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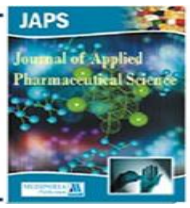

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Short Communication

GC-MS determination of bioactive components of *Canscora perfoliata* Lam. (Gentianaceae)

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ABSTRACT

In the present study, the bioactive components of *Canscora perfoliata* whole plant have been evaluated using GC-MS. The chemical compounds of the ethanol extract of *Canscora perfoliata* Lam. were investigated using Perkin-Elmer Gas chromatography-Mass spectrometry. GC-MS analysis of ethanol extracts of *Canscora perfoliata* revealed the existence of seventeen compounds. This is the first report of identification of active constituents from the whole plant of C*anscora perfoliata* Lam.

Keywords: GC-MS analysis, *Canscora perfoliata* Lam., Squalene.

INTRODUCTION

The use of plants as medicines dates from the earliest years of man's evolution (Dattner 2003). Medicinal plants serve as therapeutic alternatives, safer choices or on some cases, as the only effective treatment. People in different cultures and places have used particular plants to treat certain medicinal problems. A larger number of these plants and their extract have shown beneficial therapeutic effects, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and immunomodulatory effects (Huffman, 2003; Miller *et al*., 2004; Thatte *et al.*, 1992).

Canscora perfoliata Lam. is one of the medicinally important plant belonging to Gentianaceae. The juice prepared from the plant is given to treat any poisonous bites by Palliyar tribals of Grizzled Giant Squirrel Wildlife Sanctuary, Srivilliputhur, Western Ghats, Tamil Nadu (Muthukumarasamy *et al*., 2003). However, perusal of literature reveals that, GC-MS analysis of C*anscora perfoliata* Lam. is totally lacking and hence the present investigation was undertaken. The main objective of the present study is to analyze the various phytochemical constituents found in whole plant of *Canscora perfoliata* Lam.

MATERIALS AND METHODS

Plant material

The well grown and healthy whole plants of *Canscora perfoliata* Lam. were collected from the natural forests of Western Ghats at Thanniparai, Srivilliputhur, Virudhunagar District, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu, for further reference.

Plant sample extraction

Whole plants were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC- MS analysis

GC–MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (330mm x 0.25mm ID x 1µm df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da.

Identification of Components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The 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.

RESULTS AND DISCUSSION

The compounds present in the ethanol extract of *Canscora perfoliata* identified by GC-MS analysis are shown in Fig.1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of C*anscora perfoliata* are presented in Table -1. The prevailing compounds in ethanol extract were Azulene,1,2,3,5,6,7,8,8a- Octahydro-1,4- dimethyl-7- (1-methyl ethenyl)-, [1S-(1à,7à,8aá)]-(28.41%), Urs-12-en-24-oic acid, 3-oxo-,methyl ester, (+)-(8.99%), 6'aBicyclo [4.3.0] nonane, 5á-Odomethyl-1á-isopropenyl-4à,5à-dimethyl-,(8.94%), cedran-diol, 8S,14-(8.69%), Squalene(8.44%), Benzeneethanamine, N-[3-methoxybenzoyl]-3,4-dimethoxy-(7,80%), a-D-Glucopyranoside, methyl (6.77%), and á-Amyrin trimethylsilyl ether (5.45%). Figures 2, 3 and 4 show the mass spectra and structures of á-Amyrin trimethylsilyl ether, Azulene, 1,2,3,5,6,7,8,8a-Octahydro-1,4-dimethyl-7-(1-methylethynyl)-, [1S-(1à,7à,8aá)] and Squalene. Table 2 lists the major phytocomponents and their biological activities obtained through the GC-MS study of *Canscora perfoliata.*

Among the identified phytochemicals, Squalene has anti-oxidant activity. Recently it has been found that Squalene possesses chemo-preventive activity against the colon carcinogenesis (Rao *et al*, 1998). Amyrin is a triterpene which exhibits potential anti-fungal activity (Johann *et al*., 2007). Phytol, a bioactive principle, detected from C*anscora perfoliata* is also found to be effective at different stages of arthritis. It is found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species-promoting substances such as Phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al*., 2009). Squalene and Phytol found in the whole plant ethanol extract of *Canscora perfoliata* which are being used for the pharmacological work.

In the present study, 17 compounds have been identified from the whole plant ethanol extract of C*anscora perfoliata* by Gas Chromatography-Mass Spectrometry (GC-MS analysis). Thus, GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for detailed study in future. Further investigations in the pharmacological importance of *Canscora perfoliata* and their diversity and detailed biochemistry may add new knowledge to the information in the traditional medicinal system.

Table. 1: Components detected in *Canscora perfoliata* whole plant extract.

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	3.87	3,4-Furandiol, tetrahydro-, trans-	$C_4H_8O_3$	104	2.22
2.	11.86	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230	1.66
3.	13.34	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	362	0.42
4.	13.71	á-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	194	6.77
5.	14.58	á-Amyrin trimethylsilyl ether	$C_{33}H_{58}OSi$	498	5.45
6.	15.28	Phytol	$C_{20}H_{40}O$	296	1.39
7.	21.98	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	8.99

8.	22.38	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aá)]-	$C_{15}H_{24}$	204	28.41
9.	22.61	Cedran-diol, 8S,14-	$C_{15}H_{26}O_2$	238	8.69
10.	25.12	Squalene	$C_{30}H_{50}$	410	8.44
11.	26.11	Benzeneethanamine, N-[3-methoxybenzoyl]-3,4-dimethoxy-	$C_{18}H_{21}NO_4$	315	7.80
12.	26.69	Hesperetin	$C_{16}H_{14}O_6$	302	2.81
13.	29.28	1-Docosene	$C_{22}H_{44}$	308	2.21
14.	31.86	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280	0.94
15.	33.23	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	400	2.94
16.	34.57	6áBicyclo[4.3.0]nonane, 5á-iodomethyl-1á-isopropenyl-4à,5à-dimethyl-,	$C_{15}H_{25}I$	332	8.94
17.	35.33	4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl-	$C_{17}H_{30}O$	250	1.91

Table. 2: Activity of phytocomponents identified in the ethanol extract of the whole plant of *Canscora perfoliata*.

No.	RT	Name of the compound	Molecular Formula	Nature of compound	Activity**
1.	3.87	3,4-Furandiol, tetrahydro-, trans-	$C_4H_8O_3$	Furan compound	Antimicrobial
2.	11.86	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	Alcoholic compound	Antimicrobial
3.	13.34	1,2-Benzenedicarboxylic acid, diheptyl ester	$C_{22}H_{34}O_4$	Plasticizer compound	Antimicrobial Anti fouling
4.	13.71	á-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	Sugar moiety	Preservative
5.	14.58	á-Amyrin trimethylsilyl ether	$C_{33}H_{58}OSi$	Triterpene compound	Anticancer Antimicrobial Antiinflammatory
6.	15.28	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial Antiinflammatory Anticancer Diuretic
7.	22.38	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aá)]-	$C_{15}H_{24}$	Sesquiterpene	Antiulcer Antimicrobial Antiallergic Antiinflammatory Antipyretic Antiseptic Anticancer
8.	22.61	Cedran-diol, 8S,14-	$C_{15}H_{26}O_2$	Sesquiterpene alcohol	Antimicrobial Antiinflammatory
9.	25.12	Squalene	$C_{30}H_{50}$	Triterpene	Anticancer Antimicrobial Antioxidant Chemo preventive Pesticide Anti- tumor Sunscreen
10.	26.11	Benzeneethanamine, N-[3-methoxybenzoyl]-3,4-dimethoxy-	$C_{18}H_{21}NO_4$	Amino compound	Antimicrobial
11.	26.69	Hesperetin	$C_{16}H_{14}O_6$	Flavonoid compound	Antimicrobial Antiinflammatory Anticancer Hepatoprotective Anticholesterol Antioxidant
12.	31.86	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	Amino compound	Antimicrobial
13.	33.23	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	Steroid compound	Antimicrobial Antiinflammatory Anticancer Diuretic Antiasthma Antiarthritic
14.	34.57	6áBicyclo[4.3.0]nonane, 5á-iodomethyl-1á-isopropenyl-4à,5à-dimethyl-,	$C_{15}H_{25}I$	Iodine compound	Antimicrobial
15.	35.33	4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl-	$C_{17}H_{30}O$	Alkene compound	Antimicrobial

****Source: Dr. Duke's:** Phytochemical and Ethnobotanical Databases

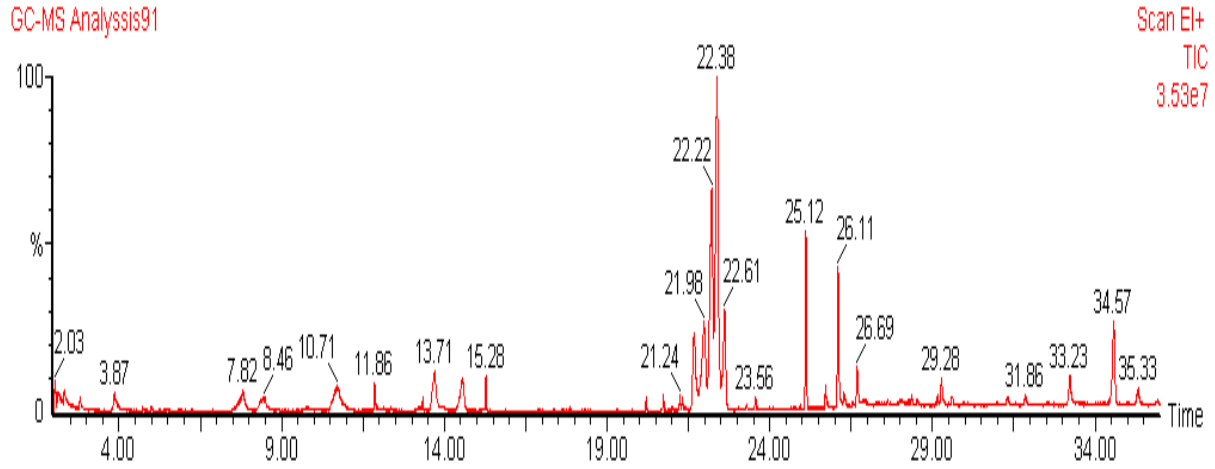

Fig. 1: GC-MS Chromatogram of ethanol extract of *Canscora perfoliata* whole plant.

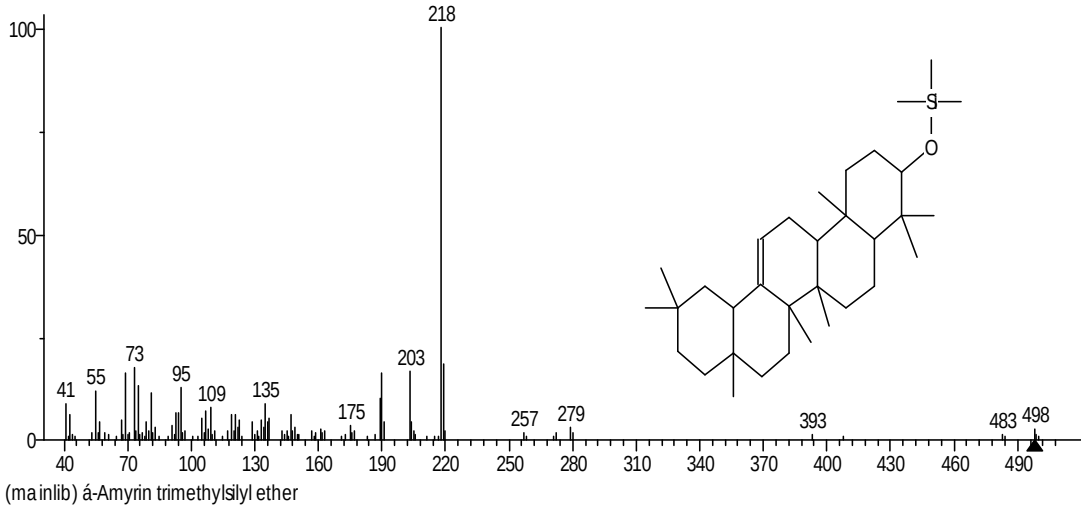

Fig. 2: Mass spectrum of á-Amyrin trimethylsilyl ether

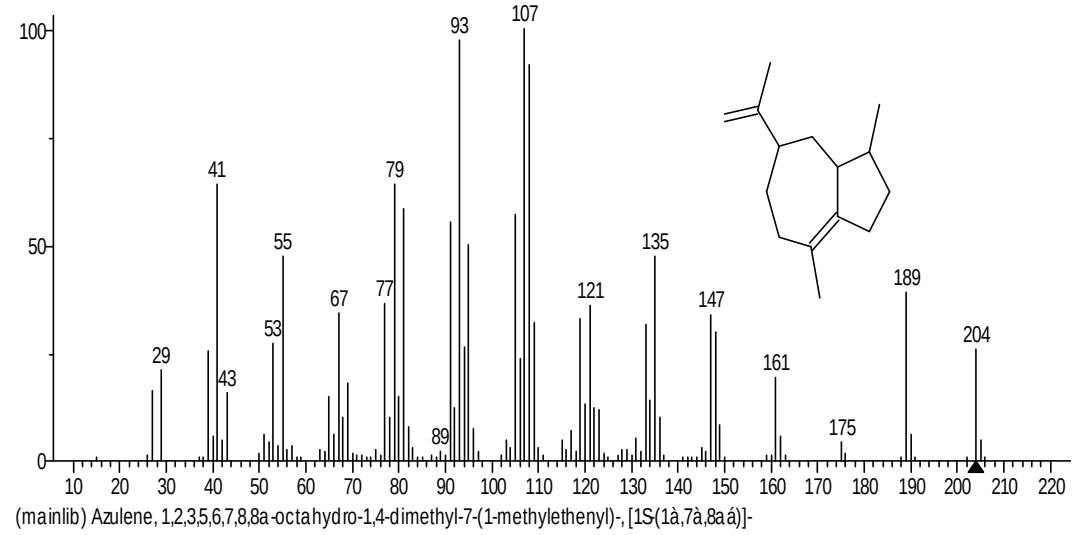

Fig. 3: Mass spectrum of Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1à,7à,8aá)]

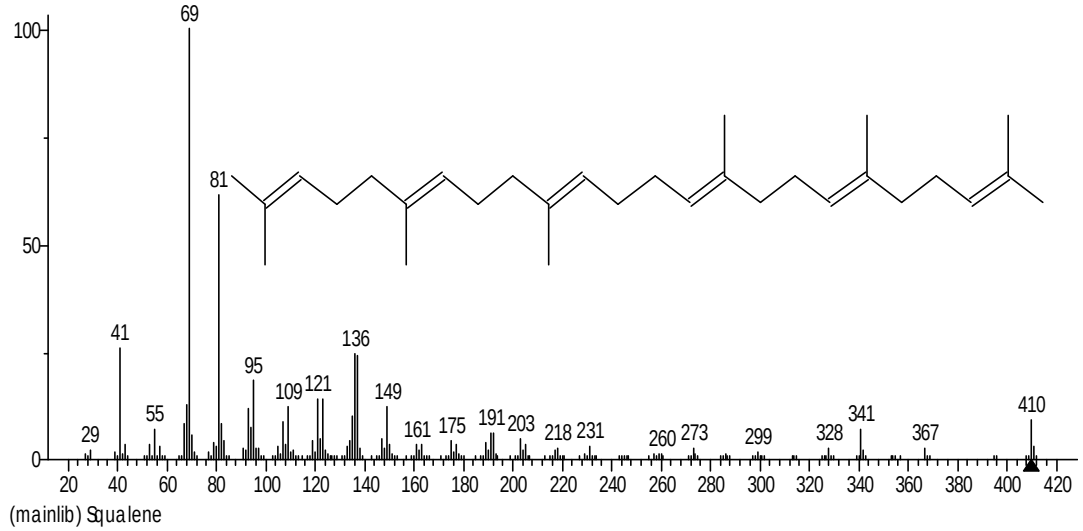

Fig. 4: Mass spectrum of Squalene

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