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Quantitative Identification of Major and Minor Constituents of Aerial Parts of *Mollugo pentaphylla* Linn. Using GC-MS

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Mollugo pentaphylla Linn. (Molluginaceae) possess affluent source of innumerable bioactive components. Analysis of chemical constituents in different fractions of methanolic extract of aerial part of the plant by Perkin-Elmer GC-MS unveiled the presence of paramount amount of stigmasterol, adipose acids, *cis*-vaccenic acid, a diterpine namely phytol were withal detected. Some minor compounds were conjointly found for the first time. This study will be helpful to understand the nature and pharmacological property of the phyto constituents in aerial part of the plant.

Keywords: *Mollugo pentaphylla* Linn., Molluginaceae, GC-MS, Stigmasterol, Phytol.

INTRODUCTION

The civilization has started banking on herbal medicines to maintain a salubrious life, that may possess reduced side effects when used both chronically and in acute cases. *Mollugo pentaphylla* Linn., a carpet wood, perennial herb, is widely distributed in all over India [1]. The herb are erect slender, 25-30 cm high branching with white, green flower, usually grow in most semi-moist place.

Flavones such as apigenin (6-*c*-arabinosyl-8-*c*-xylosyl) and mollupentin, was isolated from dried aerial parts in India [2]. Triterpenoids like mollugogenol-A [α -1-rhamnopyranosyl-(1-4)- β -d-glucopyranosyl-(1-3)], an antifungal triterpenoid and mollugogenol-B, mollugogenol-D, oleanolic acid and a steroid, sitosterol- β was isolated from dried aerial parts of the plant [3-5]. Sterol analysis on dried plant reported to contain steroid [6].

Study revealed that the aqueous extract of aerial part of *Mollugo pentaphylla* Linn. possess potent antioxidant property [1], methanolic extract of plant was reported to have potent anti-diabetic action [7] and antimicrobial activity [3]. However, till date the concrete component responsible solely or in combination for antimicrobial activity was not been identified. Hence, there may be the possibility of presence of number of components responsible for antimicrobial activity for which an investigation was intended to identify the components in the different fractions of the methanol extract of *Mollugo pentaphylla* Linn., that may unveil an in-sight for its antimicrobial property.

EXPERIMENTAL

Fresh matured plant of *Mollugo pentaphylla* Linn. was collected from the local areas of Bhubaneswar, Odisha, India and authenticated by the taxonomist, Dr. A.K. Pradhan, Professor, Department of Botany, PPD Mahavidyalaya, Tigiria, Cuttack, India. The voucher specimen of *Mollugo pentaphylla* Linn. (Regdn. No. SPS/SOAU/2008/005) has been preserved in the institution herbarium of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University for future reference. After thorough authentication, the aerial parts of *Mollugo pentaphylla* Linn. were collected in bulk, cleaned thoroughly with distilled water, followed by shade drying for 12 days. The shade dried materials were coarsely powdered in an electrical grinder and preserved in a nylon bag in a deep freezer, till further use. The powdered material was initially defatted with petroleum ether and subjected to soxhlation for 72 h using methanol. The rotary evaporator was used to concentrate the extract followed by filtration through sintered glass filter. Then different fraction of that extract was prepared by initially solubilizing the extract into water then successive partition was made using *n*-hexane, diethyl ether and ethyl acetate successively one after another in the separating funnel [8]. The different fractions prepared were; *n*-hexane fraction, diethyl ether fraction and ethyl acetate fraction of methanol extract of aerial part of *Mollugo pentaphylla* Linn.

GC-MS analysis was done by using GC Clarus 600 Perkin Elmer system and gas chromatograph interfaced with mass

detector Turbo mass gold-Perkin Elmer (GC-MS) [9]. The following programming condition has been employed.

GC Programmed - Column: TG 5 ms (30 m × 0.25 mm × 0.25 μm) Carrier gas: Helium (99.999 %) with constant flow rate of 1 mL per min, (Split ratio: 1:25), Sample injection volume 2 μL, Software: Turbomass 5.2, Oven operating in electron impact mode at 70 eV, Injection temperature was 280 °C and interface temperature was 290 °C. Oven programmed was initially 50 °C with 3 min of hold time and second step increased at a rate of 10 °C/min to 300 °C at a hold time of 7 min and total GC running time was 36 min.

MS Programmed - Library used NIST Version-Year 2011, Start Mass was 40.00 *m/z* and the End Mass was 650 *m/z* in the scan time of 1 sec. Total MS running time was 36 min.

Interpretation on mass spectrum GC-MS was done by using National Institute Standard and Technology (NIST) database 2011. The spectra of unknown compounds were compared with that of known compounds stored in the NIST library. The name, molecular weight and structure of the components of the test materials were confirmed [9]. Presence of 11 major compounds in *n*-hexane fraction, 12 major compounds in diethyl ether fraction, 9 major compounds in ethyl acetate fraction have been identified.

National Institute of Standards and Technology-11 (NIST-11) library was used to match the mass spectra of the identified

components present in the different fraction of the methanol extracts of the aerial part of *Mollugo pentaphylla* Linn. and the major and minor components has been listed in Table-1.

RESULTS AND DISCUSSION

The major component in *n*-hexane fraction were found to be *cis*-vaccenic acid (27.146 %); hexadecanoic acid, methyl ester (4.547 %); phytol, acetate (3.967 %); hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (3.656 %); octadecanoic acid (3.643 %); 9,12-octadecadienoyl chloride, (*Z,Z*) (2.498 %); 9,12,15-octadecatrienoic acid, methyl ester, (*Z,Z,Z*) (2.077 %); 9,12-octadecadienoic acid, methyl ester (1.58 %); hexadecenoic acid, *Z*-11- (1.035 %); 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.943 %); tetradecanoic acid (0.851 %) (Table-1).

Stigmasterol (37.332 %); phytol (12.652 %); *n*-hexadecanoic acid (7.902 %); (*Z*)6, (*Z*)9-pentadecadien-1-ol (7.902 %); 9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate (5.337 %); vitamin E (4.876 %); spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl (4.789 %); 1-heptatriacotanol (3.685 %); 2-cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)- (3.623 %); benzoic acid (3.423 %); 3-buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)- (2.876 %); 9-octadecenamide, (*Z*)- (2.827 %) were found as major components in diethyl ether fraction (Table-1).

TABLE-1
IDENTIFIED COMPONENTS OF METHANOL EXTRACT OF *Mollugopentaphylla* Linn. BY GAS CHROMATOGRAPH

Fraction	Compound name	Retention time (min)	Retention index	m.f.	m.w.	Peak area (%)
<i>n</i> -Hexane fraction	<i>cis</i> -Vaccenic acid	21.152	2266	C ₁₈ H ₃₄ O ₂	282	27.146
	Hexadecanoic acid, methyl ester	19.092	2045	C ₁₇ H ₃₄ O ₂	270	4.547
	Phytol, acetate	18.361	2003	C ₂₂ H ₄₂ O ₂	338	3.967
	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	23.953	2654	C ₁₉ H ₃₈ O ₄	330	3.656
	Octadecanoic acid	21.332	2277	C ₁₈ H ₃₆ O ₂	284	3.643
	9,12-Octadecadienoyl chloride, (<i>Z,Z</i>)	25.263	2851	C ₁₈ H ₃₁ ClO	298	2.498
	9,12,15-Octadecatrienoic acid, methyl ester, (<i>Z,Z,Z</i>)	20.662	2236	C ₁₉ H ₃₂ O ₂	292	2.077
	9,12-Octadecadienoic acid, methyl ester	20.622	2234	C ₁₉ H ₃₄ O ₂	294	1.58
	Hexadecenoic acid, <i>Z</i> -11-	19.292	2056	C ₁₆ H ₃₀ O ₂	254	1.035
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	18.772	2027	C ₂₀ H ₄₀ O	296	0.943
Tetradecanoic acid	17.501	1916	C ₁₄ H ₂₈ O ₂	228	0.851	
Diethyl ether fraction	Stigmasterol	29.264	3447	C ₂₉ H ₄₈ O	412	37.332
	Phytol	20.852	2248	C ₂₀ H ₄₀ O	296	12.652
	<i>n</i> -Hexadecanoic acid	19.472	2066	C ₁₆ H ₃₂ O ₂	256	7.902
	(<i>Z</i>)6, (<i>Z</i>)9-Pentadecadien-1-ol	21.042	2260	C ₁₅ H ₂₈ O	224	7.478
	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate	30.064	3606	C ₃₂ H ₅₂ O ₂	468	5.337
	Vitamin E	28.243	3254	C ₂₉ H ₅₀ O ₂	430	4.876
	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl	27.863	3233	C ₁₅ H ₂₄ O ₂	236	4.789
	1-Heptatriacotanol	28.603	3274	C ₃₇ H ₇₆ O	536	3.685
	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	17.471	1913	C ₁₃ H ₁₈ O ₃	222	3.623
	Benzoic acid	10.240	1280	C ₇ H ₆ O ₂	122	3.423
3-Buten-2-one, 4-(6,6-dimethyl-1-cyclohexen-1-yl)-	17.171	1882	C ₁₂ H ₁₈ O	178	2.876	
9-Octadecenamide, (<i>Z</i>)-	28.713	3408	C ₁₈ H ₃₅ NO	281	2.827	
Ethyl acetate fraction	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediy ester	29.474	3454	C ₃₅ H ₆₈ O ₅	568	17.277
	2-Dodecen-1-yl(-)succinic anhydride	24.733	2810	C ₁₆ H ₂₆ O ₃	266	9.657
	4-(3-Hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one	17.471	1913	C ₁₃ H ₂₀ O ₃	224	8.217
	1-Monolinoleoylglycerol trimethylsilyl ether	28.243	3214	C ₂₇ H ₅₄ O ₄ Si ₂	498	4.967
	Benzeneacetic acid	11.340	1363	C ₈ H ₈ O ₂	136	4.262
	7,22-Ergostadienol	29.684	3480	C ₂₈ H ₄₆ O	398	3.981
	Benzoic acid, 3-hydroxy-	14.921	1665	C ₇ H ₆ O ₃	138	3.829
	Octadecanal, 2-bromo-	28.703	3270	C ₁₈ H ₃₅ BrO	346	3.585
	5,5,8a-Trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	17.571	1923	C ₁₂ H ₂₀ O	180	3.031

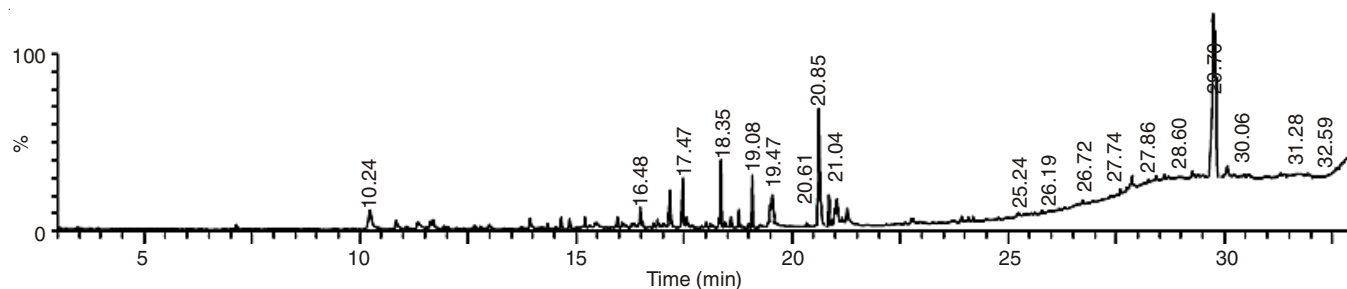


Fig. 1 Gas chromatogram of diethyl ether fraction

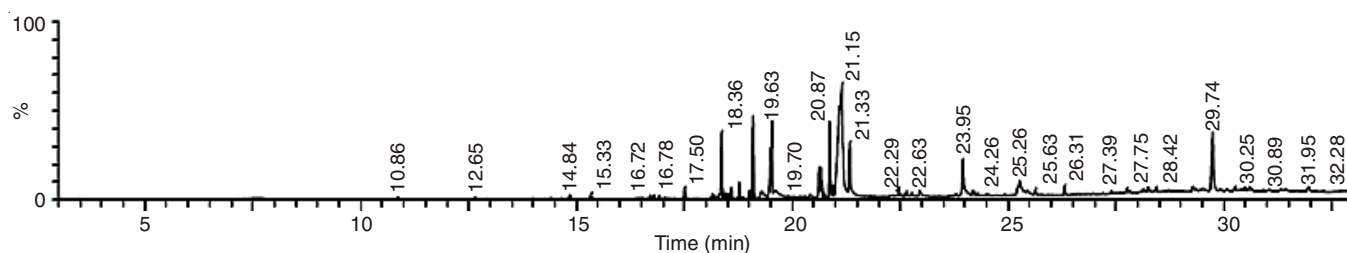
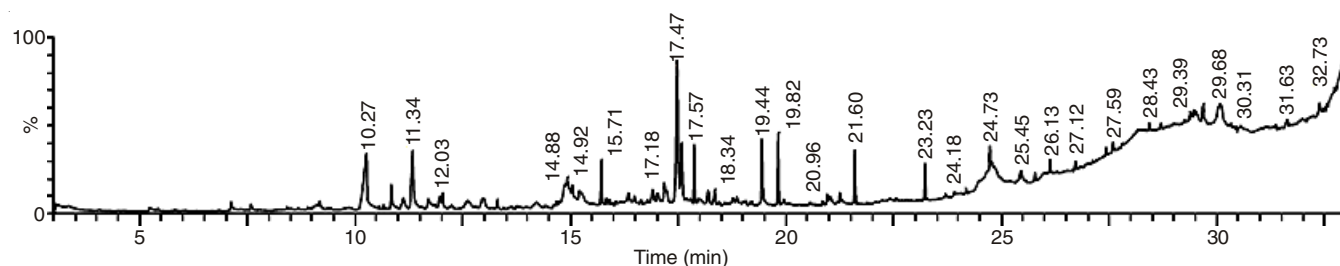
Fig. 2. Gas chromatogram of *n*-hexane fraction

Fig. 3. Gas chromatogram of ethyl acetate fraction

Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (17.277 %); 2-dodecen-1-yl(-)succinic anhydride (9.657 %); 4-(3-hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one (8.217 %); 1-monolinoleoylglycerol trimethylsilyl ether (4.967 %); benzoic acid (4.262 %); 7,22-ergostadienol (3.981 %); benzoic acid, 3-hydroxy- (3.829 %); octadecanal, 2-bromo- (3.585 %); 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2*H*-chromene (3.031 %) were found as the major and minor constituent in the ethyl acetate fraction.

The GC-MS analysis of the different fraction of methanol extract of the aerial part of *Mollugo pentaphylla* Linn. resulted many compounds having diverse use. Stigmasterol (37.332 %) was found as the major component in the extract (Fig. 1) and withal in diethyl ether fraction of methanol extract which is having antifungal/antimicrobial, anticancer, antiasthmatic, antiarthritic, diuretic, anti-inflammatory activity [10]. *cis*-Vaccenic acid, an adipose acid was found as major phyto component in *n*-hexane fraction of methanol extract (Fig. 2). Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, an adipose acid ester was found in ethyl acetate fraction of methanol extract (Fig. 3). A consequential quantity of phytol having antioxidant, antimicrobial, 5- α -reductase inhibitor, hypocholesterolemic, haemolytic, pesticide, antiandrogenic, lubricant activity [11] was additionally found in diethyl ether fraction of methanol extract. As the major compound has pharmacologically active value, the study supports the traditional utilization of aerial part of *Mollugo pentaphylla* Linn. in treating different diseases.

However, an undivided component alone or the concoction of more than one constituents like stigmasterol, *cis*-vaccenic acid, *n*-hexadecanoic acid, phytol, *etc.* found to be present in the aerial plant part may trigger the desired antimicrobial activity that has been distinguished earlier. Hence, there is the prerequisite of extensive study to identify the specific elements responsible for antimicrobial activity in the methanol extract of the aerial part of *Mollugo pentaphylla* Linn.

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