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Letter to the Editor

Quantitative variation of bioactive phyto compounds in ethyl acetate and methanol extracts of *Pergularia daemia* (Forsk.) Chiov.

Dear Editor:

Pergularia daemia Forsk (Asclepiadaceae) is a perennial twining herb commonly known as veliparuthi in Tamil. The plant has anthelmintic, laxative, antidiabetic, hepatoprotective and anti-inflammatory activities[1]. The pharmacological properties of this plant come from bioactive phytochemicals such as alkaloids, triterpenes, saponins and flavonoids. Phytochemically, the plant has been investigated for the presence of cardenolides, alkaloids, saponins and steroidal compounds[2]. In the present study, we developed a rapid method for identification and quantitative determination of putative phyto compounds in the crude extracts of ethyl acetate and methanol from whole plant of *Pergularia daemia*.

Matured *Pergularia daemia* plant was collected between August and December, 2013 from the river bank of Pudukkottai District, Tamil Nadu, India. The plant was identified and voucher specimen (ACC: 196) was deposited in the herbarium of Department of Botany, Annamalai University. The shade dried plant materials (root, stem, leaves, flower and bark) of *Pergularia daemia* of about 1,000 g were subjected for size reduction to coarse powder, which was defatted by using petroleum ether (60–80°C) and then extracted with methanol and ethyl acetate using Soxhlet apparatus for about 72 hours at 40°C. The sediment was then filtered with Whatman No. 1 filter paper[3]. Both ethyl acetate and methanolic extracts of *Pergularia daemia* were further concentrated under vacuum using rotary vacuum evaporator (Buchi R-V120, Switzerland) at 40°C and then reconstituted in dimethyl sulfoxide and stored at 4°C for further use. The percentage yield of ethyl acetate and methanol extracts were found to be 4.5 % (w/w) and 8.1% (w/w), respectively.

Preliminary phytochemical analysis revealed the presence of flavonoids, terpenoids, steroids, alkaloids, tannins and carbohydrates in ethyl acetate and methanol extracts of whole plant of *Pergularia daemia*. Gas chromatography-mass spectroscopy (GC-MS) identified a number of compounds from GC fractions of the methanol and ethyl acetate extracts of *Pergularia daemia*. The results revealed that the presence of 15 different compounds from ethyl acetate extract viz., (2S,3S)-(-)-3-propyloxiranemethanol, 4-heptanol, 3-methyl-, 1-pentanol, 4-methyl-2-propyl-, 2-decanynoic acid, dichloroacetic acid, 2,2-dimethylpropyl ester, cyclopentane undecanoic acid, 1-iodo-2-methylundecane, octadecane, 6-methyl-, heptacosane, methoxyacetic acid, 3-tetradecyl ester, 2(1H)naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-, 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-, azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1a′,7a′,8aa′)]-(e-guaiene), methoprene, 9,12-octadecadienoic acid (Z,Z)-, phenyl methyl ester (***Table 1***) and 18 different phyto compounds from methanol extract viz 8-methyl-6-nonenoic acid, vitamin D3, n-hexadecanoic acid, 4-trifluoro acetoxypentadecane, undec-10-ynoic acid, 2-cyclopentene-1-undecanoic acid, (+)-,8-nonynoic acid, didodecyl phthalate, 4-nonene, 5-nitro-, cis-Z-a′-bisabolene epoxide, 1b,5,5,6a-tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one, 1-naphthalenepropanol, a′-ethyldecahydro-5-(hydroxymethyl)-a′,5,8a-trimethyl-2-methylene [1S[1a′(S*), 4aa′, 5a′, 8aa′]]-, 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-,5a′-androstan-16-one, cyclic ethylene mercaptole, 2(1H)naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-, azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-,[1S-(1a′,7a′,8aa′)]-(e-guaiene), methoprene, 9,12-octadecadienoic acid (Z,Z)-, and phenylmethyl ester (***Table 2***) were identified.

The major compounds such as didodecyl phthalate, 9,12-octadecadienoic acid (Z,Z)-, phenylmethyl ester,

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Table 1 **Phytoconstituents identified in ethyl acetate extract of *Pergularia daemia*. using GC-MS**

No.	RT	Compounds	Molecular Formula	MW	Peak Area %
1.	16.72	(2S,3S)-(-)-3-Propyloxiranemethanol	C6H12O2	116	0.07
2.	19.09	4-Heptanol, 3-methyl-	C8H18O	130	0.07
3.	19.40	1-Pentanol, 4-methyl-2-propyl-	C9H20O	144	0.12
4.	20.80	2-Decanynoic acid	C10H16O2	168	0.05
5.	22.16	Dichloroacetic acid, 2,2-dimethylpropyl ester	C7H12Cl2O2	198	0.15
6.	23.71	Cyclopentaneundecanoic acid	C16H30O2	254	0.16
7.	24.76	1-Iodo-2-methylundecane	C12H25I	296	0.53
8.	26.06	Octadecane, 6-methyl-	C19H40	268	0.10
9.	27.26	Heptacosane	C27H56	380	3.75
10.	30.33	Methoxyacetic acid, 3-tetradecyl ester	C17H34O3	286	1.21
11.	32.88	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methy–lethenyl)-	C15H22O	218	6.88
12.	33.15	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	C15H26O	222	0.22
13.	34.12	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7- (1-methylethenyl)-, [1S-(1à,7à,8aá)]- (ë-Guaiene)	C15H24	204	86.37
14.	34.44	Methoprene	C19H34O3	310	0.12
15.	35.80	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	C25H38O2	370	0.19

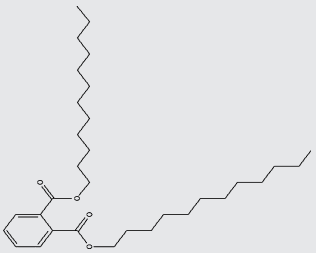
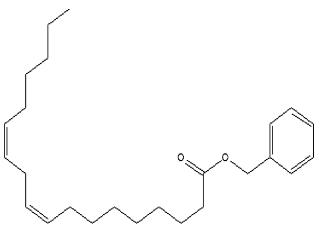
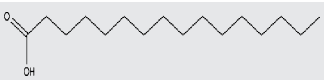
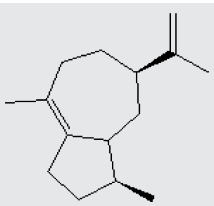
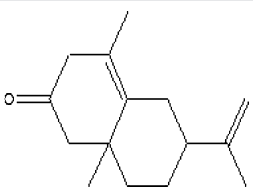
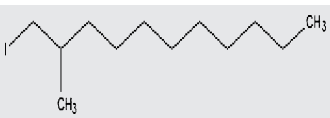
n-hexadecanoic acid, heptacosane, azulene, 1,2,3, 5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1- methylethenyl)-, 2(1H) Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methy–lethenyl)- and 1-iodo-2-methylundecane present in both extracts of this plant exert various biological activities (***Table 3***).

Phytochemical characterization of *Pergularia daemia* explored the presence of phenolics and terpenoids in ethyl acetate and methanol extracts, which may have

Table 2 **Phytoconstituents in methanol extract of Pergularia daemia. using GC-MS analysis**

No.	RT	Compounds	Molecular Formula	MW	Peak Area %
1.	11.06	8-Methyl-6-nonenoic acid	C10H18O2	170	3.07
2.	11.32	Vitamin d3	C27H44O	384	1.22
3.	12.76	n-Hexadecanoic acid	C16H32O2	256	27.10
4.	14.22	4-Trifluoroacetoxypentadecane	C17H31F3O2	324	1.32
5.	14.89	Undec-10-ynoic acid	C11H18O2	182	8.93
6.	15.94	2-Cyclopentene-1-undecanoic acid, (+)-	C16H28O2	252	5.88
7.	19.01	8-Nonynoic acid	C9H14O2	154	1.03
8.	19.97	Didodecyl phthalate	C32H54O4	502	2.50
9.	23.52	4-Nonene, 5-nitro-	C9H17NO2	171	0.32
10.	27.07	cis-Z-à-Bisabolene epoxide	C15H24O	220	5.53
11.	29.80	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	C13H20O2	208	0.60
12.	31.19	1-Naphthalenepropanol, à-ethyldecahydro-5-(hydroxymethyl)-à,5,8a-tri–methyl-2-methylene-, [1S-[1à(S*),4aá,5à,8aà]]-	C20H36O2	308	0.92
13.	32.10	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	C15H26O	222	3.19
14.	32.22	5à-Androstan-16-one, cyclic ethylene mercaptole	C21H34S2	350	1.23
15.	32.88	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methy–lethenyl)-	C15H22O	218	4.56
16.	34.02	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aá)]- (ë-Guaiene)	C15H24	204	32.28
17.	34.30	Methoprene	C19H34O3	310	0.12
18.	35.42	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	C25H38O2	370	0.22

Table 3 **Pharmacological applications of major compounds present in Pergularia daemia**

S.No	Compounds	Structure	Medicinal use
1.	Didodecyl phthalate		Antimicrobial Antifouling
2.	9,12-Octadecadienoic acid (Z,Z)-, phenyl–methyl ester		Anti androgenic Hypocholesterolemic Nematicide 5-Alpha reductase inhibitor Antihistaminic Anticoronary Insectifuge Antieczemic Antiacne Anti-inflammatory , Antiandrogenic Cancer preventive, Dermatitigenic .
3.	n-Hexadecanoic acid		Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Flavor, Lubricant, Antiandrogenic, Hemolytic 5-Alpha reductase Inhibitor.
4.	Heptacosane		Antioxidant activity
5.	Azulene, 1,2,3,5,6,7,8,8aoctahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1-methylethenyl)-		Anti allergic, Anti histaminic, Anti-inflammatory, Antipyretic, Antiseptic, Antispasmodic, Antiulcer.
6.	2(1H)Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-		Anti-inflammatory
7.	1-Iodo-2-methylundecane		Antifungal, antioxidant, anti insecticidal and antimicrobial.

an important role in maintaining good health due to their antioxidant activity. Moreover, our findings explored that the presence of biologically important active principles were highly accumulated in methanol extract when compared to ethyl acetate extract. It could be concluded that the choice of the solvent is an important criteria to retrieving more active substances. Further investigations on isolation of active principles from this plant and their possible chemopreventive mechanisms in oral carcinogenesis is currently under progress in our laboratory.

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