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Electron ionization gas chromatography-mass spectrometry (ei-gc-ms) analysis of extracted oil from *Tribulus terrestris* seeds

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Absract: The fine powdered form *T. terrestris* seeds, was extracted with n-hexane by soxhlet apparatus. The aim of the study was to analyze the *T. terrestris* seed oil (sample-A) by electron ionization Gas Chromatography-Mass Spectrometry (EI-GC-MS) using full scan method within mass range from 40-700 charge to mass ratio (m/z). Out of 102 compounds (1A-102A) 11 compounds (30A, 32A, 37A, 45A, 47A, 48A, 49A, 64A, 83A, 101A and 102A) could not be identified and 91 were identified by classical interpretation of the mass spectrum and by using NIST14 library with match factor ≥ 95 of mass spectrums. While among the 91 identified compounds 18 were found common therefore finally 73 compounds were identified in the present EI-GC-MS analysis of sample-A.

Keywords: *Tribulus terrestris*, GC-MS, seed oil.

INTRODUCTION

Phytomedicine, a substantial class of plants biochemistry. A large number of biochemical are obtained from the virtual part of plant commonly used are, flowers, bark ,roots, seeds, fruits, leaves, etc. Every part of the plant is crucial in its own perspective possessing a wonderful assets of many biological components, although fluctuating some of the medicinal properties (Makari, Haraprasad *et al.,* 2008). *T. terrestris* very frequently called as *Gokshura*, a Sanskrit word which can be define as Puncture wine. In Urdu termed as *Khar-e-khusak khurd.* It is found easily available in India and surrounding region, most commonly found in Gujrat and Rajasthan, bearing all climatic conditions extending throughout the largest part of India.

Tribulus terrestris, a traditional medicinal plant, member of Zygophyllaceae, specializing a dominant root non-woody stem, annually the plant produce flowers, survival of plant is usually more than two years. There are several seeds in each crocus with transverse partitions between them. The seeds are oily in nature. Besides, the traditional use of *T. terrestris* documented that saponins exert antifungal activity by inhibiting fungal hyphae and destroying the ultrastructure of fungi in particular (Zhang, Xu *et al.,* 2006). Insecticidal and repellent activities of methanolic extract of *T. terrestris* seeds (El-Sheikh, Bosly *et al.,* 2012) and anticancer activity of *T. terrestris* seeds extracts on human breast cells (Patel *et al.,* 2019) has also been reported in literature.

In 80's -90's the GC-MS technique was most frequently used to identify steroidal molecules in plant materials (Combaut 1986). To make the steroidal molecules volatile their TMS derivatives have to be prepared and it should be previously determined quantitatively the sample contained steroidal material or not. Later on this technique was found the best to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters, etc. in the given plant extract and Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds. Flavonoids, terpenoids, steroids, alkaloids which have greater molecular mass were difficult to identify by using peak area and retention time (Abirami and Rajendran 2011, Rukshana, Doss *et al.,* 2017). EI-GC-MS is an advance technique which can identify hydrocarbons, alcohols and esters (Hassan, Rasheed *et al.,* 2019) and as well as terpenoids (Hassan, Rasheed *et al.,* 2018), steroids and alkaloids without any derivatization and by classical interpreting the peaks and comparing with NIST14 library with match factor ≥95 of mass spectrums

Nowadays, EI-GC-MS are more efficient parameter that can identified enormous ranges of plant component. EI-GC-MS are widely involved in biopharmaceuticals and medicinal site (Jousse and Pujos-Guillot 2013). The objective of this study is to identify the phytochemical constituents present in the plant extracts and to enlighten their components on the basis of structural interpretation by the authentic technique of EI-GC-MS.

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MATERIALS AND METHODS

Collection of plant material

Seeds of the plant were purchased from the local market of Karachi, Pakistan and were identified by the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi and the voucher of specimen was deposited there.

Extraction of oil sample

T. terrestris seeds (70g) were crushed and ground to fine powder form. The fine powder was subjected to extraction by using n-hexane as solvent through soxhlet apparatus. Several cycles of syphon is processed until oil extraction perfectly completed, usually at 70°C. Finally the solvent was evaporated by rotatory evaporator to leave behind the oil sample (sample-A).

Chemicals

n-hexane used for extraction was purchased from Merck Pharmaceuticals.

Electron ionization Gas Chromatography–Mass Spectrometry (EI-GC-MS) Technique

The sample-A was introduced into Triple Quadrupole that connected with mass selective detector Agilent-HP-5MS (30m, 250um, 0.25um phase thickness) .The oven temperature was raised from 50°C to 180°C at 5°C min^{-1} and constant for 20 min at 350°C then again raised from 18°C to 290°C and remains for 20 min at 290°C. The injector temperature was 250°C with normal injection mode. MSD was operated at EI mode (not fixed, tune setting is used) and full scan data (m/z 40-700) was collected. The flow rate of carrier gas helium was 15.419 $Lmin^{-1}$. Sample (1.5ul) was inserted by auto-sampler. This analysis was carried out in H.E.J Research Institute of Chemistry, University of Karachi, Karachi, Pakistan.

RESULTS

Results obtained by GC-MS chromatograms and EI-GC-MS fragmentation pattern of sample-A are mentioned in fig. 1 and table 1 respectively.

DISCUSSION

When sample-A was analyzed by EI-GC-MS total 102 compounds were obtained. Out of these 102 compounds (1A-102A) 11 compounds (30A, 32A, 37A, 45A, 47A, 48A, 49A, 64A, 83A, 101A and 102A) could not be identified and 91 were identified by classical interpretation of the mass spectrum and by using NIST14 library with match factor ≥ 95 of mass spectrums. Among these 91 identified compounds 18 were found common therefore finally 73 compounds were identified in the present EI-GC-MS analysis of sample-A. Out of these total compounds 32 compounds (which are equivalent to 22 compounds due to repetition of the compounds in the chromatogram) 3A, 7A=13A, 16A, 19A, 24A, 25A, 27A, 31A, 38A, 39A, 40A, 43A, 44A, 52A, 54A,51A=59A=60A=65A=75A, 62A, 66A, 67A, 69A, 71A and 84A=88A=90A=91A=92A=93A (table 1 and fig. 1A, 1B and 1C) were found hydrocarbons (linear and branched)(table 2), Only one 68A (table 1 and fig.1B) was identified as conjugated diene hydrocarbon (table 2)as the fragmentation pattern m/z 41, 55 67, 81, 96, 109, 123, 137, 152, 165, 181, 195, 207,221 and 236 follows the ions formation formula $[C_nH_{2n-3}]^+$ in the lower mass range (m/z 67, 81, 96, 109 123 etc.). 6A, 18A, 36A and 57A (table 1 and fig. 1A and 1B) were identified as *alkyl cyclo-hexane* (table 2) as they have characteristics two peaks m/z =83 and m/z =55 of cyclohexane. Both the peaks are strong and in spectrum one of them is observed as base peak. 9A and 22A (table 1 and fig. 1A) were found as *alkyl cyclo-alkene* (table 2). Fragmentation pattern of 9A (table 1 and fig.1A) m/z =57, 63, 65, 71, 78, 84, 91(base peak), 105 and 120. Base peak m/z appeared due to presence of cyclo-heptadiene cation $[C_7H_7]^+$ and peaks with m/z less than 91have difference not more than 7 which indicates removal of hydrogen atoms during formation of ions but not the cleavage of the ring system with removal of carbon atoms. Peaks with m/z more than 91showed addition of 14(-CH_2-) or presence of ethyl group and therefore 9A is identified as 7-ethyl-1, 3, 5-cycloheptatriene (table 2). Fragmentation pattern of 22A (table 1 and fig.1A) m/z = 41, 55, 67 (base peak), 81, 95, 109, 123 and 138. Peak m/z 109 appeared due to presence of cyclooctene cation $[C_8H_{13}]^+$ and peaks less than m/z 109 have difference of 14 (-CH_2-) indicates breaking of ring and removal of carbon atoms one by one, on the other hand peaks m/z more than 109 showed difference of 15 (-CH_3) or substitution of two methyl groups. 22A was identified as 1, 2-dimethyl cyclooctene (table 2).

Total 14 compounds (which are equivalent to 10 due to repetition in chronogram)1A=2A, 5A, 11A=14A, 12A=17A, 15A, 21A=26A, 23A, 28A, 29A and 35A (table 1 and fig.1A) were identified as alkyl substituted benzene (table 2) since they result in a prominent peak at m/z 105 due to $[C_6H_5\text{-}CH_2CH_2]^+$ and m/z 91due to tropylium ion $[C_7H_7]^+$ formation and m/z 65 due to loss of CH≡CH from tropylium ion $[C_5H_5]^+$. 61A (table 1 and fig.1B) was found biphenyl (table 2) due to characteristics m/z 139 and 113 which were obtained by loss of methyl and ethylene group from one of the benzene ring. 42A, 55A and 63A (table 1 and fig. 1B) were identified as Naphthalene and alkyl substituted Naphthalene (table 2).

Peaks m/z 102, 87 and 75 appeared due to loss of two, three and four carbon atoms from ring B respectively. Peak m/z 113 or 115 may appeared due to loss of single carbon atom from ring B. In 55A peak m/z 126 appeared due to loss of CH_3 group from the molecular peak [142-CH_3]$^+$ similarly in 63A peaks m/z 141 and 128 appeared

due to loss of single CH_3 group and double CH_3 groups from the molecular peak respectively $[156\text{-}CH_3]^+$ and $[156\text{-}2CH_3]^+$. 70A and 72A=74A (table 1 and fig.1B) were identified as fatty alcohol and unsaturated fatty alcohol (table 2) respectively. Loss of water molecule and loss of successive methylene from the carbon chain confirm the presence of fatty alcohol. 4A and 8A=10A, 50A, 53A=56A (table 1 and fig. 1A and 1B) were found as aldehyde and unsaturated aldehydes respectively (table 2).

The base ion peak m/z 44 appeared due to McLafferty rearrangement in the molecule and formation of $[CH_2{=}CH\text{-}OH]^+$ion. 34A (table 1 and fig. 1A) was found as aromatic aldehyde (table 2), m/z 119 which is a characteristics peak of aromatic aldehyde and usually the base peak appeared due to cleavage of CHO group $[M\text{-}CHO]^+$. Only 73A (table 1 and fig.1B) was identified as methyl ketone (table 2). The base peak of m/z 43 was appeared due to formation of ion $[CH_3\text{-}C{\equiv}O]^+$ which showed 73A should be a methyl ketone. 77A, 81A and

Table 1: EI-GC-MS fragmentation pattern of sample-A with peak %

Peak	Compound	m/z (%)	Peak	Compound	m/z (%)
1	1A	106(47),91(100),77921),63(10),51(15)	52	52A	198(1),183(1),113(16),85(13),71(80),57(100)
2	2A	106(47),91(100),77921),63(10),51(15)	53	53A	152(8),123(7),109(1),95(13),81(100),67(25)
3	3A	128(9),85(35),54(100),43(89)	54	54A	184(6),155(1),141(2),127(4)113(5),57(100)
4	4A	114(3),96(23),86(18),70(100),57(66)	55	55A	142(100), 126(3),115(44),102(2),89(9),71(12)
5	5A	120(26),105(100),91(10),77(22)57(77)	56	56A	152(8),123(7),109(1),95(13),81(100),67(25)
6	6A	140(1),126(18),112(3),91(2),83(93)	57	57A	182(3),176(5),159(1),133(6),119(9), 83(100)
7	7A	142(2),113(13),85(9),71(73),57(100)	58	58A	174(1),127(10),99(15),85(26),71(30),57(100)
8	8A	112(3),97(9),83(84),79(9),65(5),57(62)	59	59A	240(1),183(2),155(15),127(10),57(100)
9	9A	120(23),105(5),91(100),78(9),65(14)	60	60A	240(1),183(2),155(15),127(10),57(100)
10	10A	112(3),97(9),83(84),79(9),65(5),57(62)	61	61A	154(100),139(4),128(10),115(11),102(7),89(5)
11	11A	120(20),105(100),91(17),77(18),51(12)	62	62A	198(5),169(1),127(4),113(6),71(60),57(100)
12	12A	120(50),91(14),77(16),65(8),51(10)	63	63A	156(100),141(89),128(21),115(28),105(9)
13	13A	142(2),113(13),85(9),71(73),57(100)	64	64A	208(1),196(9),159(10),138(3),127(10)
14	14A	120(20),105(100),91(17),77(18),51(12)	65	65A	240(1),183(2),155(15),127(10),57(100)
15	15A	120(47),105(100),91(12),77(13),51(9)	66	66A	212(5),183(1),169(1),155(3),141(12),127(4)
16	16A	142(6),128(1),85(25),71(36),57(100)	67	67A	226(3),197(1),183(1),169(2),155(4),127(6)
17	17A	120(50),91(14),77(16),65(8),51(10)	68	68A	236(10),181(1)165(1),110(28),95(47),67(100)
18	18A	152(1),140(20,123(2),111(5),95(6)	69	69A	240(3),197(1),183(2),169(2),155(2),127(6)
19	19A	268(1),98(12),85(42),71(68),57(100)	70	70A	210(2),196(4),181(4),140(8),125(30),69(81)
20	20A	174(1),127(10),99(15),85(26),57(100)	71	71A	254(4),197(3),183(3),169(4),155(3),140(8)
21	21A	134(26),119(5),105(100),91(15),77(16)	72	72A	278(14),193(4),179(4),123(46),95(72),81(100)
22	22A	138(75),134(12),123(11),109(19)	73	73A	250(5),210(2),165(7),124(13)109(20),43(100)
23	23A	134(31),119(100),105(13),91(25)	74	74A	278(14),193(4),179(4),123(46),95(72),81(100)
24	24A	156(2),112(17),71(100),57(70),43(94)	75	75A	240(1),183(2),155(15),127(10),57(100)
25	25A	156(1),112(12),71(61),57(100),43(81)	76	76A	270(8),227(10),199(5),185(6),143(16),74(100)
26	26A	134(26),119(5),105(100),91(15),77(16)	77	77A	256(20),213(3),185(12)171(12),157(15)
27	27A	156(1),126(15),85(39),71(67),57(100)	78	78A	284(9)256(4),241(10),213(8),199(7),185(6)
28	28A	134(26),119(100),91(22),77912),55(12)	79	79A	294(8),263(6),220(2),178(4),109(21),67(100)
29	29A	134(25),119(100),105(11),91(25),77(11)	80	80A	296(4),264(11),222(8),180(6),123(10),55(100)
30	30A	207(1),154(3),139(1),122(1),111(13)	81	81A	280(2),182(5),168(3),150(7),110(19),67(100)
31	31A	156(9),127(2),98(9),85(26),57(100)	82	82A	256(2),239(57),43(100)
32	32A	206(1),154(2),148(29),131(63),119(100)	83	83A	429(1),355(1),297(1),269(6),239(10)
33	33A	137(34),95(66),81(100),67(90)	84	84A	324(1),239(1),197(2),196(4),141(5),113(15)
34	34A	148(28),133(75),119(100),105(55)	85	85A	324(2),306(1),280(1),254(1),239(1)
35	35A	134(43),119(100),105(6),91(91),77(12)	86	86A	280(2),196(4),150(12),110(17),95(43),67(100)
36	36A	168(1),154(15),141(2),111(4)83(100)	87	87A	336(5),293(1),279(2),263(4)
37	37A	207(1),189(1),168(23),148(34),134(40)	88	88A	324(1),239(1),197(2),196(4),141(5),113(15)
38	38A	170(2),126(18),97(11),71(100),43(94)	89	89A	279(12),167(35),149(100)
39	39A	170(1),126(13),97(14),71(64),57(100)	90	90A	324(1),239(1),197(2),196(4),141(5),113(15)
40	40A	170(1),155(1),140(13),85(42),57(100)	91	91A	324(1),239(1),197(2),196(4),141(5),113(15)
41	41A	194(5),164(5),149(31),134(4),119(100)	92	92A	324(1),239(1),197(2),196(4),141(5),113(15)
42	42A	128(100),102(10),63(8),51(9)	93	93A	324(1),239(1),197(2),196(4),141(5),113(15)
43	43A	170(7),141(1),127(3),71(54),57(100)	94	94A	386(12),353(6),327(3),301(11),275(12)
44	44A	184(1),113(8),98(13),71(42),57(100)	95	95A	430(40),205(10),165(100),149(4),121(9)
45	45A	192(2),177(17),166(21),151(7),123(17)	96	96A	412(14),379(3),351(6)314(3),300(10),255(16)
46	46A	152(14),123(13),109(1),95(11),81(51)	97	97A	414(28),354(5),303(18),273(14)
47	47A	206(1),192(1),179(1),162(12),145(14)	98	98A	412(3),341(2),314(40),299(13),281(18)
48	48A	184(1),163(9),152(15),135(1),123(16)	99	99A	428(1),410(7),367(18),288(12),233(5),175(24)
49	49A	192(1),177(4),165(12),140(13),119(10)	100	100A	393(11),365(7),339(6),286(10),231(6),173(16)
50	50A	154(1),110(14),98(20),83(56),55(100)	101	101A	429(1),412(12),370(6),327(7)289(13)
51	51A	240(1),183(2),155(15),127(10),57(100)	102	102A	503(1),429(2),405(2),377(1),341(6)

Table 2: Total Number of Compounds Identified or Not Identified by GC-MS-EI of Sample-A

S. No.	Compound (s)	Compound(s) identified	Reference
1	3A	Nonane	(Aja, Enechi *et al*. 2016)
2	7A, 13A	2,6-dimethyl Octane	(Govindarajan, Cheekala *et al*. 2016)
3	16A	Decane	(Iwara, Igile *et al*. 2017)
4	19A	6-methyl Octadecane	(Vaithiyanathan and Mirunalini 2015)
5	24A	4-methyl Decane	(Zekeya, Chacha *et al*. 2014)
6	25A	2-methyl Decane	(Nadaf, Nasrabadi *et al*. 2012)
7	27A	3-methyl Decane	(Nadaf, Nasrabadi *et al*. 2012)
8	31A	Undecane	(Mervat, Mohamed *et al*. 2018)
9	38A	4-methyl Undecane	(Nadaf, Nasrabadi *et al*. 2012)
10	39A	2-methyl Undecane	(Mervat, Mohamed *et al*. 2018)
11	40A	3-methyl Undecane	(Govindarajan, Cheekala *et al*. 2016)
12	43A	Dodecane	(Mervat, Mohamed *et al*. 2018)
13	44A	2,6-dimethyl Undecane	(Mervat, Mohamed *et al*. 2018)
14	52A	7-methyl Tridecane	(Lim, Mo *et al*. 2018)
15	54A	Tridecane	(Mervat, Mohamed *et al*. 2018)
16	51A, 59A, 60A, 65A, 75A	2,6,10-trimethyl Tetradecane	(Ogunlesi, Okiei *et al*. 2009)
17	62A	Tetradecane	(Nadaf, Nasrabadi *et al*. 2012)
18	66A	Pentadecane	(Govindarajan, Cheekala *et al*. 2016)
19	67A	Hexadecane	(Egbung, Anosike *et al*. 2017)
20	69A	Heptadecane	(Adeyemi, Ekunseitan *et al*. 2017)
21	71A	Octadecane	(Nadaf, Nasrabadi *et al*. 2012)
22	84A, 88A, 90A, 91A, 92A, 93A	Heptacosane	(Dandekar, Fegade *et al*. 2015)
23	68A	3-methyl-Z,Z-4,6-Hexadecadiene	(Chanda, Baravalia *et al*. 2013)
24	6A	n-propyl Cyclohexane	(Morah and Ashipu 2017)
25	18A	n-butly Cyclohexane	(Mervat, Mohamed *et al*. 2018)
26	36A	n-pentyl Cyclohexane	(Nadaf, Nasrabadi *et al*. 2012)
27	57A	n-heptyl Cyclohexane	(Lattuati-Derieux, Egasse *et al*. 2013)
28	9A	7-ethyl-1, 3, 5-Cycloheptatriene	(Thangavelu, Thangavelu *et al*. 2012)
29	22A	1,2-dimethyl Cyclooctene	(Kumar, Shriram *et al*. 2019)
30	1A, 2A	p-Xylene	(Çelik, Aydınlık *et al*. 2011)
31	5A	Isopropyl Benzene	(Aiyelaagbe, Oyewole *et al*. 2010)
32	11A, 14A	1-ethyl-3-methyl Benzene	(Morah and Ashipu 2017)
33	12A, 17A	1,2,3-trimethyl Benzene	(Iwara, Igile *et al*. 2017)
34	15A	1,2,4-trimethyl Benzene	(Govindarajan, Cheekala *et al*. 2016)
35	21A, 26A	1-methyl-3-propyl Benzene	(Govindarajan, Cheekala *et al*. 2016)
36	23A	2-ethyl-1,4-dimethyl Benzene	(Govindarajan, Cheekala *et al*. 2016)
37	28A	1-methyl-4-isopropyl Benzene	(MA, SONG *et al*. 2005)
38	29A	1-methyl-3-isopropyl)	(Beckmann and Lloyd 2001)
39	35A	1,2,4,5-tetramethyl Benzene	(Morah and Ashipu 2017)
40	61A	Biphenyl	(Grob and Voellmin 1970)
41	42A	Naphthalene	(Morah and Ashipu 2017)
42	55A	1-methyl Naphthalene	(Habibi, Masoudi *et al*. 2004)
43	63A	1,3-dimethyl Naphthalene	(Habibi, Masoudi *et al*. 2004)
44	70A	3, 7, 11-trimethyl-1-Dodecanol	(Ogunlesi, Okiei *et al*. 2010)
45	72A, 74A	3,7,11,15-tetramethyl-2-Hexadecenol	(Zekeya, Chacha *et al*. 2014)
46	4A	Heptanal	(Çelik, Aydınlık *et al*. 2011)
47	8A, 10A	2-Heptenal (Z)-	(Nagalakshmi and Murthy 2015)
48	50A	2-Decenal	(Uzun, Dalar *et al*. 2017)
49	53A, 56A	2,4-Decadienal	(Uzun, Dalar *et al*. 2017)
50	34A	p-isopropyl Benzaldehyde	(Hu, Feng *et al*. 2007)
51	73A	6, 10, 14-trimethyl-2-Pentadecanone	(Nadaf, Nasrabadi *et al*. 2012)
52	77A	n-Hexadecanoic acid	(Dandekar, Fegade *et al*. 2015)
53	81A	9,12-Octadecadienoic acid (Z, Z)-	(Adeyemi, Ekunseitan *et al*. 2017)
54	86A	9, 12-Octadecadienoyl chloride (Z,Z)-	(Adeyemi, Ekunseitan *et al*. 2017)
55	76A	Hexadecanoic acid-methyl ester	(Nadaf, Nasrabadi *et al*. 2012)
56	78A	Hexadecanoic acid-ethyl ester	(Nadaf, Nasrabadi *et al*. 2012)
57	80A	9-Octadecenoic acid-methyl ester	(Yue, Shang *et al*. 2017)
58	79A	9,12-Octadecadienoic acid-methyl ester (E,E)	(Zekeya, Chacha *et al*. 2014)
59	87A	Butyl-9, 12-Octadecadienoate	(Acda 2014)
60	85A	4, 8, 12, 16-tetramethyl heptadecan-4-olide	(Batovska, Todorova *et al*. 2010)
61	89A	1, 2-Benzenedicarboxylic acid-diisoocyl ester	(Maruthupandian and Mohan 2011)
62	20A, 58A	4-Hydroxy-4-methylhex-5enoic acid-tert-butyl ester	(Asong, Amoo *et al*. 2019)
63	82A	Hexadecanoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester	(Jananie, Priya *et al*. 2011)

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S. No.	Compound (s)	Compound(s) identified	Reference
64	95A	Vitamin E	(Zekeya *et al*., 2014)
65	99A	Cycloartanol	(Rogowska *et al*., 2019)
66	100A	9, 19-Cyclolanost-24-en-3-ol-acetate (3β)-	(Noikotr *et al*., 2018)
67	96A	Stigmasterol	(Dandekar *et al*., 2015)
68	97A	γ-Sitosterol	(Abu-Lafi *et al*., 2019)
69	94A	Cholestane-3,5-diol-5-acetate	(Parthasarathy and TV 2019)
70	98A	Cholest-5-en-3-ol-24-propylidene (3β)-	(Marimuthu *et al*., 2013)
71	33A	4,5 Epoxy (Trans) Carane	(Chen-xing *et al*., 2014)
72	46A	6, 6-dimethyl-Bicyclo (3.1.1) heptane-2-carboxaldehyde	(Li *et al*., 2006)
73	41A	2, 6, 6-trimethyl-Bicyclo (3.1.1) hept-2-en-4-ol-acetate	(Wu *et al*., 2016)
S.No.	Compounds not identified		
1	30A		
2	32A		
3	37A		
4	45A		
5	47A		
6	48A		
7	49A		
8	64A		
9	83A		
10	101A		
11	102A		

Table 3: Biological activity of some important identified compounds

S. No.	Compound	Compound identified	Biological activity	Reference
1	11A	1-ethyl-3-methyl Benzene	Antimicrobial	(Morah *et al*., 2017)
2	33A	4,5 Epoxy (Trans) Carane	Cytotoxicity, In vitro Antitrypanosomal and Antiplasmodial activity	(Kpoviessi *et al*., 2014)
3	42A	Naphthalene	Antimicrobial	(Rokade and Sayyed, 2009)
4	51A	2,6,10-trimethyl Tetradecane	Antimicrobial and Cytotoxic activity	(Abdel-Hady *et al*., 2016)
5	80A	9-Octadecenoic acid-methyl ester	Antibacterial	(Liu and Tian, 2014)
6	89A	1, 2-Benzenedicarboxylic acid-diisoocyl ester	Analgesic, Antipyretic and Anti-inflammatory	(Santhi *et al*., 2012)
7	95A	Vitamin E	Anioxidant	(Burton and Ingold, 1989)
8	96A	Stigmasterol	Antimicrobial	(Yusuf *et al*., 2018)
9	97A	γ-Sitosterol	Antibacterial and Antifungal	(Burčová *et al*., 2018)
10	99A	Cycloartanol	Antihyperglycemic	(Tanaka *et al*., 2006)

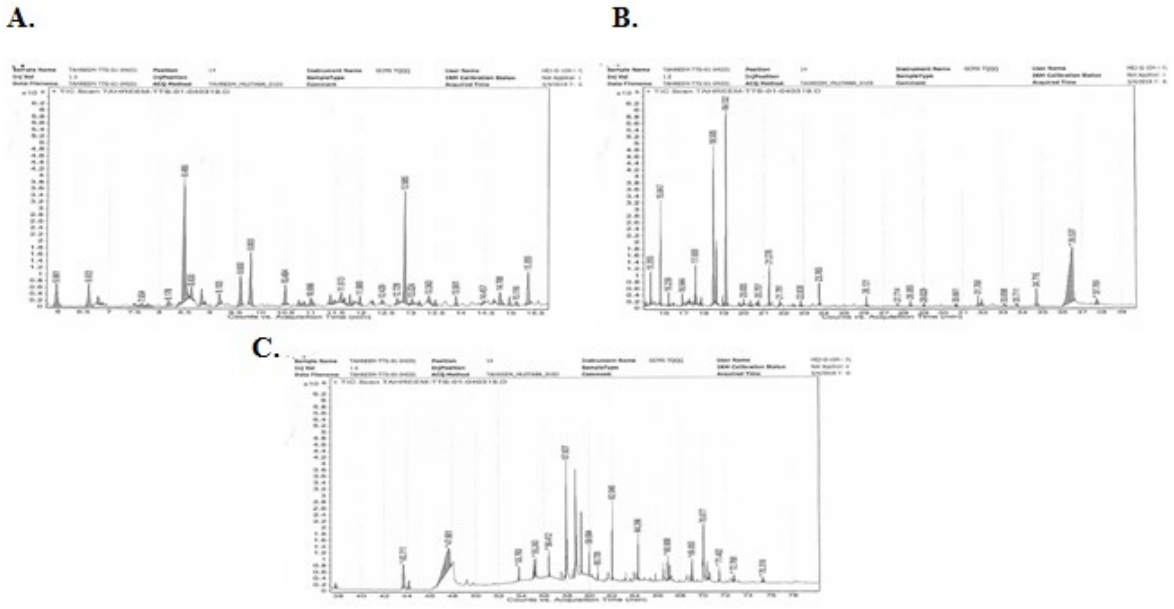

Fig. 1 GC-MS chromatograms (A, B and C) of sample-A

86A (table 1 and fig 1B and 1C) were found as free fatty acid, unsaturated fatty acid and unsaturated fatty acid chloride respectively (table 2). 76A, 78A (table 1 and fig.1B) were found as fatty acid esters (table 2), 79A, 80A and 87A (table 1 and fig. 1C) were found as unsaturated fatty acid esters (table 2), 85A and 89A (table 1 and fig. 1C) were identified as fatty acid cyclo ester and aromatic fatty acid ester respectively (table 2). Ion at m/z 74 and 88 $[CH_2=CH(OR)\text{-}OH]^+$ was appeared by McLafferty rearrangement and a long homologous series of ions m/z 115,129,143,157,171 etc. followed the general formula $[CH_3OCO(CH_2)_n]^+$ confirmed that the spectrums are of methyl ester and ethyl ester respectively. For 85A (table 1 and fig. 1C) ion peak at m/z 99 was found as base peak and was appeared due to formation of methyl lactone ion $[C_5H_7O_2]^+$and ion peaks less than m/z 99 (m/z 81, 69, 55 and 43) were found fragments of the methyl lactone confirmed presence of cyclo ester (table 2). For 89A (table 1 and fig. 1C) the molecular ion peak (m/z 390) was not observed. The first highest ion peak was appeared at m/z 279 due to loss of isooctyl from the molecule $[M\text{-}CH_2=CH\text{-}(CH_2)_3\text{-}CH(CH_3)_2]^+$ or 278 $[M\text{-}112]^+$ with addition of one hydrogen atom. Similarly m/z at 167 $[M\text{-}2(112) +H]^+$. The base peak was observed at m/z 149. 89A was identified as 1, 2-Benzenedicarboxylic acid-diisoocyl ester (table 2). 20A=58A and 82A (table 1 and fig. 1A, 1B and 1C) were found hydroxy and polyhydroxy alcohol fatty acid ester respectively (table 2).95A (table 1 and fig. 1C) was found vitamin E (table 2). 99A and 100A (table 1 and fig. 1C) were found as a cycloartenol (table 2), a triterpenoid of sterol class mostly found in plants. 96A and 97A (table 1 and fig. 1C) were found tetra cyclic triterpenoids (table 2).The higher ion peaks were appeared due to removal of water (due to presence of OH group at ring A) and alkyl groups of the hydrocarbon chain, complete hydrocarbon chain and cleavage of ring D, C and B respectively. 379 $[M\text{-}(H_2O + CH_3)]^+$, 351 $[M\text{-}(H_2O + C_3H_5)]^+$, 314 $[M\text{-}C_7H_{14}]^+$, 300 $[M\text{-}C_8H_{16}]^+$,271 [M-(side chain)-2H]$^+$ or [M-(139)-2H]$^+$, 255 [M-(139)-$H_2O]^+$, 213 [M-(side chain)-(ring D cleavage)-$H_2O]^+$, 173 [M-(side chain)-(ring D cleavage)-(C_3H_4 cleavage from ring C)-$H_2O]^+$, 159 [173-15+H]$^+$, 133 [M-(side chain)-(ring D cleavage)-(ring C cleavage)-$H_2O]^+$. 98A and 94A (table 1 and fig. 1C) were found cholastenol and cholesstane-diol derivatives (table 2) and fragmentation pattern was found very close to 96A and 97A. 33A (table 1 and fig. 1A) was found bi-cyclic monoterpene (table 2). The ion peak at m/z 137 $[M\text{-}CH_3]^+$,is believed to appear due to protonation of the monoterpene ($C_{10}H_{16}$) $[C_{10}H_{17}]^+$.Other ion peaks at m/z 109, 95, 81 and 67 were appeared by regular cleavage of the protonated monoterpene and were found to obey a general formula C_nH_{2n-3} (n = 8, 7, 6, 5). Difference between m/z 152 and 109 (= 43) confirmed presence of epoxy cyclic structure (C_2H_2O+H) of the molecule. 46A (table 1 and fig. 1B) was found a bicyclo-cyclo alkane-carboxaldehyde (table 2). 46A m/z 41, 55, 67 (base peak), 81, 95, 109,123, 137 and 152 (molecular ion). The ion peak at m/z 137 $[M\text{-}CH_3]^+$, showed presence of methyl group on the cyclic system. The second higher ion peak at m/z 123 $[M\text{-}CHO]^+$. Other ion peaks at m/z 109, 95, 81 and 67 were appeared by regular cleavage of the cycloalkane and were found to obey a general formula C_nH_{2n-3} (n = 8, 7, 6, 5). 41A (table 1 and fig. 1A) was found a bicyclo-cyclo alkene-acetate (table 2). 41A m/z 41, 55, 67, 81, 91,109, 119 (base peak), 134, 149, 164 and 194 (molecular ion). The highest ion peaks at m/z 164 $[M\text{-}2CH_3]^+$, 149 $[M\text{-}_3CH_3]^+$, 134 $[M\text{-}CH_3COOH]^+$, 119 [M-(60)-(CH_3)].The other ion peaks at m/z 109, 81 and 67 were appeared by regular cleavage of the cycloalkane and were found to obey a general formula C_nH_{2n-3} (n = 8,6, 5). The ion peak at m/z 91instead of 95 (as in 33A and 46A) was observed due to presence of double bond in the molecule. Along this classical interpretation the identification of compounds were also confirmed by using NIST14 library.

Most of the work on *T. terrestris* fruit extracts have been documented. Work on seed extracts or seed oil in limited. In our present study a number of phytoconstituents have been identified in the sample (e.g. Vitamin E) which have medicinal importance and biological activities and give idea about the use of the seed oil as medicinal agent. In future isolation of the phytochemicals should be carried out in order to more specify the reactivity of the bioactive compounds and then they can be used in pharmaceutical preparations.

CONCLUSION

EI-GC-MS analysis of *Tribulus terrestris* seeds oil makes it clear that the seed oil has medicinal importance since it contains lot of hydrocarbons, fatty acids and fatty acid esters, mono and triterpenes along with vitamin E. Work on biological activity of its phyto-constituents has been reported in the literature as phytochemicals activity of different parts of different plants and their species, which also enhance the importance of the seed oil. Work on new biological activity may also enhance the importance of the seed oil.

REFERENCES

Abdel-Hady H, Abdel-Wareth MAT, El-Wakil EA, & Helmy EA. (2016). Identification and evaluation of antimicrobial and cytotoxic activities of *Penicillium islandicum* and *Aspergillus tamarii* ethyle acetate extracts. *World J. of Pharm. Pharm. Sci.,* **5**: 2021-2039.

Abirami P and Rajendran A (2011). GC-MS Analysis of *Tribulus terrestris. Asian J. Plant Sci. Res.,* **1**(4): 13-6.

Abu-Lafi S, M Rayan, M Masalha, B Abu-Farich, H. Al-Jaas, M Abu-Lafi and A Rayan (2019). Phytochemical composition and biological cctivities of wild *Scolymus maculatus* L. *Medicines,* **6**(2): 53(1-11).

Acda MN (2014). Chemical composition of ethanolic seed extract of *Annona squamosa* L. and *A. muricata* L. (Annonaceae) using GC-MS analysis. *Philipp. Agric. Sci*., **97**(4): 422-426.

Adeyemi MA, Ekunseitan DA, Abiola SS, Dipeolu MA, Egbeyale LT and Sogunle QM (2017). Phytochemical analysis and GC-MS determination of *Lagenaria breviflora* R. fruit. *Int. J. Pharmacogn. Phytochem. Res.,* **9**(7): 1045-1050.

Aiyelaagbe OO, Oyewole AE and Oladosu AI (2010). The chemistry and cytotoxicity effects of essential oils of leaves and flowers of *Albizia lebbeck* (L.) Benth (Fabaceae) growing in Nigeria. *J. Essent. Oil Bear. Plan.*, **13**(5): 644-649.

Aja PM, Enechi OC, Ozougwu VE, Onya-Mmaghiri EA, Agu KA, Ali-Ikechukwu A and Nweke OL (2016). Phytochemical composition, Gas Chromatography-Mass Spectrometric (GC-MS) analysis and anti-bacterial activity of ethanol leaf–extract of *Ageratum conyzoides*. *Afr. J. Basic Appl. Sci.*, **8**(1): 34-40.

Asong JA, Amoo SO, McGaw LJ, Nkadimeng SM, Aremu AO and Otang-Mbeng W (2019). Antimicrobial activity, antioxidant potential,cytotoxicity and phytochemical profiling of four plants locally used against skin diseases. *Plants* **8**(9): 350(1-19).

Batovska DI, Todorova IT, Djakova GJ, Ivanova II and Popov SS (2010). GC-MS analysis of the leaf surface components of four Bulgarian grapevines grown under different conditions. *Nat. Prod. Res.*, **24**(11): 1027-1032.

Beckmann M and Lloyd D (2001). Extraction and identification of volatile organic substances (VOS) from Scottish peat cores. *Atmos. Environ*., **35**(1): 79-86.

Burcova Z, Kreps F, Greifova M, Jablosnky M, Haz A, Schmidt S and Surina I (2018). Antibacterial and antifungal activity of phytosterols and methyl dehydroabietate of Norway spruce bark extracts. *J. Biotechnol.,* **282**: 18-24.

Burton GW and Ingold KU (1989). Vitamin E as an *in Vitro* and *in Vivo* Antioxidant. *Ann. N. Y. Acad. Sci.,* **570**: 7-22.

Çelik A, Aydınlık N and Arslan I (2011). Phytochemical constituents and inhibitory activity towards methicillin resistant *Staphylococcus aureus* strains of *Eryngium* species (Apiaceae). *Chem. Biodivers*., **8**(3): 454-459.

Chanda S, Baravalia Y and Nagani K (2013). Spectral analysis of methanol extract of *Cissus quadrangularis* L. stem and its fractions. *J. Pharmacogn. Phytochem*., **2**(4): 149-159.

Chen-xing Z, Mi Z, Jing H, Ya-fang D and Bao-cai L (2014). Chemical composition and antioxidant activity of the essential oil from the flowers of *Artemisia austro-yunnanensis*. *J.Chem. Pharm. Res.,* **6**(7): 1583-1587.

Combaut G.(1986). GC-MS of plant sterol analysis. Gas Chromatography/Mass Spectrometry. Springer, pp.121-33.

Dandekar R, Fegade B and Bhaskar VH (2015). GC-MS analysis of phytoconstituents in alcohol extract of *Epiphyllum oxypetalum* leaves. *J. Pharmacogn. Phytochem*., **4**(1): 148-157.

Egbung GE, Anosike C, Utu-Baku AB, Ogar I and Nna VU (2017). Phytochemical evaluation and GC-MS analysis of *Hyptis verticillata* cultivated in Calabar Cross River State, Nigeria. *Int. J. Biol. Chem. Sci.*, **11**(5): 2548-2559.

El-Sheikh TM, Bosly HA and Shalaby N (2012). Insecticidal and repellent activities of methanolic extract of *Tribulus terrestris* L.(Zygophyllaceae) against the malarial vector *Anopheles arabiensis* (Diptera: Culicidae). *Egypt. Acad. J. Biol. Sci. A Entomol.,* **5**(2): 13-22.

Govindarajan N, Cheekala UM, Arcot S, Sundaramoorthy S, Duraisamy R and Raju I (2016). GC-MS analysis of n-hexane extract of stem bark of *Symplocos crataegoides* Buch.-Ham. ex D. Don. *Pharmacogn. J.,* **8**(6): 520-524.

Grob K and Voellmin JA (1970). GC-MS Analysis of the" Semi-Volatiles" of Cigarette Smoke. *J.Chromatogr. Sci.,* **8**(4): 218-220.

Habibi Z, Masoudi S and Rustaiyan A (2004). Essential oil of *Nepeta makuensis* Jamzad et Mozaffarian from Iran. *J. Essent. Oil Res*., **16**(3): 214-215.

Hassan A, Rasheed M, Ali M, Hassan S, Nazim U, Ishrat G, Hussain K and Ahmed M (2019). Identification of fatty acids and fatty acid esters from ethyl acetate bark extract of *Holoptelea integrifolia* (Roxb.) Planch by GC-MS. *World J. of Pharm. Pharm. Sci.,* **8**(3): 164-174.

Hassan A, Rasheed M, Ali M, Ishrat G and Ahmed M (2018). Identification of Five New Triterpenoids from Ethylacetate Bark Extract of *Holoptelea integrifolia* (Roxb.) Planch by GC-MS. *Nat. Prod. Chem. Res.,* **6**(5): 338.

Hu LF, Feng JT, Zhang X and Zhang YL (2007). Isolation and structure detection of fungicidal components from *Cuminum cyminum* seed. *Chin. J. Pest. Sci.,* **4**: 330-334.

wara IA, Igile GO, Mboso OE, Mgbeje BI and Ebong PE (2017). Evaluation of phytochemical components from ethyl acetate fraction of *Vernonia calvoana* using gas chromatography-mass spectrometry analysis and its antioxidants activities. *Afr. J. of Pharm. Pharmacol.,* **11**(42): 534-539.

Jananie RK, Priya V and Vijayalakshmi K (2011). Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. *N. Y. Sci. J.,* l**4**(4): 16-20.

Jousse C and Pujos-Guillot E (2013). Exploring metabolome with GC/MS. *Adv. Bot. Res.,* **67**: 303-329.

Kpoviessi S, Bero J, Agbani P, Gbaguidi F, Kpodonou-Kpoviessi B, Sinsin B, Accrombessi G, Frederich M,

Moudachirou M and Quetin-Leclercq J.(2014). Chemical composition, cytotoxicity and *in vitro* antitrypanosomal and antiplasmodial activity of the essential oils of four *Cymbopogon* species from Benin. *J. Ethnopharmacol.,* **151**: 652-659.

Kumar V, Shriram V, Bhagat R, Khare T, Kapse S and Kadoo N (2019). Phytochemical profile, anti-oxidant, anti-inflammatory and anti-proliferative activities of *Pogostemon deccanensis* essential oils. *3 Biotech.,* **9**(1): 31.

Lattuati-Derieux A, Egasse C, Thao-Heu S, Balcar N, Barabant G and Lavédrine B (2013). What do plastics emit? HS-SPME-GC/MS analyses of new standard plastics and plastic objects in museum collections. *J.Cult. Herit.*, **14**(3): 238-247.

Li XR, Lian YZ and Guo FQ (2006). Analysis of volatile oil in rhizoma ligustici chuanxiong-radix paeoniae rubra by gas chromatography-mass spectrometry and chemometric resolution. *Acta Pharmacol. Sin.*, **27**(4): 491-498.

Lim DK, Mo C, Lee DK, Long NP, Lim J and Kwon SW (2018). Non-destructive profiling of volatile organic compounds using HS-SPME/GC-MS and its application for the geographical discrimination of white rice. *J. Food Drug Anal.,* **26**(1): 260-267.

Liu ZX and Tian Q (2014). Analysis of chemical components of volatile oil from *Paris polyphylla* and their antibacterial activities. Zhong yao cai Zhongyaocai. *Journal of Chinese medicinal materials,* **37**(4):612-6.

MA X, PS SONG JR ZHU JB ZHAO and BC ZHANG (2005). Analysis of volatile oil from Radix Angelicae Pubescentis and Radix Heraclei in Gansu by GC-MS. *The Chinese Journal of Modern Applied Pharmacy,* **1**: 44-46.

Makari HK, Haraprasad N, Patil HS and Ravikumar S (2008). *In vitro* antioxidant activity of the hexane and methanolic extracts of *Cordia wallichii* and *Celastrus paniculata*. *The internet Journal Aesthetic and Antiaging medicine* **1**: 1-10.

Maruthupandian A and Mohan VR (2013). Phytochemical screening studies on *Melia orientalis* by GC-MS analysis. *Pharmacognosy Res.*, **5**(3): 216-218.

Maruthupandian, A. and V Mohan (2011). GC-MS analysis of some bioactive constituents of *Pterocarpus marsupium* Roxb. *Int. J. Chem Tech Res*., **3**(3): 1652-1657.

Mervat EH, Mohamed AA, Salem MZ, El-Kareem MS and Ali HM (2018). Chemical composition, antioxidant capacity and antibacterial activity against some potato bacterial pathogens of fruit extracts from *Phytolacca dioica* and *Ziziphus spina-christi* grown in Egypt. *Sci. Hortic*., **233**: 225-232.

Morah FN and Ashipu LB (2017). Chemical composition and antimicrobial activity of essential oil from *Heinsia crinita* leaf. *Am. J. Essent. Oil Nat. Prod*., **5**(2): 23-28.

Morah FN, Emehige EP and Mowang MM (2017). Chemical composition and antimicrobial activity of *Nauclea latifolia* leaf essential oil. *Int. J. Chem. Biochem. Sci.,* **11**: 44-50.

Nadaf M, Nasrabadi M and Halimi M (2012). GC-MS analysis of n-hexane extract from aerial parts of *Salvia nemorosa*. *Middle-East J. Sci. Res*., **11**(8): 1127-1130.

Nagalakshmi MA and Murthy KS (2015). Phytochemical profile of crude seed oil of *Wrightia tinctoria* R. BR. and *Wrightia arborea* (DENNST.) MABB. by GC-MS. *Int. J. Pharm. Sci. Rev. Res.*, **31**(2): 46-51.

Nan P, Peng S, Zhang Y and Zhong Y (2002). Composition of volatile oil of *Primula obconica* in Central China. *Nat. Prod. Lett*., **16**(4): 249-253.

Noikotra K, Chaveeracha A, Sudmoona R, Taneea T and Patarapadungkita N (2018). Phytochemicals, cytotoxicity, and genotoxicity of three *Artocarpus* species reveal arbutin in *A. lacucha. Sci. Asia,* **44**(3): 170-178.

Ogunlesi M, Okiei W, Ofor E and Osibote AE (2009). Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn (Euphorbiaceae), a potential medication for asthma. *Afr. J. Biotechnol.,* **8**(24): 7042-7050.

Ogunlesi M, Okiei W and Osibote EA (2010). Analysis of the essential oil from the leaves of *Sesamum radiatum*, a potential medication for male infertility factor, by gas chromatography-mass spectrometry. *Afr. J. Biotechnol*., **9**(7): 1060-1067.

Parthasarathy V and TV AK (2019). Screening of potential GCMS derived antimigraine compound from the leaves of *Abrus precatorius* Linn to target "calcitonin gene related peptide receptor using in silico analysis. *Food Science and Human Wellness* **8**(1): 34-39.

Patel A, Soni A, Siddiqi NJ and Sharma P (2019). An insight into the anticancer mechanism of Tribulus terrestris extracts on human breast cancer cells. *3 Biotech.,* **9**(58): 1-10.

Rogowska A, Styczyński M, Pączkowski C, Szakiel A and de Carvalho MÂ (2019). GC-MS analysis of steroids and triterpenoids occurring in leaves and tubers of *Tamus edulis* Lowe. *Phytochem. Lett*., **30**: 231-234.

Rokade Y and Sayyed R (2009). Naphthalene derivatives: A new range of antimicrobials with high therapeutic value. *Rasayan J. Chem.,* **2**(4): 972-80.

Rukshana M, Doss A and Kumari P (2017). Phytochemical screening and GC-MS analysis of leaf extract of *Pergularia daemia* (Forssk) Chiov. *Asian J. of Plant Sci.Res.,***7**(1): 9-15.

Santhi V, Sivakumar V, Thilaga R and Thangathirupathi A (2012). Analgesic, antipyretic and anti inflammatory activities of column fraction of Babylonia zeylanica (Bruguiere, 1789) in albino rats. *Int. J. Pharm.Biolo.l Sci.,* **2**(3):151-159.

Suárez HC, Cuéllar Cuéllar A and Pita RC (2011). Characterization of cycloartanol triterpene isolated from dichloromethane extract of *Pedilanthus tithymaloides*. *Rev. Cuba. de Plantas Medicinales* **16**(4): 347-353.

Tanaka M, Misawa E, Ito Y, Habara N, Nomaguchi K, Yamada M, Toida T, Hayasawa H, Takase M and Inagaki M (2006). Identification of five phytosterols from Aloe vera gel as anti-diabetic compounds. *Biol. Pharm. Bull.,* **29**: 1418-1422.

Thangavelu D, Thangavelu T, Vembu T, Venkatachalam K, Selladurai E and Jegadeesan M (2012). Pharmacognostic and phytochemical studies on *Gymnema sylvestre* R. Br. Hairy variant. *Int. J. Pharm. Phytopharmacol. Res*., **2**(3): 143-147.

Uzun Y, Dalar A and Konczak I (2017). *Sempervivum davisii*: phytochemical composition, antioxidant and lipase-inhibitory activities. *Pharm. Biol*., **55**(1): 532-540.

Vaithiyanathan V and Mirunalini S (2015). Quantitative variation of bioactive phyto compounds in ethyl acetate and methanol extracts of *Pergularia daemia* (Forsk.) Chiov. *J. Biomed. Res.*, **29**(2): 169-172.

Wu Y, Yan Z, Zhao J, Zhu Y. (2016). Headspace solid-phase microextraction and gas chromatography–mass spectrometry of volatile components of *Chrysanthemum morifolium* Ramat. *Trop. J. Pharm. Res*., **15**(10): 2241-2244.

Yue XF, Shang X, Zhang ZJ and Zhang YN (2017). Phytochemical composition and antibacterial activity of the essential oils from different parts of sea buckthorn (*Hippophae rhamnoides* L.). *J. Food Drug Anal.*, **25**(2): 327-332.

Yusuf AJ, Abdullahi MI, Aleku GA, Ibrahim IA, Alebiousi CO, Yahaya M, Adamu HW, Sanusi A, Mailafiya MM and Abubakar H (2018). Antimicrobial activity of stigmasterol from the stem bark of *Neocarya macrophylla*. *Journal of Medicinal Plants for Economic Development (JOMPED)*, **2**(1): 1-5.

Zekeya N, Chacha M, Shahada F and Kidukuli A (2014). Analysis of phytochemical composition of *Bersama abyssinica* by gas chromatography-mass spectrometry. *J. Pharmacogn. Phytochem*., **3**(4): 246-252.

Zhang JD, XU Z, Cao YB, Chen HS, Yan L, An MM, Goa PH, Wang Y, Jia XM and Jiang YY (2006). Antifungal activities and action mechanisms of compounds from *Tribulus terrestris* L. *J. Ethnopharmacol.,*1**03**(1): 76-84.