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PHYTOCHEMICAL SCREENING AND EVALUATION OF SEED EXTRACT OF *PSORALEA CORYLIFOLIA* LINN. BY GC-MS AND FT-IR SPECTRUM ANALYSES

B. Priyadharshini,[1] M. Prakash[2*]

[1,2*]Department of Genetics & Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu

Address for correspondence

Dr. M. Prakash

Email id: geeth_prakash@yahoo.co.in

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ABSTRACT

Background: *Psoralea corylifolia* Linn (Fabaceae), a medicinally important plant, indigenous to tropical and subtropical regions of the world and is reported in the Indian Pharmaceutical Codex, Chinese, British and American Pharmacopoeias and in different traditional systems of medicine, such as, Ayurveda, Unani and Siddha.

Objectives: The present study was conducted to investigate the phytochemical composition and functional groups of *P. corylifolia* seeds.

Methods: The shade dried seed materials were subjected to extract. The methanolic extract was subjected to gas chromatography-mass spectroscopy (GC-MS) and Fourier Transform Infra Red Spectroscopy (FT-IR) analyses.

Results: A total of forty compounds were identified from GC-MS analysis and the predominant compounds were identified as an Isopsoralen (57.45%) at the retention time of 20.967, Di-hydroxy Coumestan (12.96%) at the retention time of 18.299, Isobavachin (11.52%) at retention time of 17.654, Beta Caryophyllene Oxide (2.94%) at retention time of 15.240 and 1,7, Cyclo Octene (2.68%) at the retention time of 22.952. Furthermore, FT-IR analysis of *P.corylifolia* proved the presence of Phenols, Alkanes, Aldehydes, Alcohols, Isocynate, Aromatics, Carboxylic acid, Aliphatic amines and amines which shows major peaks(cm^{-1}) at 3412.14, 2949.39, 2842.98, 2522.06, 2075.82, 1638.46, 1512.99, 1453.39, 1412.74, 1268.98, 1111.97, 1052.14, 1032.11, 1018.75 and 667.24 respectively.

Conclusion: The plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds and to develop novel phytochemical marker to identify the medicinally important plant.

Key words: FT-IR, Functional Groups, GC-M, *P. corylifolia*, Phytochemicals

INTRODUCTION

Various plant species are mostly used in Ayurveda, and their good therapeutic effect is directly proportional to genuine raw material. They are obtained from geographical or commercial sources. Hence, correct identification of raw material becomes mandatory.[1] *Psoralea corylifolia* Linn (Fabaceae), a medicinally important plant, indigenous to tropical and subtropical regions of the world, is reported in the Indian Pharmaceutical Codex, the Chinese, British and American Pharmacopoeias and in different traditional systems of medicine, such as, Ayurveda, Unani and Siddha.[2] *P corylifolia* Linn. commonly known as 'Bakuchi' is conventionally used in Ayurvedic system of medicine for the treatment of various pathological conditions but especially for treatment of skin disorders such as psoriasis, leucoderma and leprosy in the form of internal medications as well as external applications.[3] *P. corylifolia* Linn. seed has been reported to contain several phytoconstituents including coumarins and flavone components, such as psoralen, isopsoralen, psoralidin, neobavai soflavone,[4] bavachin,[5] corylin,[6] bavachalcone[7] and possess antibacterial, anti inflammatory, antifungal,[8] antioxidant, anti filarial,[9] estrogenic, antitumor, and immune-modulatory activity. Like many other important botanical treasures, Bakuchi these days is

also getting adulterated raising a question to its genuinely.[10,11]

Thus, it is important to know the identification points on which it can be authenticated. Review of literature reveals that Bakuchi seed has not been studied in detail for its pharmacognostical characters.[12]

Considering this, an attempt has been made to establish preliminary pharmacognostical profile of seed which may be considered as a reference standard for future studies. This helps in further research on seeds and other parts of the same plant and also other plant species. Hence, the present work was undertaken to establish certain identification standards of *P. corylifolia* Linn.

MATERIALS AND METHODS

Collection and Preparation of Plant Material: The seeds of *P. corylifolia* (AUBOT0314) were collected from their natural habitats of Chidambaram, Tamil Nadu. The species have been identified with help of standard flora.[13,14] Seeds were washed three times thoroughly with running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The seeds were shade dried, ground into fine powder and stored in air tight container for further use.

Chemicals: The chemicals were purchased from Himedia, Mumbai, India and the solvents used were of analytical grade.

Equipments: Equipments used in this experiment include GC-MS and FT-IR. GC-MS was used for the comparison of samples and FT-IR was used for identification of functional groups presented in the species of *P. corylifolia.*

Extraction: Shade dried and powdered seed material of *P. corylifolia* (Linn.) was successively extracted with Petroleum ether, Chloroform, Ethyl acetate and Methanol with gentle stirring for 72 hrs separately. The extracts were filtered with Whatman No.1 filter paper and concentrated using vacuum distillation. Now the resultant samples are subjected to analysis. [15]

Sample Preparation for GC-MS analysis: The methanol seed extract is concentrated to 1ml by bubbling nitrogen into the solution. The Clarus 500 GC used in the analysis employed a column packed with Elite-1(100% Dimethyl Poly Siloxane, 3nm X 0.25nm ID X 1um df) and the components were separated using helium (1ml/min) as the carrier gas. The 2µl sample extract injected into the instrument was detected by the Turbo mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36^{th} minute GC extraction process, the oven was maintained at a temperature of $110^{\circ}C$ with a 2min holding. The injector temperature was set at $25^{\circ}C$ (Mass analyser). The MS detection was completed in 36 minutes, and the relative percentage of each component was calculated by comparing its average peak to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Version 2.0 year 2010 library. The compound prediction is based on Duke's Phytochemical and Ethno botanical databases. The identification of constituents was further supported by Kovat's Retention Indices reported in literature and NIST Library.[16,17]

FT-IR analysis: The FT-IR analysis was performed using Perkin Elmer Spectrum Version 10.03.09 system, which was used to detect the functional groups of the compound. A small amount of compound was placed directly on the zinc solenoid piece and constant pressure. Data of infrared absorbent, collected over the wave number ranged from 3500 cm^{-1} to 500 cm^{-1} using spectra software. Samples were run in triplicate and all of them were undertaken within a day period.

RESULTS

Identification of bioactive compounds through Gas Chromatography – Mass Spectroscopy: Based on GC-MS spectra, the methanolic seed extract of *P. corylifolia* showed forty phytochemical constituents. (Fig.1). The major components found to be Isopsoralen (57.45%) at the retention time of 20.967, Di-hydroxy Coumestan (12.96%) at the retention time of 18.299, Isobavachin (11.52%) at retention time of 17.654, Beta Caryophyllene Oxide (2.94%) at retention time of 15.240 and 1,7, Cyclo Octene (2.68%) at the retention time of 22.952. The active compound of *P.corylifolia* is Isopsoralen (Table.1). Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST08s) and WILEY8 and FAME having more patterns. The spectrum of unknown component was compared with the spectrum of known components stored in the NIST08s and WILEY8 library. The name, molecular formula, molecular weight and structure of the component of the test material were determined.

Identification of functional groups in *P. corylifolia* species by FT-IR spectrum analysis: The FT-IR spectrum was used to identify the functional groups of the active components based on the peak value in the region of

infrared radiation. FT-IR analysis of methanolic seed extract of *P.corylifolia* proved the presence of Phenols, Alkanes, Aldehydes, Alcohols, Isocynate, Aromatics, Carboxylic acid, Aliphatic amines and amines (Table.2). Which shows major peaks at 3412.14cm^{-1}, 2949.39 cm^{-1}, 2842.98 cm^{-1}, 2522.06 cm^{-1}, 2075.82 cm^{-1}, 1638.46 cm^{-1}, 1512.99 cm^{-1}, 1453.39 cm^{-1}, 1412.74 cm^{-1}, 1268.98 cm^{-1}, 1111.97 cm^{-1}, 1052.14 cm^{-1}, 1032.11 cm^{-1}, 1018.75 cm^{-1}and 667.24 cm^{-1} respectively (Fig.2).

DISCUSSION

The unmatched availability of chemical diversity means that natural products derived from medicinal plants, whether in the form of pure compounds or standardized extracts offer an endless supply of potential new drugs. Because of a rising interest for chemical diversity in screening programs, looking for therapeutic drugs from natural products, interest especially in palatable plants has become all through the world. The first pure compound known as psoralen was discovered by Jois and his colleagues in 1933[18], according to our knowledge. The distinguished secondary metabolites from the genus *Psoralea* including flavonoid, coumarins, phenols, benzofurans, benzopyrans, quinines, sesquiterpenoids, triterpenoids, steroids, and a few other components.

As revealed by Ji and Xu 1995,[19] the flavonoid compounds like Corylifolean, corylifolin, corylifolinin, bakuchicin, psoralidin, isopsoralidin, bavachin, isobavachin, bavachinin, bavachalcone, isobavachalcone, 7-O-me-thyl bavachin, bavachromanol, corylin, corylidin, corylinal, 4-O-methyl bavachalcone, neobavaisoflavone, bavachromene, neobavachalcone are identified from the seeds of the *P. corylifolia*. The comparative flavonoid compound isobavachin was distinguished through GC-MS analysis with the retention time of 17.654. One more significant kind of compound in *P.corylifolia* is Isopsoralen. With the retention time of 20.967 Isopsoralen compound have likewise been distinguished [20].

From *P. corylifolia*, a new benzofuran known as isocorylifonol was discovered in 2006 by Qiao et al.[21] With a retention time of 17.287, we have discovered the identical benzofuran compound known as benzosuberone through GC-MS analysis. With a retention time of 21.586, the phenolic compounds 3,3-Dimethyl-4 Phyenyl were identified, which was consistent with the previous study by Yin et al.[22] in 1989.

Timberlake[23], 1989 has revealed that, flavonoids were the most often phyto constituents of the species *Psoralea corylifolia*. He had recognized two known sesquiterpenoids, named stigmasterol and daucosterol. In our outcome, we had the equivalent phytoconstituents with the retention time of 33.033, 15.240 and 34.418 of stigmasterol, beta caryophyllene and beta sitosterol respectively.

From the FT-IR analysis, the functional groups, aromatics, carboxylic acids, aliphatic amines and amines were related to C-H stretch, C-O stretch, C-N stretch and N-H stretch individually. Siva et al.[24] in 2015 and Deepshika et al.[25] in 2006 were also reported similar functional groups. In view of the above discoveries and literary works, the methanolic seed extract of *P.corylifolia* has been generally utilized for its restorative medicinal purposes.

CONCLUSION

In the present study, we observed forty compounds from methanolic extract of seed of *P. corylifolia* through GC-MS analysis. The results of the present study confirm traditional applications of the medicinal plant *P. corylifolia*. The seed of *P. corylifolia* can be used as a food or food additives. The plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. By using FT-IR spectrum, we can confirm the functional constituents from given extract, identify the medicinal materials from the adulterate and even evaluate the quantities of medicinal materials. Many researchers applied the FT-IR spectrum as tool for distinguish closely associated plants and other organisms. The results of the present study to develop novel phytochemical marker to identify the medicinally important plant for the structural elucidation and identification of active principles present in the seeds of *P. corylifolia* await further investigations.

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TABLES

Peak#	R.Time	Area	Area%	Name
1	7.928	1945365	0.33	1,2,3-PROPANETRIOL
2	8.146	267504	0.04	CIS-SALVENE
3	8.406	512557	0.09	CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHYLID
4	8.548	274966	0.05	trans,cis-2,6-Nonadien-1-ol
5	9.612	996403	0.17	1,6-Octadiene, 3,7-dimethyl-, (S)-
6	10.418	195376	0.03	9-Dodecyn-1-ol
7	10.763	579290	0.10	Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-
8	10.926	284570	0.05	Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-
9	13.139	8519127	1.43	BICYCLO[7.2.0]UNDEC-4-ENE, 4,11,11-TRIMETHYL-8
10	15.240	17544048	2.94	(-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRI
11	15.552	1135408	0.19	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]d
12	15.883	1653431	0.28	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-o
13	16.086	1866926	0.31	(-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRI
14	16.260	2236485	0.37	(-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRI
15	16.793	757040	0.13	Coumarine, 7-formyl-4-methyl-
16	17.287	997267	0.17	1-Benzosuberone
17	17.654	68787309	11.52	Isobavachin
18	17.935	1782311	0.30	7,9-Dimethyl-8-nitrobicyclo[4.3.1]decan-10-one
19	18.014	593869	0.10	(-)-5-OXATRICYCLO[8.2.0.0(4,6)]DODECANE,,12-TRI
20	18.299	77394865	12.96	Dihydroxy Coumestan
21	18.789	2174331	0.36	PENTADECANOIC ACID, 14-METHYL-, METHYL EST
22	19.317	9322210	1.56	n-Hexadecanoic acid
23	19.925	756120	0.13	Naphthalene, 1,2,3,4-tetrahydro-2,6-dimethyl-7-octyl-
24	20.424	957829	0.16	9,12-Octadecadienoic acid, methyl ester, (E,E)-
25	20.484	1043915	0.17	(9E,12E)-9,12-OCTADECADIENOYL CHLORIDE #
26	20.967	343160074	57.45	Isopsoralen
27	21.105	10614163	1.78	[1-(3,3-DIMETHYL-OXIRAN-2-YLMETHYL)-3,7-DIME
28	21.198	2401257	0.40	SPIRO[ANDROST-5-ENE-17,1'-CYCLOBUTAN]-2'-ONE
29	21.586	2074056	0.35	3,3-Dimethyl-4-phenyl-4-penten-2-one
30	22.051	821894	0.14	2,2-Dimethylocta-3,4-dienal
31	22.426	6893946	1.15	4-[3,7-DIMETHYL-3-VINYL-1,6-OCTADIENYL]PHEN
32	22.952	16027652	2.68	1,7-CYCLOOCTENE
33	23.494	2259999	0.38	4-[3,7-DIMETHYL-3-VINYL-1,6-OCTADIENYL]PHEN
34	24.104	897560	0.15	Glycerol 1-palmitate
35	25.515	1839482	0.31	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxym
36	29.728	3163613	0.53	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hex
37	31.170	1532941	0.26	Card-20(22)-enolide, 3,14-dihydroxy-, (3.beta.,5.alpha.)-
38	32.644	1503136	0.25	B-NITROISOEUGENOL-TRIFLUOROACETYL DERIVA
39	33.033	986649	0.17	Stigmasterol
40	34.418	539087	0.09	.beta.-Sitosterol
		597294031	100.00	

Table 1: Peak Report TIC

S No	Wave No (cm^{-1})	Molecular Motion	Functional group	Absorption intensity
1	3412.14	O-H stretch	Phenols	Strong
2	2949.39	C-H stretch	Alkanes	Strong
3	2842.98	H-C=O stretch	Aldehydes	Medium
4	2522.06	O-H stretch	Alcohol	Medium
5	2075.82	C=N stretch	Isocyanate	Strong
6	1638.46	C=C stretch	Alkenes	Medium
7	1512.99	C-C stretch	Aromatics	Medium
8	1453.39	C=C stretch	Alkenes	Medium
9	1412.74	C-H stretch	Aromatics	Strong
10	1268.98	C-O stretch	Carboxylic acids	Strong
11	1111.97	C-O stretch	Carboxylic acids	Weak
12	1052.14	C-N stretch	Aliphatic amines	Medium
13	1032.11	C-N stretch	Aliphatic amines	Weak
14	1018.75	C-N stretch	Aliphatic amines	Medium
15	667.24	N-H stretch	Amines	Strong

Table 2: FT-IR absorption and functional groups of seed of *P .corylifolia*

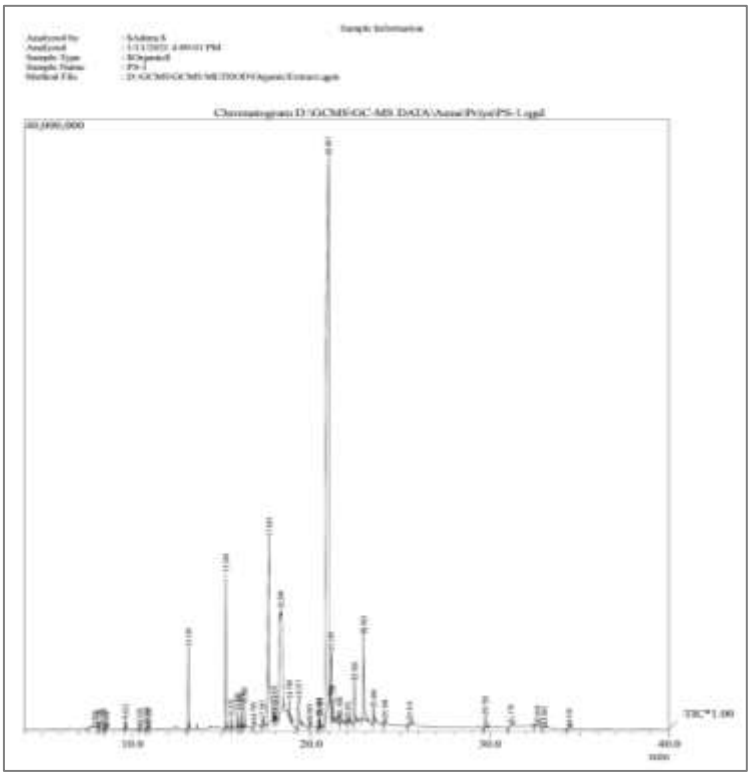

Figure 1: GC-MS chromatogram of methanolic seed extract of *P.corylifolia*

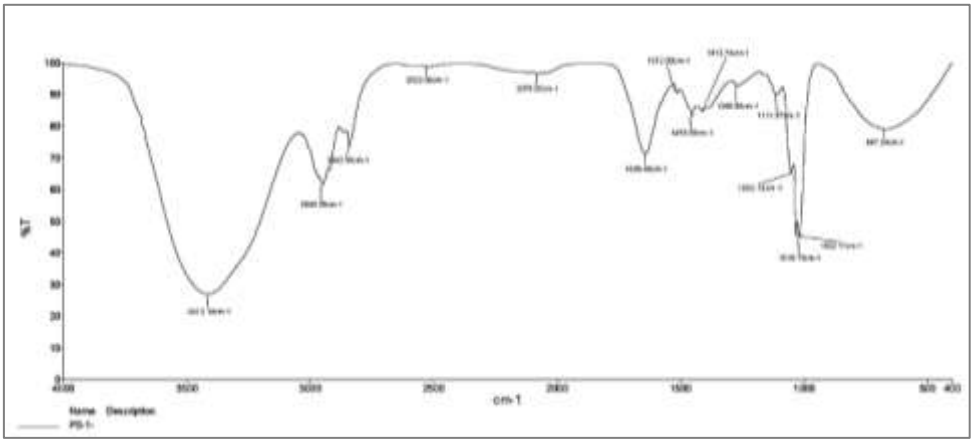

Figure 2: FT-IR Spectrum of methanolic seed extract of *P. corylifolia*