Research Article

Gas Chromatography-Mass Spectrometry Analysis and Phytochemical Screening of Methanolic Leaf Extract of *Plumbagozeylanica, Linn.*

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ABSTRACT

Plumbagozeylanica is a medicinal plant widely used in forklore medicine in India and Asia for the management of ailments such as parasitic diseases, scabies, ulcers, piles, diarrhoea, skin diseases, leprosy, fever or malaria, rheumatism and intestinal parasites. Phytochemical constituents are responsible for medicinal activity of plant species. Hence the present study was carried out to identify the phytochemical components present in themethanolic leaf extract of the *Plumbagozeylanica* by phytochemical screening methods and GC-MS analysis. The phytochemical screening showed the presence of alkaloids, tannins, steroids, flavonoids, saponins, anthraquinones, cardiac-glycosides, phlobatinnins and carbohydrates. The gas chromatography-mass spectrometry (GC-MS) analysis also identified phytochemical components like phenolics (Phenol, 3,5-bis(1,1-dimethylethyl) (RT: 10.140),1,2,4-trimethoxy -5{(1E)-1-PROPENYL} BENZENE(RT: 11.491), Tetradecanoic acid (RT:13.14 2),1-Heptadecene (RT:13.248),2(4H) –BENZOFURANONE,56,7,7A –TETRAHYDRO-6H (RT13.567:),2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECENE (RT:13.752),2-Pentadecanone,6,10,14-trimethyl(RT:13.851), 2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-{R-R (RT14.009),. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(RT 14.202), Hexadecanoicacid(RT14.658), Isophytol (RT14.883,)9,12-Octadecadienoic acid(Z,Z)- methyl ester(RT16.311), 9,12,15-Octadecatrienoic acid(Z,Z,Z)methyl ester(RT16.385),OCTADECANOIC ACID,(RT 17.0013), Cyclopentylpropionic acid(RT18.008), 4,8,12,16-Tetramethylheptadecan-4-olide(RT18.710) Hexacosane(RT18.972), 3-Cyclopentylpropionic acid,2dimethylaminoethyl ester (RT19.809),Tetracontane(20.070),1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER(RT20.896), Squalene(RT24.903),4,6-Cholestadiene-3-0ne,2,4-dinitrophenylhydrazone(RT28.271), Gamma. Sitosterol (RT32.997), FLAVONE4'-OH,5-OH,7-DI-O-GLUCOSIDE(RT 34.987). It has been reported that the presence of various bioactive compounds confirms the application of *Plumbago zeylanica* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to a novel drug.

Keywords: *Plumbago zeylanica*, gas chromatography, mass spectrometry, phytochemical screening.

INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs[1]. Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening[2]. India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani. Traditional systems of medicines are prepared from a single plant or combinations of number of plants. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug [3,4]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer and Alzheimer's diseases.[5-7]

Phytochemicals are responsible for medicinal activity of plants.[8] These are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that is primary and secondary metabolites based on the function in plant metabolism. Primary metabolites comprise carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on.[9] Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs.[8]

PlumbagozeylanicaLinn belongs to the family Plumbaginaceae and is a branched evergreen shrub reaching up to 2 m. It is distributed throughout the tropical and subtropical countries of the world. Four species of the genus *Plumbago* have been reported. They are *Plumbagoindica, Plumbagorosea, Plumbagocapensis*, and *P. zeylanica*.[10] *P. zeylanica* is known by different names in different parts of the world. Such names include white leadwort or ceylon leadwort (in English), bleiwurz or zahnkraut (in German), chitrak or chitramol (in India), ensain or enkin (in Arabsanza (in Swahili), and inabiri among the Yoruba (in South-West Nigeria).

The whole plant, roots, powder of the root, leaves and stem-bark are widely used as medicinal herbs throughout Asia and Africa. In traditional herbal medicine, the root

part of *P. zeylanica* is used for treatment of different ailments such as parasitic diseases, scabies and ulcers), piles, diarrhea, skin diseases and leprosy fever or malaria, rheumatism, intestinal parasites, anemia due to stagnant blood', internal and external trauma, toxic swelling and furunculous scabies.[11-14]

The pharmacological studies of various root extracts of *P. zeylanica* have shown that the plant has antimicrobial, antioxidant and anti-inflammatory, antipla- modial, antihyperglycemic antifungal, hypolipidaemic and antiatherosclerotic and central nervous system stimulatory, cytotoxic and anti-insecticidal properties.[15-21,10] Several active compounds previously identified and isolated from the root of *P. zeylanica* include five naphthoquinones (plumbagin, chitranone, maritinone, elliptinone and isoshinanolone), five coumarins (seselin, 5-methoxyseselin, suberosin, xanthyletin and xanthoxyletin), two plumbagic acid glucosides (3´-O-β-glucopyranosylplumbagic acid and 3´-O-β-glucopyranosylplumbagic acid methyl ester) neoisoshinanolone and 1-epineo-isoshinanolone.[22,23] In this study, investigation was carried out to identify active constituents present in the methanolic leaf extract of the plant by phytochemical screening and Gas chromatography-mass spectrometry (GC-MS) analytical methods. In the present study, volatile organic matter of the leaf sample of plant was analyzed for the first time. This work will help to identify the compounds, which may be used in body products or of therapeutic value.

MATERIALS AND METHODS

Plant Material

The young healthy leaves of *Plumbagozeylanica* L. were collected from Botanical garden of Rajasthan University, Jaipur, India. Voucher specimen of this plant material was deposited at the Herbal Museum, Department of Botany, Rajasthan University for identification. The leaves were cleaned, washed, dried and then carefully stored at -200°C for further study.

Preparation of Extract

The leaves were oven dried completely at 27 to 30°C for 1 week. The dried leaves were ground to fine powder using a local grinder.

10 g of the powdered leaf of *P. zeylanica* was exhaustively extracted in 90 mol 80% methanol with soxhlet extractor and then concentrated using rotary vacuum evaporator. 10.0 ml extract was measured out from the concentrate for phytochemical screening while the remaining extract was evaporated to complete dryness at 35°C given a dark-green color solid residue. The dried extract was stored in airtight container and placed in refrigerator.

Phytochemical Screening

The extract was subjected to preliminary phytochemical screening to test for the presence or absence of phytochemical constituents using the methods described below.[24]

Alkaloids

1.0 ml extract was stirred with 5 ml dil. Hydrochloric acid on a steam bath, filtered and 1.0 ml of each Dragendoff's/Mayer's/Wagner's reagents was added to 1.0 ml separate portions of filtrate. A cloudy orange-red/slightly yellow/turbid brown color indicates the presence of alkaloids.

Tannins

1.0 ml extract was stirred with 1.0 ml Ferric chloride. A greenish black precipitate indicates the presence of tannins; 1.0 ml extract was also stirred with 1.0 ml bromine water. A reddish brown turbid color indicates the presence of tannins.

Cardiac-Glycoside (Legal's test)

1.0 ml extract was dissolved in 5.0 ml pyridine, 2 drops 2% Sodium Nitroprusside and 2 drops 20% NaOH were added. A deep red color faded to brown indicates presence of cardenolide.

Kedde's Test

1.0 ml extract was mixed with 1.0 ml 2% 3,5-Dinitro-benzoic acid in methanol and 1.0 ml aqueous NaOH. A violet color precipitate indicates lactone ring present in cardenolide.

Steroids (Liebermann-Burchard's Test)

0.5 g extract dissolved in 2.0 ml acetic anhydride, cooled in ice and 1.0 ml conc. H_2SO_4 was carefully added. A blue-green ring indicates the presence of steroids.

Salkowski's Test

0.5 g extract was dissolved in 2.0 ml chloroform and 1.0 ml conc. H_2SO_4 was carefully added. A reddish brown color indicates the presence of steroids.

Flavonoids (Ferric Chloride test)

0.2 ml extract was added to 10% $FeCl_3$ and mixture was shaken. A wooly brownish precipitate indicates the presence of flavonoids.

Lead Acetate Test

0.2 ml extract was added to 0.2 ml 10% lead acetate and gently shaken. A dirty brownish precipitate indicates the presence of flavonoids.

Sodium Hydroxide Test

0.2 ml dil. NaOH was added to 0.2 ml extract, gently shaken. A dirty yellowish brown precipitate indicates the presence of flavonoids.

Saponins (Frothing Test)

0.2 ml extract was mixed with 5.0 ml distilled water, shaken for 20 min. Persistence of foams indicates presence of saponins.

Anthraquinones (Borntrager's Test)

2.0 g extract in 10 ml ethanol, steamed for 5 min and filtered, 2.0 ml filtrate was added to 2.0 ml chloroform, shaken thoroughly, chloroform layer was taken off and 5.0 ml distilled water added which was shaken with 5.0 ml dilute ammonia solution. A red color in ammonia upper phase indicates the presence of anthraquinones.

Phlobatinnins Test

1.0 ml extract was boiled in 2.0 ml 1% aqueous HCl. A red precipitate indicates the presence of phlobatannins.

Carbohydrate Tests

1.0 ml extract was added to 2.0 ml Fehling's solution and boiled for 5 min. A red precipitate indicates the presence of reducing sugars; 1.0 ml extract was added to 2.0 ml Barfoed' reagent and boiled for 1 min. A red precipitate indicates the presence of reducing monosaccharides; 1.0 ml extract was added to 1.0 ml Molisch's reagent and 1.0 ml conc. H_2SO_4 was carefully added. A reddish ring indicates the presence of carbohydrates.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis was carried out using a Shimadzu GCMS QP2010 Plus Spectrometer under the following conditions: DB-Polyethylene glycol coated fuse silica capillary column (30 m length × 0.25 mm ID × 0.25 µm film thickness): Helium carrier gas (1.34 ml/min); 2500C injector temperature; 2400C interface temperature; and 2000C on source temperature. 1.0 micron of extract (1 mg dissolved in 1 ml methanol alcohol); at a split ratio of 1:10 was injected. MS analysis was carried out on Shimadzu GCMS QP2010 Plus Spectrometer equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C, scanning rate of 0.5 scan/s and fragments ranging from 40 to950dalton. Identification of compound was conducted using the database of NIST08 Library. Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Library.

RESULTS AND DISCUSSION

Table 1: Results of phytochemical screening of methanolic leaf extract of P. zeylanica

Phytochemical	Result
Alkaloids	+
Tannins	+
Cardiac glycosides	+
Steroids	+
Flavonoids	+
Saponins	+
Anthraquinones	+
Phlobatinnins	+
Carbohydrates	+

+ = present.

Table 1 shows the results of the phytochemical screening of the methanolic leaf extract of *P.zeylanica.* This reveals the presence of alkaloids, tannins, steroids, flavonoids, saponins, anthraquinones, phlobatannins, cardiac-glycosides and carbohydrates. The results of the GC-MS analysis identified the various compounds present in the plant (Figure 1 and Table 2). Figure 1 shows the gas chromatogram of the extract which shows 28 distinct peaks identified by GC-MS while the compounds identified through the NIST08 L. database are listed in Table 2. The major compound present in the methanolic leaf extract of *P. zeylanica* as identified by GC-MS was Phenol, 3,5-bis(1,1-dimethylethyl) with RT: 10.140). Mass spectrum of Phenol, 3,5- bis(1,1-dimethylethyl) was shown in Figure 2. The GC-MS spectrum gives the structure of the compound (Figure2), molecular formula as C14H22O, molecular weight as 206 and biological activity as antioxidant (Table 3). Other components also identified in the leaf of P. zeylanica are 1,2,4-trimethoxy -5{(1E)-1-PROPENYL} BENZENE (RT:11.491), Tetradecanoic acid (RT:13.142), 1-Heptadecene (RT: 13.248), 2(4H) –BENZOFURANONE,56,7,7A –TETRAHYDRO-6H (RT: 13.567) 2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECENE(RT13.752), 2-Pentadecanone6,10,14-trimethyl(RT13.851)2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-{R-R(RT RT14.009),), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(RT 14.202), Hexadecanoicacid(RT14.658), Isophytol (RT14.883,)9,12-Octadecadienoic acid(Z,Z)- methyl ester(RT16.311), 9,12,15-Octadecatrienoic acid(Z,Z,Z)methyl ester(RT16.385), OCTADECANOIC ACID, (RT 17.0013), Cyclopentylpropionic acid(RT18.008), 4,8,12,16-Tetramethylheptadecan-4-olide(RT18.710) Hexacosane(RT18.972), 3-Cyclopentylpropionic acid, 2dimethylaminoethyl ester (RT19.809), Tetracontane(20.070),1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER(RT20.896),Squalene(RT24.903),4,6-Cholestadiene-3-0ne,2,4-dinitrophenylhydrazone(RT28.271), Gamma.Sitosterol(RT32.997),FLAVONE4'-OH,5-OH,7-DI-O-GLUCOSIDE(RT 34.987). It has been reported that many medicinal plants are rich in varieties of secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids.[25,26] Reports have suggested that secondary plant metabolites exert a wide range of biological activities on physiological systems.[11] The activities of some phyto-components with compound nature of flavonoids, palmitic acid (hexadecanoic acid, methyl ester and n-hexadecaonoic acid), unsaturated fatty acid and linolenic (octadecatrienoic acid) as antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary.[9]

It is therefore not unlikely that these phytochemicals found in P. zeylanica may play major roles in the reported biological activities and pharmacological properties of the plant.

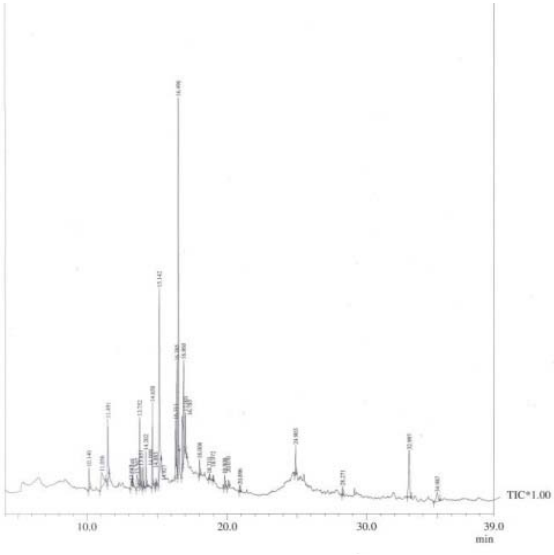

Figure 1: GC-MS chromatogram of methanolic leaf extract of P. zeylanica. Peak 2 with retention time of10.140 was identified as phenol, 3,5bis(1,1-dimethylethyl) and as the major phyto-component of the plant while other peaks were of the various phyto-components present.

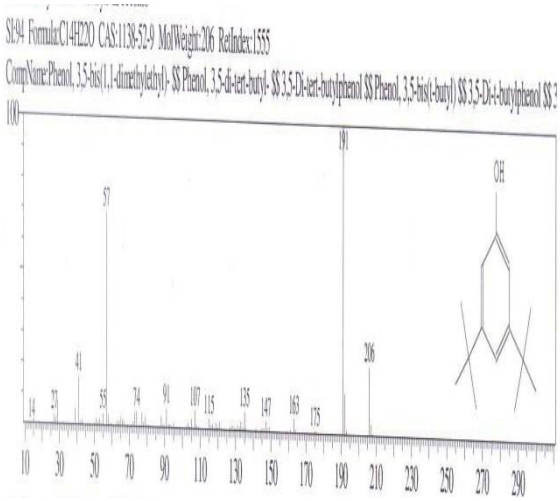

Figure2: GC- MS mass spectrum and molecular structure of phenol,3.5 – bis (1,1- dim ethyl ethyl)

Table 2: Phytochemical components identified in the methanolic leaf extract of *P. zeylanica* by GC-MS showing their RT, Area, Area %.

S.No	RT	Area	Area %	Name
1	10.140	1654601	1.56	Phenol,3.5 – bis (1,1- dimethyl ethyl)
2	11.491	6129818	5.77	1,2,4-trimethoxy -5{(1E)-1-PROPENYL} BENZENE
3	13.142	500815	0.47	Tetradecanoic acid
4	13.248	293393	0.28	1-Heptadecene
5	13.567	1895060	1.78	2(4H) – BENZOFURANONE,56,7,7A –TETRAHYDRO-6H
6	13.752	3909861	3.68	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECENE
7	13.851	845657	0.80	2-Pentadecanone,6,10,14-trimethyl
8	14.009	1276539	1.20	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-{R-R
9	14.202	2142792	2.02	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
10	14.658	4754625	4.47	Hexadecanoicacid,methyl ester
11	14.883	109601	0.10	Isophytol
12	15.142	16469497	15.50	n-Hexadecanoic acid
13	16.311	2141784	2.02	9,12-Octadecadienoic acid(Z,Z)- methyl ester
14	16.385	5450909	5.13	9,12,15-Octadecatrienoic acid(Z,Z,Z)- methyl ester
15	16.496	21506654	20.24	Phytol
16	16.785	5048319	4.75	9,12-Octadecadienoic acid(Z,Z)
17	16.860	8919217	8.39	9,12,15-Octadecatrienoic acid(Z,Z,Z)
18	17.001	2669517	2.51	OCTADECANOIC ACID
19	18.008	1252479	1.18	3-Cyclopentylpropionic acid,2-dimethylaminoetyl ester
20	18.710	200232	0.19	4,8,12,16-Tetramethylheptadecan-4-olide
21	18.972	262761	0.25	Hexacosane
22	19.809	926287	0.87	3-Cyclopentylpropionic acid,2dimethylaminoethyl ester
23	20.070	7616846	0.72	Tetracontane
24	20.896	440908	0.41	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER
25	24.903	1951079	1.84	Squalene
26	28.271	770286	0.72	4,6- Cholestadiene-3-0ne,2,4-dinitrophenylhydrazone
27	32.997	7416845	6.98	Gamma.Sitosterol
28	34.987	2040186	1.92	FLAVONE4'-OH,5-OH,7-DI-O-GLUCOSIDE

Table 3: GC- MS indicated properties ofphenol, 3.5 – bis (1,1- dimethyl ethyl)

Properties	Given as
Molecular formula	C14 H22 O
Molecular weight	206.3239
Biological activity	Antioxidant

CONCLUSION

Phenolic compounds have also been known as antioxidant agents, which act as free radical terminators and have shown medicinal activity as well as exhibiting physiological functions. It was reported that compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effects of most plants.[28] The presence of these phytochemicals in *P. zeylanica* leaf is a significant finding in this present study.

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