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Biological and toxicological evaluation of aerial parts extracts of locally grown *Cleome austroarabica*

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ABSTRACT

The current study was conducted to prepare different polarities extracts and evaluate their antimicrobial and cytotoxic activities from the aerial parts of *Cleome austroarabica* (*C. austroarabica*) which was collected from the initial campus of the University of Nizwa. The coarse powder was extracted with direct methanol by using a maceration method. The extract residue was fractionated by using various organic solvents with increasing polarity. The agar disc and brine shrimp lethality (BSL) bioassays were used to assess the antimicrobial and cytotoxic activities of the aerial extracts of *C. austroarabica.* Four concentrations (2000, 1000, 500, 250 μg/ml) of each extract were used to determine the antimicrobial activity against two Gram (+) bacteria: *Staphylococcus aurous* (*S. aurous*), *Enterococcus faecalis* (*E. aurous*) and two Gram (−) bacteria: *Haemophilus Influenza* (*H. Influenza*), *and Escherichia coli* (E. *coli*). Brine shrimp organisms were used to assess the cytotoxic activities of various polarities aerial extracts and the fractions of chloroform extract. The antimicrobial activity results showed that all polarities extracts at different concentrations did not give any activity against the tested bacteria. The cytotoxic activity of all polarities aerial extracts displayed activity within the value of LC_{50} 385.25–640.25 μg/ml in the order of chloroform > hexane > hydro alcoholic > ethyl acetate > methanol extract. However, the isolated all chloroform fractions showed high LC_{50} activity compared to control. The further extensive study will be needed to confirm the antimicrobial and cytotoxic activities of the crude extracts and to isolate the active ingredients from the highest activity aerial extracts.

1. Introduction

Cancer is an incurable disease. Recently, it is global burden and it has a major impact on communities across the world. It is a multiple disease involving abnormal cell growth and rapidly spread to the other parts of the human body (Greenwell and Rahman, 2015). The most commonly listed cancers globally available are, breast cancer, lung cancer, prostate cancer, pancreatic cancer, colorectal cancer, leukemia cancer etc. The major causes of cancer are tobacco, alcohol, obesity, poor diet, lack of physical exercise, radiation, environmental pollution, and different types of virus and bacterial infections (Roy et al., 2017). Nowadays, these diseases are creating a main health problem in the third world countries. The demand for new drugs to prevent or cure these diseases is abnormally increasing. Plants as traditional medicines are one of the basic and needful sources to provide remedies continuously to save people since the ancient times. People prefer plant derived remedies to treat those incurable diseases without side effect. Some of the plants and plant ingredients showed the potential role against cancer treatment. Therefore, the scientists are working on the natural sources to find out the cancer remedies to treat different types of cancer (Willis, 1996).

Cleome L. is a large genus included 150 species which grown well in the tropical and subtropical countries (Stevens, 2012). One of the most interesting plant family that is under the researchers lance is Cleomaceae family. They are interested, especially on

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Cleome genus because the selected genus extracts showed significant anticancer and antimicrobial activities (Bose et al., 2011).

The Cleome plant is a genus of flowering plants also belongs to Cleomaceae family. Previously, it had been placed in the same family. However, DNA studies found that the *Cleomaceae* genera is closely related to Brassicaceae family than Capparaceae family (Samout et al., 2015). The selected plant is a herbaceous sticky plant with unpleasant odor (Abdullah et al., 2016). The *C. austroarabica* is locally known as Muqabil al shams in Arabic (Ravindra, 2010). The selected plant is endemic to Southern Arabia, including Oman. In Oman, there are 12 *Cleome* species available. The height of the selected plant is about 1 m (Fig. 1). The leaves are alternate and the shape is oval to round with entire margins. It is fully covered with sticky glands (Ravindra, 2010). The flowers are a pale yellow colour and the petals folded back with yellow stamens (Ravindra, 2010). The flowering period throughout the year. The fruits and seeds are oblong and erect capsule size (Ravindra, 2010). According to the literature showed that many plant species belong to this genus are very rich content of essential oils. Several compounds which are responsible for biological activities such as terpenes, sterols, flavonoids, glucosinolates and isothiocyanates were found in various Cleome species (Jordheim et al., 2009; Chopra et al., 1972). In Oman, it is used traditionally as an eye drops to treat cataracts (Ghazanfar, 1999). Most of the *Cleome* species are generally used as folk medicine for stomach aches, scabies, inflammation, rheumatism and cytotoxic (Tsichritzis et al., 1993; Ahmad et al., 1990; Nagaya et al., 1997). However, as an extract of the entire plant is used in Southern Arabia to treat cataracts (Ghazanfar, 1999). In the literature, there are no reports available on the *C. austroarabica* for the determination of phytochemical, biological and toxicological activities. Therefore, the target of this current study is to prepare various polarities aerial extracts and to estimate their antimicrobial and cytotoxic activities by the available disc diffusion and brine shrimp lethality (BSL) bioassays.

2. Materials and method

2.1. Chemicals and materials

The standards and chemicals such as chloroform, dimethyl sulphoxide (DMSO, purity 99%) and methanol were used in this study obtained from Fisher Chemical Company, UK. Hexane was collected from Daejung, Korea. Ethyl acetate was collected from Carbon Group, Ireland. Filter papers were purchased from Whatman, UK. The control ciprofloxacin antibiotic, silica gel and TLC plate were obtained from E. Merck, Germany. Brine shrimp eggs was purchased from the USA. Sea water was collected from Wave beach, Muscat, Sultanate of Oman.

2.2. Microorganism

The clinically isolated selected microorganism such as *Escherichia coli* (*E. coli*), *Haemophilus influenza* (*H. influenza*), *Enterococcus faecalis* (*E. faecalis*), and *Staphylococcus aureus* (*S. aureus*) were obtained from one of local hospitals in Nizwa, Sultanate of Oman.

2.3. Sample collection

The aerial parts of *C. austroarabica* were collected from the initial campus of the University of Nizwa, Birkat Al Mouz, Nizwa, Oman, in November 2016. The morphological authentication was done using the microscopic method by Dr. Syed Abdullah Gilani, Department of Biological Sciences and Chemistry, College of Arts and Sciences, University of Nizwa. The collected aerial parts were cleaned and dried at ambient temperature.

2.4. Extraction

The dried plant was ground into coarse powder by using blender machines and the yield was around 414.1 gm. The powdered samples (400 gm) were macerated in about 1 L of methanol solvent for one week (Hossain et al., 2013). The extracts were filtered by using a Buchner funnel and concentrated at 40 °C using a rotary evaporator to give yield 37.35 gm. Then the residue (35 gm) was suspended in ethanol and water mixture (1:1) and finally fractioned with hexane, chloroform, and ethyl acetate consecutively to give the corresponding extracts. The mother solvent was evaporated completely and the weight of each residue was recorded.

2.5. Antibacterial activity

The antibacterial activity of each polarity aerial extract of *C. austroarabica* was evaluated by using the disc diffusion method (Abdullah et al., 2016; Matani et al., 2015). In this experiment, four

Fig. 1. Plant of *Cleome austroarabica*.

concentrations of each polarity extract (2000, 1000, 500, 250 μg/ml) were prepared by the dilution method using dimethyl sulphoxide (DMSO). Filter paper discs of 5 mm diameter size were dipped in each concentration. The discs were put on agar plates that were inoculated with the clinically isolated Gram (+) positive and Gram (−) bacteria. The solvent DMSO as a negative control and ciprofloxacin (250 μg/ml) as a positive control were used. After inoculated, the plates were kept in the incubator for 24 h at 37 °C. An antibacterial activity of each concentration of each polarity extract was evaluated manually by measuring the diameter of the inhibited zone.

2.6. Cytotoxic activity

The toxicity of each prepared aerial extract of *C. austroarabica* was done by using shrimp assay, which is usually called as sea monkeys. Shrimp invertebrate organisms about 1 mm in size with brownish colour was used for the calculation of cytotoxic activity (Lieberman, 1999).

2.6.1. Hatching of shrimp larvae

About (50 mg) of cysts (shrimp eggs) was placed into the sea water which were taken in a plastic container which was divided into two compartments with few holes. The compartment was separated into two parts by a polyethylene glycol wall. One compartment of the plastic container was covered with aluminium foil to create artificial darkness and the other compartment under light. Both the compartments were maintained at an ambient temperature. After hatching shrimp mature nauplii were attracted to the other lighted compartment that was illuminated. These nauplii was taken for the cytotoxic bioassay.

2.6.2. Brine shrimp lethality assay

The cytotoxic activity of various aerial extracts of *C. austroarabica* was evaluated by well-established BSL assay (Matani et al., 2015; Rehab and Hossain, 2016). Five different concentrations such as 10, 100, 250, and 500 μg/ml of each extract were prepared by using DMSO. After properly labelling the glass vials, 0.1 ml of each concentration was placed in each vial and the volume was adjusted to five millilitres by using the sea water. Then, ten nauplii were added to each vial with the help of a dropper. Similar concentrations of potassium permanganate were prepared in the same way without sample and used as a positive control. DMSO (0.1 ml), nauplii (10 nos) and 4.9 ml of sea water in a vial was used as a negative control. All experimental vials were kept under the light for 24 h. If the nauplii did not exhibit any internal or external movement during the time of observation then it was considered dead. Each concentration vial and positive and negative control were checked by using a magnifying glass. The number of surviving nauplii was counted after 24 hrs. The mortality (%) was calculated at each concentration and usually expressed as a median lethal concentration (LC_{50}). Probit analysis method was used to calculate the LC_{50} value (Finney, 1971). The LC_{50} value represents the concentration of the chemical that produces death in half of the subjects after a certain exposure period.

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

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

2.7. Fractionation of lowest LC_{50} by column chromatography

The chloroform aerial extract gave lowest LC_{50} value. The chloroform extract (5 gm) was subjected to column chromatography over silica gel (25 × 75 cm, 100 gm) by using *n*-hexane, *n*-hexane: chloroform, chloroform, chloroform: ethyl acetate, ethyl acetate, ethyl acetate: methanol mixtures as a mobile phase (Sohail et al., 2017). All collected fractions were on TLC and those with similar pattern were combined and screened for their cytotoxic activity.

3. Results and discussion

3.1. Yield of extracts

The aerial course powdered of *C. austroarabica* were extracted directly with methanol by maceration method. The residue was reextracted by different solvents with increasing polarity. The approximate yields and their percentage of yield of each individual extract are given in Table 1. The highest percentage of the yield of methanol extract was about 10.6% obtained from 414.1 gm of aerial parts of coarse powder of *C. austroarabica* (Table 1). After fractionation, the amount and yield of hexane and chloroform was higher than the ethyl acetate and hydro alcoholic extracts (Table 1). Therefore, the aerial parts of *C. austroarabica* contained relatively higher amounts of nonpolar ingredients compared to the polar fractions.

3.2. Antimicrobial activity

The activity of methanol extract and its derived different polarities aerial fractions of *C. austroarabica* were determined against clinically isolated Gram (+and −) bacterial strains (Najwa and Hossain, 2018). In our current experiment, two Gram (+) bacteria: *Staphylococcus aurous* (*S. aurous*), *Enterococcus faecalis* (*E. aurous*) and two Gram (−) bacteria: *Haemophilus Influenza* (*H. Influenza*), *and Escherichia coli* (*E. coli*) were used against various polarities aerial extracts at different concentrations. Unfortunately, the experimental results showed that there was no growth inhibition obtained against the tested Gram (+and −) bacteria at any concentrations (Table 2). However, the ethanol extract of leaves and flowers of other species of *C. viscosa* belongs to this family showed activity against *E. coli, P. vulgaris* and *P. aeruginosa* (Abreu et al., 2013). Another study conducted by Muhaidat et al on the essential oils of *C. droserifolia* and *C. trinervia* species belong to the same family and showed that it gave a significant inhibition against different pathogen bacterial strains (Muhaidat et al., 2015). But, the selected plant species which were grown in Oman did not give any activity, it could be due to the sensitivity of applied bacterial strains or the dose of the extract samples (Najwa and Hossain, 2018). There is no reports and data available on antimicrobial studies of the selected species in Oman. Therefore, we are unable to compare our results with the reported values.

3.3. Cytotoxic activity

The cytotoxicity of the prepared aerial extracts of *C. austroarabica* was evaluated by brine shrimp lethality (BSL) assays reported by several authors (Matani et al., 2015; Rehab and Hossain, 2016). All the prepared aerial extracts at different concentrations have shown activity against the brine shrimp larvae. Among the five

Table 1
Amount and percentage (%) of yield of each extract of *C. austroarabica*.

Extract	Amount (gm)	Yield (%)
Methanol	44.3 ± 0.09	10.6
Hexane	8.5 ± 0.19	2.05
Chloroform	11.5 ± 0.27	2.87
Ethyl acetate	0.97 ± 0.51	0.23
Hydro alcoholic	11.6 ± 0.18	2.80

Table 2
The antimicrobial activity of different polarities aerial extracts of *C. austroarabica* against the selected bacteria strains.

Extract	Conc. (μg\m)	*E. coli*	*H. influnza*	*E. faecalis*	*S. aureus*
Methanol	250	nd	nd	nd	nd
	500	nd	nd	nd	nd
	1000	nd	nd	nd	nd
	2000	nd	nd	nd	
Ciprofloxacin	250	30	22	23	23
Hexane	250	nd	nd	nd	nd
	500	nd	nd	nd	nd
	1000	nd	nd	nd	nd
	2000	nd	nd	nd	nd
Ciprofloxacin	250	31	22	20	21
Chloroform	250	nd	nd	nd	nd
	500	nd	nd	nd	nd
	1000	nd	nd	nd	nd
	2000	nd	nd	nd	nd
Ciprofloxacin	250	27	22	23	20
Ethyl acetate	250	nd	nd	nd	nd
	500	nd	nd	nd	nd
	1000	nd	nd	nd	nd
	2000	nd	nd	nd	nd
Ciprofloxacin	250	29	26	23	21
Hydro alcoholic	250	nd	nd	nd	nd
	500	nd	nd	nd	nd
	1000	nd	nd	nd	nd
	2000	nd	nd	nd	nd
Ciprofloxacin	250	29	30	23	21

nd = not detected.

prepared different polarities aerial extracts, the hexane, chloroform, ethyl acetate and hydro alcoholic extracts have displayed significant activity against the brine shrimp larvae. The mortality percentages of the shrimp larvae exposed to different aerial extracts of *C. austroarabica* are shown in Table 3. The order of activity was chloroform > methanol > ethyl acetate > hexane > hydro alcoholic extract. Moreover, there is an increase in the mean percentage of mortality with increase in concentration of aerial extract. Both the hexane and hydro alcoholic extracts gave the highest LC_{50} value 640.26 μg/ml (Table 3). According to the definition of LC_{50}, the high LC_{50} value means it has less toxicity. That means, both the hexane and hydro alcoholic extracts contained less toxic compounds (high LC_{50} value means less toxic). The minimum LC_{50} value was obtained from the chloroform extracts compared to positive control $KMNO_4$ (Fig. 2). In our present experiment, the chloroform extract showed the lowest LC_{50} value. Therefore, the chloroform extract is the most potent toxic aerial extract among the five prepared extracts from the *C. austroarabica*.

According to the LC_{50} value, chloroform extract was selected for the separation of toxic ingredients. The column chromatography method was used to separate the ingredients from the chloroform extract. After fractionation of the chloroform extract by column

Table 3
Percentage mortality (%) of brine shrimp larvae for different polarity aerial extracts of *C. austroarabica.*

Extract Conc. (μg/ml)	Mean percent mortality of brine shrimp larvae (%)						
	Standard $KMnO_4$	Methanol	Hexane	Chloroform	Ethyl acetate	Hydro alcoholic	DMSO
500	60 ± 0.19	60 ± 0.17	40 ± 0.09	60 ± 0.25	60 ± 0.17	40 ± 0.18	0
250	50 ± 0.10	30 ± 0.12	30 ± 0.18	40 ± 0.18	30 ± 0.55	30 ± 0.27	0
100	30 ± 0.23	20 ± 0.14	20 ± 0.15	20 ± 0.44	20 ± 0.21	20 ± 0.42	0
10	30 ± 0.08	10 ± 0.45	10 ± 0.29	0	10 ± 0.44	10 ± 0.76	0

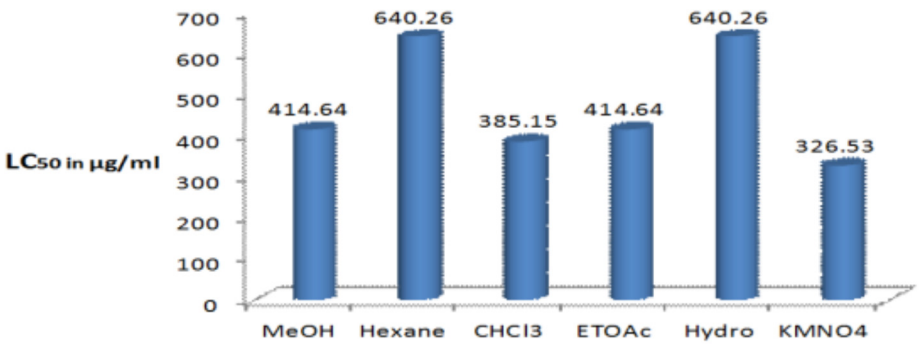

Fig. 2. Comparison of LC_{50} values of methanol (MeOH), hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), hydro alcoholic (Hydro) and potassium permanganate ($KMNO_4$).

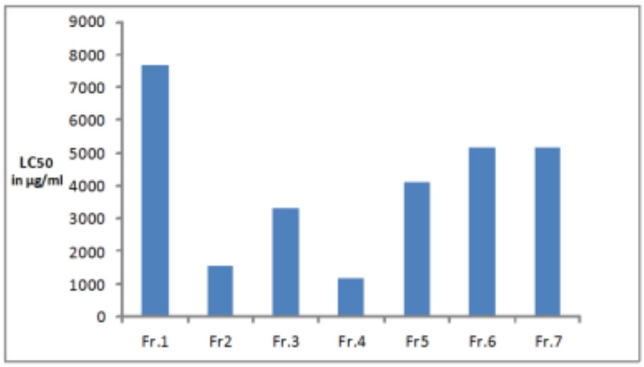

Fig. 3. LC_{50} values of different fractions of chloroform extract.

chromatography, seven fractions (Fraction 1 (0.45gm); Fraction 2 (0.15gm), Fraction 3 (0.14gm), Fraction 4 (0.47gm), Fraction 5 (0.42gm), Fraction 6 (0.48gm) and Fraction 7 (0.2 gm) were obtained by using the mobile phase e.g., *n*-hexane, *n*-hexane: chloroform, chloroform, chloroform: ethyl acetate, ethyl acetate, and ethyl acetate: methanol mixtures. All the isolated fractions from chloroform aerial extract were tested for toxicity in the same way mentioned by using brine shrimp napulii. All chloroform fractions gave cytotoxic activity at very high concentrations as compared to the original chloroform extract (Fig. 3). Some cytotoxic activity was done by the authors of the other species such as *C. droserifolia, C. viscosa* and *C. burmanni* belongs to the selected family. They reported that the *C. droserifolia* plant species showed significant activity against breast cancer cell (MGF7) and colon adenocarcinoma (HCT116) (Ezzat and Adel Motaal, 2012). Another study conducted on cytotoxic activity on the *C. viscosa* and *C. burmanni* species by Pillali and Nair. They mentioned that methanol extracts of both the plant species exhibited significant toxicity against the shrimp nauplii when compared to standard potassium permanganate (positive control) and thus is possibly a good indicator of toxicity (Pillai and Nair, 2011). 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 *C. austroarabica.*

4. Conclusion

The pharmacological (antimicrobial activity) and toxicological (cytotoxic activity) were measured by well-established bioassays. Among the five extracts from *C. austroarabica,* none of them showed antibacterial activity due to the sensitivity and the dose. However, all five aerial extracts at various doses showed cytotoxic activities against brine shrimp larvae (BSL). Among them chloroform aerial extract showed the highest toxicity. In addition, seven chloroform fractions obtained from the chloroform extract by column also showed significant activities. Therefore, further comprehensive phytochemical and pharmacological analyses are needed for the selected locally grown plant of C. *austroarabica* for the consideration of this plant as medicinal plant. It is also needed to isolate and identify the toxic ingredients which will be the pharmacological and toxicological potential for the treatment of diseases.

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