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GC-MS, HPTLC and Antimicrobial analysis of Root extracts of *Pseudarthria viscida* Wight and Arn and *Desmodium gangeticum* (Linn) DC

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Abstract

The present study was performed to evaluate the chemical composition of the methanol extract of Pseudarthria viscida (L) Wight and Arn and Desmodium gangeticum (Linn) DC. 43 compounds have been identified from P. viscida extract and the major chemical constituents are cis-Vaccenic acid (16.47%),γsitosterol (13.73%) and stigmasterol (6.24%).18 compounds have been identified from D.gangeticum and the major chemical constituents are 9,12-Octadecadienoic acid (41.71%), n-Hexadecanoic acid (9.43%) and Octadecanoic acid (5.9%). Stigmasterol was quantified from both extracts by HPTLC method (105.15 µg/ml and 20.9 µg/ml respectively). In- vitro antibacterial and antifungal activities of methanolic extracts of P.viscida and D.gangeticum was evaluated in the present study. The zone of inhibition and minimum inhibitory concentration were measured. Ampicillin (30 µg/disc) and mystatin (20 µg/disc) were used as standard for antibacterial and antifungal activity respectively.

Keywords: *Pseudarthria viscida* (L) wight and Arn., d*esmodium gangeticum* (Linn) DC., fabaceae, GC-MS analysis, HPTLC, antibacterial activity.

Introduction

In Kerala *Desmodium gangeticum* (Linn) DC. is in use as orila[1]. This plant is identified as muvila (*salaparni*) in north Indian publications. In 'The Ayurvedic Formulatory of India' the latin name cited for *salaprni* is *D. gangeticum* and *Uraria picta and Uraria lagopoides* are the Latin names given for *Prisniparni.* In several treatises *prisiniparni* is otherwise known as *prathakparni*. It is considered to be orila in Malayalam. Species of *Uraria* have four to nine leaflets and hence the name *prisniparni* or *prathakparni* do not seem to be appropriate for this plant. Similarly *Desmodium gangeticum* which has only simple leaves does not deserve the name muvila, known in Sanskrit as *salaparni* or *triparni.*

Dasamula is known to pacify pain, arthritis, fever, cough, bronchitis, general weakness, neuropathy, nervine weakness, urinary tract diseases and boosts immune power. Trade data collected over the years has indicated that the demand for different ingredients in *Dasamula* has exceeded the supply, requiring the ingredients used in the herbal preparations to be obtained in larger quantities. This being the case, it has been noted that the original species of many of the ingredients are geographically not available in the quantities required. This leads to them being substituted or even adulterated with other species that may have similarities or differences in the structural and chemical profiles. In *Dasamula Kvatha Curna, Prsniparni* is one such ingredient which is known to be substituted with other species to meet the market demand. The original *Dasamula* formulations, listed in the first edition of the Ayurvedic Formulary of India (AFI, 1978), contain roots of *Prsniparni.* Some of the different species used as substituents or adulterants for *Prsniniparni* are: i. *Pseudarthria viscida* Wight and Arn. ii. *Desmodium gangeticum* (L.) DC., iii. *Uraria lagopodioides* DC. iv. *Uraria picta Desv.*

In order to find out if the above mentioned species are of comparable action, efficacy and use to the original species *Prisniparni*, qualitative and quantitative analyses of each species have to be carried out. To start with we would like to compare *Pseudarthria viscida* Wight and Arn and *Desmodium gangeticum* (L.) DC.

Pseudarthria viscida (L) Wight and Arn. (Fabaceae) is a shrub. The roots are astringent, emollient, thermogenic, digestive, constipative, anthelmintic, anti-inflammatory, aphrodisiac, cardiotonic, febrifuge and also used as a rejuvenating tonic. They are useful in vitiated conditions of cough, bronchitis, asthma, tuberculosis, helminthiasis, cardiopathy, fever, hemorrhoids, gout, hyperthermia and general debility[2].

Desmodium gangeticum (Linn.) DC. (Fabaceae) is an eruct, diffusely branched under shrub. The roots are bitter, sweet, thermogenic, nervine tonic, aphrodisiac, diuretic, cardiotonic. They are useful in vitiated conditions of vata, anorexia, dysentery, fever, gout, caugh, asthma, cardiopathy and debility[3]. It is used in Indian system of medicine as a bitter tonic, febrifuge, digestive, anticatarrhal, antiemetic, inflammatory conditions of chest and various other inflammatory conditions due to tie 'sata' disorders[4].

The extracts of leaf, root, stem and callus obtained from *P.viscida* showed significant inhibitory activity against some fungal pathogens causing major diseases in crop plants and stored food grains[5]. The preliminary phytochemical screening

indicated the presence of alkaloids, phenolic compounds, flavonoids, tannins and saponins. The ethanolic extract of *P.viscida* showed antioxidant[6], antidiabetic[7], anti-inflammatory, diuretic effect[8], *in vitro* cytotoxic activity[9] and antidiarrhoeal activity[10]. Although *P. viscida* is commonly used in Ayurveda, scientific reports on its activity and the phytoconstituents present are very scarce. The antioxidant activities of the ethanolic extract of the whole plant material was carried out by Vijayabaskaran *et.al.* and Gincy M Mathew *et.al*.[11]. In our ongoing research work cytotoxic activity of the alkaloids and free radical scavenging activity of the aqueous extract of the root part of *Pseudarthria viscida* (L) Wight and Arn was reported[12].

In 1969 S.Ghosal and P.K. Banerjee isolated and identified 7 alkaloids from the roots of *Desmodium gangeticum*[13] and alkaloidal context of this plant possess anticholinesterase, smooth muscle stimulant, CNS stimulant,depressant responses[14]. The sterols *N, N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine, their oxides and other derivatives have been isolated from aerial parts[15]. Three pterocarpenoids gangetin, gangetinin and desmodin are the major chemical constituents of the roots[16]. Gangetin, a pterocarpan, and shows anti-fertility activity by affecting alkaline phosphatase activity in uterine fluid[17]. Phytochemical screening has revealed that *D. gangeticum* contains alkaloids such as tryptamines, phenethylamines and their *N*-oxides[18], pterocarpenoids such as gangetin, gangetinin, desmodin and desmocarpin; phospholipids[19], sterols[20] and flavanoid glycosides like 4,5,7-trihydroxy-8-prenylflavone-4'Ox-L-rhamnopyranosyl-(1-6)- β-D-glucopyranoside[21] and 8-C-prenyl-5,7,5-trimethoxy-3,4-methylenedioxy flavones[22] and also it possesses antiwrithing activity, moderate central nervous system depressant activity[23].

D.gangeticum has antioxidant activity[24], potential prophylactic and therapeutic efficacy against *Leishmania* infection[25], Ninteen compounds have been isolated by P.K.Mishra,Nasib Singh et.al.in which aminoglucosyl glycerolipid isolated from *D.gangeticum* whole plant exhibited *in vitro* antileishmanial and immunomodulatory activities[26], aqueous extract of *D.gangeticum* possesses cardio-protective effect through antioxidant activity and hypocholesterolemic action[27], alcoholic extract of *D.gangeticum* possesses a strong antioxidant activity[28]. Paste of the stem bark of *D.gangeticum* DC (Galfula II) is applied on the affected part for goiter remedy, once a day for 3-4 days[29], phytochemical examination *D.gangeticum* (Linn.) DC. root and aerial parts has resulted into lupeol, lauric acid and mixture of β-sitosterol and stigmasterol. HPLC and MS/MS showed the presence of gallic, protocatechuic, salicylic, chlorogenic, caffeic acids, rutin, quercetin and kaempferol in root and aerial parts of plant[30]. Orally administrated insulin mixed aqueous extract of *D.gangeticum* root is efficient in protecting the heart from ischemia reperfusion induced injury in diabetic rats[31] and the extracts of *D.gangeticum* have antimicrobial potential[32].

In the present work, compounds are identified by GC-MS of methanol extract of *P.viscida* and *D. gangeticum*, antibacterial activity including MRSA and antifungal activity were also carried out. Further of the compounds, stigmasterol, which is present in both the plants, was quantified by HPTLC method.

Material and Methods

Collection and identification of plant material: The plant *Pseudarthria viscida*(Linn) Wight and Arn. and *gangeticum* (Linn) DC was collected from Palakkad district, Kerala, India. The plant material was identified by the Botanical survey of India, Southern regional Centre, Tamilnadu Agricultural University Campus, Lawley Road, Coimbatore-641 003 (No.BSI/SRC/5/23/2010-11/Tech 1786and 1787).

Reagents and instruments: The gas chromatogram was recorded in Agilent make with GC 7890 with Mass detector 5975C with DB-5 column having 95% polydimethylsiloxane with 5% phenyl group. For GC/MS detection, an electron ionization (EI) with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min and injection volume was 1 µl (split ratio 10:1).Injector temperature 250°C; Ion-source temperature 260°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 25°C/min, to 150°C (hold 10 min), then 25°C/min to 260°C, ending with a 40 min. isothermal at 260°C. Total run time was 59.6 min. Software adopted to handle mass spectra and chromatogram was a Chemstation and compounds are identified from NIST library match. Pet. Ether, ethyl acetate and methanol were purchased from Finar chemicals.

Preparation of extracts: Extract for GC -MS and HPTLC studies: The roots were dried well in shade to avoid certain compounds from getting denatured in sunlight. The dried root (5 Kg) was powdered extracted with double distilled methanol by maceration process for 3 days. The methanol extract was filtered using Whatman 41 filter paper and the residue was removed. It was again filtered through sodium sulphate in order to remove the traces of moisture and the residue was used for the studies.

Test solution preparation: The methanol extract sample was dissolved in 1ml methanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

Sample application: 2µl of test solution and 2µl of standard solution was loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development: The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (steroid) and the plate was developed in the respective mobile phase up to 90mm.

Photo-documentation: The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at white light, UV 254nm and UV 366nm.

Derivatization: The developed plate was sprayed with respective spray reagent (steroid) and dried at 100°C in Hot air oven. The plate was photo-documented in day light and UV 366nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning: After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500nm. The peak table, peak display and peak densitogram were noted. The software used was winCATS 1.3.4 version.

Analysis details: Mobile phase: Petroleum ether 60-80ºC-ethyl acetate (9:1).

Spray reagent: Anisaldehyde sulphuric acid reagent.

Detection: Blue, blue-violet coloured zone at daylight mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of steroid in the given standard and may be in the sample.

Microorganisms: The following bacterial strains were employed in the screening: Gram positive *Streptococcus pneumonia* and *Bacillus cereus and* the Gram negative *Aeromonas hydrophila Vibrio cholera* and *Methicilin-resistant staphylococcus aureus* (MRSA). In the antifungal screening the following fungi were tested: *Candida albicans, M.purpureus, A.flavus, A.terreus and P.notatum.*

Antimicrobial screening: Disc diffusion method: The bacterial strains (*Streptococcus* sp*., B.cereus, A. hydrophila V. cholerae* and MRSA[33]) were inoculated in the nutrient broth under aspectic condition and incubated at 37 ^{0}C for 18 hours. After the incubation period, the test bacterial was swabbed on the nutrient agar plate using sterile cotton swab. In each of these plates, wells (10 mm) were cutout using sterile cork borer. The methanol extract was dissolved in the solvent. Controls were maintained by loading same quantity of Ampicillin into the wells. Then the petri dishes were incubated at 37 ^{0}C for 14 hours. The anti microbial activity was evaluated by measuring the zone of inhibition in diameter. The zone of inhibition in diameter was observed and recorded in millimeter.

Minimum Inhibitory concentration (MIC): The Minimum inhibitory concentration (MIC) was determined through the dilution method. Bacteria were grown in nutrient broth (NA) for 6 hrs. After this, 20 µL of 106 cells/mL were inoculated in tubes with nutrient broth supplemented with 4 different concentrations (100 µL, 150 µL, 200 µL and 250 µL) of the extracts. After 24 hrs at 37°C, the MIC of each sample was measured through optical density in the spectrophotometer (620nm) through the comparison of the sample readout with the known inoculated nutrient broth and the results are enlisted in tables, Ampicillin was used as a standard substance, DMSO as the negative control. The same method was carried out for MRSA using Ampicillin as the positive control, DMSO as the negative control.

Antifungal screening: The inoculums for the experiment were prepared in fresh sabouraud's broth from preserved slant culture. The inoculum was standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth (if excessive) or by further incubationg to get required turbidity. Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc. The standardized inoculums is inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed.

Each Petri dish is divided into 2 parts , in 2 parts extract discs such as PV and DG (250mcg) discs, (discs are soaked overnight in extract solution) and one quadrant for Std nystatin 10mcg, are placed in each quadrant with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4 º C or at room temperature for 1 hour for diffusion. Incubate at room temperature for 24 - 48 hours. Observe the zone of inhibition produced by different Antibiotics. Measure it using a scale or divider or venire calipers and record the average of two diameters of each zone of inhibition.

Results and Discussion

GC-MS analysis: The chromatogram of *P. viscida* for the GC-MS was shown in the figure-1. Forty three compounds have been identified from *P. viscida* extract in which four are phenolic compounds (2.07%), seventeen are acids (33.2%), three are plant sterols (22.13%). cis-Vaccenic acid is the major compound with 16.47%, followed by γ-sitosterol (13.73%), n-Hexadecanoicacid (8.97%), stigmasterol (6.24%) and Stigmast-4-en-3-one(5.48%). Alkaloids, alcohols, esters, ethers, hydrocarbons, terpenes etc. are also identified from NIST library match. Previously GC-MS of the methanolic extract from *P. viscida* was reported 3-O-methyl-d-glucose (61.33%) as the major compound followed by fatty acids like n-Hexadecanoic acid (12.66%), oleic acid (7.93%) and 9,12-octadecanoic acid (4.88%)[34].

The chromatogram of *D.gangeticum* for the GC-MS was shown in the figure-2. Eighteen compounds have been identified from *D. gangeticum* in which three are phenolic compounds (4.1%),

seven are acids (58.90%), two are plant sterols (5.12%). 13 common compounds are present in both extracts. 9.12-Octadecadienoic acid is the major compound with 41.71% followed by n-Hexadecanoic acid (9.43%) and octadecanoic acid (5.9%) and γ-sitosterol (3.68%). Alkaloids, alcohols, esters, ethers, hydrocarbons etc are also identified from library match. Only one report was available in the literature which indicates n-Hexadecanoic acid (34.68%) as the major compound[35].

HPTLC: The table-3 and 4 and figure- 3 and 4 indicate the presence of stigmasterol (standard Rf- 0.20cm and extract-0.20cm) in methanol extract of *P. viscida* and *D. gangeticum.* Blue, violet and pink colored zones at day light mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of stigmasterol in the given standard and in the sample. Further table 3 and 4 and fig 3 and 4 indicates the presence of atleast five steroids in *P. viscida* and four steroids in *D. gangeticum* including stigmasterol at an Rf value 0.2. Compound stigmasterol was quantified and the quantities are 105.15µg/ml and 20.9µg/ml respectively.

Antibacterial and antifungal Screening: Antimicrobial activity was conducted against a food borne pathogenic microorganisms including Gram positive and Gram negative bacteria and fungi.

The antibacterial activity and antifungal activity of the extracts of *P.Viscida* and *D.gangeticum* at different concentrations were screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The antimicrobial activity of the *P.Viscida* against gram (+ve) and gram (-ve) bacteria shown in table 5. *P.Viscida* exhibited inhibitory activity against *B.cereus* and *A. hydrophila* with narrow inhibition zones of 12.0 and 11.0 respectively and MIC value of 12, 15, 13 and 20 mg/ml for *Streptococcus pneumonia, Bacillus cereus, Aeromonas hydrophila* and *Vibrio cholera*

The methanol extract exhibited significant antifungal activity against most of the tested fungi species with zones of inhibition between 5-9 mm at the tested concentration. The antifungal activity of *P.viscida* against *Candida albicans*, *M.purpureus*, *A. flavus, A. terreus* and *P. notatum* with narrow inhibition zone 9.0, 6.0, 5.0, 7.0 and 7.0 mm respectively and MIC value of 5.0, 6.0, 8.0, 9.0 and 10.0 mg/ml and the results are comparable with the standard substance.

D.gangeticum exhibited inhibitory activity against *B.cereus* and *A. hydrophila* with narrow inhibition zones of 12.0 and 13.0 respectively and MIC value of 15, 18, 12, 10 and 20 mg/ml for *Streptococcus pneumonia, Bacillus cereus, Aeromonas hydrophila* , *Vibrio cholera* and MRSA .

The methanol extract exhibited significant antifungal activity against most of the tested fungi species with zones of inhibition between 3.5-10 mm at the tested concentration. The antifungal activity of *P.viscida* against *Candida albicans*, *M.purpureus*, *A. flavus, A. terreus* and *P. notatum*. with narrow inhibition zone 9.0, 6.0, 5.0, 7.0 and 7.0 mm respectively and MIC value of 8.0, 10.0, 7.0, 6.0 and 3.5 mg/ml and the results are comparable with the standard substance.

Anti MRSA activity: Methicillin-resistant Staphylococcusaureus (MRSA) has become endemic in most hospitals and health care facilities. The MRSA strains are broadly resistant to β-lactam and macrolide/azalide antimicrobials but responsive to certain non-β-lactam antibiotics[36]. However, resistance rates are increasing and there are other limitations in the use of those drugs. Thus given the widespread dissemination and morality caused by MRSA, the synthesis and development of new drug is imperative. *P.viscida* and *D.gangeticum* does not show any zone of inhibition against MRSA and MIC value for *P.viscida* and *D.gangeticum* is 17 and 20 mg/ml respectively.

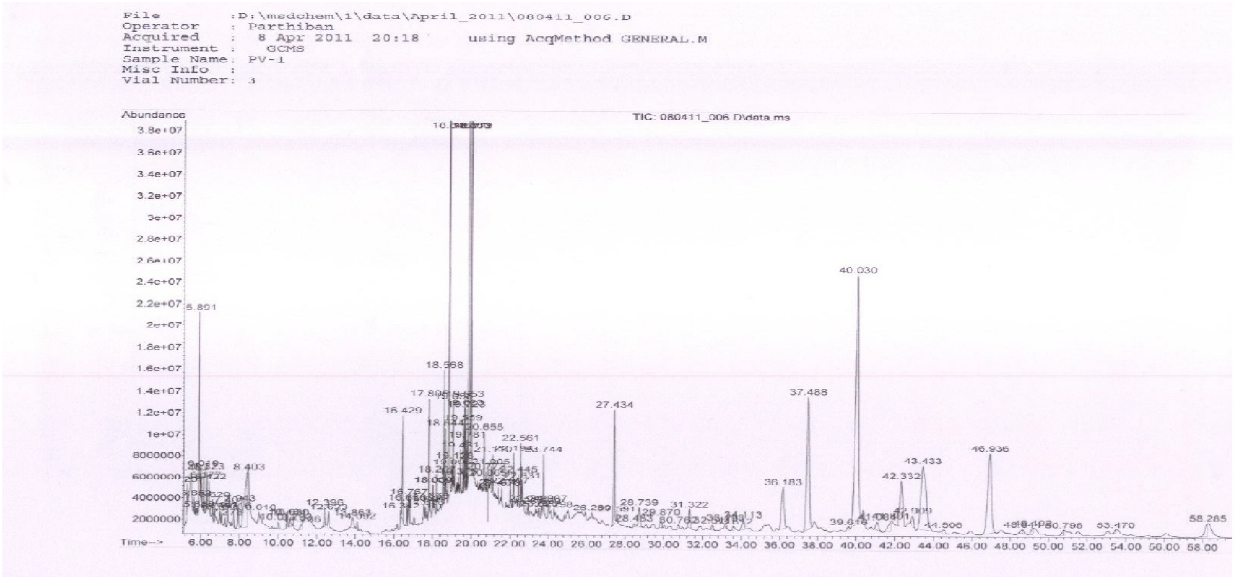

Figure-1
GC MS of *Pseudarthria viscid*

Table-1
Compounds from GC-MS of *P.Viscida*

S No	Compound name	Retention time	Area %
1.	Benzaldehyde, 4-methoxy-	6.177	0.22
2.	2-Propenal, 3-phenyl-	6.322	0.29
3.	2-Propen-1-ol, 3-phenyl	6.620	0.15
4.	Vanillin	7.716	0.33
5.	trans-Cinnamic acid	7.943	0.47
6.	Dodecanoic acid	10.484	0.17
7.	Benzoic acid, 4-hydroxy-3-methoxy-	10.630	0.28
8.	Asarone	12.396	0.38
9.	Ar.tumerone	14.092	0.19
10.	2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-	16.342	0.29
11.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	16.429	1.38
12.	2-Propenoic acid, 3-(4-methoxyphenyl)-ethyl ester	16.767	0.17
13.	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	17.548	0.23
14.	Pentadecanoic acid	17.636	0.10
15.	Hexadecanoic acid, methyl ester	18.568	0.97
16.	n-Hexadecanoic acid	18.848	8.97
17.	3,5-Dimethoxy-4-hydroxycinnamaldehyde	19.006	0.19
18.	cis-10-Heptadecenoic acid	19.315	0.10
19.	Heptadecanoic acid	19.431	0.27
20.	E-15-Heptadecenal	19.559	0.38
21.	10,13-Octadecadienoic acid, methyl ester	19.623	0.38
22.	11-Octadecenoic acid, methyl ester	19.653	0.43
23.	Phytol	19.723	0.45
24.	Octadecanoic acid, methyl ester	19.781	0.26
25.	Octadecanoic acid	19.979	2.93
26.	cis-Vaccenic acid	20.655	16.47
27.	Cyclopentadecanone, 2-hydroxy-	20.865	0.47
28.	Eicosanoic acid	21.110	0.42
29.	1-Hexacosanol	22.007	0.31
30.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	22.194	0.6
31.	Oleic acid	22.445	0.41
32.	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	22.561	0.62
33.	Docosanoic acid	22.631	0.31
34.	Rosenonolactone	22.940	0.13
35.	12-Ethylsophoramine	23.744	0.79
36.	9,12-Octadecadien-1-ol,methyl ester	24.007	0.26
37.	Piperine	27.434	2.46
38.	Campseterol	36.183	2.16
39.	Stigmasterol	37.488	6.24
40.	Gamma sitosterol	40.030	13.73
41.	Lup-20(29)-en-3-one	42.332	2.43
42.	Ergosta-4,6,8(14),22-tetraen-3-one	42.909	0.42
43.	Stigmast-4-en-3-one	46.936	5.48

Table-2
Compounds from GC-MS of *D.Gangeticum*

S. No.	Compound name	RT	Area %
1.	Benzaldehyde, 4-methoxy-	6.183	0.48
2.	1,2,3- Benzenetriol	7.296	1.06
3.	2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-	16.342	0.31
4.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	16.418	2.47
5.	N,N-Dimethyltryptamine	17.409	0.60
6.	Hexadecanoic acid, methyl ester	18.569	0.40
7.	7-Hexadecanoic acid	18.673	0.13
8.	n-Hexadecanoic acid	18.831	9.43
9.	3,5-Dimethoxy-4-hydroxycinnamaldehyde	19.006	0.57
10.	9,12-Octadecadien-1-ol,methyl ester	19.629	0.28
11.	11-Octadecenoic acid, methyl ester	19.664	0.28
12.	Heptadecanoic acid, 16-methyl-, methyl ester	19.787	0.19
13.	9.12-Octadecadienoic acid	19.886	41.71
14.	Octadecanoic acid	19.979	5.90
15.	12-Ethylsophoramine	23.750	1.31
16.	E,Z-1,3,12-Nonadecatriene	24.007	0.97
17.	Stigmasterol	37.465	1.44
18.	gamma Sitosterol	39.936	3.68

Table-3
Quantity and presence of Stigmasterol from the *P.viscida* methanol extract of dried root

Track	Peak	Rf	Height	Area	Assigned substance
SGL	1	0.20	45.8	5344.2	Stigmasterol standard
Sample *P.V*	1	0.01	175.6	1103.4	Unknown
Sample *P.V*	2	0.07	13.7	132.2	Steroid 1
Sample *P.V*	3	0.11	46.1	819.8	Steroid 2
Sample *P.V*	4	0.20	163.9	2957.8	Steroid 3 (Stigmasterol)
Sample *P.V*	5	0.22	168.3	5514.3	Steroid 4
Sample *P.V*	6	0.30	11.8	243.4	Unknown
Sample *P.V*	7	0.41	253.2	14476.6	Unknown
Sample *P.V*	8	0.77	34.1	765.9	Unknown
Sample *P.V*	9	0.81	51.2	1673.0	Steroid 5
Sample *P.V*	10	0.94	215.1	8206.5	Unknown

Table-4
Quantity and presence of Stigmasterol from the *D.gangeticum* methanol extract of dried root

Track	Peak	Rf	Height	Area	Assigned substance
Sample *D.G*	1	0.01	100.2	796.1	Unknown
Sample *D.G*	2	0.07	29.9	337.5	Steroid 1
Sample *D.G*	3	0.11	27.0	445.8	Steroid 2
Sample *D.G*	4	0.20	331.0	586.5.	Steroid 3 (Stigmasterol)
Sample *D.G*	5	0.24	163.0	2257.6	Steroid 4
Sample *D.G*	6	0.40	243.7	14466.5	Unknown
Sample *D.G*	7	0.77	25.9	1326.2	Unknown
Sample *D.G*	8	0.86	23.0	687.2	Unknown
Sample *D.G*	9	0.94	235.3	8863.4	Unknown
SGL	1	0.20	173.1	5342.8	Stigmasterol standard

Table-5
Antibacterial activity of extract of *P.viscida* and *D.Gangeticum* root

S. No.	Organism	Zone of inhibition (mm)			MIC (mg)		
		P.V.	***D.G.***	**Ampicillin**	***P.V.***	***D.G.***	**Ampicillin**
1.	*Streptococcus pneumonia*	-	-	-	12	15	-
2.	*Bacillus cereus*	12	11	15.0	15	18	5.0
3.	*Aeromonas hydrophila*	11	13	-	13	12	-
4.	*Vibrio cholera*	-	-	16.0	20	10	5.0
5.	Methicillin-Resistant *Staphylococcus aureus*(MRSA)	-	-	17.0	17	20	8.0

Table-6
Antifungal activity of *P.Viscida* and *D.Gangeticum*

Fungi	Zone of inhibition (mm)			MIC (mg)		
	P.V	***D.G***	**Nystatin**	***P.V***	***D.G.***	**Nystatin**
C. albicans	9.0	5.0	26.3	5.0	8.0	0.03
M.purpureus	6.0	4.0	18.6	6.0	10.0	1.2
A. flavus	5.0	8.0	15.6	8.0	7.0	0.004
A. terreus	7.0	8.0	14.0	9.0	6.0	0.8
P. notatum	7.0	7.0	18.0	10.0	3.5	0.3

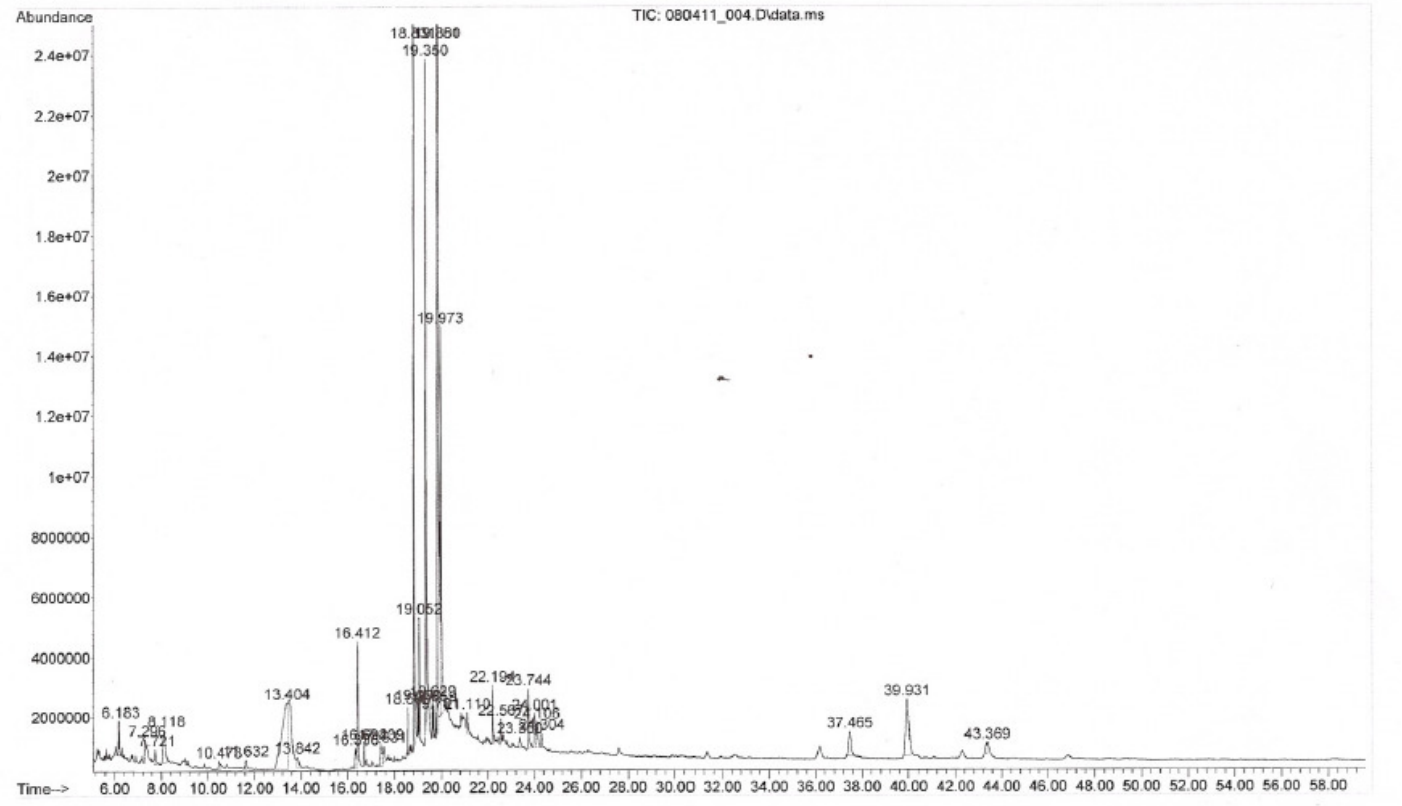

Figure-2
GC MS of *Desmodium gangeticum*

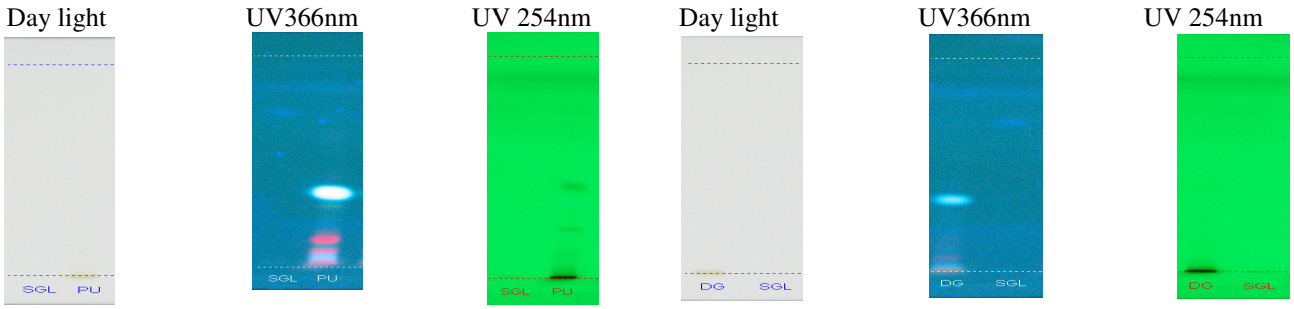

Figure-3
TLC profile of PV and DG before derivatization

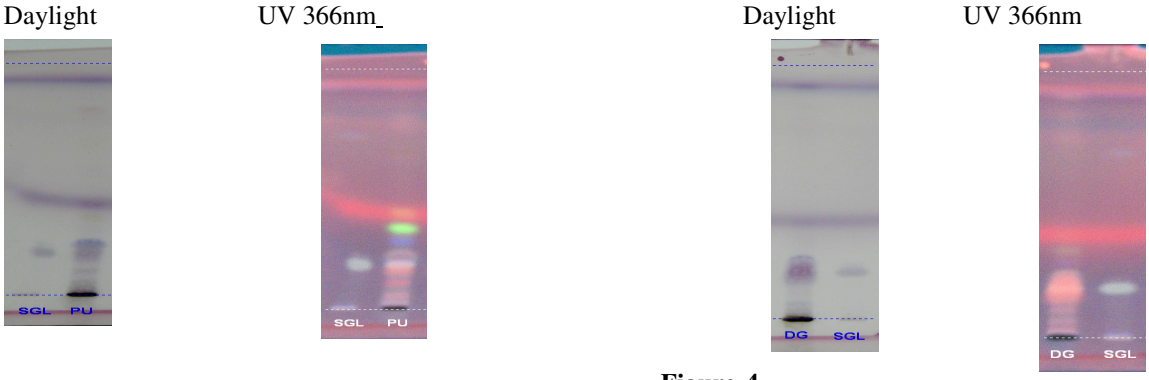

Figure-4
TLC profile of PV and DG after derivatization

Conclusion

Here we are reporting 43 compounds from *P.viscida* and 18 compounds from *D.gangeticum* using GC-MS method, antibacterial including MRSA and antifungal activities were carried out and zone of inhibition and MIC were calculated. Stigmasterol was quantified in *P.viscida* and *D.gangeticum* methanolic extracts by HPTLC method.

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