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## GC-MS ANALYSIS AND *IN VITRO* ANTILEISHMANIAL ACTIVITY OF ETHYLACETATE FRACTION OF *ALLIUM CEPA* (EAFAC)

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### Keywords:

GC-MS, Ethyl acetate fraction of *Allium cepa* (EAFAC) (band one), Leishmania major, Pentamidine, Amphotericin B

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**ABSTRACT:** In this study, GC-MS analysis shown that ethyl acetate fraction of *Allium cepa* (EAFAC) (band one) consists of 55 compounds which shown antileishmanial activity. (IC<sub>50</sub> 73.1 ± 2.1 ug/ml) is the inhibitory concentration which is observed for ethyl acetate fraction of *Allium cepa* (EAFAC) (band one). Leishmania major is the specie used for this leishmanicidal assay. In this bioassay, Amphotericin B (IC<sub>50</sub> = 0.29 ± 0.05 ug/ml) and pentamidine (IC<sub>50</sub> = 5.09 ± 0.09 ug/ml) were used as positive control drugs to compare the parasite inhibition with that ethyl acetate fraction of *Allium cepa* (EAFAC) (band one). This activity was perceived under incubation period 72 hours and incubation temperature 22°C. Methanol extract of *Allium cepa* (MEAC), Hexane fraction of *Allium cepa* (HFAC), Ether fraction of *Allium cepa* (EFAC), and Diethyl ether fraction of *Allium cepa* (DEFAC) and butanol fraction of *Allium cepa* (BFAC) exhibited no antileishmanial activities. In this research paper we represent 25 compounds out of 55 compounds of ethyl acetate fraction of *Allium cepa* (EAFAC) (band one).

**INTRODUCTION:** The medicinal values of natural products are attaining considerable significance with the perspective of grave problem created by side effects and drug resistance or multi-drug resistance of chemically originated drugs<sup>1</sup>. The family of *Allium* consists of more than 600 members which are present in Asia, North Africa, Northern America and Europe, nutraceutically, phytochemically and biochemically are closely similar but differing in color, form and taste. All the members of this family are used as medicine, vegetables, spices and ornamentals<sup>2,3</sup>.

*Allium cepa* with its complex nutritional composition is the dietary source of flavonoids in different part of the world. This medicinal plant is rich source of biologically active ingredients such as sulphur compounds, flavonoids, quercetin, fructooligosaccharides, quercetin derivatives, and kaempferols<sup>4</sup>. The phytochemicals which are present in this ethnomedicinal plant extract and fractions, have exhibited biological activities for instance antihelmintic, skin diseases, diuretic, antispasmodic, expectorant, anti-infective, carminative, antibacterial, anti-inflammatory, antifungal and antioxidant activities<sup>5-9</sup>.

The parasitic infection, leishmaniasis, is instigated by leishmania which is a protozoan, belong to Trypanosomatidae family. Cutaneous, diffuse cutaneous, mucosal and visceral leishmaniasis are the four clinically described forms of this vector transmitted disease<sup>10,11</sup>.

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<p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.9(4).1600-08">http://dx.doi.org/10.13040/IJPSR.0975-8232.9(4).1600-08</a></p>	

The thirty different vectors, phlebotomine, also known as sandflies which transmit more than twenty species of leishmania from one host to another<sup>12</sup>. The parasite of leishmaniasis, leishmania, is intracellular hemoflagellate which infects macrophages in the skin and then transfers to visceral organs and leads to visceral leishmaniasis, also known as kala-azar. The choice of drug for this communicable disease is pentavalent antimonies for instance glucantime in spite of renal and cardiac toxic effects<sup>13-15</sup>.

## MATERIAL AND METHODS:

**Plant Material:** Fresh *Allium cepa* were collected from local market of Quetta City, Balochistan, Pakistan.

### Extraction and Fractionation of *Allium cepa*:

This research of extraction and fractionation was completed in the Institute of Biochemistry University of Balochistan Quetta. 5 kg *Allium cepa* were soaked into extraction containers having methanol<sup>16</sup>. These containers containing soaked *Allium cepa* set aside for the duration of six days. Throughout six days container was shaken two times in 24 hours. After six days, the solvent such as methyl alcohol containing compounds extracted from *Allium cepa* was filtered with the help of suction filtration. The filtered methyl alcohol containing *Allium cepa* extract was with the help of rotary evaporator vaporized. Semisolid crude methanolic extract was removed and was 165.42 gm<sup>17,18</sup>.

**Formation of Fractions:** The main extract due to methyl alcohol has been separated for instance 2 portions. 1 portion (1 gm) has been screened for leishmanicidal effect whereas 2 portions (164.42 gm) relocated in the separatory funnel for the formation of different fractions with the help of solvents such as water, n-hexane, ether, diethyl ether, ethyl acetate and butanol.

In a separatory funnel with 164.42 gm extract two solvents such as water and n-hexane have been added. With thorough shaking two layers have been created n-hexane layer and water layer. Both layers have been alienated, though; water layer has been three times extracted with n-hexane. n-hexane was with the help of rotary evaporator vaporized. Semisolid n-hexane fraction was removed and

further fractionated with ether and diethyl ether resulting in ether, diethyl ether fractions.

**Fractionation of n-hexane Fraction:** n-hexane has been separated for instance 2 portions. 1 portion has been screened for leishmanicidal effect whereas 2 portion relocated in the separatory funnel for the formation of different fractions with the help of solvents such as ether and diethyl ether resulting in ether and diethyl ether fractions.

**Formation of Ether Fraction:** In a separatory funnel with n-hexane extract two solvents such as n-hexane and ether have been added. With thorough shaking two layers have been created n-hexane layer and ether layer. Both layers have been alienated, though; n-hexane layer has been three times extracted with ether. Ether was with the help of rotary evaporator vaporized. Semisolid ether fraction was removed and screened for leishmanicidal activity.

**Formation of Diethyl Ether Fraction:** In a separatory funnel with n-hexane extract two solvents such as n-hexane and diethyl ether have been added. With thorough shaking two layers have been created n-hexane layer and diethyl ether layer. Both layers have been alienated, though; n-hexane layer has been three times extracted with diethyl ether. Diethyl ether was with the help of rotary evaporator vaporized. Semisolid diethyl ether fraction was removed and screened for leishmanicidal activity.

**Fractionation of Aqueous Fraction:** Aqueous fraction is further fractionated into two fractions such as ethyl acetate and butanol fractions.

**Formation of Ethylacetate Fraction:** In a separatory funnel with aqueous extract two solvents such as water and ethyl acetate have been added. With thorough shaking two layers have been created, aqueous layer and ethyl acetate layer. Both layers have been alienated, though; aqueous layer has been three times extracted with ethyl acetate. Ethyl acetate was with the help of rotary evaporator vaporized. Semisolid ethyl acetate fraction was removed and screened for leishmanicidal activity.

**Preparatory TLC of Ethylacetate Fraction:** With the help of preparatory TLC, ethyl acetate fraction was further separated into two bands such as ethyl

acetate band one and ethyl acetate band two. This preparatory TLC was carried with the help of mobile phase methanol: chloroform (5:95).

**Formation of Butanol Fraction:** In a separatory funnel with aqueous extract two solvents such as water and butanol have been added. With thorough shaking two layers have been created, aqueous layer and butanol layer. Both layers have been alienated, though; aqueous layer has been three times extracted with butanol. Butanol was with the help of rotary evaporator vaporized. Semisolid butanol fraction was removed and screened for leishmanicidal activity. At the end of the process, different extract / fractions *i.e.*, methanolic crude extract, n-hexane, ether, diethyl ether, butanol, ethyl acetate band one and ethylacetate band two fractions were prepared for antileishmanial activities.

**Antileishmanial Bioassay:** Leishmanial promastigotes was aseptically sedimented down at 3000 rpm for 10 min, counted with the help of improved Neubaur chamber under the microscope and diluted with the fresh medium to a final concentration of  $1 \times 10^6$  parasites. In a 96 well microtiter plate, 180  $\mu$ l of the parasite culture ( $1 \times 10^6$  parasites/ml) was added in different wells in which 20  $\mu$ l of the experimental compound was added in culture and serially diluted so that minimum concentration of the compound was 1  $\mu$ g/ml. Negative control received medium with a parasite density  $1 \times 10^6$  cells/ml. The positive control contained varying concentration of standard antileishmanial compound such as Amphotericin B, Pentamidine. The plate was incubated between 21-22 °C for 72 hrs. The culture was examined microscopically on an improved Neubaur chamber and IC<sub>50</sub> values of compound possessing antileishmanial activity were counted<sup>19</sup>.

**Gas Chromatography Mass Spectrometry (GC-MS) Analysis Triple Quadrupole Acquisition Method-MS Parameters:**

**For Identification and Quantification of *Allium cepa* Compounds:** 2  $\mu$ l of *Allium cepa* extract or fraction was directly injected into the gas chromatograph mod. 6890 N Network GC System (Agilent Technologies “Palo Alto, CA”) together in the presence of mass spectrometer mod. “5973 Network Mass Selective Detector” (Agilent

Technologies “Palo Alto, CA”) and furnished in the presence of “a column HP-5MS (30 m length, 0.25 mm interior diameter, 0.25  $\mu$ m film width” Agilent Technologies, “Palo Alto, CA”). Helium gas was off. Injection was made into a split-splitless injector (split ratio 30:1) at 250 °C. The oven program was the following: “70 °C for 3 min then 6 °C /min to 180 for 5 min, then 6 °C/min to 280 °C for 10 min, then 8 °C /min to 290 °C for 20 min”. The MSD transfer line was set at a temperature of 250 °C; MSD temperature quadrupole was of 150 °C and ionization temperature was 230 °C, Mass spectra were seventy electrovolts and scan achievement was accomplished in the series between thirty five and 300 m/z. The identification of the components of the *Allium cepa* extract or fraction was assigned by matching their “mass spectra with those available in the libraries NIST 02 and WILEY”<sup>20</sup>.

**RESULTS:**

**Antileishmanial Activity of *Allium cepa* Fractions:** Antileishmanial activity of *Allium cepa* extract and fractions was performed against *Leishmania major*. Effective antileishmanial activity was observed for ethyl acetate fraction of *Allium cepa* (EAFAC) while other extract and fractions such as Methanol extract of *Allium cepa* (MEAC), Hexane fraction of *Allium cepa* (HFAC), Ether fraction of *Allium cepa* (EFAC), Diethyl ether fraction of *Allium cepa* (DEFAC) and butanol fraction of *Allium cepa* (BFAC) exhibited no antileishmanial activities. Inhibitory concentration for ethyl acetate fraction of *Allium cepa* (EAFAC) observed (IC<sub>50</sub> = 73.1  $\pm$  2.1  $\mu$ g/ml). In this bioassay, Amphotericin B (IC<sub>50</sub> = 0.29  $\pm$  0.05  $\mu$ g/ml) and pentamidine (IC<sub>50</sub> = 5.09  $\pm$  0.09  $\mu$ g/ml) were used as positive control drugs to compare the parasite inhibition with that by the *Allium cepa* extract and fractions. This activity was perceived under Incubation period 72 h and Incubation Temperature 22 °C.

**TABLE 1: ANTILEISHMANIAL ACTIVITIES OF EXTRACTS AND FRACTIONS OF *ALLIUM CEPA***

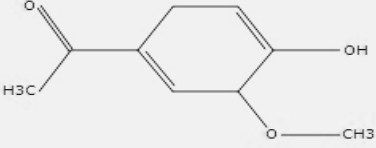
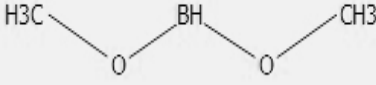
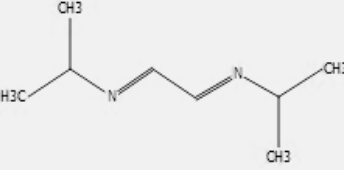
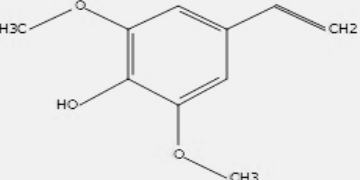
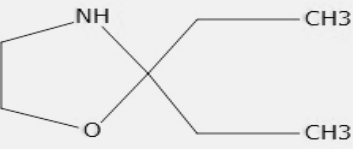
S. no	Name of extracts	IC <sub>50</sub> $\mu$ g/ml
1	MEAC	-
2	HFAC	-
3	EFAC	-
4	DEFAC	-
5	EAFAC (band one)	73.1 $\pm$ 2.1 $\mu$ g/ml
6	EAFAC (band two)	73.1 $\pm$ 2.1 $\mu$ g/ml
7	BFAC	-
8	Amphotericin B	0.29 $\pm$ 0.05 $\mu$ g/ml
9	pentamidine	5.090.09 $\mu$ g/ml

**Chemical Composition of Ethyl Acetate Fraction of *Allium Cepa* (Eafac) (Band One):**

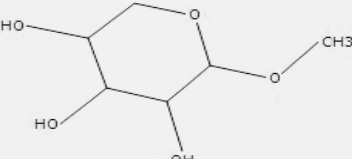
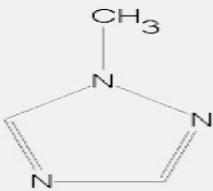
The ethyl acetate fraction of *Allium cepa* (EAFAC) (band one) which showed antileishmanial activity has been analyzed by Gas Chromatography Mass Spectrometry and results are represented in tables.

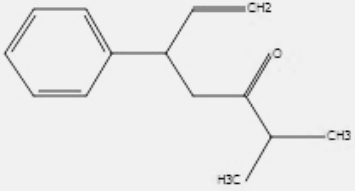
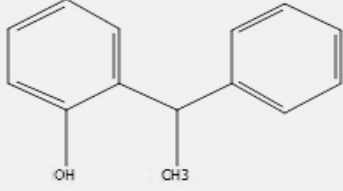
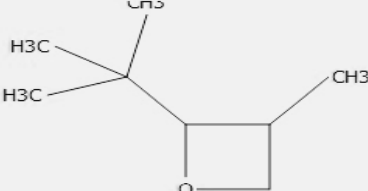
Ethyl acetate fraction of *Allium cepa* (EAFAC) (band one) consists of 55 compounds. 31 - 55 compounds are represented in this paper. 25 compounds were evaluated by RT matching with library and MS spectra.

**TABLE 2: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 31 - 35 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)**

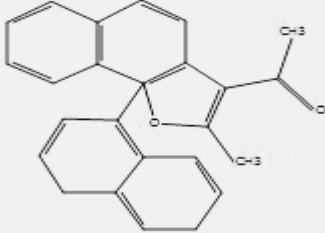

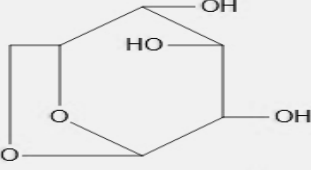

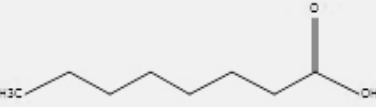
Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
31	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>	166		150.9	17.78
32	C <sub>2</sub> H <sub>7</sub> BO <sub>2</sub>	74		43.1	17.98
33	C <sub>8</sub> H <sub>16</sub> N <sub>2</sub>	140		43.1	18.9
34	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	180		180	19.34
35	C <sub>7</sub> H <sub>15</sub> NO	129		56	19.85

**TABLE 3: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 36 - 40 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)**


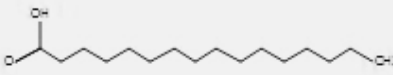
Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
36	C <sub>6</sub> H <sub>12</sub> O <sub>5</sub>	164		60	20.64
37	C <sub>3</sub> H <sub>5</sub> N <sub>3</sub>	83		84	20.69

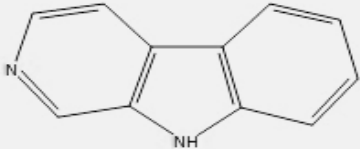
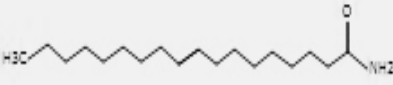
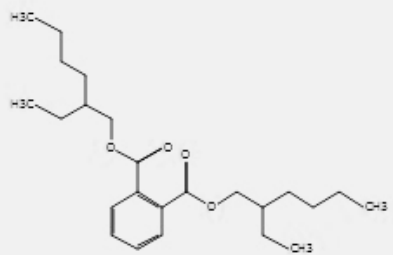
38	C <sub>14</sub> H <sub>18</sub> O	202		71	20.99
39	C <sub>14</sub> H <sub>14</sub> O	198		57.1	21.07
40	C <sub>8</sub> H <sub>16</sub> O	128		57.1	21.2

**TABLE 4: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 41 - 45 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

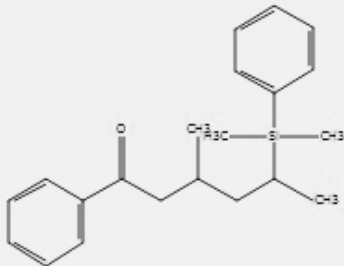
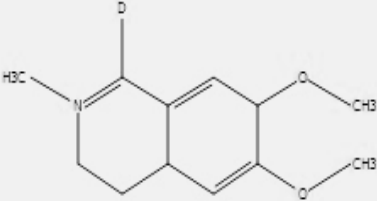
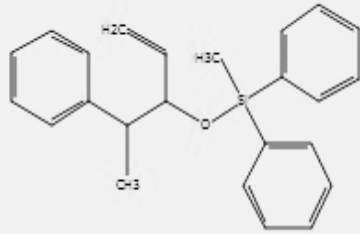
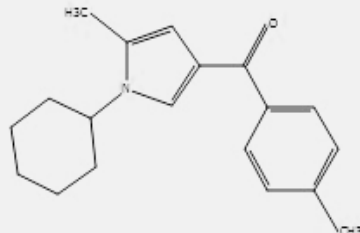
Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
41	C <sub>25</sub> H <sub>22</sub> O <sub>2</sub>	354		73	21.28
42	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296		55	21.71
43	C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>	162		57.1	22.2
44	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270		74	22.36
45	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	144		60	24.02

**TABLE 5: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 46-50 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
46	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270		74	27.97
47	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256		73	28.96

48	$C_{11}H_8N_2$	168		168	29.67
49	$C_{18}H_{35}NO$	281		59	36.77
50	$C_{24}H_{38}O_4$	390		149	39.49

**TABLE 6: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 51 - 55 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
51	$C_{21}H_{28}OSi$	324		134.9	45.27
52	$C_{12}H_{15}DNO_2$	207		207	54.51
53	$C_{24}H_{26}OSi$	358		197	56.16
54	$C_{19}H_{23}NO$	281		281	62.67

55	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> Si	284	134.9	64.24
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**TABLE 7: MASS SPECTRA OF 31 - 35 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

Compound	m/z (% Relative abundance)
31	165.9(M <sup>+</sup> ), 7326.6), 151.9(2057.7), 150.9(18016), 122.9(4967.6), 107.9(3252.1), 76.9(1955.8), 64.9(2271.7), 52(3351.8), 51(2720), 43.1(10943)
32	74(M <sup>+</sup> ), 1892.9), 73(8324.1), 69(3959.5), 68(2874.1), 57.1(7819), 56(3546.8), 43.1(9866.3), 42.1(2163.7)
33	140(M <sup>+</sup> ), 126(1150.1), 91(1158.5), 83(1841.4), 73(3450.7), 70(1812.6), 69(2441.2), 57.1(2597.2), 56(3025.3), 55(3828.1), 43.1(8935.6)
34	180(M <sup>+</sup> ), 16093), 164.9(8160.5), 136.9(7937.6), 121.9(3712.3), 93.9(2676.8), 91(3996), 76.9(6361.9), 65.9(2971.6), 64.9(4054.1), 51(3399.9)
35	129(M <sup>+</sup> ), 109.9(3180.4), 101(2209.4), 99.9(4645), 85(5947.6), 84(7387.7), 75(2333.4), 56(10647), 55(4128.9), 44.1(2424.7), 41.1(4528.6)

**TABLE 8: MASS SPECTRA OF 36 - 40 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

Compound	m/z (% Relative abundance)
36	164(M <sup>+</sup> ), 75(19445.8), 74(91658.7), 73(80074.7), 71(51001.7), 61(61534.6), 60(178910.7), 59(21150.9), 57.1(75995.2), 45.1(32745.2), 43.1(25182.1)
37	86(M+3], 5917.5), 85(M+2], 19938.9), 84(M+1], 203757.8), 83(M <sup>+</sup> ), 56(30462.1), 55(23078.9), 54(6788.4), 41.1(24366.9)
38	202(M <sup>+</sup> ), 117.9(1206.5), 116.9(3157.4), 97(2784.5), 91(4077.3), 76.9(1193.1), 75(5406.4), 71(8891.3), 70(3150.9), 68(1739.1), 56(5194.4)
39	198(M <sup>+</sup> ), 130.9(1866.3), 115.9(2042.9), 89(2260.7), 85(3995.3), 75(5406.4), 71(7296.2), 59(3641.4), 57.1(18035), 56(5039.9), 42.1(3393.5)
40	128(M <sup>+</sup> ), 97(1330), 91(1385.5), 87(1987.8), 85(3950.5), 84(5896.6), 71(7776.7), 61(11671), 57.1(17776), 56(4756.2), 42.1(3175.7)

**TABLE 9: MASS SPECTRA OF 41 - 45 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

Compound	m/z (% Relative abundance)
41	356(M+2], 691.8), 354.9(M+1], 1708.2), 354(M <sup>+</sup> ), 281(1000.9), 220.9(1273), 146.9(2055.9), 103(3049), 97(896.8), 73(19727), 69(3977.7), 41.1(3827.6)
42	296(M <sup>+</sup> ), 85(2467.1), 84(3671.7), 83(2902.8), 73(14159), 70(3433.3), 69(5657.6), 56(5406.3), 55(15066), 43.1(10662), 41.1(4577.4)
43	162(M <sup>+</sup> ), 73(4699.2), 71(3264.7), 61(3972.3), 60(7132.7), 58(2425.5), 57.1(7534.8), 55(2744.8), 45.1(6134.1), 44.1(2090.3), 43.1(5376.6)
44	270(M <sup>+</sup> ), 87(3149.8), 84(1853.5), 75(1047), 74(6656.5), 69(1500.1), 59(940.3), 55(2618), 54(1335.1), 45.1(4666.6), 41.1(1343.8)
45	149(M+5], 2669.3), 144(M <sup>+</sup> ), 103(3310.4), 85(2658.8), 76.9(2766.9), 73(3484.4), 61(1951.8), 60(5277), 403.1(2178.5), 41.1(1560.1)

**TABLE 10: MASS SPECTRA OF 46 - 50 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

Compound	m/z (% Relative abundance)
46	270(M <sup>+</sup> ), 143(3201.4), 87(17852), 75(4538.1), 74(27710), 69(4119.2), 59(3467.8), 57.1(4570.9), 55(7716.9), 43.1(7942.7), 41.1(3710.8)
47	256(M <sup>+</sup> ), 85(4067.5), 84(9135.5), 73(11214), 71(4447.7), 69(4606.7), 60(9988.1), 57.1(8901.6), 55(8235.5), 43.1(8653.4), 41.1(5269.9)
48	168.9(M+1], 3972.1), 168(M <sup>+</sup> ), 29770), 166.9(3142.8), 140.9(3327.3), 140(7509.6), 113.9(4575), 112.9(4066.2), 87.9(1906.5), 70(3739.2), 63(2834.9)

49	28(M <sup>+</sup> ), 172(16759), 69(4078.4), 67(3392.1), 60(3060.3), 59(24973), 57.1(3064.5), 55(9249.9), 44.1(3502.3), 43.1(5444.9), 41.1(4336)
50	390(M <sup>+</sup> ), 166.9(23377), 149.8(9019.3), 149(78232), 112.9(8047.6), 71(20104), 70(17979), 57.1(32572), 55(12515), 43.1(16371), 41.1(9581)

**TABLE 11: MASS SPECTRA OF 51 - 55 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)**

Compound	m/z (% Relative abundance)
51	324(M <sup>+</sup> ), 281(903.2), 252.9(575.5), 136.9(603), 134.9(1766.3), 116.9(602.4)
52	207.9(M+1], 705.6), 207(M <sup>+</sup> ], 3345.7), 120.9(644.9)
53	358(M <sup>+</sup> ), 252.9(837.6), 207.9(761), 197(992.7), 192.9(867.4), 190.9(800.8), 136.9(542.4), 120.9(826.2), 41.1(633.5)
54	281(M <sup>+</sup> ], 1590.7), 252.9(900), 197(982.6), 149(678), 118.9(513.3)
55	284(M <sup>+</sup> ), 238.9(629.6), 208.9(662.7), 134.9(2190.9), 45.1(709)

**CONCLUSION:** Finally we can conclude that Effective antileishmanial activity was observed for ethyl acetate fraction of *Allium cepa* (EAFAC) while other extract and fractions such as Methanol extract of *Allium cepa* (MEAC), Hexane fraction of *Allium cepa* (HFAC), Ether fraction of *Allium cepa* (EFAC), Diethyl ether fraction of *Allium cepa* (DEFAC) and butanol fraction of *Allium cepa* (BFAC) exhibited no antileishmanial activities. Ethyl acetate fraction of *Allium cepa* (EAFAC) band one consists of 55 compounds.

In this research paper we represent 25 compounds out of 55 compounds of ethyl acetate fraction of *Allium cepa* EAFAC (band one). In the near future, we, the researchers in the Institute of Biochemistry, University of Balochistan, Pakistan, will separate all these 55 compounds which exhibited antileishmanial activity and will lead towards drug development against leishmaniasis with least side effects.

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