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## Research Article

# Biochemical and GC-Mass analysis of *Echinococcus granulosus* hydatid cyst fluid components for humans and sheep

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**Abstract**
This study was conducted to evaluate the chemical and biochemical components of *Echinococcus granulosus* hydatid cyst fluid (HCF) isolated from infected humans and sheep. The study shows significant differences between the potassium, urea, cholesterol, uric acid, and magnesium in the HCF content of humans and sheep. In contrast, no significant differences were found in the total protein, glucose, triglyceride, creatinine, calcium, and sodium. The Urea, magnesium, calcium, creatinine, glucose, and potassium in HCF of sheep was higher than those of humans, whereas, the total protein, uric acid, triglyceride, cholesterol, and sodium content of HCF of humans were higher than in sheep. In (GC-MS), a difference in the number of peaks and components of HCF between humans and sheep was found, where the number of peaks in humans HCF was 25 peaks vs. 43 peaks in sheep.

**Keywords:** GC-MS analysis, Biochemical contents, Parasite, Human.

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## Introduction

*Echinococcus granulosus* is a common zoonotic pathogen worldwide (Jenkins et al. 2005; Deplazes et al. 2017). It infects livestock and humans as intermediate hosts and caniids especially dogs as the definitive host, causing hydatid cyst as larval stages in intermediate hosts (Jenkins 2006; Alssady & Al-Quzweeni 2019). This has severe impacts on human and animal health (Sarıözkan & Yalçın 2009; Snabel et al. 2009) and poses a significant economic and public health problem in many parts of the world (Schantz et al. 2003; Sikó et al. 2011), especially in rural areas where dogs and livestock are raised together (Driscoll et al. 2009; Groeneveld et al. 2010).

The hydatid cyst fluids (HCF) contain many biochemical components such as carbohydrates, proteins, lipids, vitamins, electrolytes, and trace elements that may have a role in the metabolism and growth of unilocular hydatid cyst (McManus & Smyth1982; Ozkan & Malazgirt 1992). Biochemical studies are useful in differentiating strain variations of *E. granulosus* in different countries (Shaafie et al. 1999; Kumaratilake et al. 1979; McManus & Macpherson 1984). Gas chromatography-mass spectrometry (GC-MS) is considered the most standardization method in analyzing metabolic components since the half of the last century, it used for investigating sugars (DeJongh et al. 1969), amino acids (Gelpi et al. 1969), sterols (Brooks et al. 1968), hormones (Gréen 1969), catecholamines (Anggard & Sedvall 1969), hydroxyl acids (Kuksis & Prioreschi 1967), fatty acids (Niehaus & Ryhage 1968), and aromatics (Coward & Smith 1969). Hence, this study was conducted to evaluate the chemical and biochemical components of *E. granulosus* HCF isolated from infected humans and sheep.

## Materials and Methods

Seven samples of HCF were collected from the liver of

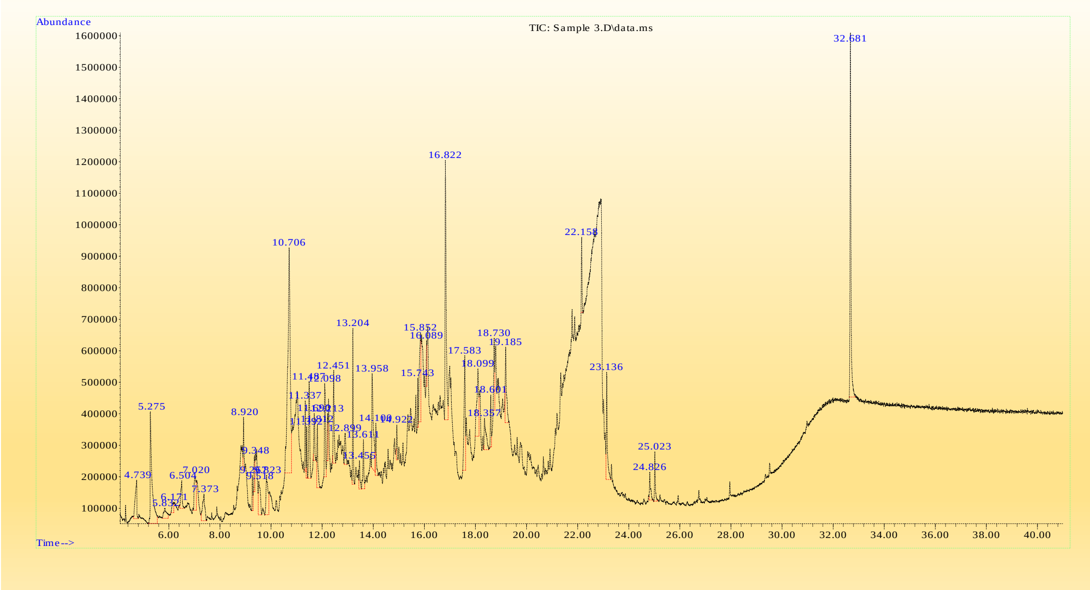

**Fig.1.** Chromatography compounds in hydatid cyst fluid from sheep diagnosed by GC-MS.

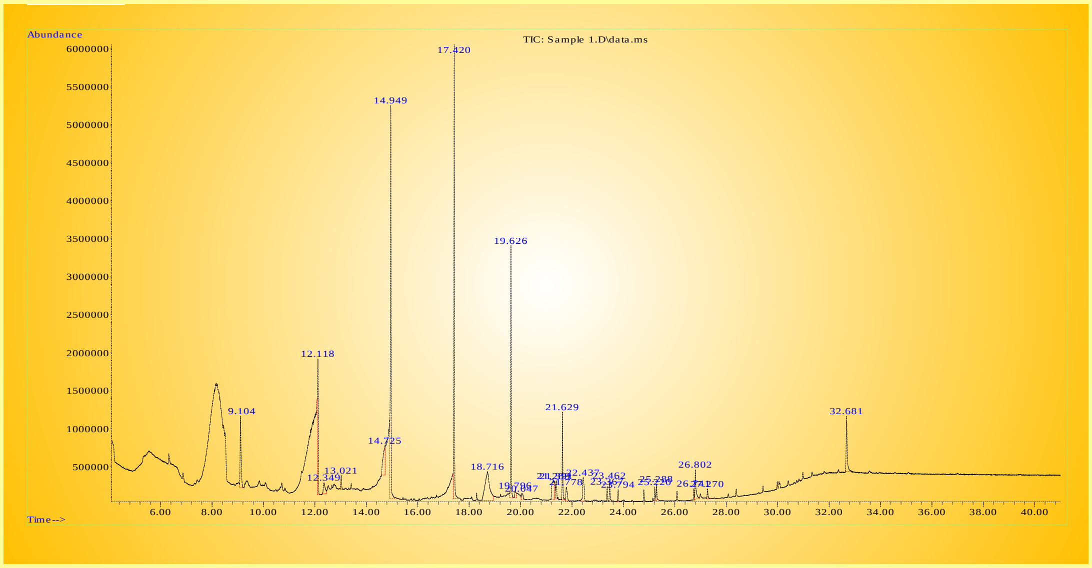

**Fig.2.** Chromatography compounds in hydatid cyst fluid from Human diagnosed by GC-MS.

infected sheep from Amara central slaughterhouse, Maysan Governorate. In addition, seven samples of human HCF were obtained after surgical removal from patients at Al-Sadder Teaching Hospital. Cyst fluid was aspirated from the cysts using sterile needles in aseptic conditions. Each cyst fluid was centrifuged at 15000rpm at 4°C for 30min. Their supernatants were analyzed for biochemical parameters, including glucose, urea, total protein, creatinine, uric acid, cholesterol, and triglycerides, using a chemistry auto-analyzer (Mindray). In addition, their electrolytes viz. sodium, potassium, magnesium, and calcium were measured using an auto-analyzer (Genex Elyte4). For GC-MS analysis, one and a half ml of each HCF was taken in a cleaned and sterilized tube with some drops of distilled water and immediately transferred to the laboratory of Basrah oil company (Nahran Omer). The HCFs were analyzed by Gas chromatography-mass spectrometry (GC-MS-Qp 2010, Shimadzu, Japan), to examine their chemical compounds, according to Al-Ataby (2022). The student's t-test was used to compare the differences between two groups in SPSS software.

**Table 1.** Biochemical content of hydatid cyst fluids of *E. granulosus* of Humans and Sheeps. (mean±SE, n = 7).

| Biochemical analysis | Units | The concentration of material between Human and sheep | | | |
|---|---|---|---|---|---|
| | | Huma | Sheep | t.test value | *P*-value |
| Total protein | g/L | 1.018±1.614 | 0.464±0.225 | 0.900 | 0.386 |
| Glucose | mg/dl | 41.528±28.549 | 68.428±32.825 | -1.636 | 0.128 |
| Urea | mg/dl | 26.285±10.942 | 65.314±20.672 | -4.415 | 0.001 |
| Uric acid | mg/dl | 4.642±3.144 | 0.961±0.467 | 3.064 | 0.010 |
| Cholesterol | mg/dl | 43.814±37.775 | 10.157±10.256 | 2.275 | 0.050 |
| Triglycerides | mg/dl | 99.442±145.328 | 59.314±91.800 | 0.618 | 0.548 |
| Creatinine | mg/dl | 0.324±0.405 | 0.387±0.332 | -.317 | 0.756 |
| Calcium | mmol/L | 8.928±6.739 | 14.628±4.611 | -1.847 | 0.090 |
| Sodium | mmol/L | 142.971±43.269 | 123.428±13.986 | 1.137 | 0.278 |
| Potassium | mg/dl | 4.285±1.181 | 6.785±1.372 | -3.652 | 0.003 |
| Magnesium | mg/dl | 1.058±0.929 | 3.158±1.353 | -3.384 | 0.005 |

**Table 2.** Peak, Retention time (RT), and concentration (Area %) of compounds in sheep hydatid cyst fluid by GC-MS.

| Peak | RT | Area% | Name |
|---|---|---|---|
| 1 | 4.739 | 1.68 | Propanoic acid |
| 2 | 5.275 | 5.63 | N, N-Dimethylaminoethanol* |
| 3 | 5.832 | 0.78 | N-Ethyl trimethylenediamine |
| 4 | 6.171 | 0.46 | 4-Piperidinamine, N,1-dimethyl |
| 5 | 6.504 | 0.99 | Butanoic acid |
| 6 | 7.020 | 1.73 | 1-Propanamine, 2-methyl-N-(2-methy lpropylidene) |
| 7 | 7.373 | 1.61 | 1H-Pyrrole, 2-methyl-1,3-Diazine |
| 8 | 8.920 | 0.92 | Pyridine, 4-butyl-, 1-oxide |
| 9 | 9.267 | 0.67 | Dimethyl sulfone |
| 10 | 9.348 | 0.82 | Pyrrolidine, Azocine, |
| 11 | 9.518 | 1.60 | Piperidine, 3-methyl- |
| 12 | 9.823 | 2.08 | Butyric acid, 3-pentadecyl ester |
| 13 | 10.706 | 14.70 | Pyrazine* |
| 14 | 11.337 | 1.27 | 1-Butanamine, 2-methyl-N-(2-methyl Nanofin |
| 15 | 11.392 | 0.75 | Pantolactone |
| 16 | 11.487 | 2.85 | Benzeneacetaldehyde |
| 17 | 11.690 | 1.09 | 3-Amino-1,7,7-trimethylbicyclo, Pyrrolidine-5-one-2-propionic acid |
| 18 | 11.812 | 1.53 | Piperazine |
| 19 | 12.098 | 2.64 | 1,2-Benzisothiazol-3-amine, TBDMS derivative |
| 20 | 12.213 | 0.87 | Pyrazine, 2-ethyl-3,5-dimethyl- |
| 21 | 12.451 | 2.31 | 4-Propyl-3-thiosemicarbazide, Pyrazine, 2-methyl-5-(1-propenyl) |
| 22 | 12.899 | 1.07 | L-Serine, methyl ester |
| 23 | 13.204 | 2.65 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- |
| 24 | 13.455 | 0.68 | Hexanoic acid |
| 25 | 13.611 | 1.28 | Ethanone, 1-(4,5-dihydro-2-thiazol |

**Table 2.** Continued.

| Peak | RT | Area% | Name |
|---|---|---|---|
| 26 | 13.958 | 4.01 | 2-Pyridinamine, 4,6-dimethyl- |
| 27 | 14.100 | 1.12 | 2-Pyridinamine, 4,6-dimethyl |
| 28 | 14.922 | 0.59 | Indolizine, 2-(4-methylphenyl)-Carbamic acid |
| 29 | 15.743 | 0.61 | Pyrazine, 2-butyl-3,5-dimethyl- |
| 30 | 15.852 | 3.57 | Ornithine |
| 31 | 16.089 | 0.63 | (Diisopropylamino)ethanol, N-Ethylpiperazine |
| 32 | 16.822 | 7.75 | Niacinamide* |
| 33 | 17.583 | 3.75 | [1,2,4]Triazolo |
| 34 | 18.099 | 3.40 | 1,4-Anhydro-d-galactitol methyl] guanine |
| 35 | 18.357 | 1.52 | Naphthalene |
| 36 | 18.601 | 0.97 | 4-Pyrimidinamine, 2-(methylthio)- Benzene, 1-fluoro-4-nitro- |
| 37 | 18.730 | 1.83 | 2,4-Imidazolidinedione, 5-(2-methy lpropyl)-, (S)-Moclobemide N,N'-Trimethyleneurea |
| 38 | 19.185 | 2.36 | 1-Naphthalenemethanamine, Quinoline, 2-ethyl-3-Methyl-4-phenyl-1H-pyrrole |
| 39 | 22.158 | 1.31 | Scyllo-Inositol, l-Inositol |
| 40 | 23.136 | 2.75 | n-Hexadecanoic acid |
| 41 | 24.826 | 0.78 | Octadec-9-enoic acid, Oleic Acid |
| 42 | 25.023 | 1.05 | Octadecanoic acid |
| 43 | 32.681 | 9.31 | Cholesterol * |

## Results

The results showed that the content analysis of the hydatid cyst fluid caused by *E. granulosus* larvae has differences in its components between sheep and humans (Table 1). The results showed significant differences in concentration of potassium, urea, cholesterol, uric acid, and magnesium in HCFs, whereas no significant differences were found in total protein, glucose, triglyceride, creatinine, calcium and sodium. The results also showed that the concentration of total protein, uric acid, triglyceride, cholesterol, and sodium of hydatid cysts fluid isolated from humans was higher than in sheep. The concentration of glucose, urea, magnesium, calcium, creatinine, and potassium in hydatid cysts fluid was higher in sheep (Table 1).

GC-MS results found 43 peaks in sheep hydatid fluid (Fig. 1, Table 2) compared to 25 peaks in human (Fig. 2, Table 3). In sheep, the highest percentage of contents were Pyrazine (14.70%) and Niacinamide (7.75%). It has several effective compounds such as Ornithine (3.57%), N, N-Dimethylaminoethanol (5.63%), and many other fatty acids (Table 2). In human, the compounds were Formamide, DL-Allothreonine, 1,5-Anhydroglucitol, and Cyanogen chloride as 19.35, 18.93, l9.48, and 4.97%, respectively. In addition, Chloromethyl cyanide (6.60%), Carbonic acid (4.30), cholesterol (3.94%), and many other compounds (Table 3).

## Discussion

Some studies have analyzed hydatid cyst fluid's chemical analysis in humans and animals (McManus1981; Çelik 1989). Chemical substances in the hydatid cyst fluid of *E. granulosus* have an essential role in parasites' metabolism and immunological functions (Thompson & Lymbery 1995; Garippa et al. 2004). These chemical compositions can protect and provide nutritional material. Knowledge of parasite nutrition can identify a new way to prevent hydatid disease by changing the nutrient composition of cyst fluid or blocking nutrition, and metabolic pathways of these elements in the cyst are strictly controlled to meet the requirements of parasite growth (Juyi et al. 2013).

The results indicated the variation in concentrations of some biochemicals of HCFs that may be a result of an increase or decrease in this biochemical component, and this may explain the ability of the parasite to convert some harmful components into unharmful to evade the body's

**Table 3.** Peak, Retention time (RT), and concentration (Area %) of compounds of Human HCF by GC-MS.

| Peak | RT | Area% | Name |
|---|---|---|---|
| 1 | 9.104 | 4.97 | Cyanogen chloride* |
| 2 | 12.118 | 6.60 | Chloromethyl cyanide* |
| 3 | 12.349 | 1.44 | Oxirane, trimethyl- N- Benzenediamine, 4-methoxy-N,.al pha.-dimethyl-, ydrochloride Methyl-3,4 methylenedioxyampheta mine |
| 4 | 13.021 | 0.71 | Butane, 2,2'-thiobis- Diglycerol Pentane, 2-[(1-methylethyl)thio]- |
| 5 | 14.725 | 4.30 | Carbonic acid* |
| 6 | 14.949 | 19.35 | Formamide* |
| 7 | 17.420 | 18.93 | DL-Allothreonine* |
| 8 | 18.716 | 9.48 | 1,5-Anhydroglucitol* |
| 9 | 19.626 | 10.14 | Benzene, (2,2-dimethoxyethyl)- |
| 10 | 19.796 | 0.66 | 2,5-Dihydroxybenzoic acid, 3TMS derivative |
| 11 | 20.047 | 0.73 | Trimethylsilyl [2-(4-chlorophenyl) -4-phenyl-1,3-thiazol-5-yl]acetate 3-Isopropoxy-1,1,1,7,7,7-hexamethy l-3,5,5-tris(trimethylsiloxy)tetra Siloxane Heptasiloxane, 1,1,3,3,5,5,7,7,9,9 ,11,11,13,13-tetradecamethyl |
| 12 | 21.289 | 3.00 | Fumaric acid |
| 13 | 21.391 | 1.10 | Phthalic acid |
| 14 | 21.629 | 3.68 | Ethane, 1,1'-[oxybis(methylenethio)]bis- Benzeneacetonitrile, 4- fluoro- Benzamide, 3-methoxy-N- methyl- |
| 15 | 21.778 | 1.39 | Cyclononasiloxane, octadecamethyl- |
| 16 | 22.437 | 2.52 | 6-(2-Aminophenyl)-1,2,4-triazine-3,5(2H,4H)-dione tritms1,7-Di hexamethyl-1,3,5,7-tetraoxa-2,4,6- trisilaheptane N1-(1 Adamantyl)-4-aminobenzene-1- sulfonamide - |
| 17 | 23.367 | 0.72 | Cyclononasiloxane |
| 18 | 23.462 | 1.06 | Benzamide, 3-methoxy-N-methyl- |
| 19 | 23.794 | 0.60 | Benzothiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-(1-adamantoyl amino)-6-methyl-, ethyl ester |
| 20 | 25.220 | 0.72 | Hexadecanamide |
| 21 | 25.288 | 0.74 | Benzamide, 3-methoxy-N-(3-methoxybenzoyl)- N-butyl-Benzamide |
| 22 | 26.741 | 0.59 | Naphthalene-1-sulfonamide, 4-chlor o- N-(adamantan-1-yl) methyl- Bisphenol A, TBDMS derivative |
| 23 | 26.802 | 2.06 | 9-Octadecenamide, (Z)- |
| 24 | 27.270 | 0.58 | 1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane) |
| 25 | 32.681 | 3.94 | Cholesterol***** |

immunity or some medication (Agosin & Repotto 1967; Nash & Al-Janabi 1980; Aziz 1987; Bowles & McManus 1993). The results showed differences in components of HCFs; these findings do not agree with McManus (1981), who found the similarity between the components of the HCF in sheep and a human.

In GC-MS analysis, the results showed a difference in the number of peaks and components of cystic fluid between humans and sheep, 25 vs. 43 peaks. These differences between the HCF components from different hosts were also reported in previous studies (Jeffs 1988; Huang & Xu 1994). This may be due to the absence of some biochemical receptors in the cyst membrane (Juyi et al. 2013), and our finding does not agree with Aziz et al. (2011) and Al-Ataby (2022). In addition, the differences may be due to the variation of *E. granulosus* strains or the intermediate host, animal age, infection period, history of treatment, geographical location, and food or food behavior. The GC-MS technique has been used before in insects (Thamer 2012), essential oils of some seeds (Al-Maliki 2016), fatty acids, and essential oils of stevia (Al-Tamimi 2021), and bioactive substances extracted from some algae (Khalaf 2012).

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