate
26	20.250	7.82	1-nonadecene
27	20.299	1.24	Eicosane
28	21.121	3.08	Dodecylcyclohexane
29	22.082	7.32	1-tricosene
30	22.828	1.54	n-heptadecylcyclohexane
31	23.557	3.43	1-tricosene
32	24.328	1.99	cyclohexane, eicosyl
33	24.803	1.21	di-n-octyl phthalate
34	24.992	1.73	Cyclooctacosane

Table 8: Compounds identified in leaves of *Datura metel* L. by GC-MS

Peak	Retention time	Area%	Name of compound
1	14.515	1.45	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
2	14.812	4.25	1-Pentadecene
3	17.816	10.81	1-Heptadecene
4	17.900	1.55	Octadecane
5	18.557	1.74	2-Pentadecanone, 6,10,14-trimethyl-
6	18.922	2.23	8-Octadecanone
7	19.506	2.90	2- Bromopropionic acid, pentadecyl ester
8	20.058	4.90	Dibutyl phthalate
9	20.222	9.96	1-Nonadecene
10	21.206	0.93	2(3H)-Furanone, 5-Heptyldihydro
11	21.348	7.34	9,12,15-octadecatrienoic acid, methyl ester, (z,z,z)-
12	21.452	14.17	Phytol
13	21.883	1.37	(e)-9-octadecenoic acid ethyl este
14	22.062	3.57	1-nonadecene
15	22.822	1.85	decane 4-cyclohexyl-, 4-cyclohexyl
16	23.545	1.59	1-nonadecene
17	24.291	1.36	1-octadecanol
18	24.795	5.91	1,2-benzenedicarboxylic acid
19	27.551	1.98	furan, 4,5-diethyl-2,3-dihydro-2,3-dimethyl
20	27.786	3.00	17-pentatriacontene
21	28.189	13.69	4-tert-butylcalix[4]arene
22	33.675	3.45	ergost-5-en-3-ol

Bharathi *et al*, 2010 reported antibacterial activity and phytochemical analysis of *D. metel* against bacterial pathogens associated with HIV like *P. aeruginosa, K. pneumoniae, E. coli, S. aureus and S. typhi*. Antibacterial activity of the plant extracts (Fresh leaves, stems and root) show different inhibition spectrum against the isolated opportunistic bacterial pathogens. Among them leaf methanol extract shows good antibacterial activity.

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