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Phytochemical screening of various extracts of leaves of *Stereospermum personatum* (Hassk.) Chatterjee with a focus on GC-MS analysis of bioactive compounds in methanolic extract

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ABSTRACT

Stereospermum personatum (Hassk.) Chatterjee is a medicinal plant of high therapeutic value, which belongs to the family Bignoniaceae. Various parts of this plant like roots, stem, bark, leaves, flowers and seeds are used to cure inflammation, pain, fever, malaria, infections, cardiac disorders and asthma. Literatures shown that the different parts of the plant contain various bioactive constituents such as alkaloids, cardiac glycosides, saponins, steroids, phenolic compounds, tannins and flavonoids. Till date, there are no published reports on the phytochemical profile of the methanolic extract of the leaves of this plant. Therefore the proposed study aimed to carry out the Phytochemical evaluation of different extracts and Gas Chromatographic Mass Spectrometric (GC-MS) characterization of methanolic leaf extract of this plant. In the present study, Phytochemical screening of various leaf extracts revealed the presence of many active phytoconstituents like phenolic compounds, terpenoids, alkaloids, steroids, tannins, saponins flavonoids and glycosides which correlates with the published reports. GC-MS analysis of methanolic extract of leaves showed the presence of thirteen volatile constituents which justifies the therapeutic potential and traditional uses the plant. The present study could be exploited for development of plant based chemotherapeutic agents.

INTRODUCTION

The plants have been used as a major source for discovering new drugs. Now a days drugs obtained from plants have made huge contributions towards the health and well-being of human[1,2]. Medicinal plants and plant products are used as health care products long ago . Allopathic drugs have rapid and better therapeutic action but herbal drugs have high therapeutic potential, less side effects and are cheaper than allopathic drugs. They could be used as best starting material for the development of new drugs[3]. *Stereospermum personatum* (Hassk.) Chatterjee is one of the highly valued medicinal plant .Various parts of the plant is used traditionally to cure diseases like wounds , fever, kidney stones, inflammation, cardiac disorders, malaria and cough .It is known as' Trumpet flower' and 'Yellow Snake tree' in English 'Padiri' in Hindi'Pathiri' in Tamil.'and Karingazha' in Malayalam[4-5]. It is one of the ingredient of the famous Ayurvedic formulation 'Dasamoolarishtam'[6]. The preliminary phytochemical screening of various parts of this plant showed the presence of secondary metabolites such as alkaloids, steroids, tannins, phenolic compounds, flavonoids, terpenoids, cardiac glycosides and saponins which substantiates the existing study reports[7]. These secondary metabolites might contribute therapeutic activity to the plant.

Gas chromatography-Mass spectrometry (GC-MS) is a highly established and best method for finding the chemical profile of plant species[8]. Literature review has shown that there is no documented report of the chemical profile of methanolic leaf extract of this plant and also it showed good antioxidant activity[9]. Hence this study is designed to carry out the preliminary Phytochemical evaluation of various extracts prepared by successive solvent extraction of dried leaves and investigation of the Phytochemical characterization of the methanolic extract of the leaves by GC-MS technique.

MATERIALS AND METHODS

Collection of the Plant Material

The mature leaves of the plant were collected from a single plant from Vithura, Thiruvananthapuram district. This specimen was identified and authenticated by Dr. Kiran Raj , Department of Botany ,Sree Narayana College ,Kollam (voucher number: 2812).The collected leaves were washed, shade dried and powdered. The coarse powder was used for soxhlet extraction.

Preparation of Extracts [10]

50g of dried coarse powder of leaves packed in a soxhlet-extractor and extracted successively with solvents like petroleum ether (60-80°C), chloroform, ethyl acetate and methanol. Each time, the extraction was continued till the solvent in the thimble of extractor become clear .The marc obtained after the methanol extraction was dried and macerated with chloroform water for 24 hrs and filtered to obtain the water extract. All the extracts collected were evaporated in a water bath and the dried extracts were stored in a desiccator till required .

Preliminary Phytochemical screening[11]

All the extracts were subjected to Phytochemical screening using standard procedures .All the extracts were subjected to preliminary phytochemical screening and evaluated for in-vitro anti-oxidant activity. Methanolic extract showed good antioxidant activity and more number of secondary metabolites compared to the other extracts. So this extract was selected for GC-MS analysis.

GC-MS analysis

Methanolic extract obtained from *Stereospermum personatum* (Hassk.) Chatterjee was subjected to GC-MS spectrometric analysis .The instrument used was shimadzu GC-MS Model Number QP2010S, Shimadzu equipped with column Rxi-5si/MS, 30.0m length, 0.25mm internal diameter, 0.25 micrometer thickness. The settings were as follows ,column oven temperature: 60.0 °C, injection temperature: 260.00 °C, injection mode: split ,flow control mode: linear velocity , pressure: 57.4kPa, total flow : 24.0 ml /minute, column flow: 1.00 ml /minute, linear velocity: 36.5 cm /sec ,oven temperature program :60 °C holds for 2min: 10 °C to 260 °C holds for 10 min and 5 °C /minto 280 °C holds for 6 minutes. In the Mass Spectrometer part, start time :7.00 min, end time: 42.00 minute, ACQ Mode: Scan , event time: 0.50 sec, Scan speed: 1000, start m/z: 500.00.software used to handle Mass spectra and Chromatograms were GC-MS Software: GC-MS Solution. Interpretation of mass spectra of methanolic extract of leaves of *Stereospermum personatum* was conducted using the data bases of National Institute Of Standard and Technology (NIST) and WILEY 8 libraries.

RESULTS

The preliminary Phytochemical screening of the leaf extracts of *Stereospermum personatum* (Haask.) Chatterjee showed the presence of secondary metabolites like flavanoids, steroids, alkaloids, glycosides, terpenes, phenolic compounds and tannins. The results of phytochemical screening is shown in the Table 1.

Table 1 : Preliminary Phytochemical Screening of extract of *Stereospermum personatum* (Haask.) Chatterjee

Sl No	Test	Pet Ether	Chloroform	Ethyl acetate	Methanol	Water
1	Alkaloids	-	+	+	+	-
2	Carbohydrates	-	-	-	+	+
3	Proteins and Amino acids	-	-	-	-	-
4	Steroids	+	+	+	+	-
5	Glycosides	-	-	-	+	-
6	Fixed oils and fats	+	-	-	-	-
7	Tannins and phenolic compound	-	-	-	+	+
8	Flavanoids	-	-	-	+	-
9	Terpenoids	+	+	-	-	-
10	Saponins	-	-	-	+	+

Table 2 : GC-MS analytical report of methanolic extract of *Stereospermum personatum* (Haask.) Chatterjee leaves

Peak Report TIC

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	11.565	11098977	3.77	435687	1.45	COUMARAN	120.05
2	22.693	4622426	1.57	236402	0.78	1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXYLIC ACID	60.00
3	24.384	982453	0.33	161697	0.54	GAMMA.-HYDROXYISOEUGENOL	137.05
4	31.439	873253	0.30	252911	0.84	LINOLENIC ACID, METHYL ESTER	79.05
5	31.649	2474434	0.84	923445	3.06	Phytol	71.05
6	33.390	7261847	2.47	2591195	8.60	CYCLONONASILOXANE, OCTADECAMETHYL-	73.05
7	34.395	3529584	1.20	278813	0.92	PAULOWNIN	149.00
8	37.828	25301664	8.60	2662314	8.83	CYCLONONASILOXANE, OCTADECAMETHYL-	73.05
9	38.405	20135300	6.84	1752994	5.82	GLYCEROL .BETA.-PALMITATE	57.05
10	38.794	8880944	3.02	1031609	3.42	ISOGMELINOL	402.10
11	38.983	21027055	7.15	2497373	8.29	2H-PYRAN-2-ON, 5,6-DIHYDRO-4-(2,3-DIMETHYL-2-BUTEN-4-YL)-	180.00
12	39.135	27784515	9.44	3898735	12.93	N-(9,10-DIOXO-9,10-DIHYDRO-1-ANTHRACENYL)-3,4-DIMETHOXYBENZAMIDE	165.00
13	39.195	26640932	9.06	4458659	14.79	NEOGMELINOL	165.05
14	39.292	10450925	3.55	3549825	11.78	PHOSPHONIC ACID, (4-OXO-2-BUTENYL)-, DIETHYL ESTER, (E)-	177.05
15	39.681	123135596	41.85	5411530	17.95	ISOGMELINOL	151.05
		294199905	100.00	30143189	100.00		

Table 3 **:** Biological activity, molecular weight and molecular formula of isolated compounds.

Sl No.	NAME OF COMPOUND	BIOLOGICAL ACTIVITY	MOLECULAR FORMULA	MOLECULAR WEIGHT g/mol
1	COUMARAN	Antitubercular, Anticancer, Antiprotozoal, Anti-HIV	C_8H_8O	120
2	PHYTOL	Antioxidant, Antimicrobial, Antinociceptive	$C_{20}H_{40}O$	294
3	CYCLONONASILOXANE,OCTA DECA METHYL	Antioxidant, Antimalarial, Antitumor	$C_{18}H_{54}O_9Si_9$	667
4	PAULOWNIN	Antioxidant, Antibacterial, Cytotoxic	$C_{20}H_{18}O_7$	370
5	ISOGMELINOL	Antitumour	$C_{22}H_{26}O_7$	402
6	NEOGMELINOL	Antifungal	$C_{22}H_{26}O_7$	402

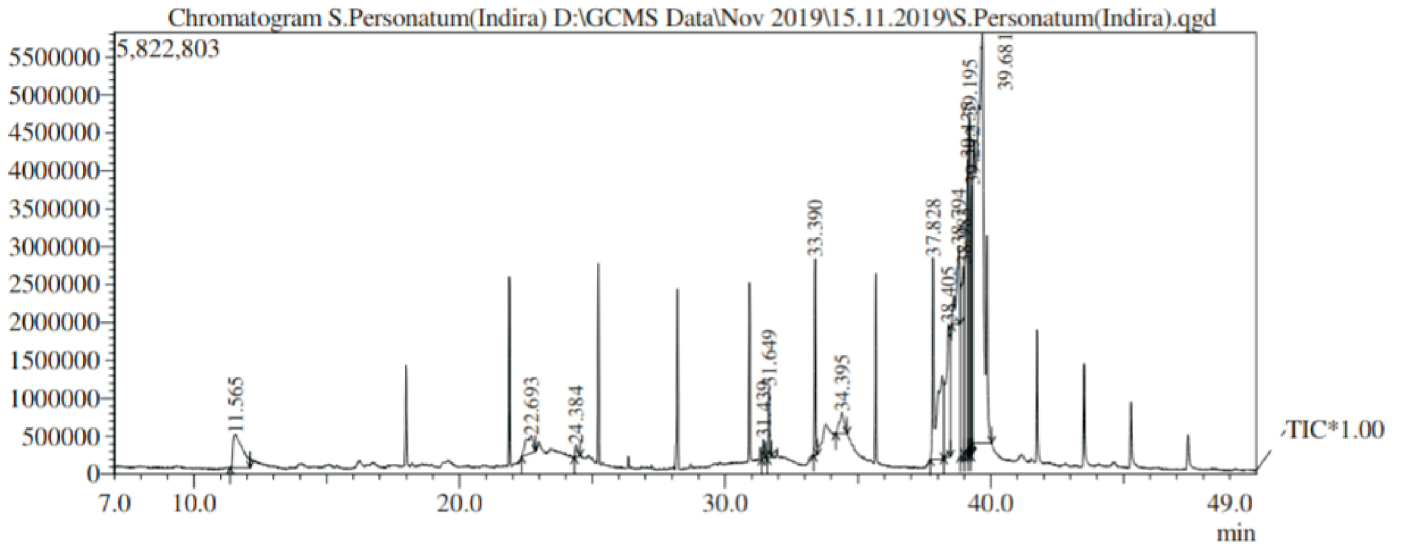

Fig 1 : GC-MS Chromatogram of methanolic extract of leaves of *Stereospermum personatum* (Hassk.) Chatterjee.

The methanolic extract contain more number of active constituents and better antioxidant activity compared to other extracts and therefore it was taken for GC-MS spectrometric analysis .The GC-MS analysis revealed presence of 13 volatile compounds in the crude methanolic leaf extract. The compounds with their retention time (RT) and Peak Area (%) are shown in Table 2.

The major compounds present in extract were identified based on percentage peak area in the chromatograph (Fig 1)

The compounds identified are coumaran (RT:11.565, Peak percentage:3.17), 1,3,4,5-tetra hydroxy cyclohexane carboxylic acid (RT:22.693, Peak percentage:1.57), gamma hydroxyl isoeuginol (RT:24.384,Peak percentage:0.33), linoleic acid, methylester (RT:31.439 peak percentage: 0.30), phytol (RT:31.649, Peak percentage:0.84), cyclonona siloxane, octadeca methyl (RT:37.828, Peak percentage:8.60), paulownin (RT: 34.395, Peakpercentage: 1.20), glycerol beta palmitate (RT:38.405, Peak percentage: 6.84), isogmelinol (RT:39.681, Peak percentage: 41.85), 2-Hpyran-2-ON,5,6-dihydro 4(2,3-dimethyl-2-butenyl) (RT:38.983, Peak percentage: 7.15), N-(9,10-dioxo 9,10-dihydro-1-anthracenyl)3,4-dimethoxy benzamide (RT:39:135, Peak percentage:9.44), neogmelinol (RT:39.195, Peak percentage: 9.06) and phosphonic acid (4 oxo-2-butenyl diethyl ester (€)(RT:39.292, Peak percentage:3.55)

DISCUSSION

Plant derived drugs have made major contribution to both traditional and modern medicine. The preliminary phytochemical evaluation of various extracts of the leaves of *Stereospermum personatum* revealed the presence of large number of secondary metabolites such as alkaloids, glycosides, flavanoids, tannins, terpenes, phenolic compounds and steroids .These secondary metabolites might be responsible for traditional claims and therapeutic potential of this plant. From different literatures, it was reported that the plant has antioxidant, antimicrobial, wound healing, analgesic, anti-inflammatory and antidiabetic activity which might be due to these secondary metabolites[12-15]. The GC-MS analysis of crude methanolic extract of leaves of this plant showed 13 compounds. Some of the bioactive constituents have potential Pharmacological activities which is shown in table 3. The retention time showed that coumaran eluted first, and isogmelinol eluted last. The peak area and peak height revealed that isogemelinol, and N-(9,10-dioxo-9,10-dihydro-1-anthracenyl)-3,4-dimethoxy benzamide are present in large quantities. Literature review also showed that coumaran possess anti tubercular, anticancer and antiprotozoal activities[16] phytol and paulownin has antioxidant, antibacterial and cytotoxic activity, gmelinol has antifungal activity, isogmelinol has antitumour activity, cyclosiloxane octamethyl has antioxidant and antitumour properties[17-19]. Further studies are required to isolate and purify these compounds which would serve as suitable leads for new drugs.

CONCLUSION

Phytoconstituents present in the plants may be responsible for their therapeutic potential. The Phytochemical characterisation by GC-MS analysis of methanolic extract of leaves of *Stereospermum personatum* (Hassk.) Chatterjee revealed the presence 13 compounds and some of them have potential pharmacological activities. This study justifies its traditional use as a herbal remedy for curing many ailments and it serves as a tool for identification and standardization of this plant species. This plant could be exploited for the development of new drug molecules of future use. Further studies to isolate the bioactive constituents are continuing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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