ISSN : 2249-5746

International Journal of Ayurvedicand Herbal Medicine 7:4 (2017) 2672–2684

Journal homepage: http://www.interscience.org.uk

DOI: 10.18535/ijahm/v7i4.06

Impact factor: 4.415

Gc-Ms Analysis Of Root And Aerial Parts Ethanolic Extract Of *Phyllanthus Vasukii* Parthipan *Et Al.*, Sp. Nov. (Phyllanthaceae)

Jemimma, H. L*., Arumugasamy,K , Nantha Kumar. R. , Abdul Kaffoor H.,

Department of Botany, Kongunadu Arts and Science College (Autonoumous), Coimbatore, Tamilnadu, India

Abstract

Objective: The objective of this research is to determine the possible bioactive components of the root and aerial parts of *Phyllanthus vaukii* using GC-MS analysis.

Methods: The GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a BR-5MS, fused silica capillary column (30mm × 0.25mm 1D × 0.25 µMdf, composed of 5% Diphenyl / 95% Dimethyl poly siloxane).

Results: From *P. vasukii* root and aerial parts, thirty (30) and twenty seven (27) components were identified respectively. Of the 30 compounds eluted from the root extract, stigmasterol had the highest peak area of 18% and the lowest was n-Propyl 11-octadecenoate showing 0.38%. In the aerial extract 1,2,3-Benzenetriol had the highest peak area of 31.65% and Z,Z-3,15-Octadecadien-1-ol acetate with the lowest peak area of 0.19%. These results indicate the ethanol extract of *P. vasukii* aerial and root parts possess potent antioxidant, hepatoprotective, anti-inflammatory, antiarthritic, antioxidant, anticancer, Immunostimulant, antitumour, cancer preventive, antiarthritic, antidiabetic, antimicrobial effects so that it can be recommended as a plant of pharmaceutical importance.

Conclusion: However, isolation of individual phytochemical constituents may proceed to find a novel drug or lead compound.

Keywords: *Phyllanthus vasukii* (*P.vasukii),* Gc-Ms Analysis, Bioactive Compound, root and aerial parts, ethanolic extract

Introduction

The use of plants as medicines by man has been in existence since a long time and we still continue to search for plants as drug for a particular disease. Herbal medicines are safe than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway[1]. The plant *Phyllanthus vasukii* belongs to the family Phyllanthaceae. The genus *Phyllanthus* is large and distributed widely in tropical and subtropical countries of the world. It has been in use as herbal medicine for a long time in China, India, Brazil and South-East Asian nations. The beneficial medicinal effects of plant materials are the nature of secondary metabolites for an example, alkaloids, flavonoids, lignin, phenols and terpenes The approval of traditional medicine as an alternative form of health care and the improvement of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds[2]. The medicinal actions of plants unique to particular plant species or groups are consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct[3,4]. These are used in traditional medicine practices in particularly, antibacterial, hepatoprotective, antidiabetic, antihypertensive, analgesics, anti-inflammatory, hepatoprotective and antimicrobial properties[5]. 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 disease[6,7]. The *Phyllanthus* species are employed by the

local people of Thailand, Latin America and Africa to cure jaundice, renal calculi and malaria etc.[8-10]. More than several hundreds of phytoconstituents were reported from different species of *Phyllanthus*, which mainly constitute lignins, triterpenoids, flavonoids and tannins. GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids esters, alkaloids, steroids, amino acid and nitro compounds[11].

Material and methods

Collection of Plant material

Roots of plant *Phyllanthus vasukii* was collected from P. vellore, Namakkal District, Eastern Ghats, Tamilnadu, India. The plant was described by Parthiban *et al.* (2017). A voucher specimen was deposited at the Herbarium of Botany Department, Bharathiar University, Coimbatore, India.
The roots and healthy aerial parts (without seed) of *P. vasukii* species were collected from Namakkal,Southern Eastern Ghats of Tamil Nadu state, Republic of India. They were thoroughly washed there itself with running water, brought to lab and shade dried. The plant was identified by Prof. Rajendran at the department of Botany, Bharathiar University, Coimbatore, India and authenticated. The voucher specimens were deposited at the Herbarium of Botany Department, Bharathiyar University, Coimbatore.

Preparation of powder and extracts

The shade dried materials were pulverized separately to fine powders. These powders were defatted with petroleum ether and then extracted with ethanol in a soxhlet apparatus. After cooling, the extracts were evaporated to dryness and kept under refrigeration for further study.

GC-MS Analysis

The GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a BR-5MS, fused silica capillary column (30mm × 0.25mm 1D × 0.25 µMdf, composed of 5% Diphenyl / 95% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1mLmin^{-1} and an injection volume of 2µLwas employed (split ratio of 10:1); Injector temperature 280°C; Ionsource temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°Cmin^{-1}, to 200°C, then 5 °C min^{-1}to 280°C, ending with a 12min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 50 to 500 amu. Total GC running time was 40.50 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The biological activities of the components were referred from Dr. Dukes' Ethanobotanical database.

Result

GC-MS analysis

GC-MS analysis of root and aerial parts of *P. vasukii* showed the presence of steroids, nitrogen and sulphur compounds. A total of 30 and 27 phytocompounds were detected in the ethanol extract of root and aerial parts respectively (Table 1 and 2). The first eluted compound was phenol, 2-methoxy-3-(2-propenyl) with a retention time of 7.92 in the root extract. Similarly levoglucosenone, a chiral compound eluted first with a retention time of 4.24 in the aerial extract. The major peak area of 18.95 and 14.81% were occupied by stigmasterol and β- sito sterol respectively in the root extract. The aerial parts of *P. vasukii* ethanol extract showed that 1, 2, 3- benzene triol, a polyhydroxy phenolic compound occupied a major peak area of 31.65%
.

Table1. GC-MS analysis of the root ethanol extract of *P. vasukii*

S No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	7.92	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164	12.01
2.	9.94	α-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-cyclic methyl	$C_{13}H_{26}NO_6$	331	0.79
3.	11.32	5,6,7,8,9,10-Hexahydro-9-methyl-spiro[2H-1,3-benzoxazine-4,1'-cyclohexane]-2-thione	$C_{14}H_{23}NOS$	253	0.68
4.	11.64	Cubedol	$C_{15}H_{26}O$	222	2.54
5.	12.40	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_3$	194	2.63
6.	15.08	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.13
7.	15.52	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	1.22
8.	15.85	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324	0.38
9.	16.01	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	6.33
10.	17.39	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.35
11.	17.49	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.45
12.	18.34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	2.57
13.	18.44	Ethyl Oleate	$C_{20}H_{38}O_2$	310	3.44
14.	18.83	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	1.13
15.	20.26	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	0.43
16.	21.04	Curan, 16,17-didehydro-, (20.xi.)-	$C_{19}H_{24}N_2$	280	0.72
17.	22.32	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	0.45
18.	23.23	Heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$	438	1.57
19.	23.83	Z,Z-3,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	2.63
20.	24.82	Bufa-20,22-dienolide, 3,14-dihydroxy-, (3β,5β)-	$C_{24}H_{34}O_4$	386	1.55
21.	25.99	Androst-4-en-9-thiocyanomethyl-11-ol-3,17-dione	$C_{21}H_{27}NO_3S$	373	1.47
22.	27.28	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	1.54
23.	27.75	Squalene	$C_{30}H_{50}$	410	2.48
24.	28.95	9,12,15-Octadecatrienoic acid, 2,3-bisoxy propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4$	496	1.70
25.	30.54	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	$C_{27}H_{44}O_3$	416	1.65
26.	30.88	Stigmasta-5,22-dien-3-ol, acetate, (3β)-	$C_{31}H_{50}O_2$	454	3.68
27.	31.98	β-Sitosterol acetate	$C_{31}H_{52}O_2$	456	2.82

28.	34.87	Campesterol	$C_{28}H_{48}O$	400	5.89
29.	35.59	Stigmasterol	$C_{29}H_{48}O$	412	18.95
30.	37.19	β-Sitosterol	$C_{29}H_{50}O$	414	14.81

Table.2 GC-MS analysis of the aerial part ethanol extract of *P. vasukii*

S No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	4.24	Levoglucosenone	$C_6H_6O_3$	126	4.42
2.	6.17	2(5H)-Furanone, 4-methyl-5-(2-methyl-2-propenyl)-	$C_9H_{12}O_2$	152	3.49
3.	8.60	1,2,3-Benzenetriol	$C_6H_6O_3$	126	31.65
4.	9.86	α-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-cyclic methyl	$C_{13}H_{26}NO_6$	331	4.56
5.	11.04	4,4-Diacetamido-1,2,5-trimethylpiperidine	$C_{12}H_{23}N_3O_2$	241	0.71
6.	11.62	Methiocarb-anisole	$C_{10}H_{14}OS$	182	1.73
7.	13.95	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	7.07
8.	14.26	7-Hexadecyn-1-ol	$C_{16}H_{30}O$	238	1.67
9.	14.49	Phytol, acetate	$C_{22}H_{42}O_2$	338	2.35
10.	15.06	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.72
11.	15.50	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.37
12.	15.95	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324	1.13
13.	16.01	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.25
14.	17.37	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.33
15.	17.60	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.30
16.	18.33	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	0.52
17.	18.42	Ethyl Oleate	$C_{20}H_{38}O_2$	310	1.15
18.	18.81	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.21
19.	21.11	Curan, 16,17-didehydro-, (20.xi.)-	$C_{19}H_{24}N_2$	280	0.23
20.	23.77	Z,Z-3,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	0.19
21.	27.71	Squalene	$C_{30}H_{50}$	410	4.49
22.	29.53	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	$C_{27}H_{46}O_2$	402	3.36
23.	31.26	γ-Tocopherol	$C_{28}H_{48}O_2$	416	3.65
24.	32.77	Vitamin E	$C_{29}H_{50}O_2$	430	6.40
25.	34.87	Campesterol	$C_{28}H_{48}O$	400	2.97
26.	35.53	Stigmasterol	$C_{29}H_{48}O$	412	5.42
27.	37.20	β-Sitosterol	$C_{29}H_{50}O$	414	9.67

Discussion

The GC-MS chromatogram in root and aerial part ethanol extract of *P. vasukii* showed a total of 30 and 27 phytocompounds (Fig 1 and 2). The peaks in the chromatogram were compared with the database of the spectrum of known components stored in the GC-MS library. The detailed tabulation of GC-MS analysis is

given in Table 2 and 4 . The identification of phytochemical compounds was based on the peak area, retention time, molecular weight, molecular formula and its activiy. The major bioactive compounds in the ethanol extract of *P. vasukii* were identified as Stigmasterol (18.95%), β-Sitosterol (14.81%) and Phenol, 2-methoxy-3- (2-propenyl)- (12.01%) in the root whereas the aerial parts showed the presence of 1,2,3-Benzenetriol (31.65%) and β-Sitosterol(9.67%).The above compounds have been reported to possess many biological activities such as antioxidant, anti-inflammatory, hepatoprotective, antiviral, antimicrobial and analgesic as referred from Dr. Duke's Phytochemical and Ethanobotanical Databases.

Table 3. Activity of bioactive compounds identified in the GCMS study of root samples of *P. vasukii*

S No.	RT	Name of the compound	Molecular Formulae	M W	Peak Area %	Compound Nature	**Activity
1.	7.92	Phenol, 2-methoxy-3-(2-propenyl)-	$C_{10}H_{12}O_2$	164	12.01	Phenolic compound	Antioxidant Antimicrobial Anti-inflammatory
2.	9.94	α-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-cyclic methyl	$C_{13}H_{26}NO_6$	331	0.79	Amino compound	Antimicrobial
3.	11.32	5,6,7,8,9,10-Hexahydro-9-methyl-spiro[2H-1,3-benzoxazine-4,1'-cyclohexane]-2-thione	$C_{14}H_{23}NOS$	253	0.68	Sulfur compound	Antimicrobial
4.	11.64	Cubedol	$C_{15}H_{26}O$	222	2.54	Sesquiterpene alcohol	Anti-tumor, Analgesic Antibacterial, Anti-inflammatory Sedative, Fungicide
5.	12.40	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	$C_{11}H_{14}O_3$	194	2.63	Phenolic compound	Antioxidant Antimicrobial Anti-inflammatory
6.	15.08	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.13	Palmitic acid methyl ester	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic
7.	15.52	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	1.22	Plasticizer compound	Antimicrobial Anti-fouling
8.	15.85	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324	0.38	Unsaturated compound	No activity reported
9.	16.01	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	6.33	Palmitic acid ethyl ester	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic
10.	17.39	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	1.35	Linoleic acid	HypocholesterolemicNematicideAntiarthriticHepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase inhibitor Antihistaminic

							AnticoronaryInsectifugeAntieczemicAntiacne
11.	17.49	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.45	Oleic acid ester	Cancer preventive Flavor Hypocholesterolemic 5-Alpha reductase inhibitor Antiandrogenic Perfumery Insectifuge Anti-inflammatory Anemiagenic Dermatitigenic Choleretic
12.	18.34	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	2.57	Linoleic acid methyl ester	HypocholesterolemicNematicideAntiarthriticHepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase inhibitor Antihistaminic AnticoronaryInsectifugeAntieczemicAntiacne
13.	18.44	Ethyl Oleate	$C_{20}H_{38}O_2$	310	3.44	Oleic acid ester	Cancer preventive Flavor Hypocholesterolemic 5-Alpha reductase inhibitor Antiandrogenic Perfumery Insectifuge Anti-inflammatory Anemiagenic Dermatitigenic Choleretic
14.	18.83	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	1.13	Stearic acid ethyl ester	No activity reported
15.	20.26	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	0.43	Unsaturated fatty acid	No activity reported
16.	21.04	Curan, 16,17-didehydro-, (20.xi.)-	$C_{19}H_{24}N_2$	280	0.72	Nitrogen compound	Antimicrobial
17.	22.32	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	0.45	Acetate compound	No activity reported
18.	23.23	Heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$	438	1.57	Ester compound	No activity reported
19.	23.83	Z,Z-3,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	2.63	Acetate compound	No activity reported
20.	24.82	Bufa-20,22-dienolide, 3,14-dihydroxy-, (3β,5β)-	$C_{24}H_{34}O_4$	386	1.55	Hydroxy compound	No activity reported
21.	25.99	Androst-4-en-9-thiocyanomethyl-11-ol-3,17-dione	$C_{21}H_{27}NO_3S$	373	1.47	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic

22.	27.28	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	1.54	Amide compound	Antimicrobial Anti-inflammatory
23.	27.75	Squalene	$C_{30}H_{50}$	410	2.48	Triterpene	Antibacterial Antioxidant Antitumor Cancer preventive Immunostimulant Chemo preventive Lipoxygenase-inhibitor Pesticide
24.	28.95	9,12,15-Octadecatrienoic acid, 2,3-bisoxy propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4$	496	1.70	Linolenic acid ester compound	HypocholesterolemicNematicideAntiarthriticHepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase inhibitor Antihistaminic AnticoronaryInsectifugeAntieczemicAntiacne
25.	30.54	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	$C_{27}H_{44}O_3$	416	1.65	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic
26.	30.88	Stigmasta-5,22-dien-3-ol, acetate, (3β)-	$C_{31}H_{50}O_2$	454	3.68	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic
27.	31.98	β-Sitosterol acetate	$C_{31}H_{52}O_2$	456	2.82	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic
28.	34.87	Campesterol	$C_{28}H_{48}O$	400	5.89	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic
29.	35.59	Stigmasterol	$C_{29}H_{48}O$	412	18.95	Steroid	Antioxidant Anti-inflammatory Sedative Antihepatotoxic Caner-preventive Antiviral OvulantHypocholesterolemic Estrogenic Artemicide
30.	37.19	β-Sitosterol	$C_{29}H_{50}O$	414	14.81	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic

Table 4. Activity of bioacive compounds identified in the GCMS study of aerial part samples of *P. vasukii*

S No.	RT	Name of the compound	Molecular Formulae	M W	Peak Area %	Compound Nature	**Activity
1.	4.24	Levoglucosenone	$C_6H_6O_3$	126	4.42	Chiral	Natural product

						compound	synthesizing
2.	6.17	2(5H)-Furanone, 4-methyl-5-(2-methyl-2-propenyl)-	$C_9H_{12}O_2$	152	3.49	Furan compound	No activity reported
3.	8.60	1,2,3-Benzenetriol	$C_6H_6O_3$	126	31.65	Polyhydroxy phenolic compound	Antioxidant Anti-inflammatory Analgesic Antimicrobial
4.	9.86	α-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-cyclic methyl	$C_{13}H_{26}NO_6$	331	4.56	Glucose moiety	Preservative
5.	11.04	4,4-Diacetamido-1,2,5-trimethylpiperidine	$C_{12}H_{23}N_3O_2$	241	0.71	Amino compound	Antimicrobial Anti-inflammatory
6.	11.62	Methiocarb-anisole	$C_{10}H_{14}OS$	182	1.73	Sulfur compound	Antimicrobial
7.	13.95	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	7.07	Terpene alcohol	Antimicrobial Anti-inflammatory
8.	14.26	7-Hexadecyn-1-ol	$C_{16}H_{30}O$	238	1.67	Unsaturated alcoholic compound	No activity reported
9.	14.49	Phytol, acetate	$C_{22}H_{42}O_2$	338	2.35	Diterpene compound	Antimicrobial Anti-inflammatory Anticancer Diuretic
10.	15.06	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.72	Palmitic acid methyl ester	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic
11.	15.50	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.37	Plasticizer compound	Antimicrobial Anti-fouling
12.	15.95	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324	1.13	Ester compound	No activity reported
13.	16.01	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.25	Palmitic acid ethyl ester	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic
14.	17.37	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.33	Linoleic acid	Hypocholesterolemic NematicideAntiarthriticHepatoprotective Antiandrogenic Hypocholesterolemic 5-Alpha

							reductase inhibitor Antihistaminic AnticoronaryInsectifugeAntieczemicAntiacne
15.	17.60	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.30	Oleic acid methyl ester	Cancer preventive Flavor Hypocholesterolemic 5-Alpha reductase inhibitor Antiandrogenic Perfumery Insectifuge Anti-inflammatory Anemiagenic Dermatitigenic Choleretic
16.	18.33	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	0.52	Linoleic acid methyl ester	Hypocholesterolemic NematicideAntiarthriticHepatoprotective Anti androgenic Hypocholesterolemic 5-Alpha reductase inhibitor Antihistaminic AnticoronaryInsectifugeAntieczemicAntiacne
17.	18.42	Ethyl Oleate	$C_{20}H_{38}O_2$	310	1.15	Oleic acid ester	Cancer preventive Flavor Hypocholesterolemic 5-Alpha reductase inhibitor Antiandrogenic Perfumery Insectifuge Anti-inflammatory Anemiagenic Dermatitigenic Choleretic
18.	18.81	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.21	Stearic acid ethyl ester	No activity reported
19.	21.11	Curan, 16,17-didehydro-, (20.xi.)-	$C_{19}H_{24}N_2$	280	0.23	Nitrogen compound	Antimicrobial
20.	23.77	Z,Z-3,15-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308	0.19	Unsaturated alcoholic compound	No activity reported
21.	27.71	Squalene	$C_{30}H_{50}$	410	4.49	Triterpene	Antibacterial Antioxidant Antitumor Cancer preventive Immunostimulant

							Chemo preventive Lipoxygenase-inhibitor Pesticide
22.	29.53	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	$C_{27}H_{46}O_2$	402	3.36	Flavonoid fraction	Antimicrobial Anti-inflammatory
23.	31.26	γ-Tocopherol	$C_{28}H_{48}O_2$	416	3.65	Vitamin E compound	Antiageing, Analgesic, Antidiabetic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, .Antibronchitic, Anticoronary
24.	32.77	Vitamin E	$C_{29}H_{50}O_2$	430	6.40	Vitamin E compound	Antiageing, Analgesic, Antidiabetic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic Antiulcerogenic, Vasodilator, Antispasmodic, .Antibronchitic, Anticoronary
25.	34.87	Campesterol	$C_{28}H_{48}O$	400	2.97	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic
26.	35.53	Stigmasterol	$C_{29}H_{48}O$	412	5.42	Steroid	Antioxidant Anti-inflammatory Sedative Antihepatotoxic Caner-preventive Antiviral OvulantHypocholesterolemic Estrogenic Artemicide

27.	37.20	β-Sitosterol		$C_{29}H_{50}O$	414	9.67	Steroid	Antimicrobial Anti-inflammatory Anticancer Antiasthma Hepatoprotective Diuretic

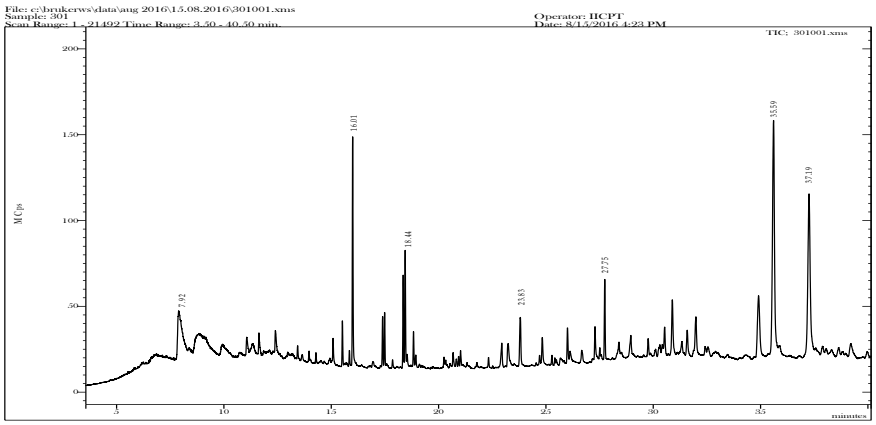

Fig. 1: GC- MS Chromatogram of *P. vasukii* root

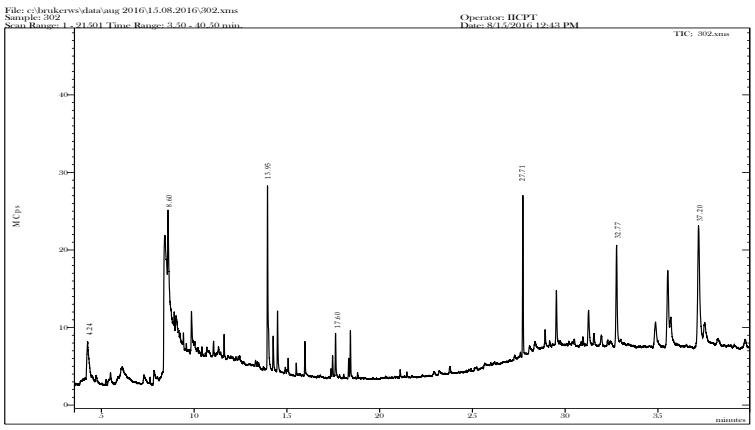

Fig. 2: GC- MS chromatogram of aerial parts of *P.vasukii*

The identified phytochemicals, hexadecanoic acid ethyl ester, squalene have the property of antioxidant activity12. Phenolic compounds are a class of antioxidant agents which act as free radical terminators13. Phytoconsituents such as flavonoids, terpenoids and alkaloids are known to possess hepatoprotective activity. The presence of these compounds in our extract may be responsible for its antioxidant and thus hepatoprotective activity. This activity was attributed to high reactivity of the hydroxyl groups being correlated with ROS scavenging capability14. Thus in the present study the hepatoprotective effect of ethanol extracts of P. vasukii may be due to antioxidant defense system. Thus this type of GC-MS analysis was the first step towards understanding the nature of active principles in this medicinal plant and it was helpful for further pharmacological studies. It has been reported that the bioactive components of leaves of P. amarus using GC-MS analysis and nine components from P. amarus leaves were identified15. The prevailing components in the ethanolic extract of leaves were 3,5-di-tri-butylphenol, methyl 14-methyl

pentadecanoate, palmitic acid (hexadecanoic acid), 10-octadecanoate, 9-hexadecenal, glycerol 1, 3-dipalmitate, 2, 13-octadecadiene-1-ol, dioctytl ester and heptanoic acid (9-dece-1-yl ester). The presence of various bioactive compounds confirms the application of P. amarus for various ailments by traditional practitioners.

Phyllanthus species have been reported to have extensive history in medicine systems16,17. They have listed the various classes of phytochemicals found in Phyllanthus species18. It has the maximum reports of pharmaceutically important compounds isolated from aqueous or organic solvent extracts. The lignin sphyllanthin, hypophyllanthin, niranthin, nirtetralin, virgatusin, and heliobupthalmin lactone are common to P. amarus, P. maderaspatensis, P. urinaria, and P. virgatus19. Phyllanthin phytochemical compound which had been studied to the most extent was considered to be correlated with antiinflammatory, immunomodulatory, antitumor, and hypotensive activities20. Reports have been published on the absence of phyllanthin and hypophyllanthin from P. maderaspatensis and P. urinaria21. Both phyllanthin and hypophyllanthin are present in P. amarus and P. fraternus but the concentration of these two lignans varies substantially in the two species22. Presence of the lignan, phyltetralin, is common to P. amarus, P. fraternus, P. maderaspatensis, P. virgatus, and P. urinaria. The lignanhinokinin has been reported from P. amarus, P. tenellus, and P. virgatus23. Flavonoids such as rutin, quercitrin, quercetin, kaempferol, and astragalin are present in both P. amarus and P. urinaria24. Presence of several ellagitannins such as geraniin, corilagin, and phyllanthusiins are also common to P. amarus as well as P. urinaria25.

Conclusion

In the present study, thirty and twenty nine bioactive compounds from the root and aerial parts of *P. vasukii* were identified by GC-MS analysis. The presences of various bioactive compounds justify that this plant can be used for treating various ailments. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. Therefore, it could be said that *P. vasukii* contains various bioactive compounds. Hence, it is recommended as a plant of phytopharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

Reference

1. Zaidan M, Rain N, Badrul A. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop. Biomed. 2005; 22:165-170.
2. Parvathi S. Antimicrobial activity of some traditional medicinal plants. J. Med. Plant Res. 2010; 4:316-321.
3. Wink D, Vodovotz Y, Grisham M, DeGraff W, Cook J, Pacelli R. Antioxidant effects of nitric oxide. Methods Enzymol. 1999; 301:413-424.
4. Janakiraman N, Johnson M, Sahaya SS. GC-MS analysis of bioactive constituents of Peristrophe bicalyculata (Retz.) Nees (Acanthaceae) Asian Pac. J. Trop. Biomed 2012; S46–S49.
5. Kokwaro JO, Medicinal plants of east Africa, First Edition, East Africa Literature Bureau, Nairobo, 1976, 58-59.
6. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth in vivo by Andrographis paniculata extract and andrographolide. Imunopharmarcol immunotoxicol. 2007; 29:81-93.
7. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian Medicinal Plants for acetyl cholinesterase inhibitory activity. Phytother. Res. 2007; 21:1142-1445.
8. Poompachee K and Chudapongse N. Comparison of the antioxidant and cytotoxic activities of Phyllanthus virgatus and Phyllanthus amarus extracts. Medical Principles and Practice, 2011; 21(1): pp. 24–29.
9. Moreira J, Klein-Júnior LC, CechinelFilho V and De Campos Buzzi F. Anti-hyperalgesic activity of corilagin, a tannin isolated from Phyllanthus niruri L. (Euphorbiaceae). Journal of Ethnopharmacology, 2013;146(1): 318–323.
10. Omulokoli E, Khan B and. Chhabra SC. Antiplasmodial activity of four Kenyan medicinal plants. Journal of Ethnopharmacology, 1997;56(2):133–137.

11. Muthulakshmi A, Margret JR, Mohan VR. GC-MS Analysis of Bioactive components of Feonia elephantum correa (Rutaceae). J. App. Pharm. Sci. 2012; 2 (2):69-74.
12. Kalay E, Yigit G, Aslan Y, Brown KE, Pohl E, Bicknell LS et al. CEP152 is a genome maintenance protein disrupted in Seckel syndrome. Nat Genet. 2011;43:23-6.
13. Om Prakash Tiwari, Yamini B, Tripathi. Antioxidant properties of different fractions of Vitex negundo Linn, Food Chem. 2007; 100(3): 1170- 1176
14. Akram, N.A., M. Ashraf and F. Al-Qurainy. Aminolevulinic acid-induced regulation in some key physiological attributes and activities of antioxidant enzymes in sunflower (Helianthus annuus L.) under saline regimes. Sci. Hort. 2012; 142: 143-148.
15. Mamza, U.T., Sodipo, O.A. and Khan, I.Z. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of bioactive components of Phyllanthus amarus leaves. Int. Res. J. Plant Sci. 2012; 3 (10):pp.208-215.
16. Unander DW, Webster GL, Blumberg BS. Records of usage or assays in Phyllanthus (Euphorbiaceae). I. Subgenera Isocladus, Kirganelia, Cicca and Emblica. J Ethnopharmacol. 1990; 30: 233-264.
17. Unander DW, Webster GL, Blumberg BS. Uses and bioassays in Phyllanthus (Euphorbiaceae): a compilation II. The subgenus Phyllanthus. J Ethnopharmacol. 1991; 34: 97-133.
18. L. Nahar, S. D. Sarker, and A. Delazar, Phytochemistry of the genus Phyllanthus in Phyllanthus Species. Scientific Evaluation and Medicinal Applications, R. Kuttan and K. B. Harikumar, Eds., , Taylor and Francis Group, CRC Press, London, UK, 2011. pp. 119–138
19. K. Shanker, M. Singh, V. Srivastava, R. Verma, A. Gupta, and M. Gupta. Simultaneous analysis of six bioactive lignans in Phyllanthus species by reversed phase hyphenated high performance liquid chromatographic technique. Acta Chromatographica. 2011; vol. 23, no. 2, pp: 321–337.
20. D. F. P. Leite, C. A. L. Kassuya, T. L. Mazzuco et al. The cytotoxic effect and the multidrug resistance reversing action of lignans from Phyllanthus amarus. Planta Medica. 2006; vol. 72, no. 15, pp: 1353–1358.
21. S. K. Sharma, S. M. Arogya, D. H. Bhaskarmurthy, A. Agarwal, and C. C. Velusami. Hepatoprotective activity of the Phyllanthus species on tert-butyl hydroperoxide (t-BH)-induced cytotoxicity in HepG2 cells. Pharmacognosy Magazine. 2011; vol. 7, no. 27, pp: 229–233.
22. K. Tripathi, R. K. Verma, A. K. Gupta, M. M. Gupta, and S. P. S. Khanuja. Quantitative determination of phyllanthin and hypophyllanthin in Phyllanthus species by high-performance thin layer chromatography. Phytochemical Analysis. 2006; vol. 17, no. 6, pp: 394–397.
23. Huang ST, Yang RC, Yang LJ, Lee PN, Pang . JHS: Phyllanthus urinaria triggers the apoptosis and Bcl-2 down-regulation in Lewis lung carcinoma cells. Life Sci 2003;72: 1705–1716.
24. T. K. Nara, J. Glyeye, E. L. Cerval et al., Flavonoids of Phyllanthus niruri, Phyllanthus urinaria, Phyllanthus orbiculatus. Plantes Médicinales et Phytothérapie. 1977; vol. 11: pp: 82–86.
25. L. Yeap Foo and H. Wong. Phyllanthusiin D, an unusual hydrolysable tannin from Phyllanthus amarus. Phytochemistry. 1992; vol. 31, no. 2: pp: 711–713.