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GC-MS analysis of non-polar fractions of leaves, stems and roots of *Pisonia grandis* R.br.

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Abstract

The present investigation was designed to explore the secondary metabolites present in the petroleum ether extract of leaves, stems and roots of *Pisonia grandis* R.Br. using gas chromatography-mass spectrometry (GC-MS). Known quantity of shade dried and pulverized parts of Pisonia grandis were extracted with pet-ether at reflux temperature for 6 h. The extracts were concentrated under vacuum and the concentrated extracts designated as PGSP, PGRP and PGLP were subjected to GC-MS analysis. The GC-MS analysis revealed the most prevailing phytoconstituents as *n*-hexadecanoic acid (palmitic acid), 6-octadecenoic acid (petroselenic acid), 9-octadecenoic acid (oleic acid), 2,3-bis-[(9*E*)-9-octadecenoyloxy]propyl (9*E*)-9-octadecenoate (9-octadecenoic acid 1,2,3-propanetriyl ester) and phytol in the extracts. The presence of various therapeutically important metabolites justifies the use of the plant for various ailments by tribals and traditional healers.

Keywords: Pisonia grandis, GC-MS Analysis, n-Hexadecanoic acid, Oleic acid, Phytol.

Introduction

Interest in medicinal plants for health aid has re-emerged. During the past 20 years there has been a tremendous advance in medicinal plant research. About 80% of the population of the developing countries depends on medicinal plants for their primary healthcare [1]. The family *Nyctaginaceae* is small but well-known for its ornamental and medicinal values. Plants of the *Nyctaginaceae* family have been found to possess immense medicinal potential particularly hypoglycemic potential [2, 3]. *Pisonia grandis* R.Br. is one such medicinal plant of *Nyctaginaceae* family and is used in Indian traditional medicine as an anti-diabetic agent [4, 5]. The medicinal value of various parts of this plant has been duly validated by a number of scientific findings [6-11]. This study aims to explore the secondary metabolites present in the petroleum ether extracts of leaves, stems and roots of *Pisonia grandis* R.Br. using gas chromatography-mass spectrometry (GC-MS) technique.

Materials and Methods

Collection and Preparation of Plant Material

Healthy, fresh and matured parts (leaves, stems and roots) of *Pisonia grandis* were collected from local areas of Coimbatore, Tamilnadu, India. The plant parts were cleaned, shade dried and cut into small pieces.

Preparation of Extract

Shade dried parts of *Pisonia grandis* were extracted with petroleum ether at reflux temperature for about 6 h. The extracts were concentrated under vacuum. The concentrates designated as PGSP (petroleum ether extract of stems of *Pisonia grandis*), PGRP (petroleum ether extract of roots of *Pisonia grandis*) and PGLP (petroleum ether extract of leaves of *Pisonia grandis*) were analysed by GC-MS technique.

GC-MS Analysis

Hewlett–Packard 6890 gas chromatograph (Agilent Technologies, CA) connected to a HP5973 mass selective detector was used. Separations were performed on an Agilent Ultra 2 fused silica capillary column (12 m length, 0.2 mm internal diameter). Helium was used as the carrier gas at a flow rate of 1 ml/min. Samples were injected in splitless mode. Initial column temperature was 100ºC and it was increased to 400ºC. Ions were generated by the electron-ionization mode at 70 eV. Diluted samples of 1 ppm concentration were injected. Total GC running time was 36 min. Interpretation of mass spectrum was done with reference to National Institute of Standard and Technology (NIST) database. The relative percentage of the chemical constituents in crude extracts was expressed as percentage by peak area normalization.

Results

The phytoconstituents present in the petroleum ether extract of PGSP, PGRP and PGLP have been identified by GC-MS analysis. Figures 1-3 depict the GC chromatogram of the extracts PGSP, PGRP and PGLP. The various constituents present in extracts PGSP, PGRP and PGLP expressing peak area more than 1%,

have been listed with their retention time (R_t), molecular weight (MW), molecular formula (MF) and molecular structure in Tables 1-3. Chart 1 represents the number of constituents in the extracts exhibiting more than 1% peak area in the chromatogram. The most prevailing phytoconstituents present in the extracts PGSP, PGRP and PGLP are illustrated in Table 4 and their individual mass fragmentation patterns represented by figures 4A-H. The therapeutical potential of some of the identified constituents is listed (Table 5) with reference to Dr. Duke's phytochemical and ethnobotanical databases [12].

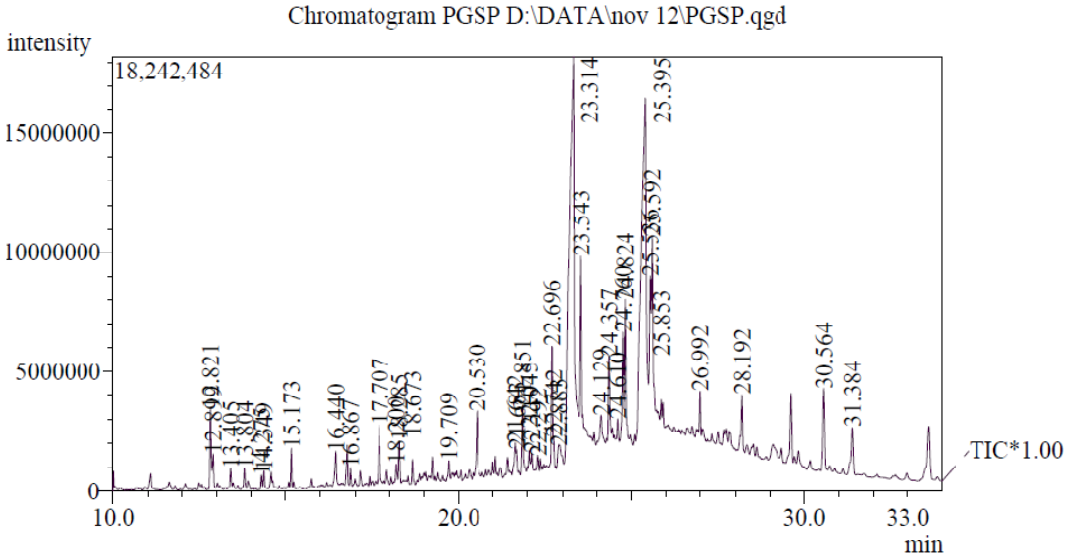

Fig. 1 GC-MS chromatogram of PGSP

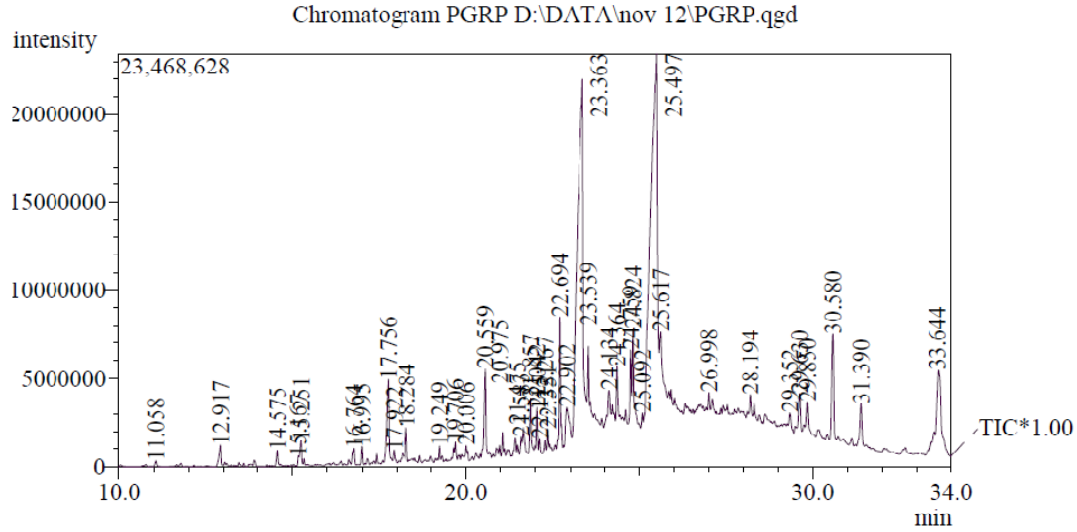

Fig. 2 GC-MS chromatogram of PGRP

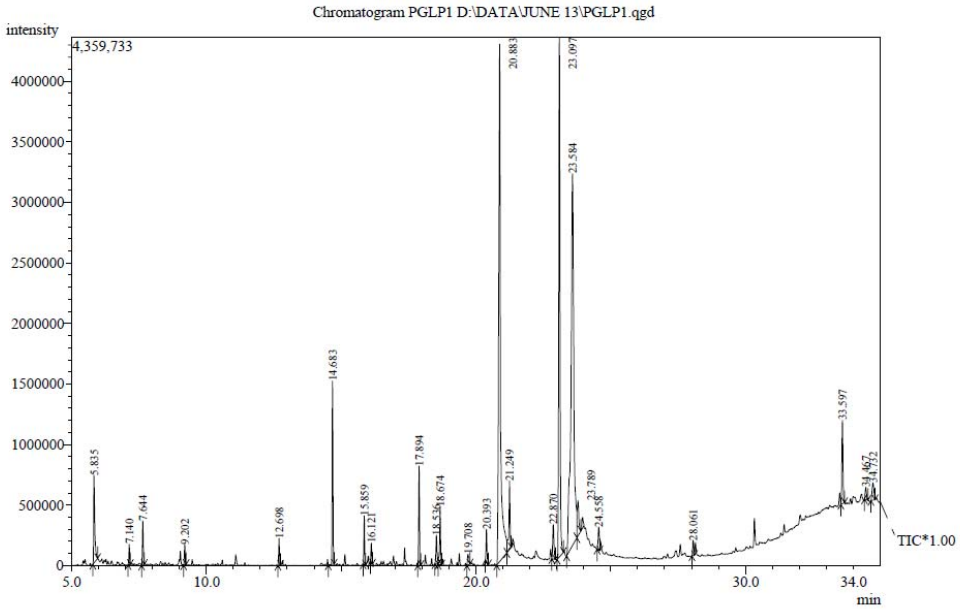

Fig. 3 GC-MS chromatogram of PGLP

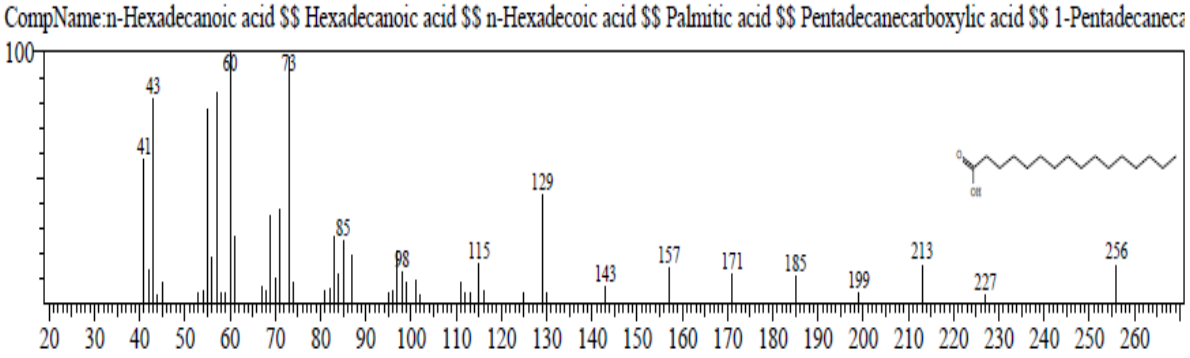

Fig.4A Mass spectrum of n-hexadecanoic acid

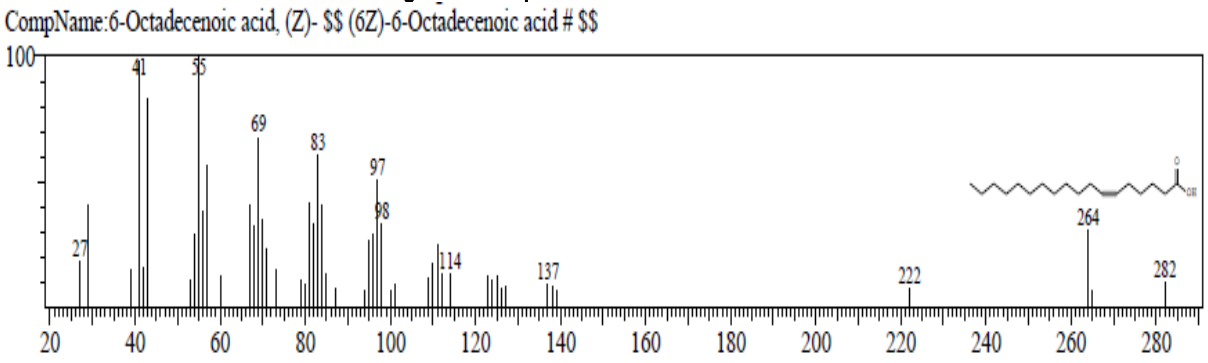

Fig.4B Mass spectrum of 6-octadecenoic acid

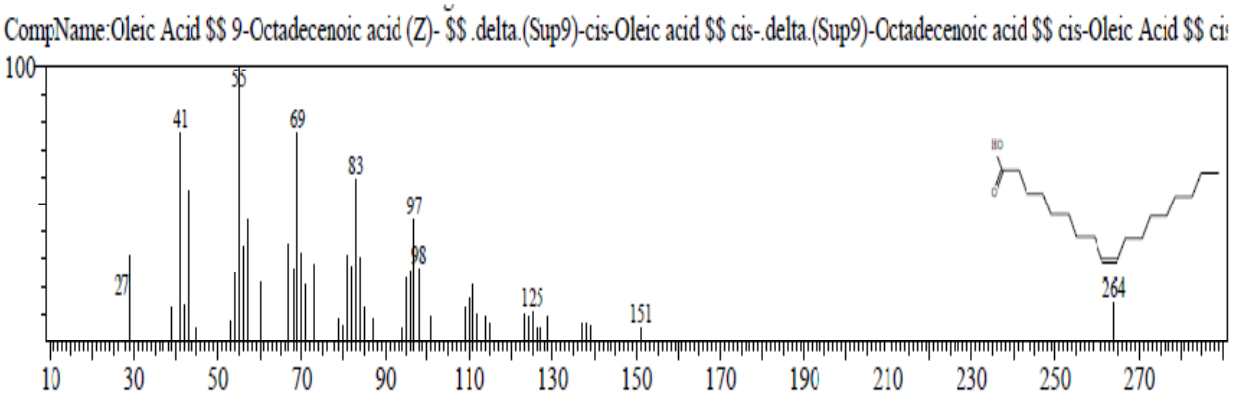

Fig.4C Mass spectrum of 9-octadecenoic acid

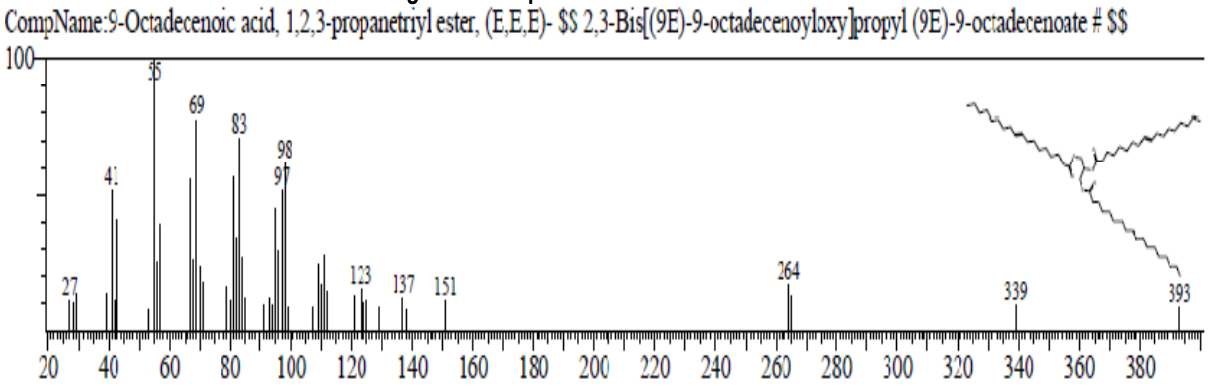

Fig.4D Mass spectrum of 2,3-bis[(9E)-9-octadecenoyloxy]propyl(9E)-9-octadecenoate

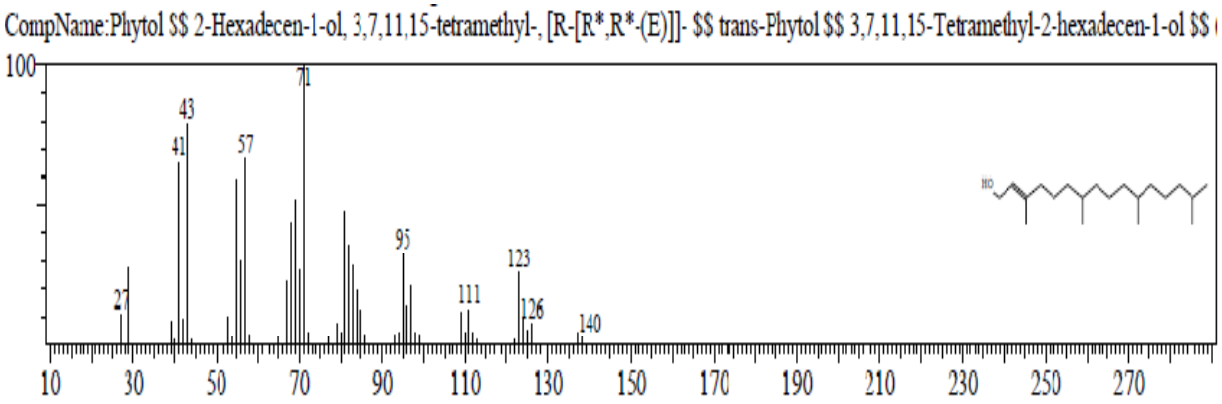

Fig.4E Mass spectrum of phytol

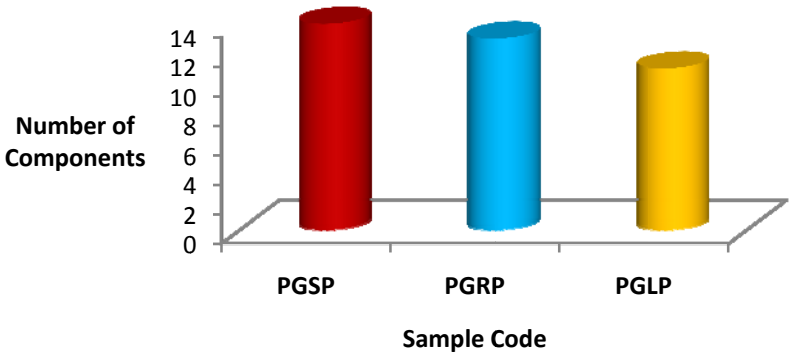

Chart 1
Number of metabolites identified in PGSP, PGRP and PGLP

Table.1. Metabolites identified in PGSP by GC-MS

RT	Area %	Molecular Weight	Molecular Formula	Name of the Compound
23.31	30.60	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid (Palmitic acid)
25.39	25.88	282	$C_{18}H_{34}O_2$	6-octadecenoic acid (Petroselenic acid)
25.59	5.11	310	$C_{20}H_{38}O_2$	9-octadecenoic acid ethyl ester (Ethyl oleate)
25.53	4.11	322	$C_{21}H_{38}O_2$	n-prpopyl-9,12-octadecadienoate
23.54	3.78	284	$C_{18}H_{36}O_2$	Ethyl hexadecanoate (Palmitic acid ethyl ester)
24.82	2.84	296	$C_{19}H_{36}O_2$	9-octadecenoic acid methyl ester (Methyl elaidate)
24.76	2.30	294	$C_{19}H_{34}O_2$	Methyl-10-trans,12-cis-octadecadienoate
22.69	2.28	270	$C_{17}H_{34}O_2$	Methyl hexadecanoate (Palmitic acid methyl ester)
30.56	2.27	279	$C_{16}H_{22}O_4$	Mono-(2-ethylhexyl)phthalate
25.85	1.84	312	$C_{20}H_{40}O_2$	Methyl-17-methyl-octadecanoate
24.35	1.71	270	$C_{17}H_{34}O_2$	Heptadecanoic acid (Margaric acid)
21.85	1.46	242	$C_{15}H_{30}O_2$	Pentadecanoic acid
17.70	1.23	200	$C_{12}H_{24}O_2$	Dodecanoic acid (Lauric acid)
20.53	1.07	228	$C_{14}H_{28}O_2$	Tetradecanoic acid (Myristic acid)

Table.2. Metabolites identified in PGRP by GC-MS

RT	Area %	Molecular Weight	Molecular Formula	Name of the Compound
25.49	35.50	282	$C_{18}H_{34}O_2$	9- octadecenoic acid (Oleic acid)
23.36	30.30	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid (Palmitic acid)
30.58	3.38	390	$C_{24}H_{38}O_4$	di-isooctylphthalate
33.64	3.35	404	$C_{25}H_{40}O_4$	Oxalic acid-hexadecyl 2-methylphenyl ester
17.75	2.65	200	$C_{12}H_{24}O_2$	Dodecanoic acid (Lauric acid)
22.69	2.53	270	$C_{17}H_{34}O_2$	Methyl hexadecanoate (Palmitic acid methyl ester)
20.55	2.03	228	$C_{14}H_{28}O_2$	Tetradecanoic acid (Myristic acid)
24.82	1.47	296	$C_{19}H_{36}O_2$	9-octadecenoic acid methyl ester(Methyl elaidate)
24.75	1.31	294	$C_{19}H_{34}O_2$	Methyl-10-trans,12-cis octadecadienoate(Linoleic acid methyl ester)
31.39	1.25	506	$C_{36}H_{74}$	Hexatriacontane
24.36	1.24	270	$C_{17}H_{34}O_2$	Heptadecanoic acid (Margaric acid)
21.85	1.12	242	$C_{15}H_{30}O_2$	Pentadecanoic acid
23.53	1.10	284	$C_{18}H_{36}O_2$	Ethyl hexadecanoate (Palmitic acid ethyl ester)
22.04	0.95	278	$C_{16}H_{22}O_2$	di-isobutyl phthalate

Table.3. Metabolites identified in PGLP by GC-MS

RT	Area %	Molecular Weight	Molecular Formula	Name of the Compound
5.83	2.64	99	C_5H_9NO	Methyl pyrrolidin
7.64	1.02	138	$C_9H_{14}O$	- isophorone (Isoacetophorone)
14.68	3.76	206	$C_{14}H_{22}O$	2,4 di-tert-butylphenol
18.67	1.44	266	$C_{19}H_{38}$	1-nonadecene
20.39	0.98	270	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester (n-hexadecanoic acid methyl ester)
20.88	26.22	256	$C_{16}H_{32}O_2$	n-hexadecanoic acid (Palmitic acid)
21.24	2.18	354	$C_{24}H_{50}O$	Lignoceric alcohol (1-tetracosanol)
22.87	1.19	296	$C_{19}H_{36}O_2$	9-octadecenoic acid, methyl ester
23.09	18.36	296	$C_{20}H_{40}O$	Phytol
23.58	29.35	884	$C_{57}H_{10}4O_6$	2,3-bis-(9E)-9-octadecenoyloxy]propyl(9E)-9-octadecenoate (9-octadecenoic acid, 1,2,3-propanetriyl ester)
23.78	1.55	340	$C_{20}H_{36}O_4$	2-Ethylhexyl maleate
33.59	2.45	410	$C_{30}H_{50}$	trans-Squalene (2,6,10,14,18,22tetracosahexaene,2,6,10,15,19,23-hexamethyl)

Table.4. Major metabolites identified in PGSP, PGRP and PGLP by GC-MS

Sample Code	R_t	Area(%)	MW	MF	Name of the Compound
PGSP	23.31	30.60	256	$C_{16}H_{32}O_2$	*n*-hexadecanoic acid (palmitic acid)
	25.39	25.88	282	$C_{18}H_{34}O_2$	6-octadecenoic acid (petroselenic acid)
PGRP	25.49	35.50	282	$C_{18}H_{34}O_2$	9-octadecenoic acid (oleic acid)
	23.36	30.30	256	$C_{16}H_{32}O_2$	*n*-Hexadecanoic acid (palmitic acid)
PGLP	23.58	29.35	884	$C_{57}H_{10}4O_6$	2,3-bis[(9E)-9-octadecenoyloxy]propyl (9E)-9-octadecenoate (9-octadecenoic acid, 1,2,3-propanetriyl ester)
	20.88	26.22	256	$C_{16}H_{32}O_2$	*n*-hexadecanoic acid (palmitic acid)
	23.09	18.36	296	$C_{20}H_{40}O$	Phytol

Table.5. Therapeutic applications of the metabolites of *Pisonia grandis* R.Br.

Metabolite	Therapeutic application
n-hexadecanoic acid (palmitic acid)	anti-oxidant , anti-alopecic, anti-androgenic, anti-fibrinolytic, hypercholesterolemic, anti-inflammatory,anti-tumor effects[13-17]
9-octadecenoic acid (oleic acid)	5- -Reductase-Inhibitor, allergenic, anti-alopecic, anti-androgenic, anti-inflammatory, anti-cancer, choleretic, dermatitigenic nature [18-22]
Phytol	Cancer-preventive and anti-microbial effect [23-25]

Discussion

GC-MS analysis is the first step towards exploring the phytoconstituents present in medicinal plants. Gas chromatogram of petroleum ether extracts of stems of *Pisonia grandis* revealed that n-hexadecanoic acid and 6-octadecenoic acid are the most prevailing phytoconstituents. 9-octadecenoic acid and *n*-hexadecanoic acid are the most substantial phytoconstituents observed in the chromatogram of roots. Unfussy appearance in the chromatogram of leaves of *Pisonia grandis* revealed that 9-octadecenoic acid-1,2,3-propanetriyl ester, phytol and *n*-hexadecanoic acid are the major phytoconstituents. Among the most prevailing phytoconstituents, *n*-hexadecanoic acid, 9-octadecenoic acid and phytol are therapeutically significant molecules. Occurrence of these molecules in the extracts of *Pisonia grandis* validates the use of this plant in the treatment of various ailments by tribals and traditional healers.

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References

[1]. Prabhadevi V, Sahaya Sathish S, Johnson M, Venkatramani B, Janakiraman N. Phytochemical studies on *Allamanda cathartica* L. using GC-MS. Asian Pacific Journal of Tropical Biomedicine 2012; S550-S554.

[2]. Narayanan CR, Joshi DD, Mujumdar AM. Hypoglycemic action of *Bougainvillea spectabilis* leaves. *Curr. Sci.* 1984; 53: 579-581.

[3]. Pari Land Amarnath Satheesh M. Antidiabetic activity of *Boerhaavia diffusa* L. effect on hepatic key enzymes in experimental diabetes. Journal of Ethnopharmacology 2004; 91(1):109-13.

[4]. Shubashini K.Sripathi, Poongothai G, Lalitha P. Insulinomimetic Pinitol from the Leaves of *Pisonia grandis* (R.Br.). Journal of Natural Remedies 2011; 11(1):39-43.

[5]. Sunil C, Latha PG, Suja SR, Shine VJ, Shyamal S, Anuja GI, Sini S, Rajasekharan S, Ajastian P, Ignacimuthu S, Kaliselvan V. Effect of ethanolic extract of *Pisonia alba* Span leaves on blood glucose levels and Histological changes in tissues of Alloxan-induced diabetic rats. International journal of Applied Research in natural products 2009; 2(2):4-11.

[6]. Habibur Rahman A, Elumalai M, Chinna Eswaraiah, Dipankar Bardalai. Evaluation of anxiolytic activity of ethanolic extract of *Pisonia grandis* R. Br leaves in mice. J Chem Pharm Res 2011; 3(5):646-652

[7]. Shubashini K. Sripathi, Poongothai G. Bioassay-guided fractionation and anti-fungal activity studies on *Pisonia grandis* R.Br. International Journal of Current Research 2010; 10 :035-037

[8]. Jayakumari S, Arthanareswaran A, Vijayalakshmi A, Malarkodi Velraj, Ravichandran V. Free Radical Scavenging Activity of *Pisonia grandis* R.Br Leaves. Ind J Pharm Edu Res 2012; 46(1) : 34-37

[9]. Prabu D, Nappinnai M, Ponnudurai K, Prabu K. Evaluation of Wound-Healing Potential of *Pisonia grandis* R.Br: A Preclinical Study in Wistar Rats. The International Journal of Lower Extremity Wounds 2008; 7: 21-27.

[10]. Anbalagan N, Rajinikanth KN, Kishore Gnanasam S, Thomas Leonard J, Balakrishna K, Ramachandran S, Sridhar SK. Analgesic, Anti-inflammatory and Diuretic Activities of *Pisonia grandis*. Natural Product Sciences 2002; 8:97-99

[11]. Radha R, Arokiyaraj S, Agastian P, Balaraju K, Mohan Kumar R, Bula, P. Phytochemical analysis and anti-inflammatory activity of *Pisonia grandis* R.Br. Journal of Biomedical and Pharmacology 2008;1:1.

www.ars-grin.gov/duke.

[12]. Chan P, Cheng JT, Tsao CW, Niu CS, Hong CY. The in vitro antioxidant activity of trilinolein and other lipid-related natural substances as measured by enhanced chemiluminescence. Life Sci. 1996; 59(24):2067-73.

[13]. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 2002; 22(5):2587-90.

[14]. http://www.amberswaves.com/prickly_lettuce.html

[15]. Liu J, Shimizu K, Kondo R. Anti-androgenic activity of fatty acids. Chem Biodivers. 2009;6(4):503-12.

[16]. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des. 2012; 80(3):434-9.

[17]. Sheba DW, Saxena RK, Gupta R . The fungistatic action of oleic acid. *Current Science* 1999; 76 (8): 1137-1139.

[18]. Carrillo C, M. del M. Cavia, Alonso-Torre S. R. Antitumor effect of oleic acid; mechanisms of action. A review. Nutr Hosp. 2012;27(5):1860-1865.

[19]. Joseph J. Collins N.D. Phytotherapeutic Management of Endocrine Dysfunctions. Nutri News – Recent Health and Nutrition Information From Douglas Laboratories , Winter 2006.

[20]. Carrillo C, M. del M. Cavia S. Alonso-Torre. Role of oleic acid in immune system; mechanism of action; a review. Nutr Hosp. 2012;27(4):978-990.

[21]. Liang T, Liao S. Inhibition of steroid 5 - reductase by specific aliphatic unsaturated fatty acids. Biochem J. 1992; 15; 285(Pt 2): 557–562.

[22]. Lee KL, Lee SH, Park KY. Anticancer activity of phytol and eicosatrienoic acid identified from perilla leaves. J Koean Soc. Food Sci. Nutr. 1999; 28:1107-1112.

[23]. Komiya T, Kyohkon M, Ohwaki S, Eto J, Katsuzaki H, Imai K, Kataoka T, Yoshioka K, Ishii Y, Hibasami H. Phytol induces programmed cell death in human lymphoid leukemia Molt 4B cells. International journal of Molecular Medicine 1999;4(4): 377-457.

[24]. Inoue Y, Hada TA, Shiraishi K, Hirore, H Hamashima, Kobayashi S: Biphasic effects of Geranylgeraniol, Terpenone and Phytol on the growth of *Staphylococcus aureus*. *Antimicrobial agents and Chemother*2005; 49(5): 1770-1774.