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Toxicological study of various crude extracts of *Hyoscyamus gallagheri* native to Oman

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ABSTRACT

Cancer is considered a major health issue all over the world for its high morbidity and mortality. Naturally obtained anticancer compounds have comparatively less side-effects, thus it remains a major focus for research. *Hyoscyamus gallagheri* (*H. gallagheri*), is one of the plants which have a useful source of bioactive metabolites. The present study is based on preparing plant extracts from the aerial parts of the herbal drug *H. gallagheri* with various polarities, determining their cytotoxicity and isolating the major cytotoxic compound from the highest active extract. A methanol extract was prepared by the Soxhlet extraction method. Then, it was processed with solvents of increasing polarities. Later, the extracts were evaluated for their cytotoxic activity by brine shrimp lethality assay (BSLA). The highest mass obtained was ethyl acetate and the highest cytotoxicity activity was observed in the ethyl acetate extract, While the lowest was water extract. Ethyl acetate extract was processed for isolation of cytotoxic compounds by different chromatographic methods to give nine fractions which were evaluated by BSLA. Among all, fractions No. 2, 3 and 4 showed significant activity against BSLA and the fractions were purified using preparative thin-layer chromatography (PTLC) to give four compounds (1–4). Only compound 1 showed high purity and appropriateness for NMR analysis. The compound was identified, and the structure was elucidated using 1D and 2D spectroscopy and was found to be 5,7,2′,3′,4′ pentahydroxyflavanone (1). In conclusion, the isolated bioactive compound could be used as an anticancer agent.

1. Introduction

Plants have always had a fundamental role in the cure of many diseases all through history. We only need a glimpse at the ancient civilizations to realize the significance of plants in medicine. An obvious example of this is the use of Unani and Hindu remedy systems which were effective in treating certain diseases and symptoms [1]. However, those systems proved to be less effective with the passage of time. Then synthetic chemistry emerged, as a pressing human need, to satisfy people's growing necessity for medication [2]. That is when plants were introduced to be studied deeply in an attempt to make use of all its chemicals.

The aim is to focus on those plants in different aspects, for instance, botanical, chemical, biological, *etc*. After this, it will be processed *in vitro* assay for identification, extraction, isolation of the chemical compounds and evaluation of its possibility to be used as a medication. Plant-derived drugs have a critical role as anticancer agents and there is a variety of products in the market such as vincristine (Oncovin®) and paclitaxel (Taxol®). Such drugs were discovered when processed *in vitro* for analyzes. Some of which are extremely complex and so it is isolated from other plant materials for better activity. Plant-derived anticancer agents are drawing attention as they are proving to be more effective [3,4].

When attempting to observe the medical development in the recent years, we can obviously state that the medical community is firmly approving that the natural-derived drugs are a preferable option compared to others, thus a strong approach toward plant analysis is noticed due to its diversity, availability, cost-effectiveness, and safety [4].

Ever since the research community has been involved in this approach, the working load is increasing day after day because of the huge number of plants to be analyzed and the continuous discovery of new species. This clearly explains the reason behind the modicum work processed on the selected species. Besides, being recently discovered and geographically restricted. Although, other species belonging to the same family were processed *in vitro* as well as *in vivo* and have proven to be pharmacologically active in many different aspects. A good example is the *Hyoscyamus albus* which, when investigated, was found to have an anticancer activity against different cell lines and also was traditionally

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used as nervous sedative, parasympatholytic, mydriatic, anticholinergic and analgesic and lately its anti-diabetic activity has been confirmed [5,6] while *H. Niger* have a good number of non-alkaloidal substances [7] which possess various pharmacological activity such analgesic, anti-inflammatory, antipyretic, anticonvulsant, spasmolytic, anti-diarrheal, anti-secretory, bronchodilatory, urinary bladder relaxant, hypotensive, cardio depressant, vasodilator, antitumor, and feeding deterrent properties [8–10]. *Hyoscyamus senecionis* has proven to have antimicrobial activity [11]. Generally, *Hyoscyamus* species are known to have a pseudotropine-derived group of alkaloids which have a strong inhibitory activity on ß-glycosidases which is important for cell recognition and can possibly be used in the treatment of some types of cancer [5].

Hyoscyamus gallagheri (*H. gallagheri*) belongs to the family of Solanaceae. It has 14 different species: *H. albus L.* (containing both var.*desertorum*, var. *canariensis*), *H. arenarius Dun.*, *H. aureus L.*, *H. ceratophyllus Fisch.*, *H. niger L.* (containing var. *agrestis*, var. *bohemicus*, var. *pallidus*) *H. physaloides L.*, *H. pseudophysaloides Roth.*, *H. pussilus L.*, *H. reticulatus L.*, *H. senecionis*. *Hyoscyamus* species are found in the Mediterranean region, the Middle East and in Central Asia. Only three species belonging to this family have been found and registered in Dhofar, Oman [2,12,13]. *H. gallagheri* is originated from Oman, Salalah. All the previously mentioned plants' species, including *H. gallagheri* contain a high concentration of hyoscyamine and hyoscine (scopolamine) or both as the major compounds [14]. Regarding the chemicals, they are also found in different natural sources such as Datura herb which consists of up to 0.5 % of total alkaloids, including hyoscine as the main alkaloid while L-hyoscyamine is found but in minor quantities. Because the main chemicals are known to have a sedative effect [10]. It is used in cerebral examinations, preoperative, treatment of asthma and cough. While the hyoscine hydrobromide helps with motion sickness and gastric or duodenal ulcer. Also, *Hyoscyamus* has a decent amount of alkaloids ranging from 0.05 % up to 0.15 % from which 75 % are hyoscyamine as the main chemical in the form of (-) hyoscyamine and atropine. While hyoscine is found only in minor quantities. Therefore, it is used to counteract absorbing because of its purgative effect [10]. It is also effective in treating spasms of the urinary tract. Besides its sedative effect, it has an expectorant, antispasmodic and anti-asthmatic effect [15].

H. gallagheri is an Omani plant that grows in the coastal regions of Salalah. The information of the selected plant species is very limited and the plant is not found in many places around the world. Sir Michael Gallagheri, who works as a Biology counselor for the Omani Museum, discovered it [14]. The first occurrence was on 10th May 1983 from Oman, Plateau above Sharbithat specifically, as a preservative specimen and the data was published by Royal Botanic Garden Edinburgh [12]. College of Pharmacy and Nursing in University of Nizwa is working on the indigenous medicinal plant for the discovery of new drugs which may be used as treatment in various diseases. Traditionally, the Omani people have always been using the selected plant to increase hair density, alleviate dental pain, to relieve snake bite and scorpion sting pain [14]. Because the plant is not found all over the world and it has been discovered recently, there is no evidence that it has been analyzed for cytotoxicity previously. Therefore, the objective of the present study is to evaluate the cytotoxic activity of the prepared crude extract and its fractions of *H. gallagheri* by brine shrimp method. Finally, to isolate the cytotoxic compounds from the significant activity of the crude extract.

1.1. Plant description

H. gallagheri, locally known as Zgaf, is a rare plant that is found in the low sand dunes and gravel desert in the farthest places on the Omani coast between Shuwaymiyah and Sherbithat (two coastal villages). It is a very inhospitable area with rare rain. The highest level of humidity comes from the Indian Ocean dense fog on the beach. It is was described as a long-life fleshy plant that can be found in groups of 1 m wide 25 cm high. The leaves are oval, thick, gray-green with straight edges. Leaves grow densely on the stem. The flowers are attractively purple and conical in shape with yellow spots inside the anther. Flowers are short aged, but the bell-shaped cups with brown lively colors on the plant lasts throughout the whole year. The stem has a few branches, upright or stretched on the ground with a tendency to grow upward. Its lower parts are covered by old leaves. Its pats are furry with short-branched hairs and gland hairs. Leaves follow each other thickly. They are whitish-green, oval, between 3 and 8.5 mm in length. (including the petiole) with sharp blades. Its base rolls up to form indistinguishable petioles. They are thickly covered with silky fur. Flowers upright clusters of about 10 cm. Sepals are tubular in the shape of a bell- 22–33 mm in length. They get on the bigger in the stage of fruiting. It has five unequal segments of which the upper are the biggest. They could be circular or triangular. It ranges from 2 to 5 mm in length and from 3 to 8 mm in width. It could be acute or convex in shape. They are covered with short silky fur. The fruitful sepal has the shape of a wide bell of about 2.5–4 cm. The coriander is pale purple with yellow spots. It is conical in shape. It ranges between 4 and 5 cm in length. The fruit is an oval capsule, $8-15$ mm in length and $5-8$ mm in diameter. It consists of many oval compressed seeds about $1–1.5 \times 0.8-1$ mm and reddish pale brown in color [5,14,16].

1.2. Problem statement

Nowadays, many pieces of research are focusing on finding new medications from natural sources because of their advantages when compared to synthetic drugs. Oman is blessed with a reasonable number of rare species that can make a good base to be used in researches of new medicines. *H. gallagheri* is one of the rarest medicinal plants which is found in Oman and has no evidence that it was analyzed for cytotoxic activity before. Although the plant has been used traditionally and well-known in this region, it is not widely recognized by a large number of people due to its limited geographical distribution. The ethnic communities of Oman use medicinal plants and formulated products as herbal medicine to treat curable and incurable diseases. Throughout history, it has proven to be effective on many different levels. Therefore, aim is to test the cytotoxicity of one of these plants and introduce it to be recognized globally to be utilized and manufactured as a remedy. This study may be considered as a small contribution from an Omani institution to the research community worldwide. There is no recorded evidence of any cytotoxic compounds found in *H. gallagheri* growing in Oman. Therefore, our team is directly involved to develop the method for the extraction, identification and quantification of indigenous medicinal plants that will be used for standardizing, fingerprinting and commercializing the new plant-related drugs.

2. Materials and methods

2.1. Sample collection

The selected plant sample was collected from Salalah, near Yemen borders at the end of October 2018 in the morning time around 8 a.m.. The aerial parts of the plant were separated instantly from the roots, then they were kept in a plastic bag and transported to the research laboratory. The collected plant was identified by Dr. Syed Abdullah Gilani, College of Arts and Sciences, at the University of Nizwa. They were kept at room temperature, washed and dried [17].

2.2. Extraction

The dried aerial parts of *H. gallagheri* (700 gm) were milled into coarse powder form and then extracted with methanol (2 L) by using a Soxhlet extractor for 36 h. Later, the Methanol extract was processed

for the solvent to be evaporated with the aid of the rotatory evaporator at 24 °C by reduced pressure to give the extract (59.34 gm, yield 8.47 %). The paste of methanol extract (31 gm) was suspended in water and extracted successively with hexane, chloroform, ethyl acetate, and butanol to give the corresponding: hexane, chloroform, ethyl acetate, and butanol-soluble fractions, respectively. Finally, the part of the water fraction was also evaporated to give a water extract [17,18].

2.3. Cytotoxic activity bioassay

The cytotoxic activity of the prepared plant extracts was measured by using brine shrimp lethality (BSL) bioassay technique [19]. A stock concentration of 1000 µg/mL in dimethyl sulfoxide (DMSO) was prepared and diluted to make different serial concentrations (500, 100, 50, 25 and10 µg/mL). DMSO is considered the least toxic solvent to be used in cytotoxic experiments thus it will not have a negative impact on the experiment. Seawater was prepared by dissolving 38.2 gm of sea salt into 1 L of distilled water. Then brine shrimp eggs were hatched in the previously prepared seawater and kept in a closed container for 24 -hs from nauplius to be hatched. That active nauplius was used for the cytotoxicity bioassay of the various extracts. Ten nauplii (10) were placed in each test tube along with 500 µl of a diluted solution of each polarity extract and 4.5 mL of seawater. Next, it was kept for 24 h for incubation. The next day, the active shrimp was counted in each tube to calculate the lethality percentage by the standard protocol described by Weli et al. [19].

The mortality (%) of each experimental dose and the positive and negative controls were evaluated by using the formula:

$$\text{Mortality (\%)} = \frac{\text{No. of dead nauplii}}{\text{Total number}} \times 100$$

2.4. Isolation of cytotoxic compounds by column chromatography

The highest active extract, ethyl acetate, was used for the separation of compounds using different chromatographic techniques. Initially, the column was packed by silica gel with a non-polar solvent of hexane. The ethyl acetate extract (1.5 gm) was subjected to column chromatography on silica gel for separating compounds. The eluted was ethyl acetate: hexane (6:4) solvent system. Then we increased polarity by adding methanol to the previous system (10:100), the eluent was collected in 169 test tubes.

2.5. Cytotoxic activity of the isolated fraction

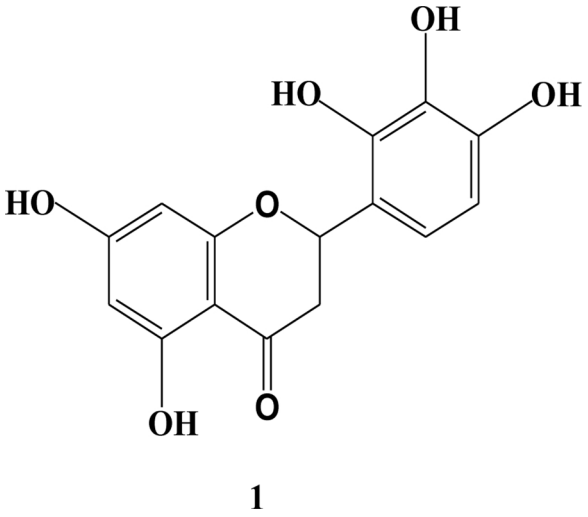
The ethyl acetate derived from different fractions obtained from the column was measured for their cytotoxicity by using brine shrimp lethality (BSL) bioassay technique [5]. Each fraction was by dimethyl sulfoxide (DMSO). Similarly, ten nauplii (10) were placed in each concentration test tubes along with 500 µl of the diluted solution of each fraction and 4.5 mL of seawater. Finally, all the samples test tubes were incubated for 24 h. Finally, the alive shrimp was determined by counting the surviving nauplii in each tube to calculate the lethality percentage by the standard protocol.

2.6. Separation of compounds by PTLC

Fraction 2, 3 and 4 were combined and purified by preparative TLC over silica gel GF_{254} using hexane/ethyl acetate (1:1) as the developing solvent to give 4 compounds **1** (19.3 mg), compound **2** (27.7 mg), compound 3 (17.7 mg), and compound 4 (39.3 mg).

2.7. Compound 1

The purified compound was tried to crystallize by using different solvents. However, it was not crystallized by any solvent. It is a white solid (11.3 mg); R_f 0.59 (ethyl acetate-hexane; 6:1); ^{1}H-NMR (δ values, DMSO-d_6, Bruker, USA): 2.25 (dd, 1H, J = 12.9 &17.1 Hz, H-2), 3.25 (dd, 1H, J = 2.88 & 17.1 Hz, H-3), 5.42 (dd, 1H, J = 2.7 & 12.7 Hz, H-2), 5.43 (s, 1H, H-6), 5.87 (s, 1H, H-8), 6.78 (d, 1H, J = 8.4 Hz, H-2′), 7.30 (d, 1H, J = 8.4 Hz, H-6′), 9.59 (s, 1H, 4′−OH), 10.83 (s, 1H, 5′−OH), 12.13 (s, 1H, 5−OH). ^{13}C–NMR (δ values, DMSO-d_6): 196.4 (C-4), 166.67 (C-5), 163.51 (C-7), 162.96 (C-10), 157.74 (C-4′), 128.28 (C-6′), 128.89 (C-5′), 115.20 (C-1′), 101.81 (H-9), 95.82 (C-8), 78.45 (C-2), 42.00 (C-3), 95.82 (C-2′), 95.00 (C-3′). On the basis of spectral data, compound 1 was identified as 5,7,2′,3′,4′-pentahydroxyflavanone (1, Fig. 1).



1

Fig. 1. Structure of compound 1.

3. Results

3.1. Yield of extracts

The powders, obtained from the aerial parts, were extracted with methanol by a hot extraction method for 36 h and the solvent-free residue was partitioned by different solvents with increasing polarity. The approximate yields and their percentage of the yield of each individual extract are given in Table 1.

3.2. Cytotoxic activity

All extracts of *H. gallagheri* were evaluated for their cytotoxic activity by brine shrimp lethality (BSL) assays reported by several authors [5,19]. Among the aerial extracts at different concentrations of each extract have shown activity against the brine shrimp larvae. The percentage mortality and LD_{50} values against shrimp larvae exposed to various extracts at different concentrations of *H. gallagheri* are shown in Table 2,3.

Table 1
Amount and percentage (%) of yield of each extract of *H. gallagheri*.

Extract	Amount (gm)	Yield (%)
Methanol	31.05	8.47
Hexane	3	9.66
Chloroform	11.2	36.06
Ethyl acetate	6.8	21.90
Butanol	4.88	15.71
Water	3.88	12.49

Table 2
The percentage of mortality against shrimp larvae exposed to various extracts at different concentrations of *H. gallagheri*.

Conc. (µg/mL)	Hexane	Chloroform	Ethyl acetate	Butanol	Methanol	Water	DMSO	Sea water
10	10	0	0	0	10	10	0	0
25	10	10	10	20	10	10	0	0
50	30	20	20	20	20	10	0	0
100	30	20	40	30	30	20	0	0
500	40	30	50	30	30	20	0	0
1000	50	40	60	50	40	30	0	0

Each value is a mean of three biological replicates.

Table 3
IC_{50} values against shrimp larvae exposed to various extracts.

Extract	IC_{50} values (µg/mL)
Methanol	5714.79 ± 0.43
Hexane	1119.48 ± 0.21
Chloroform	4226.68 ± 0.97
Ethyl acetate	421.69 ± 0.10
Butanol	2523.48 ± 0.09
Water	261035.80 ± 0.28

Each value is a mean of three biological replicates.

3.3. Isolation cytotoxic compounds by column chromatography

All the test tubes were checked by TLC and the test tubes of the same R_f value were combined together and a total of nine fractions were obtained [Fraction 1 (309 mg); Fraction 2 (122 mg); Fraction 3 (83 mg); Fraction 4 (76 mg) Fraction 5 (182 mg); Fraction 6 (21 mg); Fraction 7 (54 mg); Fraction 8 (361 mg); Fraction 9 (53 mg)].

3.4. Cytotoxic activity of various fractions

All the fractions isolated from the column were checked for cytotoxicity against the brine shrimp lethality method described before [5,19]. Among the fractions, only fractions 2, 3,4 and 9 gave a promising activity against cytotoxicity which is presented in Table 4.

3.5. Purification of cytotoxic compounds

After column chromatography, the ethyl extract gave nine fractions (1–5). All the isolated fractions were impure. On the basis of TLC, we selected fractions 2, 3 and 4 for further separation due to the number of compounds and as the weight of the tired and fourth compound is very low. All three fractions were combined together and separated by PTLC and characterized on the basis of ^{l}H-NMR, ^{13}C-NMR, DEPT 90 and 135, and HMBC.

Table 4
The percentage of mortality against shrimp larvae exposed to different fractions of *H. gallagheri*.

Fractions	Mortality percentage
1	0
2	100
3	100
4	80
5	0
6	0
7	0
8	0
9	70
DMSO	0
Sea water	0

Each value is a mean of three biological replicates.

4. Discussion

A considerable percentage of the drugs currently available in the medical markets for the treatment of different stages of cancer are naturally obtained. The statistics show that about 60 % of the new chemical ingredient (NCIs) presented between the years 1981 and 2002 in this field was naturally obtained [20]. The traditional medicinal plant is one of the essential sources to obtain a new drug. So, the screening of medicinal plants has great importance to establish new plants and to isolate new ingredients to manage life-threatening diseases like cancer [17]. Brine shrimp lethality (BSL) bioassay is one of the rapid, simple and cheap methods for screening the cytotoxic activity.

The highest yield was obtained from methanol extract and it was about 8.47 % from 700 gm of aerial powder of *H. gallagheri* (Table 1). After the solvent-solvent partition, the highest amount was obtained from chloroform and the lowest was in hexane (Table 1). Therefore, the aerial parts of *H. gallagheri* contained high amounts of relatively polar constituents present in the chloroform extract.

Among the six prepared different polarities aerial extracts: hexane, chloroform, ethyl acetate, butanol, methanol, and water extracts. Ethyl acetate has displayed the most significant activity against the brine shrimp larvae. The mortality percentages of the shrimp larvae exposed to different aerial extracts of *H. gallagheri* are shown in Table 2. The order of activity was ethyl acetate > hexane > butanol > chloroform > methanol > water extract. An obvious observation, there was an increase in the average percentage of mortality when increasing the concentration of aerial extract. Ethyl acetate extracts gave the highest LD_{50} value = 421.69 µg/mL (Table 3). According to the definition of LD_{50}, a higher LD_{50} value means it is less toxic. That means water and hydro-alcohol extracts contain less toxic compounds. The highest LD_{50} value was obtained from the water extract compared to negative control DMSO While the ethyl acetate extract is the most potent toxic obtained from the aerial extract among the six prepared extracts from the *H. gallagheri*. (Table 2 and 3). Later, the ethyl acetate extract was subjected to column chromatography over silica gel for the isolation of compounds. The column chromatography was initiated by the addition of silica powder and hexane. Then the extract was introduced after dissolving it in a system of hexane: ethyl acetate (4:6). When the solvent started moving, the collection of compounds in the test tubes was started. Approximately 3 mL in each test tube or depending on color intensity. Later the polarity was increased by the addition of methanol to the previous system (10:100). A number of 169 test tubes were collected and checked by TLC to combine the test tubes of the same R_f values. The BSL method was reapplied to the combined fractions and only 5 fractions gave promising results. Fraction 2 was chosen for purification as it gave 100 % mortality (Table 4). Fraction 2 was further purified by preparative TLC and gave 4 compounds. Out of which only compound 1 turned out to be pure and proper for analysis after TLC examination. Thus, it was introduced to NMR for analysis.

The ^{1}H NMR spectrum of compound **1** revealed the presence of a chelated hydroxyl signal at δ 12.13 for the hydroxyl group connected to C-5. Two sharp singlets at δ 10.83 and δ 9.59 which indicated for two hydroxyl protons at the carbon position C-4′ and C-5′. Two singlets at δ

5.43 and 5.87 which indicated the presence for one proton each are characteristic of phenyl ring protons of the flavonoid nucleus (H-6 and H-8). Three doublets of doublets at the position of δ 5.42 (J = 2.7 & 12.7 Hz), δ 2.25 (J = 2.7 & 12.7 Hz), δ 3.25 (J = 2.88 & 17.1 Hz), indicated the presence of a methine proton (H-2) and two germinal protons H-3a and H-3b, respectively. In addition, two doublet signals at δ 6.78 and δ 7.30 were attributed to H-2′and H-3′protons in the aromatic ring. Furthermore, the analysis of ^{1}H-^{1}H COSY spectrum showed all anticipated correlations for the suggested structure. The ^{13}C NMR spectrum showed fifteen signals that mean it contains 15 carbons. The signal at δ 196.4 indicates characteristic absorption of the carbonyl carbon at C-4. The signal at δ 78.45 indicated the presence of C-2 carbon of flavonoid. The other characteristic signals were observed at δ 162.96 and δ 101.81 which is indicated the C-9 and C-10 carbons, respectively. The aromatic phenolic carbons were shown at the position δ 166.67, 163.51, 157.74, 128.89, and 95.00 was indicated the presence of respectively C-5, C-7, C-5′, C-4′ and C-3′ of the flavonoid skeleton. The DEPT 90 and DEPT 135 spectra that showed the presence of five CH groups and a single methylene carbon. The methine signals were attributed to C-2, C-6, C-8, C-2′, and C-3′while the methylene was assigned to C-3 carbon. The HMBC spectrum contained many correlations that could be attributed to the isolated molecule. For instance, the methine proton resonating at δ 5.42 (1H, dd, J = 2.7 & 12.7 Hz) showed an HMBC correlation to carbons signals appeared at δ 196.4 (C-4), 95.00 (C-3), 115.20 (C-1′) and 95.82 (C-2′). On the basis of the above spectra data, the isolated compound 1 was tentatively identified as 5, 7, 3′, 4′, 5′-pentahydroxyflavanone. This structure will be confirmed once we obtain its HRMS. There are no reports and data available on the selected species. Therefore, we are unable to compare our results with the reported data due to the lack of literature on the toxicological activity of *H. gallagheri* but *Hyoscyamus* family members have remarkable results. For example, cannabisins D and G obtained from *H. Niger* seeds have proven to have moderate cytotoxic activity in cultured human prostate cancer cells and high activity against human lung cancer with LD_{50} of 66 μg/mL. While other research was conducted by *H. niger* is grown in Iraq found all plant parts to be very toxic [21,22]. While *H. reticulates* have clinical records to be toxic as there were several cases of *H. reticulates* poisoning that was introduced to the hospitals among which 19 Bedouin children between the age of 4–8 years and 6 female cases between the age of 19–49 [23]. Withanolides, a group of C28 ergostane-type steroids found in the Solanaceae family, including *Hyoscyamus,* upon research proved to have selective antitumor activity against the MG-63 cell line [24]. *H. albus* was found to have significant anticancer activity [24].

5. Conclusion

The cytotoxic activity of *H. gallagheri* was measured by well-established bioassays. All the six aerial extracts of *H. gallagheri* showed various cytotoxic activity against brine shrimp larvae (BSL). Among them, ethyl acetate aerial extract showed the highest toxic activity which upon further analysis and purification using different chromatographic system, spectral data analysis gave 5,7,2′,3′,4′-pentahydroxyflavanone (1). Therefore, the isolated compound could be used as an anticancer agent.

CRediT authorship contribution statement

Doha M. Aboalola: Data curation, Investigation. **Afaf M. Weli:** Resources, Writing - original draft. **Mohammad A. Hossain:** Conceptualization, Project administration, Supervision. **S. Al Touby:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they do not have any competing financial interests or institutional and personal relationships that could have appeared to influence the work reported in this paper.

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