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SESAMIN - AN ACTIVE LIGNIN COMPOUND FROM *PSILANTHES TRAVENCORENSIS*, A MEMBER OF RUBIACEAE

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ABSTRACT

Psilanthustravancorensis (Wt. & Arn.) Leroy (Rubiaceae), a medicinal plant, as it popularly called 'Pushkaramulla', is distributed in southern Western Ghats in India and Sri Lanka. The root is primarily used in Indian Systems of Medicine and folk medicines. However, no systematic documentation of the phytochemical constituents of the plant has been made till date. This paper reports the phytochemical screening of *Psilanthustravancorensis*(root) by qualitative and gas chromatography-mass spectroscopy (GC-MS) analysis for the first time. The qualitative analysis of root has revealed the presence of alkaloids, flavonoids, glycosides, phenols, terpenoids and steroids. The GC-MS analysis of isolated fractions of the plant extract through column chromatography revealed the presence of so many active compounds such assantalol,eugenol,oleic acid, etc. An active e lignancompound namely sesamin{5, 5' -(tetrahydro 1H, 3H -furo [3,4-c-] furan-1, 4-diyl) bis-, [1S- (1α, 3α, 4 β, 6α)] 1,3-benzodioxole} was isolated, which is established as an inhibitor of pregnane X receptor (PXR) and reports show that this compound may be useful for modulating drug-metabolizing enzymes (DMEs) expression and drug efficacies. Besides these compounds, so many hydrocarbons, fatty acids, fatty acid esters, alcohols, ketones, aldehydes and phenolic compounds were identified. These different phytochemical constituents may be responsible for pharmacological activities of the plant.

Keywords: *Psilanthustravancorensis*, phytochemical analysis, GC-MS.

INTRODUCTION

Psilanthustravancorensis (Wt. & Arn.) Leroy belonging to the family Rubiaceae, whichis of the major group Angiosperms (Flowering plants), is a medicinal plant popularly known as '*Pushkkaramulla*'. The plant is distributed in southern Western Ghats in India and in Sri Lanka. The plant, a rare and endemic species, was abundant in the Kerala forest in the past, but now very scarce[2]. The plant is also known as *cherumulla* and *kattu-mulla*[1]. The root is used in Indian systems of medicines and folk medicines. It is reported to cure anaemia, cardiac diseases, skin diseases, oedema and also diseases due to kapha and vata. A paste of the root is applied to indo-

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lent ulcers and inflammatory swellings[3.4]. Tribal people of Kerala use the roots to treat rheumatic pain[5]. *Psilanthustravancorensis* is an unexplored plant and is the need of the hour to explore the plant in all aspects including comparative clinical studies which are essential to substantiate its therapeutic powers. No systematic documentation of the phytochemical constituents of the plant has been made till date. This paper deals the phytochemical screening of *Psilanthustravancorensis* (root) by qualitative analysis and gas chromatography-mass spectroscopy (GC-MS) analysis for the first time.

GC-MS is an instrumental technique by which complex mixtures of volatile organic compounds are separated, identified and quantified. Generally, compounds that are sufficiently volatile and thermally stable may be analyzed by GC-MS method. MS detection enables the determination of molecular masses of target compounds and the elucidation of their molecular structure. The molecular structure is determined from the specific fragmentation patterns, when the compounds are bombarded with fast electrons in an MS ion source. The sample molecules lose an electron resulting in a molecular ion (M+., radical cation) and further smaller ions called daughter ions with characteristic relative abundances. This provides a fingerprint for the structure of the molecule. Using searchable data bases, compounds are identified and molecular structure is determined. The combined GC-MS procedure is very useful when dealing with a sample that is a mixture of two or more different compounds, because the various compounds are separated from one another before being subjected individually to MS analysis. In GC, a very small amount of a liquid sample is vaporized, injected into a long, coiled metal column, and pushed through the column by helium gas. Along the way, different compounds in the sample stick to the walls of the column to different extents, and thus travel at different speeds and emerge separately from the end of the column. In GC-MS, each purified compound is sent directly from the end of GC column into the MS instrument, so in the end we get a separate mass spectrum for each of the compounds in the original mixed sample. Because a compound's MS spectrum is a very reliable and reproducible 'fingerprint', we can instruct the instrument to search an MS database and identify each compound in the sample. The extremely high sensitivity of modern GC-MS instrumentation makes it possible to detect and identify very small trace amounts of organic compounds. GC/MS analysis is considered as a tool for conclusive proof of identity.

MATERIALS AND METHODS

Plant material

The plant material selected for study is the root of*Psilanthustravancorensis* (Wt. & Arn.) Leroy (Figure 1)

Figure 1: *P. travancorensis* plant (From Wikimedia Commons,the free media repository; Photo taken from Botanical garden of Kariyavattam University Campus, Trivandrum)

Collection of plant material

Root of *P. travancorensis* collected from Thiruvananthapuram District of Kerala, India.

Preparation of plant powder and extract

The plant material (root) was dried in shade, finely powdered and the powder was passed through 40 mesh sieve and stored in airtight container at room temperature.

Successive extraction

About 100gms of the powder was taken in a Soxhlet apparatus and extracted successively with five solvents, viz. hexane, ethyl acetate, acetone, and methanol. The mother liquor remaining is noted as the aqueous fraction. The residue was concentrated, dried and were stored in tightly sealed dark glass containers at 5^0C for phytochemical analysis.

Phytochemical analysis

The five successive extractives were tested for different secondary metabolites – alkaloids, flavonoids, glycosides, phenols, saponins, tannins, steroids, terpenoids and coumarins[6].

Column chromatography of petroleum ether extract

The petroleum ether extract was subjected to column chromatography on silica gel G (60-120 mesh). The residue (1 g) was adsorbed on silica gel (2 g) and packed on a silica gel column (25 g) which was preequilibrated with petroleum ether. Elution was carried out using different solvents of increasing polarity in the following order: petroleum ether, a mixture of petroleum ether and ethyl acetate in different ratios and ethyl acetate. Aliquots of 25 ml were collected and each of these was subjected to thin layer chromatography (TLC) on silica gel G plates of 0.5 mm thickness and in solvent systems of different polarity. The fractions which gave similar R_f values in the same solvent systems were mixed together. The solvent system which gave maximum separation for each fraction was noted. Four major fractions were obtained. This was taken for further analysis. To identify the compounds present, each fraction was subjected to Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis: Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National InstituteStandard and Technology

(NIST) having more than 62,000 patterns. The mass spectrum of theunknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular formula, molecular weight % peak area and structure of the components of the test materials were ascertained[7,8].

RESULTS ANDDISCUSSION

The yield obtained for petroleum ether fraction was 26%.The major fractions on column chromatography was obtained for solvent systems; Petroleum ether-ethyl acetate (98:2)- 1 fraction, Petroleum ether-ethyl acetate (95:5)-2 fractions and Petroleum ether-ethyl acetate (70:30)- 1 fraction.

The successive extracts of the root of *P. travancorensis* revealed the presence of alkaloids, flavonoids, glycosides, phenols, terpenoids and steroids (Table 1). Preliminary screening tests may be useful in the detection of bioactive principle and may subsequently lead to drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

Table1. Preliminary screening of secondary metabolites from *P. travancorensis*

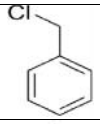
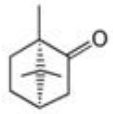
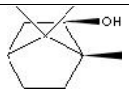
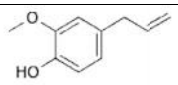
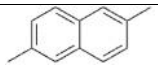
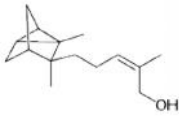
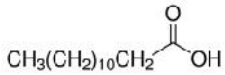
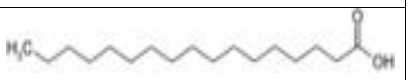
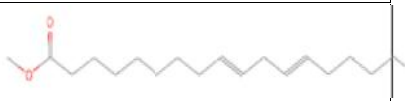
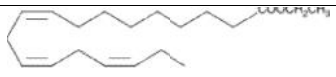
Sl.NO.	Secondary metabolites	Root				
		I	II	III	IV	V
1.	Alkaloids	+	+	+	+	+
2.	Flavonoids	+	+	+	+	+
3.	Glycosides	-	+	+	+	-
4.	Phenols	+	+	+	+	+
5.	Saponins	-	-	-	-	-
6.	Tannin	-	-	-	-	-
7.	Steroids	-	-	-	-	-
8.	Terpenoids	+	+	+	+	+
9.	Coumarins	+	+	+	+	+

'+' Present '-' absent; I- Hexane; II – Ethyl acetate; III – Acetone; IV – Methanol; V- Aqueous

The petroleum ether extract was subjected to column chromatography. 4 major fractions were obtained. These 4 fractions were subjected to GC-MS analysis.GC-MS chromatogram of the petroleum ether extract of *P. travancorensis* (Root) peaks indicates the presence of 16 important/ major compounds in fraction I, 7 compounds in fraction II, 8 compounds in fraction III and one compound, sesamin, in fraction IV. The chemical compounds identified in fraction I are presented in Table 2, fraction II in Table 3, fraction III in Table 4 and fraction IV in Table 5.

Sesamin, { 5, 5' -(tetrahydro 1H, 3H -furo [3,4-c-] furan-1, 4-diyl) bis-, [1S- (1α, 3α, 4 β, 6α)] 1,3-benzodioxole} an active lignan compound was found to be a major compound was isolated and identified.Sesamin is established as an inhibitor of pregnane X receptor (PXR) and reports show that this compound may be useful for modulating drug-metabolizing enzymes (DMEs) expression and drug efficacies.

Table 2: Phytochemicals present in the fraction I of petroleum ether extract of *P. travancorensis* (Root)

Sl.No.	Retention time(R_T)	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structural formula
1.	3.698	Benzyl chloride	C_7H_7Cl	126.58	0.10	
2.	5.168	Camphor	$C_{10}H_{16}O$	152.23	0.09	
3.	5.341	Isoborneol	$C_{10}H_{18}O$	154.25	0.07	
4.	5.452	Borneol	$C_{10}H_{18}O$	154.25	0.09	
5.	7.895	Eugenol	$C_{10}H_{12}O_2$	164.20	0.02	
6.	8.919	2,6-dimethyl naphthalene	$C_{12}H_{12}$	156.22		
7.	13.066	α- santalol	$C_{15}H_{24}O$	220.35	0.47	
8.	18.561	Tridecanoic acid	$C_{13}H_{26}O_2$	214.34	6.95	
9.	20.416	9,12- octadecanoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	0.25	
10.	20.517	Heptadecanoic acid (margaric acid)	$C_{17}H_{34}O_2$	270.45	0.11	
11.	20.963	Octadecanoic acid methyl ester	$C_{19}H_{34}O_2$	294.47	0.06	
12.	21.592	Linoleic acid ethyl ester	$C_{20}H_{34}O_2$	306.5	3.06	
13.	21.693	9-eicosyne	$C_{20}H_{38}$	278.51	6.74	

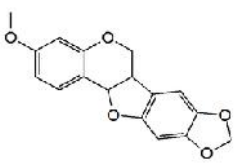
14.	22.149	Eicosanoic acid	$C_{20}H_{40}O_2$	312.53	4.70	
15.	22.271	Octadeca-noic acid (Stearic acid)	$C_{18}H_{36}O_2$	284.48	5.22	
16.	27.806	Pterocarpin	$C_{17}H_{14}O_5$	298.29	22.37	

Table 3: Phytochemicals present in the fraction II of petroleum ether extract of *P. travancorensis* (Root)

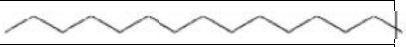
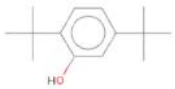
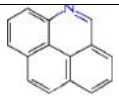
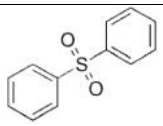
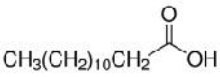
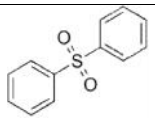
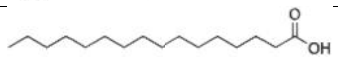
Sl. No.	Retention time (R_T)	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structural formula
1.	6.892	Tridecane	$C_{13}H_{28}$	184.36	1.60	
2.	8.240	Tetradecane	$C_{14}H_{30}$	198.39	0.97	
3.	9.741	Pentadecane	$C_{15}H_{32}$	212.41	0.11	
4.	10.238	2,5-bis(1,1-dimethylethyl) phenol	$C_{14}H_{22}O$	206.32	0.58	
5.	11.302	4-Azapyrene	$C_{15}H_9N$	203.24	1.26	
6.	17.821	Diphenylsulfone	$C_{12}H_{10}O_2S$	218.27	29.27	
7.	18.307	Tridecanoic acid	$C_{13}H_{26}O_2$	214.34	17.14	

Table 4: Phytochemicals present in the fraction III of petroleum ether extract of *P. travancorensis*(Root)

Sl. No.	Retention time (R_T)	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structural formula
1.	17.831	Diphenyl-sulfone	$C_{12}H_{10}O_2S$	218.27	8.42	
2.	18.297	Hexadeca-noic acid (Palmitic acid)	$C_{16}H_{32}O_2$	256.42	2.69	

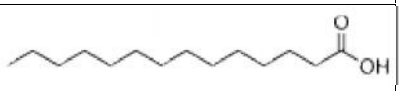
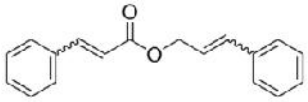
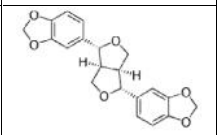
3.	21.572	Oleic acid	$C_{18}H_{34}O_2$	282.46	2.37	
4.	21.866	Octadeca-noic acid (Stearic ac-id)	$C_{18}H_{36}O_2$	284.48	5.22	
5.	22.058	Tetradeca-noic acid (Myristica-cid)	$C_{14}H_{28}O_2$	228.37	0.36	
6.	28.141	2-octyl-cyclopropa-neoctanal	$C_{19}H_{36}O$	280.48	4.53	
7.	28.841	Cinnamyl-cinnamate	$C_{18}H_{16}O_2$	264.32	11.35	
8.	30.219	Methyl es-ter(E)-9-octadeca-noic acid	$C_{19}H_{36}O_2$	296.48	2.98	

Table 5: Phytochemicals present in the fraction IV of petroleum ether extract of *P. travancorensis*(Root)

Sl. No.	Retention time(R_T)	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structural formula
1	24.937	5, 5' -(tetrahydro 1H, 3H -furo [3,4-c-] furan-1, 4-diyl) bis-, [1S-(1α, 3α, 4 β, 6α)] 1,3-benzodioxole(Sesamin)	$C_{20}H_{18}O_6$	354.35	72.51	

GC-MS analysis revealed that linoleic acid ethyl ester, 9-eicosyne, Eicosanoic acid, octadecane, Pterocarpin, Diphenylsulfone, Palmitic acid, Oleic acid, Stearic acid, Tridecanoic acid and Sesamin are the major compounds present in the extract.The GC-MS analyses revealed that the petroleum ether extract mainly contains hydrocarbons, fatty acids, fatty acid ester, alcohols, ketones, aldehydes and phenol.These phytochemicals along with other polar phytochemicals which are to be explored may be responsible for various pharmacological actions reported. This study is only a preliminary study of only the petroleum ether extract. Anin-depth study on the plant is going on which may provide a good concrete base-for all the biochemical and phytochemical properties shown by the plant.

CONCLUSION

The successive extracts of root, stem and leaves have revealed the presence of alkaloids, flavonoids, glycosides, phenols, terpenoids and steroids. GC-MS analysis

revealed that the petroleum ether extract mainly contains hydrocarbons, fatty acids, fatty acid ester, alcohols, ketones, aldehydes and phenol. These phytochemicals along with other polar phytochemicals which are to be explored may be responsible for various pharmacological actions reported. This study is only a preliminary study of only the petroleum ether extract. An in-depth study on the plant is going on which may provide a good concrete base for all the biochemical and phytochemical properties shown by the plant.

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