Int. J. Chem. Sci.: 13(3), 2015, 1295-1304
ISSN 0972-768X
www.sadgurupublications.com

PHYTOCHEMICAL AND GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *PISONIA GRANDIS* R. Br

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ABSTRACT

Pisonia grandis R. Br (Nyctaginaceae) is widely distributed throughout India and is a widespread evergreen tree commonly known as 'Leechaikottaikeerai'. Leaves, stems and roots of this species are extensively used by the tribals in the preparation of several folk medicines. The plant is traditionally used as anti-rheumatic and antifungal. This plant is also pharmacologically studied for its anti-fungal, anti-oxidant, anti-microbial, anti-inflammatory, anti-diabetic, diuretic, analgesic and wound healing properties. This paper includes the GC-MS analysis of methanolic extract of the plant extract and phytochemical preliminary analysis of the plant *Pisonia grandis* R. Br.

Key words: *Pisonia grandis* R. Br, Preliminary screening of secondary metabolites, GC-MS Analysis of methanolic extract.

INTRODUCTION

The species *Pisonia grandis* R. Br (*Nyctaginaceae*) is widely distributed throughout India and is a widespread evergreen commonly grown lettuce tree and is especially adapted to sea coasts and grows well in gardens in Chennai and other places near the sea, on both east and west coasts[1]. Leaves are useful in chronic rheumatism, wound healing and also used as vegetable[1-3]. The plant is used in tribal folklore since long time. Chewing two leaves of this tree has been traditionally followed to control diabetes. A Plethora of pharmacological activities of *Pisonia grandis* R. Br has been documented[4-10]. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently envogue in parts of the world[11]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region[12]. The present study includes the qualitative and GCMS analysis of phytochemicals

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and phyto constituents present in *Pisonia grandis* R.Br, which can be further separated and analysed for its medicinal value.

EXPERIMENTAL

The plant with flower were collected from aromatic and medicinal plant department, Agriculture University, Coimbatore (district), Tamil Nadu, India and authenticated in Botanical Survey of India, located in Coimbatore, Tamil Nadu, India. The leaves of *Pisonia grandis* R. Br where cleaned and dried in shade. The shade dried leaves were cut in to small pieces and then used for the study.

Extraction[13]

5 g portions of powdered plant materials were dispersed in 50 mL of 100% methanol. The solution was left to stand at room temperature for 24 hrs and was filtered with Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening using the following tests.

Phytochemical screening

The following tests were carried out for various metabolites with methanolic extract.

Test for alkaloids (Wagner's test)[14]

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL water) and observed for the formation of reddish brown colour precipitate. There was a formation of reddish brown colour confirming the presence of alkaloid.

Test for flavonoids (NaOH test)[15]

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour. An yellow orange colour got formed, so it was concluded that the plant extract contains Flavonoids.

Test for phenols (Ferric chloride test)[16]

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour. A deep blue colour was observed indicating the presence of phenols.

Test for quinones[17]

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate. A green precipitate got formed instead of yellow therefore, the methanolic extract of *Pisonia grandis* R.Br does not contain Quinones.

Test for sterols (Liebermann-Burchard test)[17]

Extract (1 mL) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour. No dark pink or red colour precipitate, absence of sterols.

Test for tannins (Braymer's test)[18]

2 mL of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution. A greenish colour solution got formed indicating the presence of Tannins.

Test for terpenoids (Salkowki's test)[17]

1 mL of chloroform was added to 2 mL of each extract followed by a few drops of concentrated sulphuric acid and observed for formation of reddish brown precipitate. No reddish brown precipitate absence of Terpenoids.

Test for saponins (Foam test)[18]

2 mL of extract was added in 6 mL of water in a test tube. The mixture was shaken vigorously and observed for theformation of persistent foam that confirms the presence of saponins. No foam gets generated, which confirms the absence of saponin.

Test for oxalate[18]

To 3 mL portion of extracts a few drops of ethanoic acid glacial were added. A greenish black colouration indicates presence of oxalates. Green colour got formed, which shows the presence of oxalates.

Test for coumarin[18]

To 2 mL of the extract few drops of 10% NaCl were added. A yellow colour indicates the presence of coumarin. No Yellow colour was observed, which shows the absence of coumarin.

GC-MS Analysis

Fresh leaves of *Pisonia grandis* R.Br were collected, washed in water and air dried under shade. Dried leaves were powdered using an electric pulverizer. 40 g of the leaf powder was weighed and subjected to extraction with 500 mL of methanol solvent for 8 hr (64-80°C) using Soxhlet apparatus. The leaf extract so obtained was concentrated by distillation. Then the crude extract was taken to, Chemistry laboratory South Indian Textile Research Association (SITRA), located in Coimbatore, Tamil Nadu, India. The GC-MS analysis of methanolic extract of *Pisonia grandis* R. Br was carried out under the following conditions.

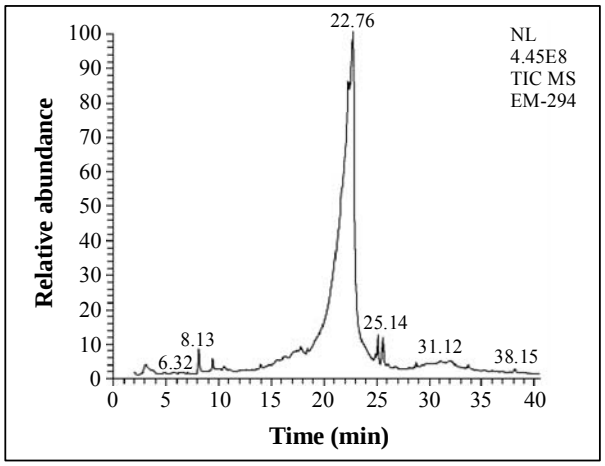

Fig. 1: GC-MS spectrum of methanolic extract of *Pisonia grandis* R.Br

Instrument name	: DSQ
Equipment	: Thermo GC - trace ultra ver: 5.0, Thermo MS DSQ II
Column	: ZB 5 - MS capillary standard non-polar column
Dimension	: 30 Mts, ID : 0.25 mm, FILM : 0.25 μm
Carrier gas	: He, flow : 1.0 ML/Min
Temperature program	: Oven temp 70 C raised to 260 C at 6 C/min
Injection volume	: 1 Micro Liter
Library search software	: Nist Willy

RESULTS AND DISCUSSION

The phytochemical screening results of the *Pisonia grandis* R.Br indicated the presence of phytoconstituents-alkaloid, flavonoids, phenols, tannins and oxalate. The details are given in Table 1.

Table 1: Result of phytochemical screening of the ariel parts of *Pisonia grandis* R. Br

S. No.	Phytochemicals	Leaves extract (100% methanol)
1	Alkaloids	+
2	Flavonoids	+
3	Phenols	+
4	Quinones	–
5	Sterols	–
6	Tannins	+
7	Terpenoids	–
8	Saponins	–
9	Oxalate	+
10	Coumarin	–

(+ = present) (– = absent)

The GC-MS analysis of the methanolic extract of *Pisonia grandis* R.Br from the NIST inbuilt, Library Data Bank showed the presence of 38 compounds. Of the 38 compounds obtained, 11 compounds had the higher matching probability above 50% with the standard compounds of the NIST Library data Bank. Out of which, 9 compounds found to be in lower proportion with the percentage peak area less than 1. Two of the compounds 2,3-dihydro benzofuran and phytol were in a significantly higher proportion with the percentage peak area 3.7. Two compounds of the remaining 27 compounds moderately match the library Bank at the range 40 to 50% and both the compounds were in lower proportion with the peak area less than 1 percent. The probability of matching with that of the compound in Data Bank, NIST of another set of 4 compounds of the remaining 25 is found to be 30-40 percent. Out of these four compounds, 3 compounds are found to be in lesser proportion with the peak area less than 1 percent, and the other compound, 2-methoxy-4-vinylphenol is in greater proportion with the percentage peak area 2.3. Of the remaining 21 compounds, 12 compounds found to match the Data Bank at the probability range of 20-30% and 11 of these compounds found to be in lesser proportion with the percentage peak area less than 1. But one compound, 9-Octadecenoic acid (Z)- (CAS) of the 12 are found to be in higher proportion with the peak area 3.7%. And all the remaining 9 compounds of the 38 compounds obtained in methanolic extract of of *Pisonia grandis* R.Br were found to match with the data bank compounds at the range between 5-20% and of which 2 compounds are present in higher

proportion with the peak area greater than 1 and the remaining 7 compounds were found to be in lesser proportion with the peak area percent less than 1.

Table 2: Phytocompononents in methanolic extract of *Pisonia grandis* R.Br in GC-MS report library search results

S. No.	SI	RSI	Compound name	Probability	Molecular formula	Molecular weight	Area (%)
1	662	861	Exomethylenecyclohex-2-yl (4-methyl-phenyl) sulfone	12.37	$C_{14}H_{18}O_2S$	250	1.80
2	737	824	2(3H)-Furanone, dihydro-3-methylene- (CAS)	34.98	$C_5H_6O_2$	98	0.21
3	696	904	2H-Pyran-2,6(3H)-dione	56.24	$C_5H_4O_3$	112	0.31
4	631	650	1-Dodecanol, 3,7,11-trimethyl-(CAS)	20.84	$C_{15}H_{32}O$	228	0.36
5	735	737	2,2,3,3,4,4-Hexa deutero Octadecanal	43.92	$C_{18}H_{30}D_6O$	268	0.27
6	723	726	Deoxyspergualin	54.55	$C_{17}H_{37}N_7O_3$	387	0.13
7	631	635	Strychane, 1-acetyl-20à-hydroxy-16-methylene-	9.95	$C_{21}H_{26}N_2O_2$	338	0.02
8	903	913	2,3-Dihydro benzofuran	51.01	C_8H_8O	120	3.67
9	901	926	2-Methoxy-4-vinylphenol	30.13	$C_9H_{10}O_2$	150	2.32
10	800	835	2-Methoxy-4,5,6-trimethylpyrimidine	72.41	$C_8H_{12}N_2O$	152	0.74
11	716	758	Desulphosinigrin	20.95	$C_{10}H_{17}NO_6S$	279	0.26
12	712	714	Demycarosylturimycin H	23.08	$C_{43}H_{74}N_2O_{14}$	842	0.01
13	725	737	L-Serine, O-(phenylmethyl)-(CAS)	51.70	$C_{10}H_{13}NO_3$	195	0.40
14	674	803	(3R, 5S)-3-azido-5-methyl-2(3H)-furanone	13.92	$C_5H_7N_3O_2$	141	0.21
15	709	720	3-Oxo-20-methyl-11-à-hydroxyconanine-1,4-diene	20.34	$C_{22}H_{31}NO_2$	341	0.09
16	730	749	Desulphosinigrin	28.85	$C_{10}H_{17}NO_6S$	279	0.05

Cont…

S. No.	SI	RSI	Compound name	Probability	Molecular formula	Molecular weight	Area (%)
17	751	761	Dodecanoic acid (CAS)	20.95	$C_{12}H_{24}O_2$	200	0.52
18	687	755	Mome Inositol	21.40	$C_7H_{14}O_6$	194	0.11
19	671	683	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	17.39	$C_{12}H_{22}O_{11}$	342	0.33
20	645	674	1H-Benzotriazole, 5-nitro-(CAS)	19.65	$C_6H_4N_4O_2$	164	0.08
21	940	948	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]-(CAS) Or phytol	65.51	$C_{20}H_{40}O$	296	3.68
22	841	841	9-Octadecenoic acid (Z)-(CAS)	22.71	$C_{18}H_{34}O_2$	282	3.72
23	719	719	Hexadecanoic acid, 2,3-dihydroxypropyl ester (CAS)	21.13	$C_{19}H_{38}O_4$	330	0.19
24	671	734	3-Oxo-20-methyl-11-à-hydroxyconanine-1,4-diene	19.82	$C_{22}H_{31}NO_2$	341	0.46
25	716	725	Isoquinolin-6,7-diol-1-carboxylic acid, N-acetyl-1-methyl-	62.74	$C_{13}H_{15}NO_5$	265	0.17
26	884	912	2-Propenoic acid, 3-(4-methoxy-phenyl)-, 2-ethylhexyl ester	65.94	$C_{18}H_{26}O_3$	290	0.55
27	559	952	3-Hexylindole	50.49	$C_{14}H_{19}N$	201	0.13
28	568	602	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis- (CAS)	8.10	$C_{28}H_{44}O_4$	444	0.45
29	803	893	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	46.58	$C_{19}H_{38}O_4$	330	0.64
30	705	714	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde	16.01	$C_{23}H_{32}O$	324	1.11

Cont…

S. No.	SI	RSI	Compound name	Probability	Molecular formula	Molecular weight	Area (%)
31	716	749	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl) oxy] propyl ester, (Z,Z,Z)- (CAS)	32.03	$C_{27}H_{52}O_4Si_2$	496	0.02
32	756	770	DI-(9-Octadecenoyl)-glycerol	23.86	$C_{39}H_{72}O_5$	620	0.54
33	734	746	Quercetin 7,3',4'-trimethoxy	61.26	$C_{18}H_{16}O_7$	344	0.05
34	758	769	Isochiapin B %2<	21.96	$C_{19}H_{26}O_6$	350	0.10
35	789	790	Ethyl iso-allocholate	38.03	$C_{26}H_{44}O_5$	436	0.10
36	806	845	1-Heptacosanol (CAS)	4.20	$C_{27}H_{56}O$	396	0.47
37	503	608	Hexacosanoic acid, methyl ester (CAS)	28.86	$C_{27}H_{54}O_2$	410	0.04
38	857	871	Mome inositol	82.11	$C_7H_{14}O_6$	194	0.01

CONCLUSION

From Table 1, the present study concludes that the methanolic extract of *Pisonia grandis* R.Br consists of alkaloid, flavonoids, phenols, tannins and oxalates. From Table 2, it is concluded that the 6 compounds namely 2,3-dihydro benzofuran, phytol, 9-octadecenoic acid (Z)-(CAS), 2-methoxy-4-vinylphenol, exomethylenecyclohex-2-yl (4-methyl-phenyl) sulfone, 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde, are in higher proportion with the percentage peak area greater than 1. Further, these compounds can be separated and isolated to know about its medicinal value in Future.

ACKNOWLEDGEMENT

I thank Principal, St. Joseph's College, Trichy, Tamil Nadu for providing a great support to do research in the college. Thanks to Dr. V. Alex Ramani (Associate Professor in Chemistry, St. Josephs College Trichy, Tamil Nadu) for his good support and guidance for the success completion of the work. Thanks to Mrs B. Renuga (Analytical Chemist) South Indian Textile Research Association, Coimbatore for her support to carry out GC-MS analysis at SITRA Lab. Thanks to the Principal SNS College of Technology for providing good lab equipment's to do the research work.

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Revised : 17.06.2015 *Accepted : 19.06.2015*