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GC-MS analysis of phytochemical components of *Pseudoglochidion anamalayanum* Gamble: An endangered medicinal tree

V. Anand Gideon

Department of Botany, Bishop Heber College (Autonomous), Tiruchirappalli, India

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ABSTRACT

Plants of the genus Pseudoglochidion belongs to the family Euphorbiaceae have been widely employed for controlling the various diseases. Objective of the present study was to carry out phytochemical analysis of Ethanolic extract of leaves. Phytochemical analysis of this plant confirms the presence of various phytochemicals like sterols, terpenoids, alkaloids, tannins, and glycosides. Thus further studies can be conducted to investigate the unexploited potential of P. anamalayanum. So as a part and as a basis for further utilization, the phytochemicals of P. anamalayanum were identified, such as Propane, 1,1,3- triethoxy (44.89 %), Tetradecanoic acid (6.72%), N,N-Dimethyltryptamine (5.98 %), Oleic acid (5.78 %), (E)-9- Octadecanoic acid ethyl ester (5.68 %), 1-Hexyl-2-nitrocyclohexane (4.61 %), Diisooctyladipate (4.46%), Hexadecanoic acid, ethyl ester (3.39%) and Squalene (3.16%) by GCMS analysis and the activity of each compound was discussed in this paper. These are reported for the first time in P. anamalayanum.

Keywords: Phytochemical, *Pseudoglochidion anamalayanum*, Ethanolic Extract, GC-MS analysis.

INTRODUCTION

Natural medicine from plant source is well-known to be safe and effective. Plant species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries (Bobbarala et al., 2011). However, they could be exploited for significant pharmacological purposes. For example *Pseudoglochidion anamalayanum* is medium sized tree of 20-35ft height belonging to the family Euphorbiaceae. It is found along the road side fragments in Anaimalai Hills of Western Ghats. Phytochemicals or the secondary metabolites, play an important role in the protective activities of plants which are utilized for medicinal properties. Hence, the present study is carried out using GC-MS Analysis for Phytochemical components from Ethanolic extract of *Pseudoglochidion anamalayanum* Gamble. For the widespread acceptance of herbal medicines, standardization, quality control of the herbal materials, as well as evaluation of efficacy, safety and quality of the phyto-pharmaceuticals are indispensable (Huie, 2002). Identification of individual components of complex mixtures of phytochemicals requires the use of several techniques. One of the most popular methods of studying phytochemical composition is GC-MS, which allows the identification of the specific natural compounds found in a plant extract by comparing their relative retention times and indices and their mass spectra (Yani et al., 2005; Adams, 2007).

MATERIALS AND METHODS

The leaf sample of the plant was extracted with ethanol and analysed for bioactive components in GC-MS. Quantitative analysis of the different compounds were performed on a GC Clarus 500 Perkin Elmer gas chromatograph equipped with Column: Elite-1 (100 % Dimethyl poly siloxane), 30m x 0.25 mm ID x 1.0 µ df at the oven temperature programme of 110 °C -2 min hold up to 280 °C at the rate of 5 °C/min-9 min hold. 1µl of each sample was injected in triplicate splits and quantities represented relative area percentage as derived from the intergrator. Injector temperature was 250 ºC and the split ratio was 10 :1. Helium was used as a carrier gas with a

constant flow at 1 ml/min and the detector was Mass detector-Turbo mass gold- Perkin Elmer. The inlet line temperature was 200 ºC and the source temperature was 200 ºC. The instrument was operated at 70 eV in electron impact mode. Full-scan analyses were performed in the mass range 45 – 400 m/z at1 scan. Data were evaluated by Software- Turbo mass 5.1. The total GC time was 45 minutes and the MS time was 46 minutes.The relative amounts of individual components of the total composition was expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds (NIST Ver.2.1) or by retention indices and mass spectra (Mondello et al., 1995).

RESULTS AND DISCUSSION

The phytochemical study was carried out by GC/MS analysis of *Pseudoglochidion anamalayanum* Gamble and the chromatogram identified eighteen phytochemicals as constituents (Plate 1). Of these compounds, Propane, 1,1,3-triethoxy was the major compound (44.89 %) followed by Tetradecanoic acid (6.72%),N,N-Dimethyltryptamine (5.98 %), Oleic acid (5.78 %), (E)-9- Octadecanoic acid ethyl ester (5.68 %), 1-Hexyl-2-nitrocyclohexane (4.61 %), Diisooctyladipate (4.46%), Hexadecanoic acid, ethyl ester (3.39%) and Squalene (3.16%). Remaining constituent chemical compounds were less than three percentages. The components, their retention times and their activities are summarized in Table 1. Of the 18 compounds identified, 11 compounds were already reported with different activities in various other plants by several studies. These compounds with noted activities are summerised along with their synonyms.

Table 1. Components identified from the Ethanolic extract of the leaves of *Pseudoglochidion anamalayanum* in GC-MS

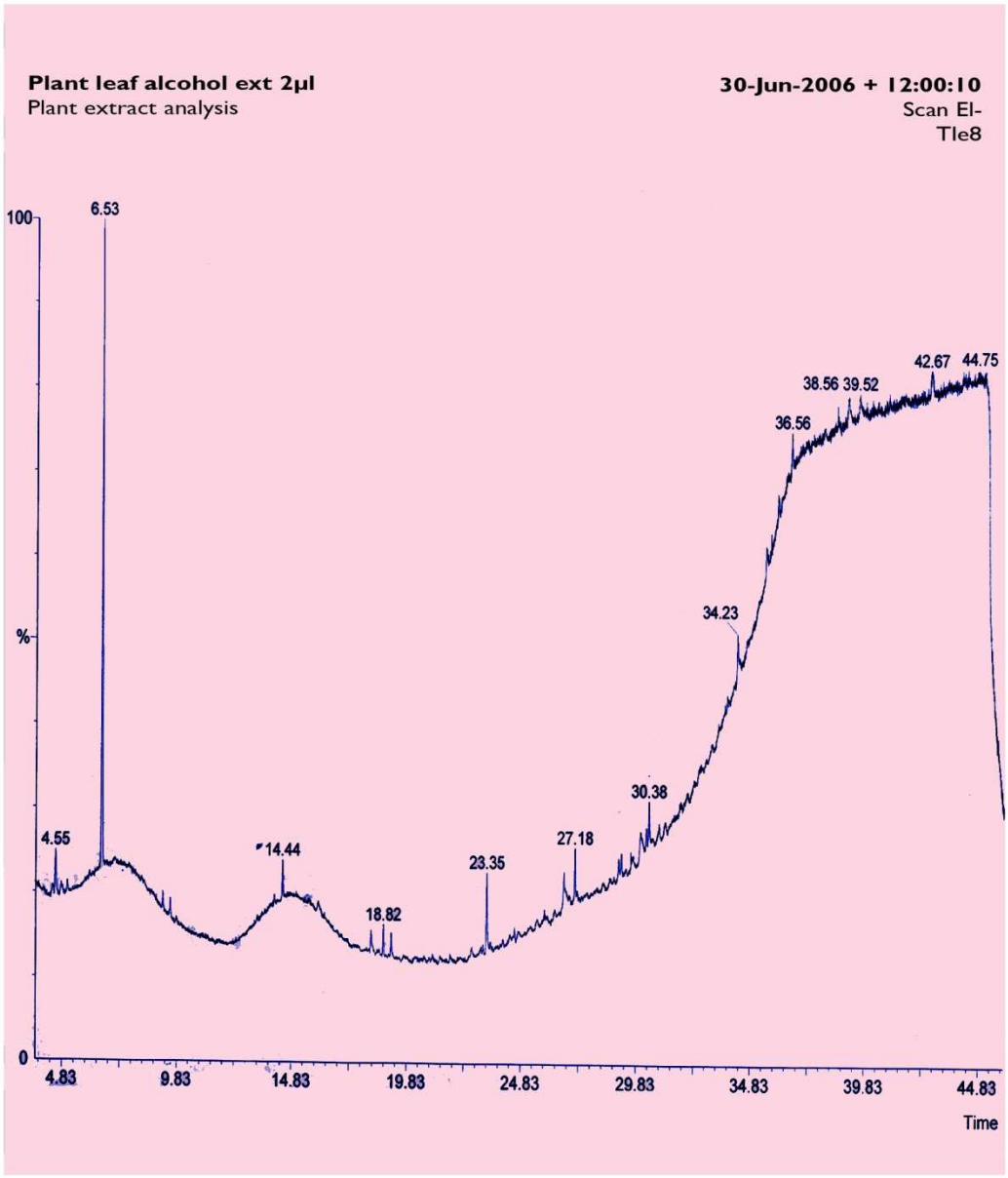
Sl.	RT	Name of the Compound	Mol. Wt.	%	Activity
1	6.53	Propane, 1,1,3-triethoxy	176	44.9	No activity reported
2	9.22	Octanoic acid, ethyl ester	172	1.02	Flavor
3	9.54	Decane, 2,3,5,8-tetramethyl-	198	1.48	No activity reported
4	14.44	Heptadecane,2,6,10,14-tetramethyl-	296	2.78	No activity reported
5	18.28	Undecanoic acid	186	2.11	No activity reported
6	18.82	Dodecanoic acid, ethyl ester	228	1.91	Antibacterial, Antiviral, Antioxidant, Candidicide, Hypercholesterolemic
7	19.16	Hexadecane	226	1.63	Antibacterial
8	23.18	Tetradecanoic acid, ethyl ester	256	Trace	Antioxidant, Antimicrobial, Cancer preventive, Cosmetic, Weedicide, Hypercholesterolemic
9	23.34	N,N-Dimethyltryptamine	188	5.98	Insecticide,Antibacterial
10	26.7	Tetradecanoic acid	228	6.72	Lubricant, Nematicide, Antibacterial
11	27.18	Hexadecanoic acid, ethyl ester	284	3.39	Hypercholesterolemic, Lubricant, Antimicrobial, Flavor, Cosmetic, Perfumery
12	29.19	9-Octadecenoic acid(Z)-, methyl ester	296	1.63	Flavour, Cancer preventive, Anti-inflammatory
13	30.01	Oleic acid	282	5.78	Hypercholesterolemic, Dermatitigenic, Anti-inflammatory
14	30.38	(E)-9- Octadecanoic acid ethyl ester	310	5.63	Perfumery
15	34.23	Diisooctyladipate	370	4.46	No activity reported
16	39.03	1-Hexyl-2-nitrocyclohexane	213	4.61	No activity reported
17	39.52	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester	568	2.83	No activity reported
18	42.67	Squalene	410	3.16	Antibacterial, Antitumor, Antioxidant, immunostimulant, Lipoxygenase-inhibitor

Dodecanoic acid, ethyl ester ($C_{14}H_{28}O_2$)
Synonyms: Ethyl laurate; Lauric acid; ethyl ester; Ethyl dodecanoate (Plate 5-A).This compound was reported with antibacterial, antioxidant, antiviral, hypercholesterolemic and candidicide activity (Mitova et al., 2003).

Tetradecanoic acid ($C_{14}H_{28}O_2$)
Synonyms: Myristic acid; n- Tetradecanoic acid; n- Tetradecoic acid; Neo-Fat14;Crod acid; 1-Tridecanecarboxylic acid; n-Tetradecan-1-oic acid (Plate 5-B).The compound was reported to be a lubricant and nematicide (Zheng et al., 1992) and also showed antibacterial activity against Gram-positive and Gram-negative bacteria (Salman et al., 2006; Yayli et al., 2006).

Tetradecanoic acid, ethyl ester ($C_{16}H_{32}O_2$)
Synonyms: Myristic acid, ethyl ester; Ethyl myristate; Ethyl tetradecanoate; Ethylester of tetradecanoic acid; Ethyl N-tetradecanoate (Plate 5-C).This compound was reported with antioxidant, antimicrobial, cancer preventive, cosmetic, weedicide and hypercholesterolemic activity (Harborne and Baxter, 1983; Ross, 2003).

Plate 1. GC-MS Chromatogram showing the Phytochemical Components from the leaf of *Pseudoglochidion anamalayanum*



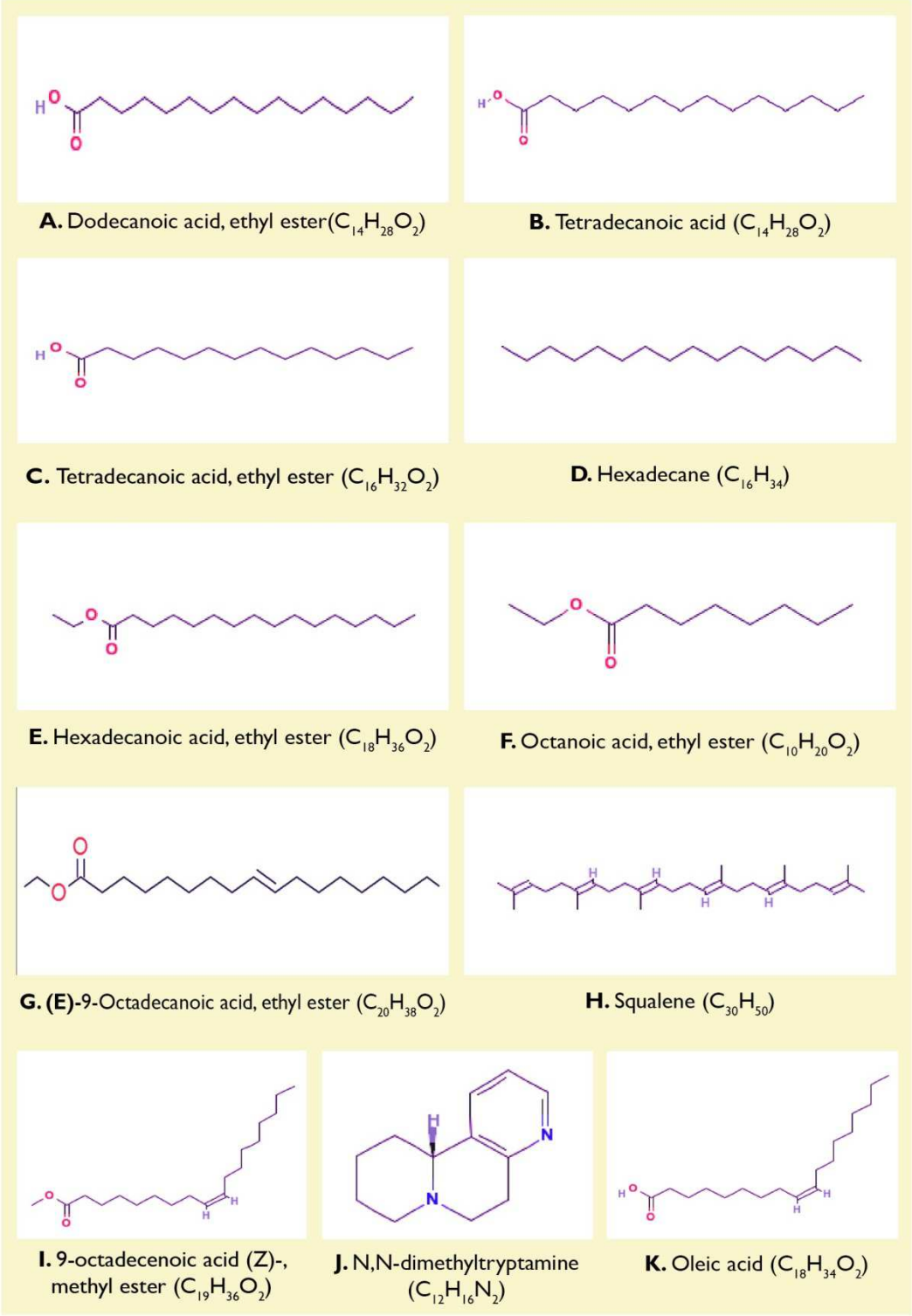
Hexadecane ($C_{16}H_{34}$)
Synonyms: n-Cetane; n-Hexadecane; Cetane (Plate 5-D). Antibacterial activity of this compound was reported by (Slantchev et al., 2002; Yayli et al., 2006).

Hexadecanoic acid, ethyl ester ($C_{18}H_{36}O_2$)
Synonyms: Palmitic acid, ethyl ester; Ethyl hexadecanoate; Ethyl palmitate (Plate 5-E). This compound was reported with following activities like flavour, antibacterial (Kujumgiev et al., 1993), hypercholesterolemic, lubricant (Ivanova et al., 2002), cosmetic and perfumery (Kroes et al., 1991).

Octanoic acid, ethyl ester ($C_{10}H_{20}O_2$)
Synonyms: Ethyl caprylate; Ethyl octanoate; Ethyl octoate; Ethyl n-octanoate; Caprylic acid ethyl ester; n-Caprylic acid ethyl ester; Ethyl octylate (Plate 5-F). This compound was reported with flavour properties (Rocha et al., 2005).

(E)-9- Octadecanoic acid ethyl ester ($C_{20}H_{38}O_2$)
Synonyms: 9-Octadecenoic acid, ethyl ester, (E)- ; Ethyl 9-octadecenoate, (E)- ; Ethyl (9E)-9-octadecenoate (Plate 5-G).This compound was reported to have perfumery activity (Harborne and Baxter, 1983; Ross, 2003).

Plate 2. Chemical structure of Bioactive compounds isolated from *Pseudoglochidion anamalayanum* Gamble



Squalene ($C_{30}H_{50}$)

Synonyms: 2, 6, 10, 14, 18, 22-Tetracosahexane, 2, 6, 10, 15, 19, 23-hexamethyl-; Skvalen; Spinacene; supraene; (6E, 10E, 14E, 18E)-2,6,10,15,19,23-Hexamethyl- 2,6,10,14,18,22-tetracosa hexaene (Plate 5-H).This compound was reported with following potential properties like antibacterial (Newmark, 1997), antioxidant, antitumor and cancer preventive properties (Kelly, 1999; Smith, 2000), immuno stimulant (Salman et al., 2006) and also as a lipoxygenase inhibitor (Wei and Shibamoto, 2007) by several studies.

9-Octadecenoic acid(Z)-, methyl ester ($C_{19}H_{36}O_2$)
Synonyms: Oleic acid, methyl ester, Methyl cis-9-octadecenoate, Methyl oleate, (Z)-9-octadecenoic acid methyl ester, Emery, Methyl (9Z)-9-octadecenoate (Plate 5-I). Flavour (Omolosa and Vagi, 2001), cancer preventive (Simin et al., 2000), anti-inflammatory(Yunfeng et al., 2007) and antimicrobial activity (Wagh et al., 2007) properties of this compound were already reported.

N,N-Dimethyltryptamine ($C_{12}H_{16}N_2$)
Synonyms: 1H-Indole-3-ethanamine; N-N-demethyl-; Indole, 3-[2-(dimethylamino) ethyl]-, Dimethyl tryptamine, DMT; 3-(2-Dimethylaminoethyl) indole; 2-(1H-Indol- 3-yl)-N,N-dimethyl ethanamine #, Alkaloid, (DMT, Bufetonina) (Plate 5-J).This compound was reported with insecticidal (McKenna et al., 1984), antibacterial (Rodriguez, 2007) and hallucinogenic properties (Strassman, 1995).

Oleic acid ($C_{18}H_{34}O_2$)
Synonyms: (9E)-octadec-9-enoic acid; (9E)-Octadecenoic acid; (9Z)-octadec-9- enoate; (9Z)-octadec-9-enoic acid; (9Z)-Octadecenoic acid; (E)-Oleic acid; (Z)-2- (Methyloleylamino) ethanesulphonic acid; (Z)-,(Z)-Octadec-9-enoic acid; delta.9-cis- Oleic acid; 9,10-Octadecenoic Acid; 9-CIS-Octadecenoic acid; 9-elaidic acid; 9- Octadecenoic acid (Plate 5-K).The compound possesses antimicrobial (Novak et al., 1961), hypercholesterolemic (Natali et al., 2007), dermatitigenic (Newmark, 1997), anti-inflammatory and anti-tumor activity (Kimura, 2002; Yunfeng et al., 2007).

The GC-MS analysis showed the presence of 18 phytochemical compounds of which 11 compounds were reported to possess various bioactivities. Of the 11compounds, seven compounds were reported to have antimicrobial activities (dodecanoic acid, ethyl ester; hexadecane; tetradecanoic acid, ethyl ester; N,N-dimethyltryptamine; tetradecanoic acid; hexadecanoic acid, ethyl ester andsqualene). Hypercholesterolemic activity was reported in five compounds(dodecanoic acid, ethyl ester; tetradecanoic acid, ethyl ester; hexadecanoic acid,ethyl ester; oleic acid and squalene). Anticancer properties were reported in three compounds (tetradecanoic acid, ethyl ester; 9-octadecenoic acid (Z)-, methylester; and squalene). Anti-inflammatory activities were reported in two compounds, namely, 9-octadecenoic acid (Z)-, methyl ester and oleic acid.

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