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PHYTOCHEMICAL AND GC-MS ANALYSIS OF LEAF EXTRACTS OF *PSEUDARTHRIA VISCIDA* (LINN.) WIGHT & ARN.

M. Rajina*, P. Ratheesh-Chandra and K. M. Khaleel

Research Centre in Botany, Sir Syed College, Taliparamba, Kannur University, Kannur - 670142, Kerala, India.

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Correspondence to Author:
M. Rajina

Research Scholar
Research Centre in Botany
Sir Syed College, Taliparamba,
Kannur University, Kannur - 670142,
Kerala, India.

E-mail: rajinam98@gmail.com

ABSTRACT: The present study elucidates the secondary metabolite profile of leaves of *Pseudarthria viscida*, one of the main ingredient of dasamula in Ayurveda. Alkaloids, phenolic compounds and flavonoids were estimated in different solvent-extracts. Acetone extract of leaves showed highest content of phenols and flavonoids. The leaves are quite rich in alkaloids and is found to be 1.9 mg g^{-1}. GC-MS analyses revealed the presence of 20 major volatile organic compounds and were identified by their mass fragmentation pattern in comparison with NIST library. Most of the compounds have antioxidant, anti-inflammatory, antimicrobial and anticancer properties. The present study has identified a major compound Neophytadiene which is a well-known enzyme inhibitor.

INTRODUCTION: Nature has been a source of medicinal agents for thousands of years and a striking number of modern drugs have been isolated from natural sources, many based on their use in traditional medicines or phytomedicines.[1] Scientific interest in medicinal plants has flourished due to an increased efficiency of new plant derived drugs, growing interest in natural products and rising concerns about the side effects of conventional medicine. The use of various herbal remedies and preparations are described throughout human history representing the origin of modern medicine. Many conventional drugs originate from plant sources.[2] *Pseudarthria viscida* is a shrub, belonging to the family Fabaceae, known as 'muvila' in Malayalam and 'triparni' in Sanskrit.

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The roots are astringent, emollient, thermogenic, digestive, constipative, anthelmintic, anti-inflammatory, aphrodiasiac, cardiotonic, febrifuge and also used as rejuvenating tonic. They are useful in vitiated conditions of cough, bronchitis, asthma, tuberculosis, helminthiasis, cardipathy, fever, hemorrhoids, gout, hyperthermia, and general debility.[3] In Ayurveda system of medicines it is used as an ingredient of *Dasamula* that is known to pacify pain, arthritis, fever, cough, bronchitis, general weakness, neuropathy, nervine weakness, urinary tract diseases and boosts immune power. The plant has shown to possess antifungal [4] antioxidant [5] and analgesic and anti-pyretic effects. [6] In the present study, some of the medicinally important secondary metabolites are quantitatively estimated and their active phytochemical constituents are identified by GC-MS analysis.

MATERIALS AND METHODS:

Collection of Plant Materials and Preparation of Extracts: Fresh seedlings of *Pseudarthia viscida* was collected from Government Ayurvedic

Medical College, Parassinikadavu, Kannur and cultivated in field under greenhouse conditions. Healthy, disease-free plant leaves were collected at maturity and washed thoroughly under running tap water followed by double distilled water and were dried at room temperature (34 °C) for one week. Then the dried leaves were powdered in a laboratory grinder and stored in air tight containers.

Twenty five grams of powdered sample was successively extracted by cold maceration technique using solvents acetone methanol and water. The extracts were dried at room temperature and stored at 4 °C. The crude extracts thus prepared were used for the estimation of phenolics and flavonoids.

Determination of total alkaloids: The total alkaloid contents in different plant samples were quantified according to the method of Harborne, 1973. Five gram of powdered sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the supernatant was concentrated on a water bath at 100 $^{\circ}$C to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Determination of total phenolic content: Total phenolic content in each crude extract was estimated using Folin–Ciocalteu method as described by Makkar *et al.,* 2000. Hundred milligram of crude extract was dissolved in 100 ml of respective solvent. 1 ml of this solution was transferred to a test tube, added 0.5 ml of 2 N Folin-Ciocalteu reagent followed by 1.5 ml of 20% Na_2CO_3 solution and the volume was made up to 8 ml with distilled water.

The mixture was subjected to vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm with a spectrophotometer (Labtronics model LT-290). The total phenolic content was calculated using gallic acid as standard and the results were expressed as gallic acid equivalents (GAE).

Determination of total flavonoids: The total flavonoid content of different crude extracts were estimated using a slightly modified colorimetric assay described by Zhishen *et al.,* 1999. 0.5 ml of diluted extract was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes- added 0.15 ml of 10% $AlCl_3$ solution and allowed to stand for 6 minutes, followed by 2 ml of 4% NaOH solution. Finally added distilled water to bring the final volume to 5 ml and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined spectrophotometrically at 510 nm. The analysis was performed in triplicates and the total flavonoid content was estimated using rutin as standard and the results were expressed as rutin equivalents.

GC-MS Analysis: GC-MS analysis of *P. viscida* leaf extract was performed using a Themo Scientific Trace 1300 gas chromatograph with TG-5MS column (30 m × 0.25 mm ID ×0.25 µm) and ISQ- QD Single quadrupole mass spectrometer. Helium (99.999%) was used as the carrier gas at constant flow rate of 1 ml/minute and an injection volume of 1 µl was employed.

An injection port temperature of 280 ºC and an ion-source temperature of 200 ºC was set. The oven temperature was programmed from 60 ºC for 3 minutes with an increase of 5ºC /minute to 240 ºC with a hold time of 3 minutes. Then temperature was increased at a rate of 35 ºC/min till 280 ºC with a hold time of 5 minutes. Scan interval was programmed for 0.2 seconds with a mass range of 40-450 amu. Total GC running time was 45 minutes. The peaks were identified by comparing their mass fragmentation pattern with that of NIST library data.

Statistical Analysis: All experiments were repeated for a minimum of 5 times and the mean values ± standard error is given in tables.

RESULTS AND DISCUSSION: Secondary metabolites in acetone, methanol and water extracts of leaves are analyzed and the results are tabulated in **Table 1.** The acetone extract showed highest level of phenols (25.74 mg g^{-1}). The higher amount of phenol is important in regulation of growth, development and disease resistance. The flavonoid

content was also higher in acetone extract (65.29 mg g^{-1}).

TABLE 1: SECONDARY METABOLITES IN DIFFERENT EXTRACTS OF *P. VISCIDA* LEAVES

Secondary metabolites	Acetone	Methanol Aqueous	
	mg g^{-1}		
*Total phenols	25.74±0.03	20.39±0.49	6.16±0.31
**Flavonoids	65.29±0.06	64.45±0.38	8.69±0.04
***Alkaloids		1.9±0.29	

*gallic acid equivalent
**rutin equivalent
***In 1 g dried leaf powder

GC-MS analysis: GC-MS chromatogram of *P. viscida* leaf extract clearly revealed the presence of 20 major peaks indicating the presence of 20 organic volatile compounds. The major compounds identified by GC-MS analysis is Neophytidiene with maximum peak area of 19.92. Neophytadiene is a well-known enzyme inhibitor found to be effective in inhibition of cyclooxygenase or lipoxygenase leads to decreased production of prostaglandins and leukotriene's.[10] Other major compounds identified are 10-Heneicosene (c,t), 1-Heptatriacotanol, 3,7,11,15 – Tetramethyl - 2-hexa decen-1-ol. etc. 2,4-Di-tert-butylphenol is a phenol that uses as a chemical intermediate for the synthesis of UV stabilizers or antioxidants. 1-Hexadecanol is acetyl alcohol also known as palmityl alcohol, is a solid organic compound and a member of the alcohol class of compounds and is used as emulsifying agent in pharmaceutical preparations. It possesses potential uses in cosmetics as emollients, emulsifying agents, foam boosting agents and surfactants.

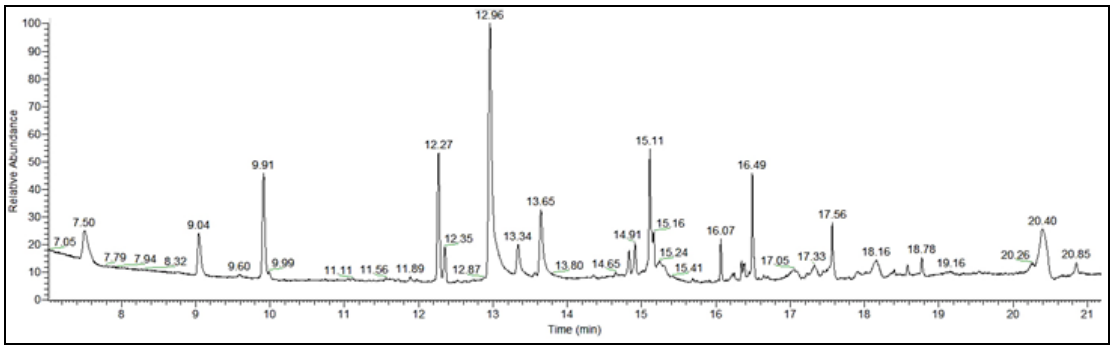

FIG. 1: GC- MS ANALYSIS OF *P. VISCIDA* LEAF EXTRACT

TABLE 2: COMPOUNDS IDENTIFIED IN THE GC - MS ANALYSIS

Sl. no.	RT	Name of the Compound	Peak area %	Cas #
1.	7.51	Hexadecen-1-ol, trans-9-	3.75	64437-47-4
2.	9.04	2,4-Di-tert-butylphenol	3.40	96-76-4
3.	9.59	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid]	0.35	35871-54-6
4.	9.92	1-Hexadecanol	7.34	36653-82-4
5.	11.11	1,3-di-iso-propylnaphthalene	0.15	NA
6.	12.27	E-15-Heptadecenal	7.20	NA
7.	12.35	Heptadecane,2,6,10,14-tetramethyl-	1.90	18344-37-1
8.	12.96	Neophytadiene	19.92	504-96-1
9.	13.34	Phytol, acetate	2.49	NA
10.	13.65	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5.27	102608-53-7
11.	14.35	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	0.23	NA
12.	14.66	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	0.29	108511-85-9
13.	14.83	n-Hexadecanoic acid	1.31	57-10-3
14.	14.91	Phthalic acid, 8-bromoctyl butyl ester	1.60	NA
15.	15.11	10-Heneicosene (c,t)	8.31	95008-11-0
16.	15.24	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate	2.09	NA
17.	16.07	1,2-15,16-Diepoxyhexadecane	1.50	NA
18.	16.38	6,9,12,15-Docosatetraenoic acid, methyl ester	1.57	17364-34-0
19.	17.56	1-Heptatriacotanol	3.76	105794-58-9
20.	18.16	4,22-Stigmastadiene-3-one	2.11	20817-72-5

CONCLUSION: Medicinal plants are the richest bio-resources of drugs of traditional system of medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical

intermediates and chemical entities for synthetic drugs. Some traditional medicines are highly equipped with more qualities in therapeutical basis, majority of the people in developing countries have resorted to the use of medicinal plants as an alternative treatment. Herbal medicines are rich in active ingredients and are safe with the body chemistry of man. [11] The results obtained from the present study indicates *P. viscida* have the potential to act as a source of useful drugs because of the presence of different active compounds.

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CONFLICT OF INTEREST: The authors declare no known conflict of interest.

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