



Received on 18 February, 2017; received in revised form, 22 April, 2017; accepted, 27 May, 2017; published 01 October, 2017

IN VITRO ANTILEISHMANIAL ACTIVITY AND GC-MS ANALYSIS 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. (IC₅₀ 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 (IC₅₀ = 0.29 ± 0.05 ug/ml) and pentamidine (IC₅₀ = 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 30 compounds out of 55 compounds of ethyl acetate fraction of *Allium cepa* (EAFAC) (band one).

INTRODUCTION: Leishmaniasis is a disease triggered by Leishmania, a parasite belongs to family Trypanosomatidae. Cutaneous, Mucocutaneous, Visceral and post kala azar dermal leishmaniasis (PKDL) are the four types of Leishmaniasis which have been explained^{1, 2}. Complexity and diversity are the two characters which categorized this tropical disease³. Normally skin with nodule or ulcer in a patient presents cutaneous leishmaniasis.

Macrophages in the skin are infected by diverse types of leishmania which leads to prognosis and clinical presentation^{4, 5}. Mucosa with destructive ulcers spread from the anterior station of digestive and respiratory systems for instance mouth and nose to posterior station such as pharynx and larynx in mucocutaneous leishmaniasis^{6, 7}.

Second leading blood sucking for instance parasitic disease in the entire world is visceral leishmaniasis, also known as kala azar⁸. Nodular rash or a macular, maculo-papular is the character for leishmaniasis such as post kala azar dermal leishmaniasis (PKDL)⁹. 1.5-2 million fresh cases are assessed worldwide, 350 million people are in danger of this tropical infectious disease and 70000 deaths occur annually¹⁰. For this syndrome, chemotherapy is the real treatment.

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.8(10).4354-62
	Article can be accessed online on: www.ijpsr.com
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8(10).4354-62	

Though it is costly in addition to frequently needs long standing invasive and venomous intrusion. In the course of recent decades, research has revealed that compounds isolated from plants may possibly act as new-fangled drugs for the treatment of Leishmaniasis¹¹⁻¹³. *Allium* family consists of enormous number of plants which have biological activities. One of the members of this family is *Allium cepa* which has been used as anti-infective agent, diuretic, anthelmintic, expectorant, carminative, stomachic and antispasmodic^{14, 15}. This medicinal plant has also shown antibacterial and antifungal activities^{16, 17}. Numerous experimental studies have revealed that *Allium cepa* and its extracts reduce platelet aggregation, lower blood pressure, reduce blood lipid levels and increase fibrinolysis¹⁸⁻²⁰.

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. Five kg *Allium cepa* were soaked into extraction containers having methanol²¹. 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^{22, 23}.

Formation of Fractions: The main extract due to methyl alcohol has been separated for instance 2 portions. 1 portion (1gm) 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 portions 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 were aseptically sedimented down at 3000 rpm for 10 min, counted with the help of improved Neubauer chamber under the microscope and diluted with the fresh medium to a final concentration of 1×10^6 parasites. In a 96 well microtiter plate, 180 μ l of the parasite culture (1×10^6 parasites/ml) was added in different wells in which 20 μ l of the experimental compound was added in culture and serially diluted so that minimum concentration of the compound was 1 μ g/ml. Negative control received medium with a parasite density 1×10^6 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 Neubauer chamber and IC_{50} values of compound possessing antileishmanial activity were counted²⁴.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis Triple Quadrupole Acquisition Method-MS Parameters: For identification and quantification of *Allium cepa* compounds: 2 μ l of

Allium cepa extract or fraction was directly injected into the gas chromatograph mod. 6890N 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 μ 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 electro volts 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"²⁵.

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 ($IC_{50} = 73.1 \pm 2.1 \mu$ g/ml). In this bioassay, Amphotericin B ($IC_{50} = 0.29 \pm 0.05 \mu$ g/ml) and pentamidine ($IC_{50} = 5.09 \pm 0.09 \mu$ 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 hr and Incubation Temperature 22 °C.

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

S. no	Name of extracts	IC ₅₀ ug/ml
1	MEAC	-
2	HFAC	-
3	EFAC	-
4	DEFAC	-
5	EAFAC (band one)	73.1 ± 2.1 ug/ml
6	EAFAC (band two)	73.1 ± 2.1 ug/ml
7	BFAC	-
8	Amphotericin B	0.29 ± 0.05 ug/ml
9	pentamidine	5.090.09 ug/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. 1-30 compounds are represented in this paper. 30 compounds were evaluated by RT matching with library and MS spectra.

TABLE 2: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 1-5 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)

Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
1	C ₆ H ₈ O ₄	144		43.1	5.955
2	C ₄ H ₁₀ O ₄	122		43.1	6.089
3	C ₄ H ₈ O ₂	88		43.1	6.55
4	C ₈ H ₁₈ O	130		57.1	7.104
5	C ₇ H ₁₄ O	114		43.1	7.82

TABLE 3: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 6-10 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)

Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
6	C ₅ H ₆ N ₂ O ₂	126		43.1	8.376
7	C ₅ H ₁₀ O ₂	102		55	8.514

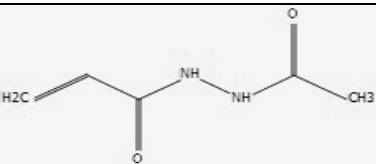
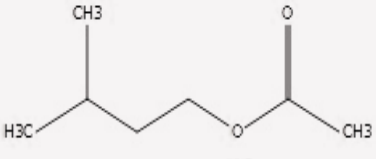
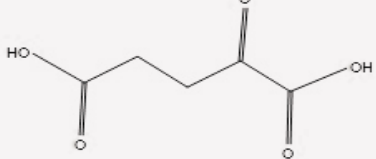
8	$C_5H_8N_2O_2$	128		55	8.516
9	$C_7H_{14}O_2$	130		43.1	8.874
10	$C_5H_6O_5$	146		101	9.011

TABLE 4: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 11-15 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)

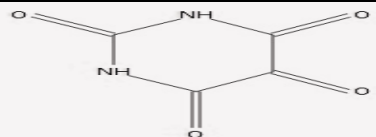
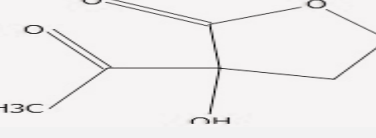
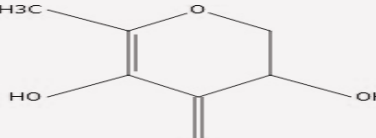
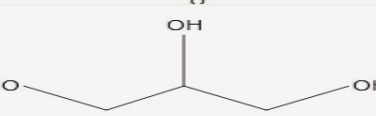
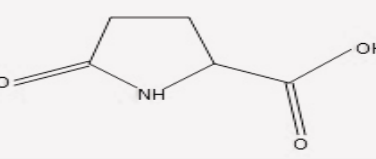
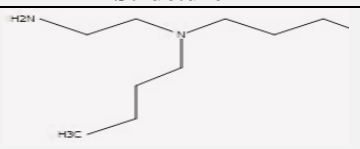
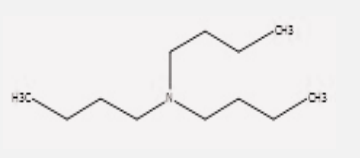
Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
11	$C_4H_2N_2O_4$	142		86	9.515
12	$C_6H_8O_4$	144		43.1	9.805
13	$C_6H_8O_4$	144		43.1	10.01
14	$C_3H_8O_3$	92		43.1	10.73
15	$C_5H_7NO_3$	129		84	10.81

TABLE 5: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 16-20 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)

Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
16	$C_{10}H_{24}N_2$	172		142.1	11.55
17	$C_{12}H_{27}N$	185		142.1	11.62

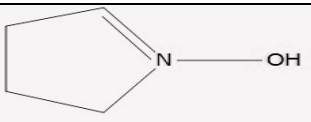
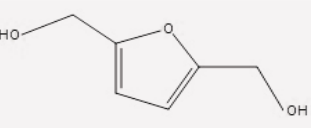
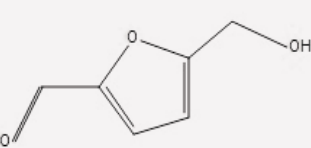
18	C_4H_7NO	85		85	11.8
19	$C_6H_8O_3$	128		97	11.89
20	$C_6H_6O_3$	126		97	12.06

TABLE 6: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 21-25 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)

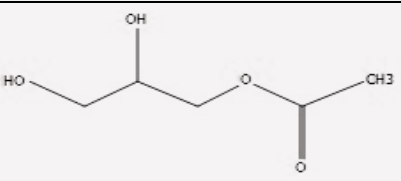
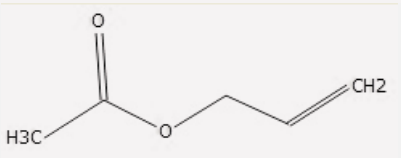
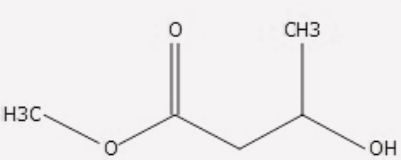
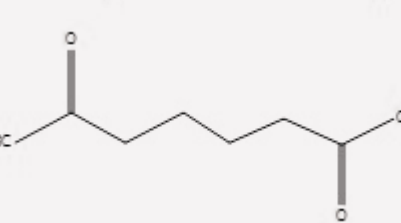
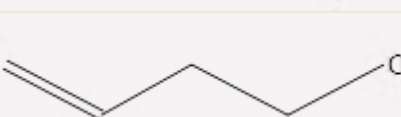
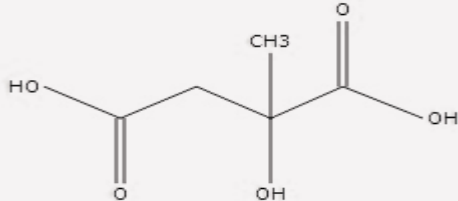
Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
21	$C_5H_{10}O_4$	134		43.1	12.42
22	$C_5H_8O_2$	100		43.1	12.71
23	$C_5H_{10}O_3$	118		43.1	13.27
24	$C_7H_{12}O_3$	144		43.1	13.53
25	$C_3H_6O_2$	74		43.1	13.87

TABLE 7: MOLECULAR FORMULA, MOLECULAR MASS, STRUCTURE, m/z AND RT OF 26-30 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)

Compound	Molecular Formula	Molecular Mass	Structure	m/z	RT
26	$C_5H_8O_5$	148		43.1	14.76

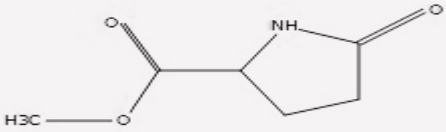
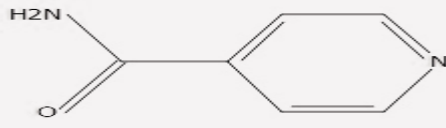
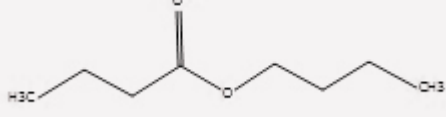
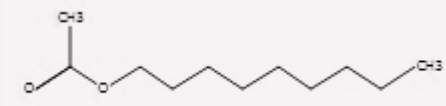
27	C ₆ H ₉ NO ₃	143		84	15.54
28	C ₆ H ₆ N ₂ O	122		43.1	16.09
29	C ₈ H ₁₆ O ₂	144		43.1	17.21
30	C ₁₁ H ₂₂ O ₂	186		43.1	17.44

TABLE 8: MASS SPECTRA OF 1-5 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)

Compound	m/z (% Relative abundance)
1	144(M ⁺ 4659.3), 101(10469), 75(1411), 73(9925.8), 57.1(2009.3), 56(2177.4), 55(9232.3), 45.1(4919.6), 43.1(20802), 41.1(1750.5)
2	122(M ⁺), 75(2119.6), 74(6376), 61(16691), 60(3511), 56(2363.7), 47(2101.5), 45.1(4095.8), 44.1(13011), 43.1(19851), 42.1(3376.9)
3	88(M ⁺), 73(8626.6), 60(1939.9), 58(1818.3), 57.1(2490), 56(1592.9), 55(921.9), 53(938.4), 45.1(5245.1), 44.1(6979.2), 43.1(9487.6)
4	130(M ⁺), 84(7636.1), 83(14885), 70(15437), 69(7197.7), 57.1(76935), 56(16885), 55(21177), 43.1(27162), 42.1(5347.3), 41.1(18587)
5	128(M ⁺ 14],4868), 114(M ⁺), 113.9(6252.4), 85(5852.1), 72(6061.8), 58(3307.3), 57.1(9735.3), 55(2801.1), 45.1(2494.3), 43.1(14751), 41.1(2762)

TABLE 9: MASS SPECTRA OF 6-10 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)

Compound	m/z (% Relative abundance)
6	126.9(M ⁺], 3157.6), 126(M ⁺], 43523), 83(5235), 70(2999.1), 55(36644), 54(4955.6), 53(6959), 45.1(2963.4), 43.1(65292), 42.1(6925.2)
7	128(M ⁺ 26], 7530.4), 102(M ⁺), 101(3473.2), 95(14774), 72(4336.6), 57.1(3754.7), 55(28788), 45.1(4414.6), 44.1(11313), 43.1(23472), 42.1(3470.4)
8	128(M ⁺],7530.4), 101(3473.2), 72(4336.6), 58(1958.2), 57.1(3754.7), 55(28788), 45.1(4414.6), 44.1(11313), 43.1(23472), 42.1(34704)
9	130(M ⁺), 89.9(2701), 74(10330), 73(4670.9), 70(10509), 56(3021.1), 55(20964), 45.1(5888.3), 44.1(15379), 43.1(30322), 42.1(4886.1)
10	146(M ⁺), 101(9636), 74(2234.4), 73(3071.5), 68(8414.1), 59(3276.7), 56(1808.9), 55(9313.9), 45.1(6166.7), 44.1(6645.6), 42.1(2736.8)

TABLE 10: MASS SPECTRA OF 11-15 COMPOUNDS IN ETHYL ACETATE FRACTION OF ALLIUM CEPA (EAFAC) (BAND ONE)

Compound	m/z (% Relative abundance)
11	142.1(M ⁺], 16010), 103(17569), 99.9(13127), 86(33136), 71(16279), 61(9092.8), 44.1(10801), 43.1(23049), 42.1(10244), 41.1(9459.9)
12	144(M ⁺), 102(14371), 85(2399.7), 60(1236), 58(1546.4), 57.1(6052), 56(15455), 55(3070), 53(1526.5), 45.1(4707.1), 43.1(30454)
13	144(M ⁺], 104671.8), 101(100924.5), 73(68117.2), 72(82772.5), 58(12219), 55(76747.2), 45.1(66366.9), 44.1(175889.8), 43.1(252345), 42.1(15657.7)
14	92(M ⁺), 73(1731.6), 72(10198), 71(1811.1), 62(1865.6), 61(32920), 60(6840.8), 58(2012.7), 44.1(17215), 43.1(47510), 42.1(9478.7)
15	129(M ⁺), 91(1391.1), 86(1680.9), 85(2157.5), 84(24308), 83(2681.5), 71(1163.3), 56(5068.7), 55(8548.2), 54(2388.6), 41.1(2341.4)

TABLE 11: MASS SPECTRA OF 16-20 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)

Compound	m/z (% Relative abundance)
16	172(M ⁺), 142.1(36060), 99.9(19242), 87.9(14716), 58(6323.3), 57.1(8926), 55(10169), 45.1(17409), 44.1(7350), 43.1(15961), 42.1(6981.8)
17	185(M ⁺), 143(3705.7), 142.1(32744), 99.9(18992), 98(3098.3), 58(5774.2), 57.1(5176.5), 45.1(3943), 44.1(6075.7), 42.1(5942.7), 41.1(4504.8)
18	86(M+1], 2089.8)85(M ⁺], 37058), 68(2461.9), 63.9(1442.9), 58(1627.5), 57.1(7040), 56(5220.7), 55(11809), 53(1680.9), 42.1(3274.7)
19	128(M ⁺],7690), 110.9(2974), 108.9(2601.8), 97(16569), 83(2290.8), 69(7486.2), 53(4067), 52(2436.3), 51(3127.9), 41.1(5454.1)
20	126(M ⁺], 79715.1), 97(169332.7), 69(65788.8), 53(41752.5), 52(20384.5), 51(33720.4), 50(22178), 42.1(14969), 41.1(93511.3), 39(15748)

TABLE 12: MASS SPECTRA OF 21-25 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)

Compound	m/z (% Relative abundance)
21	134(M ⁺), 103(60497.1), 89.9(4814.3), 72(9218.5), 61(20936.1), 45.1(8038.6), 44.1(19474.2), 43.1(236534.5), 42.1(9482.8)
22	99.9(M ⁺], 1926.7), 61(2746.7), 59(1375.9), 58(2098.3), 57.1(1756.4), 56(1683.6), 55(2411.3), 45.1(3023.8), 43.1(25744), 42.1(3307.7)
23	118.9(M+1], 6699.4), 118(M ⁺), 97(2929.4), 75(4331.1), 74(3027.5), 71(2970.3), 61(3810.4), 55(3399.7), 45.1(8433.1), 43.1(23739), 42.(2694.5)
24	144(M ⁺], 3309.8), 101(2643.9), 61(4926.8), 58(3153.9), 56(3067.6), 55(9933.6), 45.1(5190.5), 44.1(2463.2), 43.1(30047), 42.1(3594.5)
25	74(M ⁺], 2719.7), 73(2966.1), 70(1645.6), 69(1426.7), 59(2190.7), 57.1(2817.3), 43.1(11187), 42.1(3943.9)

TABLE 13: MASS SPECTRA OF 26-30 COMPOUNDS IN ETHYL ACETATE FRACTION OF *ALLIUM CEPA* (EAFAC) (BAND ONE)

Compound	m/z (% Relative abundance)
26	148(M ⁺), 104(3865.5), 103(17792), 86(2383.6), 85(10235), 61(11920), 57.1(4755.4), 45.1(8460.2), 44.1(2756.1), 43.1(27301), 42.1(4231)
27	143(M ⁺], 12035.9), 85(13170.1), 84(275329.5), 59(9525.1), 56(38547.4), 55(13756.4), 54(10236.4), 42.1(6716.1), 41.1(44016.2)
28	133.9(M+12], 919.5), 121.9(M ⁺], 6049.8), 105.9(4458), 87.9(876.9), 77.9(6403.8), 52(1726.3), 51(4283.3), 50(2185.9), 44.1(1288.6), 43.1(6859.5)
29	114(M ⁺), 89(17404), 73(6395.6), 72(5339.2), 71(16474), 69(5870.1), 57.1(18257), 56(6554.6), 44.1(5176.9), 43.1(18429), 41.1(5882.3)
30	186(M ⁺), 97(1986.6), 83(2085.9), 74(2309.4), 70(2193.1), 69(3182.9), 56(4817.3), 55(4477.9), 43.1(12101), 42.1(2649.1), 41.1(2116.7)

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 30 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.

ACKNOWLEDGEMENT: The authors are thankful to Hussain Ebrahim Jamal (HEJ), Research Institute of Chemistry, University of Karachi, Karachi, Pakistan, for providing antileishmanial facilities and GC-MS analysis.

CONFLICT OF INTEREST: Authors do not have any conflict of interest.

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How to cite this article:

Achakzai JK, Panezai MA, Kakar AM, Agha Z, Khan J and Kakar A: *In vitro* antileishmanial activity and GC-MS analysis of ethylacetate fraction of *Allium cepa* (EAFAC). *Int J Pharm Sci Res* 2017; 8(10): 4354-62. doi: 10.13040/IJPSR.0975-8232.8(10).4354-62.

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