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GC-MS STUDY OF HYDATID CYST ISOLATED FROM HUMAN, SHEEP AND CAMEL WITH / WITHOUT EXPOSURE TO SOME PLANT EXTRACTS, TINIDAZOLE AND BENDAZOLE IN BASRAH PROVINCE, SOUTHERN IRAQ

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Abstract

Gas chromatography-mass spectrometry (GC-MS) methods were utilized to provide a comprehensive characterization of the chemical composition of plant or other organic compounds. Collected eighty-one, four samples of hydatid cyst from human, seventy samples from sheep and seven samples from camel during the period (October 2019 to April 2020) in Basrah and ThiQar province. This study show different between chemical compound in liquid of hydatid cyst and germinal layer in human, sheep and camel, these chemical compound contain amino acid, oil, cholesterol, organic and other chemical compound in each plant and drug. Our study designing for mention chemical material that present in hydatid cyst with germinal layer and the differences between three hosts hoses are human, camel and sheep. The current study recorded differences between plants (*Ceratonia siliqua, Moringa oleifera* and *Capparis spinosa* and two drugs Tindazol and bendazol).

Key words: GC-MC, analysis of chemical compounds, hydatid cyst parasite

Introduction

Echinococcosis, is a zoonotic disease caused by the larval stage of Echinococcus, affects humans and other mammals, such as sheep, dogs and camels. Hydatidosis causes harmful, slowly enlarged cysts in the liver, lungs, and other organs (Eslami A and Hosseini 1998).

Cystic Echinococcosis caused a significant economic losses and public-health concern in Iraq (Hama *et al*., 2015). The pathogenicity of hydatidosis depends on the extent and severity of infection and the organs on which it is situated, when occur rupture of hydatid cysts often leads to sudden death due to anaphylaxis, hemorrhage and metastasis (White *et al*., 2004).

The chromatography is wide field of applications. and main area of use in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons (Balamurugan V., Balakrishnan, 2016).

Mass Spectroscopy, a system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose.

(Kadhim *et al*., 2016). Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of substances within a test sample (Kell *et al*., 2005).

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Materials and Methods

GC-MS analysis was used in current study to identify the chemical compounds existing in the parasite sample . GC-MS technique was carried out at GC- MS Lab. Basra Oil Company (Nahran-Omer) by using GC Shimadzu QP 2010 system and gas chromatograph interfaced to Mass Spectrometer (GC-MS).

It was equipped with fused silica capillary column (DB5MS) (Length: 30m, Diameter: 0.32 mm, Film thickness: 0.25 µm, composed of 95% methyl poly siloxane and 5% phenyl) and Helium gas Injection was conducted in split mode, and the column temperature was programmed at 40°C for 5 minute with an increase at a rate of 28 °C/minute until reach to final temperature 280 °C, 1 µl of sample was injected into the capillary column with fixing the injector and detector temperature at 40°C.

Sample collection

Human samples were collection from surgery unit in hospital from patient that suffering from hydatid cyst.

Table 1: Qualitative compound report by GC-Mass to all compound for human liquid hydatid cyst.

Peak	Name	Formula	RT
1	3-Trifluoroacetoxydodecane	C14H25F3O2	5.532
2	Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	5.908
3	3-Octyn-2-o	C8H14O	6.992
4	1-(5-Bicyclo[2.2.1]heptyl)ethylamine	C9H17N	7.485
5	Oxime-, methoxy-phenyl-	C8H9NO2	8.083
6	(5-Tert-butyl-2,4-dihydroxyphenyl) ethanone	C12H16O3	9.014
7	cyclotetrasiloxane, octamethyl-	C8H24O4Si4	9.834
8	N-Methyladrenaline, 3TMS derivative	C19H39NO3Si3	12.544
9	Tetraacetyl-d- xylonic nitrile	C14H17NO9	18.929
10	[1,1'-Bicyclopropyl]- 2-octanoic acid, 2'-hexyl-, methyl ester	C21H38O2	19.756
11	Benzoic acid, 4-methyl-2-trimethyl-silyloxy-, trimethylsilyl ester	C14H24O3Si2	27.219
12	Hexasiloxane, 1,1,3,3,5,5,7,7, 9,9,11,11-dodecamethyl-	C12H38O5Si6	29.867
13	Cholesterol	C27H46O	31.937

While the animal samples (sheep and camel) were collected from slaughter or butcher after remove the cyst from infected organ, the cyst was washed three times with normal saline and then separated.

Collected the fluid from germinal layer then put in clean tube about 1.5 ml and add some drops from distal water and then transport immediately to the lab.

Basra Oil Company to work GC-MS. Put the germinal layer after wash in freezer for one day and ,then grind in mill or mortar until it turns into liquid form then add some drops from distal water and transport immediately to the Basra Oil Company to work GC-MS. This method innovation because no study by Gc mass of parasite.

Preparation of plant and extraction: the plants were collected from the Natural plant nursery in Thi-Qar province during the period from October 10/10/2019 to April 1/4/2020.These plants wash and dire and then it was ground by a mill or mortal. Then he took 100 grams of the plant and put it in a baker containing 200 ml distilled water and mixed it with a magnetic motor for 30 minutes, then separated from the precipitator by using centrifuge at 3000rpm for 15 minutes, then distributed it with glass plates and placed inside the oven at a degree of 60to dry and then took a weight. (Al-Haidari. 2005).

Results

The present study show different in compound by GC-Mass, table 1 explain qualitative compound report by GC-Mass to all compound for human liquid hydatid cyst, table 2 show qualitative analysis report to only ten largest compound by GC-Mass for human

Table 2: Qualitative analysis report to only ten largest compound by GC-Mass for human liquid hydatid.

Peak	Start	RT	End	Height	Area	Area%
1	5.256	5.532	5.866	20868.29	429907.51	15.64
2	5.866	5.908	5.967	31663.74	72322.07	2.63
3	8.049	8.083	8.277	158731.29	587044.84	21.36
4	9.848	9.868	9.896	18852.62	31289.34	1.14
5	11.307	11.341	11.432	122749.5	250521.5	9.12
6	13.621	13.676	13.746	19261	40222.41	1.46
7	14.795	14.823	14.879	67594.7	92782.14	3.38
8	16.889	16.914	16.941	21048	29438.57	1.07
9	26.934	27.219	27.309	6345097	383977	13.97
10	31.869	31.937	32.111	858579.39	2748203.61	100

Table 3: Qualitative compound report by GC-Mass to all compound for sheep liquid hydatid cyst.

Peak	Name	Formula	RT
1	Silane, triethyl(2-phenylethoxy)	C14H24OSi	6.394
2	Propanone, 1-(1-adamantyl)-3-dimet-hylamino-	C15H25NO	7.853
3	2-Pentanol, 3-chloro-4-methyl-, (R*, R*)-(.+/-.)-	C6H13ClO	9.715
4	1-(5-Bicyclo[2.2.1]heptyl)ethylamine	C9H17N	7.485
5	d-Glycero-d-ido-heptose	C7H14O7	11.383
6	Imidazole, 2-amino-5- [(2-carboxy) vinyl]-	C6H7N3O2	11.925
7	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	12.321
8	l-Gala-l-ido-octose	C8H16O8	12.439
9	N-[3-[N-Aziridyl]propylidene]tetrahy-drofurfurylamine	C10H18N2O	13.155
10	Dodecanoic acid, 3-hydroxy-	C12H24O3	14.607
11	Pterin-6-carboxylic acid	C7H5N5O3	14.802
12	Paromomycin	C23H45N5O14	15.121
13	Acetamide, N-methyl-N-[4-(3-hydrox-ypyrrolidinyl)-2- butynyl]	C11H18N2O2	16.865
14	Pyrrolizin-1,7-dione-6-carboxylic acid, methyl(ester)	C9H11NO4	19.972
15	2-Myristynoyl pantetheine	C25H44N2O5S	22.209
16	Oleic Acid	C18H34O2	23.863
17	Cholesterol	C27H46O	32

Table 4: Qualitative analysis report to only ten largest compound by GC-Mass for sheep liquid hydatid.

Peak	Start	RT	End	Height	Area	Area%
1	9.146	9.715	10.362	610664.46	18230749.5	32
2	10.362	11.383	11.869	1253887.07	48807780.74	85.68
3	12.265	12.321	12.418	538592.73	3063025.48	5.38
4	13.106	13.155	13.204	658841.81	1189521.19	2.09
5	14.565	14.607	14.76	511258.02	2014000.51	3.54
6	19.819	19.972	20.062	566128.63	3016690.94	5.3
7	22.167	22.209	22.417	498744.15	2040906.01	3.58
8	23.828	23.863	24.002	1172918.13	3408669.26	5.98
9	24.016	24.071	24.092	625962.55	1301282.86	2.28
10	31.87	32	32.09	15202834.81	56963088.63	100

Table 6: Qualitative analysis report to only ten largest compound by GC-Mass for Camel liquid hydatid.

Peak	Start	RT	End	Height	Area	Area%
1	4.782	4.914	4.955	3794681.87	16716822.87	40.38
2	4.955	5.018	5.032	5389657.98	16423284.99	39.67
3	5.032	5.108	5.164	5621087.61	16544242.64	39.97
4	17.143	17.345	17.505	1156562.71	4807259.42	11.61
5	18.554	18.79	20.062	1540817.88	41396709.34	100
6	22.16	22.209	22.341	2171882.98	4632020.58	11.19
7	23.821	23.856	23.974	1261931.23	3612553.31	8.73
8	24.016	24.071	24.148	2570139.55	6104412.99	14.75
9	27.17	27.219	27.282	1542761.71	2523902.54	6.1
10	31.868	31.965	32.097	10838287.3	34349553.97	82.98

Table 5: Qualitative compound report by GC-Mass to all compound for camel liquid hydatid cyst.

Peak	Name	Formula	RT
1	Acetic acid	C2H4O2	4.914
2	2-Propanone, 1-hydroxy-	C3H6O2	5.108
3	Propanoic acid, 3- (acetylthio)-2-methyl-	C6H10O3S	5.581
4	4,4-Ethylenedioxy-pentanenitrile	C7H11NO2	6.165
5	2,4,6,8-Tetraazabicyclo[3.3.0]octan-3-one,		
	7-nitroimino-	C4H6N6O3	7.283
6	d-Glycero-d-ido-heptose	C7H14O7	8.569
7	2(3H)-Furanone, 5-heptyldihydro	C11H20O2	13.231
8	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	C6H7N3O2	13.697
9	R-Limonene	C10H16O3	14.406
10	Dodecanoic acid, 3-hydroxy-	C12H24O3	15.156
11	Phenol, 2,6-bis(1,1-dimethylethyl)-	C14H22O	17.345
12	Desulphosinigrin	C10H17NO6S	18.79
13	Acetamide, N-methyl-N-[4-(3-hydroxypyr-		
	rolidinyl)-2-butynyl]-	C11H18N2O2	20.18
14	n-Hexadecanoic acid	C16H32O2	22.209
15	Hexadecanoic acid, ethyl ester	C18H36O2	22.508
16	9-Hexadecenoic acid	C16H30O2	23.342
17	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	C20H38O2	23.8
18	Oleic Acid	C18H34O2	23.856
19	Octadecanoic acid	C18H36O2	24.071
20	1H-2,8a-Methanocyclopenta[a]cyclopropa		
	[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10		
	aoctahydro-5,5a,6-trihydroxy-1,4-bis(hydr-		
	oxymethyl)-1,7,9-trimethyl-, [1S- (1.alpha.,		
	1a.alpha.,2.alpha.,5.beta.,5a.beta.,6.beta.,		
	8a. alpha.,9.alpha.,10a.alpha.)]-	C20H28O6	25.266
21	1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa		
	[8,9]cycloundec[1,2-b]oxiren-5(1aH)-one,		
	2,7,9,10-tetrakis(acetyloxy)decahydro-3,6,		
	8,8,10a-pentamethyl	C28H38O11	25.774
22	Di-n-octyl phthalate	C24H38O4	27.219
23	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,1		
	-dodecamethyl-	C12H38O5Si6	29.866
24	Cholesterol	C27H46O	31.965

liquid hydatid cyst, but the table 3 qualitative compound report by GC-Mass to all compound for sheep liquid hydatid cyst, table 4 analysis for sheep.

Table 5 show qualitative compound report by GC-Mass to all compound for camel liquid hydatid cyst while the table 6 analysis report for camel, table 7 Show qualitative compound report by GC-Mass to all compound for human germinal layer of hydatid cyst, table 8 show analysis report for human but table 9 show qualitative compound report by GC-Mass to all compound for camel germinal layer of hydatid cyst, table 10 explain qualitative compound report by GC-Mass to all compound for plant *Ceratonia siliqua* but the table 11 qualitative compound report by GC-Mass to all compound for *Moringa oleifera* , table 12 shoe qualitative compound report by GC-Mass to all compound for *Capparis spinosa* while the table 13 qualitative compound report by GC-Mass to all compound for Tindazol, table 14 qualitative compound report by GC-Mass to all compound for Albendazol.

Discussion

The current study was the first study in Iraq and world in G C-Mass of parasite hydatid cyst, so the little references in the world about this subject most study of G C-Mass on plant or chemical compound. (Lima *et al*., 2017) designing biochemical pathways such as Gc-Mass that may clarify the processes of parasite life cycle, helminthes infection and host-parasite interaction, providing targets to further

Table 7: Qualitative compound report by GC-Mass to all compound for human germinal layer of hydatid cyst.

Peak	Name	Formula	RT
1	Dipipanone	C24H31NO	4.259
2	Cyclotrisiloxane, hexamethyl-	C6H18O3Si3	5.33
3	Ethanone, 1-(3-thienyl)-	C6H6OS	6.508
4	Arsenous acid, tris(trimethylsilyl) ester	C9H27AsO3Si3	7.257
5	Silicic acid, diethyl bis(trimethylsilyl) ester	C10H28O4Si3	8.886
6	Silane, dimethyl(dimethyl(3-phenylpro-2-enyloxy)silyloxy)(3-phenylpro-2-enyloxy)	C22H30O3Si2	9.599
7	Di-sec-butyl phthalate	C16H22O4	21.214
8	2-Butenenitrile, 2-chloro-3-(4-methoxyphenyl)	C11H10ClNO	22.466
9	2-Butenenitrile, 2-chloro-3-(4-methoxyphenyl)-	C11H10ClNO	22.466
10	Cholan-24-oic acid, 3,6,7-tris(acetyloxy)-,methyl ester, (3.alpha.,5.beta.,6.alpha.,7.alpha.)-	C31H48O8	31.856

Table 8: Qualitative compound report by GC-Mass to all compound for sheep germinal layer of hydatid cyst.

Peak	Name	Formula	RT
1	Arsenous acid, tris(trimethylsilyl) ester	C9H27AsO3Si3	5.567
2	Benzoic acid, 2- (methylamino)-, 2-methylpropyl ester	C12H17NO2	7.574
3	Oxime-, methoxy-phenyl-	C8H9NO2	7.965
4	Cyclotetrasiloxane, octamethyl-	C8H24O4Si4	9.762
5	zuleno[4,5-b]furan-2,9-dione, decahydro-6a-hydroxy-6,9a-dimethyl-3-methylene-, [3aS-(3 a.alpha.,6.beta., 6a.alpha., 9a.beta.,9b.alpha.)]	C15H20O4	20.878
6	1H-Indene, 2-butyl-3-hexyl-	C19H28	22.159
7	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	C12H38O5Si6	29.458

Table 9: Qualitative compound report by GC-Mass to all compound for camel germinal layer of hydatid cyst.

Peak	Name	Formula	RT
1	Cyclotrisiloxane, hexamethyl	C6H18O3Si3	5.204
2	Oxime-, methoxy-phenyl-_	C8H9NO2	8.142
3	Silicic acid, diethyl bis(trimethylsilyl) ester	C10H28O4Si3	8.938
4	Cyclotetrasiloxane, octamethyl-	C8H24O4Si4	9.762
5	Arsenous acid, tris(trimethylsilyl) ester	C9H27AsO3Si3	11.307
6	Stannane, dimethyl-	C2H8Sn	12.033
7	2,6-Dihydroxybenzoic acid, 3TMS derivative	C16H30O4Si3	12.494
8	1H-Pyrrole, 2-ethyl-3,4,5-trimethyl-	C9H15N	12.941

interference for parasite control or even infection treatment.

In Iraq thy are no study about parasite by GC-Mass. This study show chemical compound that different in each host human, sheep and camel and the germinal layer also different compound in each host. The current study show present cholesterol, oil, alkaline material and amino acid in liquid or germinal layer of parasite some of them benefit the chemical compound used in the manufacture of soap and cosmetics, and some of the substances are dangerous and ignite and irritate the skin.

This study mention the liquid of human cyst was similar with germinal layer of human cyst only in (Cyclotrisiloxane, hexamethyl),

Table 10: Qualitative compound report by GC-Mass to all compound for *Ceratonia siliqua*

Peak	Name	Formula	Arean	RT
1	Acetic acid	CH*f*COOH	24.10	4.156
2	2-Propanone, 1-hydroxy	$C_3H_6O_2$	9.52	4.525
3	4-Amino-1-butano	C4H11NO	1.25	5.220
4	Butanoic acid, 4-hydroxy-	$C_4H_8O_3$	2.52	8.263
5	Benzeneacetaldehyde	C_8H_8O	1.86	10.494
6	N,N-Dimethyl-1-propanamine	C5H13N	3.54	10.633
7	2,5-Dimethylfuran-3,4(2H,5H)-dione	C6H8O3	2.21	11.383
8	Propanenitrile	CHCHCN	1.20	11.473
9	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-	C6H8O4	19.55	12.384
10	L-Alanine, TMS derivative	$C_6H_{15}NO_2Si$	1.05	12.724
11	Mephenesin	$C_{10}H_{14}O_3$	3.97	22.939

Table 11: Qualitative Compound Report by GC-Mass to all compound for *Maringa oleifera*

Peak	Name	Formula	Arean	RT
1	etraacetyl-d-xylonic nitrile	C14H17NO9	3613115.78	4.198
2	Acetic acid	C2H4O2	24071395.65	4.302
3	2,3-Butanediol, [R- (R*,R*)]-	C4H10O2	18822326.22	4.393
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	1998508.64	12.335
5	Imidazole, 2-amino-5-[(2-carboxy)vinyl]	C6H7N3O2	1906502.12	13.996
6	Melezitose	C6H7N3O2	7438991.33	16.921
7	3-O-Methyl-d-glucose	C7H14O6	143111711.7	21.083
8	Sulfurous acid, 2-ethylhexyl hexylester	C14H3003S	165212.45	22.43
9	Desulphosinigrin	C10H17NO6S	2356580.01	22.251
10	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13, 13-tetradecamethyl	C14H44O6Si7	3011428.94	30.471
11	Tetradecanoic acid, 10,13 -dimethyl-, methylEster	C17H3402	301256.92	31.84

Table 12: Qualitative compound report by GC-Mass to all compound for *Capparis spinosa*

Peak	Name	Formula	Arean	RT
1	methyl .alpha.-D-Glucopyranoside,	C7H14O6	82.36	18.297
2	Imidazole, 2-amino-5-[(2-carboxy)v inyl]-	C6H7N3O2	3.64	21.903
3	Atropine	$C_{17}H_{23}NO_3$	9.50	24.794
4	Atomoxetine	$C_{17}H_{21}NO$	4.50	25.857
5	2H-Benzotriazole , 2-methyl-	C7H7N3	5.60	26.970
6	2,5-Octadecadiynoic acid , methyl ester	C19H30O2	6.30	27.980
7	Propionic acid	C*f* H† O,	8.32	29.16
8	Dimethyl fumarate	C6H8O4	11.57	31.20

Table 13: Qualitative compound report by GC-Mass to all compound for Tindazol.

Peak	Name	Formula	Arean	RT
1	4-FluorohistamineMetaraminol 3,3-Dimethyl-4-methylamino-butan- 2- one	C7H15NO	8.90	12.655
2	Pterin-6-carboxylic acid	C7H5N503	11.34	16.143
3	Propanamide dl-3-Aminoisobutyric acid, N-methy l-, methyl ester Paradrine	C5H11NO2	10.54	20.993
4	Cathine	$C_9H_{13}NO$	5.81	22.549

Table 14: Qualitative compound report by GC-Mass to all compound for Albendazol.

Peak	Name	Formula	Arean	RT
1	Methylpent-4-enylamine	C6H13N	2.52	11.557
2	1-Ethyl-3-piperidinol	C7H15NO	2.40	15.893
3	1,4-di-iso-propylnaphthalene	C16H20	1.76	19.374
4	2-(5-Aminohexyl)furan	C10H17N0	1.46	19.874
5	9,12-Octadecadienoic acid, methyl ester	C19H34O2	8.29	23.487
6	Tridecanoic acid, 12-methyl-, methyl ester	C15H30O2	5.16	23.786
7	sarcosine, N-isobutyryl-, tetradecyl ester	C21H41NO3	1.95	23.946
8	Tetrasiloxane	$H_{10}O_3Si_4$	2.23	26.420

but different in other compound while in sheep the germinal layer was different in all compound with liquid cyst in sheep as well as in camel the germinal layer was different in all compound with liquid cyst in camel. But there are some similarity between germinal layer of sheep with germinal layer of human only in (Arsenous acid, tris (trimethylsilyl ester), also between the germinal layer of human and germinal layer of camel only in (Cyclotetrasiloxane, octamethyl and Arsenous acid, tris (trimethylsilyl) ester. The similarity between germinal layer of sheep with germinal layer of camel only in Arsenous acid, tris (trimethylsilyl) ester and Oxime-, methoxy-phenyl.

Gc.Mass of plant study compound for *Moringa oleifera* was agree with our study in Bharath University Rao., *et al.,* (2019) in two compound but they study in seed while current study in levels., the study also show agree with study in some compound for *Capparis spinosa* with (Altameme. 2016).

There are some study in same plant but no agree may be because the different in conditions of the working or the way of work such as study (Aliyazicioglu1 *et al.,* 2015).

Also in our the study show (GC-MS) Study for Analysis of chemical compounds of Tinedazol and Bendazol and show some organic and amino acid material that different in result from other studies such as (Anumolu *et al.,* 2014). And other study of Bendazol such as (D.M. Rathod *et al*., 2016).

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