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IDENTIFICATION OF FATTY ACIDS AND FATTY ACID ESTERS FROM ETHYL ACETATE BARK EXTRACT OF HOLOPTELEA INTEGRIFOLIA (ROXB.) PLANCH BY GC-MS

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ABSTRACT

It is an ornamental plant with certain medicinal characteristics due to many valuable and active Phytoconstituents in various parts of the plant. Ethyl acetate extract of the bark was triturated with warm n-hexane and warm n-hexane fractions were pooled in order to get Holoptelea *integrifolia* Bark-Ehyl Acetate-Hexane Soluble (HIB-EA-HS) which was analyzed on GC-MS. The identified fatty acids and fatty acid esters included: octadecanoicacid, methyl ester (1), hexadecanedioic acid,dimethyl ester (2), eicosanoic acid, methylester (3), octadecanedioic acid, dimethyl ester (4), Cis-13-eicosenoic acid (5), docosanoicacid, methyl ester (6), 1,2-benzenedicarboxylicacid, diisooctyl ester (7), methyl- 21-methyldocosanoate (8), and tetracosanoicacid, methyl ester (9). Not any saturated fatty acid was found in the present study. A number of fatty acids and fatty acid esters have been identified from the stem extract of *H. integrifolia* but these fatty acids and fatty acid esters were identified the first time from the bark of the plant.

KEYWORDS: Fatty acids, Fatty acid esters, bark, *Holoptelea integrifolia*, GC-MS.

INTRODUCTION

Nature has been a source of medicinal plants for thousands of years and the use of medicinal plants for relief from illness can be traced back to five thousand years of written documents of early civilization in India, China and the near east. *Holoptelea integrifolia*, vernacular name is Nemali chettu (Telugu) and commonly known as Indian Elm tree. *Holoptelea integrifolia* belongs to the family of *Ulmaceae* and it is widely distributed all over tropical and temperate regions of northern hemisphere including Indian peninsula, China, Burma and Srilanka. The leaves and stem of this plant are used by local people for gastritis, skin diseases, obesity (Bambhole, Jiddewar, 1985), cancer (Graham *et al*., 2000). The fresh material, stem or leaves of plant is applied as paste externally twice or thrice a day for wound healing purpose (Sidhu *et al*., 1999). The bark and leaves of the plant are reported to contain anti-inflammatory, carminative, anthelmintic, depurative, astringent, dyspeptic and antidiabetic properties (Warrier, Nambiar, 1993). The ethanol crude extracts of stem bark of *H. integrifolia* was found anti-oxidant which is comparable to the standard vitamin E (Saraswathy, Devi, Ramasamy, 2008). A number of 1,4-naphthalenedione (a phytochemical of the plant) derivatives have been found to have anti-bacterial activity (Vinod *et al*., 2010). Benzene, chloroform, methanol and aqueous extracts of stem bark of *H. integrifolia* showed anthelmintic activity against adult earth worm *Pheretima posthuma* (Durga, Padmaa, 2010). Fatty acids like 6-Octadecenoic acid, (Z)-, Tridecanoic acid, n- Hexadecanoic acid, Tetradecanoic acid, 9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)- were found in good quantities in most of the extracts of the plant and these components were found to have anti-inflammatory, antimicrobial activities (Allin, Mangamoori, 2014). In our present study *n*-hexane soluble ethyl acetate bark extract of the plant was subjected to GC-MS to identify the new fatty acids and fatty acid esters.

MATERIAL AND METHODS

Plant material and chemicals

The bark of the *Holoptelea integrifolia*plant was collected from the premises of the University of Karachi, Pakistan during January-April 2014 and identified by Prof. Dr. Ghazal Hafeez Rizwani Department of Pharmacognosy, University of Karachi. A herbarium voucher specimen No.0045 was deposited in the museum of the Department of Pharmacognosy at University of Karachi, Pakistan (Guo *et al*., 2013). The purified chemicals and all other reagents used during the research work were commercially purchased from Merck (Germany).

Standardization

Standardization of *Holoptelea integrifolia* (Roxb) Planch was performed using internationally acceptable assays and analysis (Kumar *et al*., 2012; Singh *et al*., 1992) i.e. for pharmacognostic identification microscopic, macroscopic, sensory, and histological examinations were performed. Physiochemical examinations were determined by measuring extractive value, moisture contents and ash value. For microbiological examination total viable count (TVC) test was conducted. While, pre-physiochemical examination were performed using wet test or dipped reagent and spectroscopic analysis (Guo *et al*., 2013).

Fractionation and isolation

Holoptelea integrifolia plant bark (10 kg) were cleaned and then chopped into small pieces. These pieces were percolated in 80% methanol at room temperature for 15 days. The percolate was filtered thrice separately by using Whatman filter paper No.1. Thereafter, under reduced pressure and controlled temperature 40°C, the filtrate was evaporated to dryness and the methanolic extract was lyophilized to a powdered form. Lyophilized powder (300g) was partitioned with an equal quantity of distilled water (450ml) and ethyl acetate (450ml) 1: 1,ethylacetate layer was evaporated under reduced pressure and temperature 40°C to obtain ethylacetate extract (Guo *et al*., 2013). The ethyl acetate extract was triturated with warm *n*-hexane to obtain *n*-hexane soluble fraction (HIB-EA-HS).

GC-MS for identification of the constituents

n-hexane soluble fraction (HIB-EA-HS) was subjected to GC-MS to identify the compounds. For gas chromatography-mass spectrometric detection (GC-MS) a Hewlett Packed 5890 gas chromatograph was combined with a Jeol, JMS-600 mass spectrometer operating in EI mode with ion source at 250°C and electron energy at 70 eV was used. Injector was set at 260°C with splitting ratio 1:30. Mass spectral survey was performed using MS libraries (NIST, 2005).

EIMS was performed on JEOL, the MS route JMS-600H. It is important to mention that the GC-MS and EIMS analyses were performed from an outsource laboratory (H.E.J Research Institute of Chemistry, University of Karachi, Karachi, Pakistan).

RESULTS AND DISCUSSION

The early peaks (1-21) of GC-MS of the HIB-EA-HS (Table-1), consists of free fatty acids and their methyl esters. Fatty acids are commonly occurring important phytochemicals of the

medicinal plants. They are widely occurring in natural fats and dietary oils and they play an important role as nutritious substances and metabolites in living organisms (Cakir, 2004). Many fatty acids are known to have antibacterial and antifungal properties (Russell, 1991). Certain fatty acids such as omega-3 fatty acids are used for the prevention of cardiovascular diseases (Kris-Etherton, Harris, Appel, 2003). It has also been observed that these fatty acids show selective behavior of inhibition for tumor cell growth (Zhu, Su, Li, 1989). These are also found to possess antioxidant, anticancer, and hypotensive properties (Zhao *et al*., 2013). In our present study out of 21 peaks 9 peaks were identified as fatty acids and fatty acid esters. A number of fatty acids and esters have been identified from the ethyl acetate stem extract of *H. integrifolia* (Alli, Mangamoori, 2014) but these 9 compounds are a new addition into them.

Peaks-1, 2, 3, 4, 5, 6, 9, 11, 16, 18, 20, and 21 were not identified. The spectrum of peak-7 (scan 07442) (Table-2) showed molecular peak [M, $C_{19}H_{38}O_2]^+$of medium intensity at *m/z* 298. *α*-Cleavage resulted in a diagnostic methoxy radical loss $[M-31]^+$ generating the acylium fragment ion $[H_3C(CH_2)_{16}CO]^+$ at *m/z* 267. McLafferty rearrangement ion $[H_2C{=}C(OH)OCH_3]^+$ appeared as base peak at *m/z* 74. The series of ions of general formula $[(CH_2)_n COOCH_3]^+$ separated by 14 mass units were also identified. It is typical fragmentation of saturated fatty acid methyl esters. This fragmentation pattern when compared with electronic mass spectral library (NIST, 2005) confirmed the presence of *n*-octadecanoic acid methyl ester **(1)** (Table-3). On the same basis peak-10 (scan 08282), peak-14 (scan 08847), peak-17 (09085) and peak-19 (09305) (Table-2) were identified as *n*-eicosaonic acid methyl ester **(3)**, *n*-docosanoic acid methyl ester **(6)**, Methyl, 21-methyldocosanoate **(8)** and *n*-tetracosanoic acid methyl ester **(9)** respectively (Table-3) (Figure-1).

Fatty acid methyl esters (FAMEs) are usual constituents of plants. These are particularly present in seed and seed oils. For example **1**, **3**, **6** and **9** have been reported as natural constituents in the extracts and essential oils of *Plumbago zeylanica* Linn (Ajayi *et al*., 2011); *Satureja thymbra* and *Satureja cuneifolia* (Goren *et al*., 2003); *Rorippa indica* L (Ananthi, Kumari, 2013); *Carduus pycnocephalus* L (Al-Shammari, Hassan, Al-Youssef, 2012).; *Plastrum Testudinis* (a Chinese traditional medicine) (Wang *et al*., 2012); *Nelumbo nucifera* (Jeon *et al*., 2009), and *Phoenix dactylifera* L (Azmat *et al*., 2010). Compound **1** was found to exhibit inhibitory activity against *Staphylococcus aureus*, *Escherchia coli* and *Candida*

albicans (Mubarakali *et al*., 2012) and compound has been found to possess anti-diabetic properties (Shilpa *et al*., 2009).

Peak-13 (scan 08747) (Table-2) was identified in the present study as a unsaturated fatty acid, *Cis*-13-eicosenoic acid **(5)** (Table-3), it has also been identified as a phytochemical in *Brassica hirta* seeds (Krishnaveni, Saranya, 2016). The unsaturated fatty acids play a vital role in the human body. For example these are responsible for the normal growth of cells, nerves and blood vessels inside a body. These are reported to regulate energy requirement and oxygen transportation in the body and decrease the level of cholesterol in blood (Liu *et al.*, 2009; Igwe, Okwu, 2013) (Figure-2).

For peak-8 (scan 08026) (Table-2) molecular ion peak $[M]^+$ was not observed in GC-MS. Loss of methoxy radical gives medium intensity peak at *m/z* 283 $[M-31]^+$. Fragment ions *m/z* 250 $[M-64]^+$, 241 $[M-73]^+$, 209 $[M-105]^+$ and 191 $(M-123]^+$ were also observed respectively. A second series of abundant ions was also found as *m/z* 98 (84 +14) (base peak), 112 (84 +14x2), 125 (84 + 14x3). McLafferty fragment ion peak was also identified at *m/z* 74. This fragmentation is typical for dimethyl esters of saturated dicarboxylic acids. This fragmentation pattern when compared with electronic mass spectral library (NIST, 2005) confirmed the presence of hexadecanedioic acid methyl ester **(2)** (Table-3). On the same basis peak-12 (scan 08661) was resolved as octadecanedioic acid, dimethyl ester **(4)** (Table-3) (Figure-3).

For peak-15 (scan 08890) (Table-2) molecular ion peak $[M]^+$ was not observed in GC-MS. Loss of isooctyl group $[CH_2=CH-(CH_2)_3-CH(CH_3)_2]$ should give medium intensity peak at *m/z* 278 $[M-112]^+$but it was observed with difference of one hydrogen at *m/z* 279. $[M]^+$. Fragment ions *m/z* 166 $[M-(112x2)]^+$ was also observed at *m/z* 167. The characteristic peak of phthalic acid ester was observed as base peak at *m/z* 149. Other peaks were also observed with difference of one hydrogen at *m/z* 104 and 76. This fragmentation pattern when compared with electronic mass spectral library (NIST, 2005) confirmed the presence of 1, 2-benzenedicarboxylic acid, diisooctyl ester **(7)** (Table-3). Compound **(7)** has also been identified in aerial parts of *Gmelina asiatica Linn* by GC-MS (Merlin *et al*., 2009), and as a bioactive constituents of *Pterocarpus marsupium Roxb* (Maruthupandian, Mohan, 2011). Its antimicrobial and antifouling activity has been reported (Duke, Beckstrom-Sternberg, 1994). It is also characterized by GC-MS with its antimicrobial activity as a alkaloid constituent of *Solanum nigrum* (Jasim *et al*., 2015) (Figure-4).

Fatty acids and their esters are generally obtained in large amount in seed extracts of the plants. In stem and bark extracts they found in small amount. Wound healing property of the stem extract of *H. integrifolia* has been confirmed by the presence of fatty acids and their esters in the extract as antimicrobial agents. In present study nine fatty acids and esters were identified, biological activity of most of them has been reported as a constituent of the extracts of some other plants but properties of some have to be evaluated.

Table 1: Data obtained by GC-MS of the fraction (HIB-EA-HS).

Peak #	Scan #	R.T	M. F	M.Wt	Width	Area	Area Sum	AreaSum %
1	00304	6.309	$C_9H_{15}NO_2$	169	0.049	829125	357689120	0.2318
2	00378	6.859	C_2HBrF_2	142	0.044	658721	357689120	0.1841
3	00577	8.312	$C_3H_4Cl_2O_2$	142	0.045	412871	357689120	0.1154
4	00624	8.669	$C_{13}H_{22}O_2$	210	0.051	215291	357689120	0.0601
5	05931	47.52	$C_{17}H_{24}O_3$	276	0.068	1211148	357689120	0.3386
6	07303	57.558	$C_{14}H_{18}N_2O$	230	0.125	1453850	357689120	0.4064
7	07442	58.57	$C_{19}H_{38}O_2$	298	0.069	1012665	357689120	0.2831
8	08026	62.852	$C_{18}H_{34}O_4$	314	0.064	6981839	357689120	1.9519
9	08225	64.3	$C_{14}H_{16}N_2O_3$	260	0.1	2770558	357689120	0.7745
10	08282	64.726	$C_{21}H_{42}O_2$	326	0.051	2467839	357689120	0.6899
11	08575	66.873	$C_{20}H_{35}O_2$	308	0.041	1959682	357689120	0.5478
12	08661	67.499	$C_{20}H_{38}O_4$	342	0.025	1413484	357689120	0.3951
13	08747	68.128	$C_{20}H_{38}O_2$	310	0.054	3945691	357689120	1.1031
14	08847	68.866	$C_{23}H_{46}O_2$	354	0.048	35935429	357689120	10.0465
15	08890	69.178	$C_{24}H_{38}O_4$	390	0.05	15474151	357689120	4.3261
16	09040	70.277	$C_{16}H_{34}O$	242	0.044	1644295	357689120	0.4596
17	09085	70.605	$C_{24}H_{48}O_2$	368	0.043	1210955	357689120	0.3385
18	09096	70.686	$C_{26}H_{44}O_5$	436	0.044	1254125	357689120	0.3506
19	09305	72.211	$C_{25}H_{50}O_2$	382	0.046	9226477	357689120	2.5794
20	09405	72.95	$C_{22}H_{43}NO$	337	0.043	2506510	357689120	0.7007
21	09969	77.077	$C_{27}H_{40}O_3$	412	0.045	7731039	357689120	2.1613

Table 2: Data obtained by GC-EI-MS of the fraction (HIB-EA-HS).

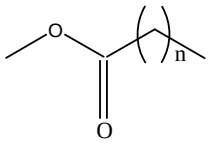
Peak #	Scan #	Fragment ions *m/z*
1	00304	167 (10), 143 (3), 131 (12), 107 (2), 97 (13), 95 (23), 93 (3), 85 (60), 82 (100), 77 (3), 67 (3), 62 (3), 60 (8), 55 (3).
2	00378	206 (3), 179 (10), 142 (100), 128 (3), 121 (3), 106 (10), 93 (3), 83 (50), 72 (18), 66 (4), 60 (4), 55 (4).
3	00577	207 (2), 153 (7), 119 (3), 104 (6), 95 (3), 91 (5), 82 (8), 77 (100), 69 (3), 60 (3), 55 (10), 51 (4).
4	00624	140 (4), 121 (4), 117 (4), 109 (23), 107 (50), 105 (5), 93 (100), 90 (6), 85 (25), 82 (28), 77 (25), 68 (6), 63 (6), 57 (32), 55 (22), 52 (4).
5	05931	276 (20) 261 (21), 232 (25), 217 (53), 205 (100), 189 (52), 175 (50), 135 (37), 91 (42), 77 (28), 57 (80).
6	07303	230 (100) 215 (32), 201 (50), 187 (25), 173 (10), 159 (22), 141 (13), 115 (25), 91 (20), 77 (23), 55 (23).

7	07442	298 (20), 267 (10), 255 (20), 241 (10), 213 (10), 199 (19), 185 (10), 157 (5), 143 (23), 129 (20), 111 (10), 97 (21), 87 (75), 74 (100), 55(30).
8	08026	283 (30), 250 (4), 241 (21), 209 (12),191 (10), 168 (12), 154 (12),125 (12),112 (30), 98 (100), 84 (45), 74 (72), 69 (45), 59 (30), 55 (72).
9	08225	260 (80), 245 (8), 229 (100), 217 (25), 201(21), 185 (21), 172 (17), 159 (19), 115 (30), 77 (20).
10	08282	326 (25), 295 (15) 283 (20), 227 (15), 213 (5), 199 910), 143 (23), 129 (12), 97 (15), 87 (75), 83 (20), 74 (100), 55 (30).
11	08575	308 (20), 276 (40), 261 (8), 207 (12), 175 (12), 165 (18), 123 (21), 109 (25), 95 (40), 81 (60), 69 (65), 55 (100).
12	08661	311(25), 269 (20), 237 (17), 219 (12), 154 (16), 125 (18), 112 (35), 98 (100), 84 (48), 74 (70), 55 (95).
13	08747	290 (5), 262 (3), 248 (5), 234 (3), 220 (5), 207 (9), 192 (6), 166 (9), 150 (11), 136 (17), 125 (25),111 (52), 97(90), 83 (96), 69 (93), 55(100).
14	08847	354 (28), 332 (3), 311 (20), 255 (12), 199 (10), 157 (5), 143 (25), 129 (10), 97 (13), 87 (78), 74 (100), 55 (30).
15	08890	279 (12), 167 (28), 149 (100), 132 (5), 113 (8), 104 (8), 93 (5), 83 (7), 76 (5), 71 (20), 57 (22).
16	09040	281 (10), 267 (5), 259 (12), 242 (8), 199 (8), 185 (8), 139 (10), 125 (28), 111 (48), 97 (95), 83 (92), 69 (92), 57 (100).
17	09085	368 (30), 355 (5), 337 (5), 325 (21), 281 (6), 255 (5), 227 (3), 207 (22), 199 (10), 185 (10), 143 (27), 87 (80), 74 (100), 69 (52).
18	09096	396 (3), 375 (2), 355(3), 327 93), 307(5), 275 (22), 267 (8), 207 (25), 199 (40), 190 (12), 181 (23), 167 (60), 139 (25), 121 (52), 111 (25), 81 (60), 69 (70), 55(100).
19	09305	382 (40), 368(2), 351 (4), 339 (20), 283 (10), 157 (8), 143 (25), 129 (13), 111 (10), 97 (21), 87 (78), 74 (100), 55 (42).
20	09405	337 (5), 320 (4), 294(6), 281 (5), 274 (3), 253 (3), 235(2), 221 (2), 207 (15), 189 (4), 165 (4), 147 (4), 126 (10), 97 (25), 83 (28), 72 (75), 59 (100).
21	09969	488 (2), 428 (2), 394 (100), 379 (4), 355 (2), 327 (2), 309 (2), 275 (23), 253 (21), 223 (3), 207 (30), 177 (20), 135 (80), 119 (52), 105 (47), 91 (47), 81 (53), 69 (48), 55 (53).

Table 3: Fatty acids and Fatty acid esters identified through GC-MS.

Peak #	Scan #	R.T	M. Formula	M. Wt	Name of Compound (number)
1	00304	6.309	$C_9H_{15}NO_2$	169	UN-1
2	00378	6.859	C_2HBrF_2	142	UN-2
3	00577	8.312	$C_3H_4Cl_2O_2$	142	UN-3
4	00624	8.669	$C_{13}H_{22}O_2$	210	UN-4
5	05931	47.52	$C_{17}H_{24}O_3$	276	UN-5
6	07303	57.558	$C_{14}H_{18}N_2O$	230	UN-6
7	07442	58.57	$C_{19}H_{38}O_2$	298	Octadecanoicacid,methyl ester **(1)**
8	08026	62.852	$C_{18}H_{34}O_4$	314	Hexadecanedioic acid,dimethyl ester **(2)**
9	08225	64.3	$C_{14}H_{16}N_2O_3$	260	UN-7
10	08282	64.726	$C_{21}H_{42}O_2$	326	Eicosanoic acid,methylester **(3)**
11	08575	66.873	$C_{20}H_{35}O_2$	308	UN-8
12	08661	67.499	$C_{20}H_{38}O_4$	342	Octadecanedioic acid,dimethyl ester **(4)**

13	08747	68.128	$C_{20}H_{38}O_2$	310	Cis-13-Eicosenoic acid **(5)**
14	08847	68.866	$C_{23}H_{46}O_2$	354	Docosanoicacid,methylester **(6)**
15	08890	69.178	$C_{24}H_{38}O_4$	390	1,2-Benzenedicarboxylicacid, diisooctyl ester**(7)**
16	09040	70.277	$C_{16}H_{34}O$	242	UN-9
17	09085	70.605	$C_{24}H_{48}O_2$	368	Methyl, 21-methyldocosanoate **(8)**
18	09096	70.686	$C_{26}H_{44}O_5$	436	UN-10
19	09305	72.211	$C_{25}H_{50}O_2$	382	Tetracosanoicacid,methyl ester **(9)**
20	09405	72.95	$C_{22}H_{43}NO$	337	UN-11
21	09969	77.077	$C_{27}H_{40}O_3$	412	UN-12

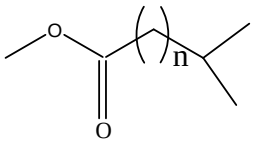


n = 16, *n*-Octadecanoic acid methyl ester **(1)**, $C_{19}H_{38}O_2$

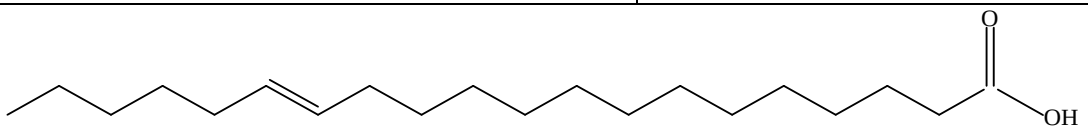
n = 18, *n*-Eicosaonic acid methyl ester **(3)**, $C_{21}H_{42}O_2$

n = 20, *n*-Docosanoic acid methyl ester **(6)**, $C_{23}H_{46}O_2$

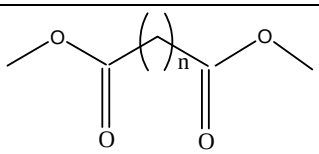
n = 22, *n*-Tetracosanoic acid methyl ester **(9)**, $C_{25}H_{50}O_2$



n = 19, Methyl, 21-methyldocosanoate **(8)**, $C_{24}H_{48}O_2$

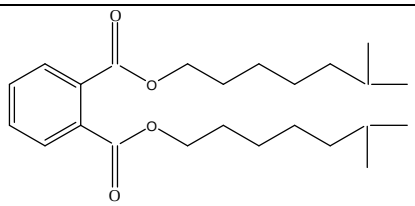


Cis-13-Eicosenoic acid **(5)**, $C_{20}H_{38}O_2$



n = 14, Hexadecanedioic acid, dimethyl ester **(2)**, $C_{18}H_{34}O_4$

n = 16, Octadecanedioic acid, dimethyl ester **(4)**, $C_{20}H_{38}O_4$



1,2-Benzenedicarboxylic acid, diisooctyl ester **(7)**, $C_{24}H_{38}O_4$

Figure 1: Structures of identified fatty acids and fatty acid esters 1- 9.

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