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Original Article

PHYTOCHEMICAL, ANTI-INFLAMMATORY, ANTIOXIDANT, CYTOTOXIC AND ANTIBACTERIAL STUDY OF *CAPPARIS CARTILAGINEA* DECNEFROM YEMEN

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ABSTRACT

Objective: To investigate phytochemicals and biological activities of *Capparis cartilaginea* extracts.

Methods: The methanolic extracts of leaves, stem and twigs of *C. cartilaginea* were screened for their phytochemicals. The essential oil of the leaves was hydrodistilled by a Clevenger apparatus and analyzed by gas chromatography-mass spectrometry (GC-MS). The leaves extract of *C. cartilaginea* was evaluated for its anti-inflammatory effect, using formalin-induced paw edema. The leaves, stem and twigs extracts were assessed for their antioxidant activity, using free radical scavenging assay, cytotoxic activity, using 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay and antibacterial activity, using the microdilution method.

Results: All extracts of *C. cartilaginea* contained alkaloids, carbohydrates, protein, coumarin, phytosterols, bitter principles, phenols and tannins. The essential oil of the leaves was mainly composed of isopropyl isothiocyanate (69.4%), butane,1-isothiocyanate (26.97%) and isobutyl isothiocyanate (3.26%). The leaves extract at doses of 200 and 400 mg/kg, significantly inhibited paw edema at the 3rd h (49.1%, 54.0%, respectively) and this effect was comparable to that of diclofenac (58.87%). The leaves extract showed the highest antioxidant activity with IC_{50} value of 91.71 µg/ml. The twigs extract exhibited the highest cytotoxic activity against human lung carcinoma (A549) with IC_{50} of 57.5 µg/ml. The leaves and stem extracts exhibited antibacterial activity against *Staphylococcus aureus* with minimum inhibitory concentration (MIC) of 5.0 mg/ml.

Conclusion: The leaves extract of *C. cartilaginea* is a potential source of bioactive compounds that could have a role in anti-inflammation. Twigs extract of the *C. cartilaginea* possesses a potential cytotoxic effect on human lung cell line.

Keywords: *Capparis cartilaginea,* Phytochemical, Essential oil, Anti-inflammation, Antioxidant cytotoxicity, Antibacterial

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INTRODUCTION

Capparis are the largest genera of Capparidaceae which are shrubs, trees and woody climbers that comprise about 250-400 species. They are found in tropical and subtropical zones of Southern America, Europe, Africa, Madagascar, Asia, Australia, and the Pacific Islands [1]. In Yemen, four species were found, *Capparis cartilaginea* Decne, *C. spinosa, C. deciduas* and *C. tomentosa* [2].

Capparis cartilaginea have various traditional uses in the Arab region. It is used for easing bruises, childbirth, earache, headache, paralysis, swelling, skin and joint inflammation, knee problems; tendinitis and snakebites [3, 4]. In Yemen, it is called lattssaf, laşaf or nişaf [4] and used to treat itching, shortness of breath, head cold, tumors, wounds, boil and for painful knees [5-7]. Phytochemical studies of *C. cartilaginea* have resulted in isolating flavonoids [8, 9] and isothiocyanates [3]. Other phytochemicals, such as carbohydrates, saponins, polyphenols, flavonoids, tannins, triterpenes, sterols, amino acid and protein have also been found in the leaves of *C. cartilaginea* from Yemen [10].

A previous investigation has reported that *Capparis* species extracts, such as *C. spinosa* and *C. decidua* from Saudi Arabia has a significant anti-inflammatory activity [11]. Recently, the mechanism of anti-inflammatory effect of *C. spinosa* was suggested by El Azhary *et al.* [12] and involved inhibition of cellular infiltration and cytokine gene expression. Rutin, a flavonoid, obtained from *C. acutifolia* Sweet exhibited a potent anti-inflammatory effect [13]. Other studies have reported the antioxidant, cytotoxic, larvicidal, antimicrobial, hypotensive and bradycardiac activities of *C. cartilaginea* [3, 9, 10, 14-19].

Although previous studies have reported the phytochemical screening of Yemeni *C. cartilaginea* leaves from various places [9, 10], in this study, the essential oil component, acute toxicity and anti-inflammatory effects of *C. cartilaginea* leaves extract were determined; which, to the best of our knowledge have never been investigated. Taking into account the environmental effects on the quality and quantity of chemical components of plant species and the consequent difference in biological effects, our study investigated the phytochemical screening of leaves, stem and twigs of *C. cartilaginea* and their biological activities, including the antibacterial, antioxidant and cytotoxic activities.

MATERIALS AND METHODS

Chemicals, reagents and drugs

Solvents used in plant extraction and chemical tests, including methanol (99.8%), diethyl ether, toluene, dimethylsulfoxide (DMSO) (Scharlae, Spain), l0% ammonia solution, glacial acetic acid (WinLab, UK), ethyl acetate (HiMedia, India), formic acid (Fluka, Switzerland), chloroform (Sigma, Germany), anhydrous sodium sulphate, n-hexane (Univar, USA)and tween 80 (Uni-Chem, Beograd). Formalin was obtained from BDH Chemicals, UK. Reagents, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Aldrich, Germany. The standard drug such as diclofenac sodium was purchased from Denk Pharma, Germany. Vitamin C was purchased from Sigma, USA. Ceftriaxone (Ceftix-3®) was obtained from Trifecta pharma, China. Amikacin (likacin®) was purchased from Lisapharma, Italy. All the chemicals and solvents used were of standard analytical grades.

Plant material

The plant *C. cartilaginea* Decne was collected from AL-Madan, Emran province, Yemen in November 2016. The plants were identified by Dr. Abdul Wali Al Khulaidi, Public Authority for

Research and Agricultural Extension, Dhamar, Yemen. A voucher specimen (CCD 16) was prepared and deposited in the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

Preparation of the methanol extracts

The leaves (2827.51 g), twigs (1188.26 g) and stem (464.88 g) of the plant were cleaned, air-dried in the shade and then grounded. The parts were extracted with methanol and filtered. The filtrates were dried using a rotary evaporator. The final percentage yield of leaves, twigs and stem was then calculated. Subsequently, the extracts were stored in airtight containers at room temperature until use.

Experimental animals

Mature male Albino rats, weighing 150-250 g, were obtained from the animal house of Faculty of Science, Sana'a University. The rats were used for determination of acute oral toxicity, median lethal dose (LD_{50}) and anti-inflammatory activity. The animals were housed in polypropylene cages under controlled temperature (23±2 °C) and light (light-dark cycle of 12 h). They were fed with food and water *ad libitum*. The rats were acclimatized in the laboratory at least 48 h before the experiments. The experiments were approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana`a University, (01/FPh Sana'a/2016).

Phytochemical screening

Chemical tests

Chemical tests were performed according to Banu and Cathrine [20] to identify alkaloids, carbohydrates, fixed oils and fats, glycosides, anthraquinones, phenolic compounds and tannins, phytosterols, proteins, saponins and gum and mucilage.

Thin layer chromatography

Components of the extracts and their retention factor (Rf) values of each part of *C. cartilaginea* were identified using thin layer chromatography (TLC) using a conventional one-dimensional ascending method using silica gel 60 F254, 20 x 20 cm (Merck, Germany) [21]**.** Each of the three extracts (1 g) was individually dissolved in 5 ml of methanol on a water bath for 10 min. To extract alkaloids, the methanol extracts (l g) were moistened with 1 ml of l0% ammonia solution and then 5 ml of methanol was added and the alkaloids were then extracted for 10 min on a water bath. Each extract solution was spotted for TLC analyses.

Different solvent systems were used to identify the components of the extracts of the plant. Solvent system I; ethyl acetate: methanol: water (100:13.5:10) was used for the analysis of polar compounds, including anthraglycosides, alkaloids, cardiac glycosides, bitter principles, and flavonoids. The solvents were allowed to run for a distance of 6 cm. Solvent system II; ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) was used for flavonoids identification. Solvent system III; chloroform: glacial acetic acid: methanol: water (64:32:12:8) was used for saponins. Solvent system IV; diethyl ether: toluene (1:1) saturated with 10% acetic acid was used for coumarins. The developed chromatograms were first inspected under UV light (VilberLourmat, French) at wavelengths of 254 nm and 365 nm. Each chromatogram was then analyzed for the presence of bioactive constituents by spraying with appropriate reagents.

Isolation of essential oil

The dried leaves of *C. cartilaginea* Decne (317.4 g) were hydrodistilled for 6 h using a Clevenger apparatus. The oily layer was measured, collected and dried, over anhydrous sodium sulphate. The percentage of yield was also calculateded. The oil was placed in a vial and kept in a cool place until used.

Gas chromatographic-mass spectroscopy analysis (GCMS)

The composition of the essential oil of the plant was determined using a Shimadzu GCMS-QP2010 SE System operating in EL mole (70Ev), equipped with a flamed ionizing detector (FID), and aRTx-5 (30 m× 0.25 mm, 0.25 µm film thickness), capillary column. One drop of oil was dissolved in 5-6 drops of n-hexane and placed in the specific vial used for GC analysis. The diluted sample (1 µl) was injected automatically in spilt mode (auto-injector Shimadzu AOC-20i), using pressure-controlled nitrogen as a carrier gas at a linear velocity of 42.9 cm^3/s.

The temperature of the injector and detector was maintained at 250 °C. The oven temperature was programed at 45 °C for 2 min and gradually increased to 300 °C at a rate of 5 °C/min and held for 5 min. The peak areas and retention times were measured via computerized integration. The relative amounts of the individual components were calculated based on the peak area obtained without a flame ionization detector (FID) response factor correction. A homologous series of n-alkane standards ($_{28}C$-$_{8}C$) was also prepared and analyzed under the same conditions used for essential oils. The constituents of essential oil were determined by comparing their relative retention indices with those in the literature and their mass spectral data with the existing Wiley library [22]**.**

Acute oral toxicity

Acute oral toxicity and LD_{50} of the methanolic extract of *C. cartilaginea* Decne leaves were determined in albino rats as described in Al-Mahbashi *et al.* [23]. In a pilot experiment, five groups (six rats each) received the leaves extract dissolved in distilled water with 3% tween 80 at doses of 500, 1000, 2000, 3000 and 5000 mg/kg body weight (b. w), respectively. Signs of toxicity and number of deaths per dose in the following 48 h were also recorded.

Anti-inflammatory assay

To determine the anti-inflammatory effect of the dried leaves extract in the rat, a formalin-induced inflammation test was carried out as described by Hosseinzadeh and Younesi [24] and Shripad *et al.* [25].

Formalin-induced inflammation

Rats were divided into four groups of 6 and food was held overnight before the day of the experiment. Treatment groups received 200 and 400 mg/kg orally (P. O.) of the extract. The reference group received oral diclofenac sodium 20 mg/kg, while the control group were given only distilled water in 3% tween 80 (2 ml/kg), orally. Treatment of rats was 1 hour before administration of inflammatory agent. The acute inflammation was induced by intraplantar administration of 0.1 ml of 2% freshly prepared formalin solution into the right hind paw of each rat in each group. Paw size of rats was measured prior to administration of the inflammatory agent. The changes in paw size were measured using a plethysmometer to every 1 h for the following 4 h. Edema was assessed in terms of the difference in the zero-time linear diameter of the injected hind paw and its linear diameter at time t (i.e. 1, 2, 3, 4 h) following the administration of 2% formalin solution.

The anti-inflammatory effect of the extract was calculated using the following equation:

$$\text{Inhibition } (\%) = \frac{\text{Ec} - \text{Et}}{\text{Ec}} \times 100$$

Ec = represents the average difference in paw size of the control group.

Et = the average difference in paw size after the extract was administered to the rats.

Antioxidant activity

The antioxidant activity of *C. cartilaginea* Decne extracts was carried out according to the method described by Blois [26] and Bains and Tripathi [27] with a few modifications. The stock solution of each extract (10 mg/ml) was diluted into 6 concentrations (2000, 1000, 500, 250, 125 and 62.5 µg/ml) in methanol. An aliquot (100 µl) of the extract was placed in a microtiter plate before 100 µl of DPPH solution (0.1 mmol) was added to each well to get a serial concentration of 1000, 500, 250, 125, 62.5 and 31.25 µg/ml. The mixture was shaken vigorously for 1 min and allowed to stand at room temperature for 30 min. The reduction of the DPPH free radical was measured by reading the absorbance at 492 nm using spectrophotometer (microplate reader) (LiNEAR GEA, Spain). Vitamin C (100-3.125 µg/ml) was used as a positive control and the

methanol solvent as a negative control. Extracts without DPPH were also used as blank solutions. Each experiment was carried out in triplicates. The percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Ab c} - (\text{Ab1} - \text{Ab0})}{\text{Abc}} \times 100$$

Abc = absorbance of DPPH, Ab1 = absorbance of extract with DPPH and Ab0 = absorbance of extracts without DPPH (sample blank).

Cytotoxic activity

Cell culture

Human breast adenocarcinoma cell line (MCF-7), human lung cancer cell line (A-549) and human colon adenocarcinoma (HCT-116), were originally purchased from the American type culture collection (ATCC, Wesel, Germany) and grown in the tissue culture lab of the Egyptian company for production of vaccines, sera and drugs (Vacsera, Giza, Egypt). The cells were maintained in Roswell Park Memorial Institute medium (RPMI1640) in a humidified, 5% (v/v) CO_2 atmosphere at 37 °C.

Cytotoxicity assay

Exponentially growing cells were trypsinized, counted and seeded at appropriate densities (5000 cells/0.33 cm^2well) into 96-well microtiter plates. Cells were incubated in a humidified atmosphere at 37 °C for 24 h. Then, cells were exposed to different extracts at the desired concentrations, (0.1, 1, 10, 100 and 1000 µg/ml) for 72 h. At the end of the treatment period, the media was removed and cells were incubated with 200 µl of 5% MTT solution/well to allow to metabolize the dye into a coloured-insoluble formazan complex for 2 h. The medium was discarded from the wells and the formazan crystals were dissolved in 200 µl/ well-acidified isopropanol for 30 min, covered with aluminium foil and with continuous shaking using a MaxQ 2000 plate shaker (Thermo Fisher Scientific Inc., MI) at room temperature. Absorbance was measured at 570 nm using an Epoch microplate reader (BioTek instruments, Winooski VT). The cell viability was expressed relative to the untreated control cells and the concentrations induced 50% growth inhibition (IC_{50}) were calculated from the concentration-response curve using GraphPad prism 5 Software (GraphPad Software, Inc., CA, USA) [28, 29].

Antibacterial activity

The methanol extracts of leaves, stem and twigs and essential oil of *C. cartilaginea* Decne were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* using broth microdilution method. All bacteria under investigation were sub-cultured on human blood agar (HuBA) (Rapid laps, UK). The bacteria used were obtained from the Microbiology department of YemenLab, Sana'a, Yemen.

Preparation of bacterial suspensions

Bacterial suspensions were prepared according to standard methods recommended by the National Committee for Clinical Laboratory Standard [30] with minor modifications. The bacterial cultures were grown on HuBA at 35 °C for 24 h. About 4-5 isolated bacteria colonies were transferred into a sterile screw cap bottle containing 5 ml of sterile normal saline. The suspension was mixed and the turbidity was standardized to 2-5×10^8 colony forming units/ml (CFU/ml), that match the turbidity of McFarland 0.5 standard at 530 nm. The resulting bacterial suspension (2-5×10^8 CFU/ml) was diluted in Mueller-Hinton broth (MHB) (Oxoid, UK) to obtain the test inoculum of 2-5×10^4 CFU/ml.

Broth microdilution assay

The quantitative assay of the antibacterial activity of the plant extracts was performed according to the reference method recommended by [30]. Sterility condition was maintained throughout the experiments. The stock solution (500 mg/ml) of each extract was dissolved in 1% DMSO: methanol (1:1) and serially diluted in MHB into 6 serial concentrations (10-0.31 mg/ml). Aliquots (100 µl) of each resultant solution were then transferred into 96-well microtiter plates. Each well was later filled with 100 µl of the bacterial suspension (2-5×10^4 CFU/ml), which achieved the desired final concentrations of the test material 5, 2.5, 1.25, 0.63, 0.31 and 0.16 mg/ml.

Five controls were used; negative control [DMSO: methanol (1:1), growth control (100 µl of bacterial suspension was mixed with 100 µl of MHB), positive control (Ceftriaxone was used for *P. aeruginosa* and *E. coli* while Amikacin was used for *S. aureus*), sterile control of plant extracts (100 µl of plant extracts and 100 µl MHB broth) and sterile broth 200 µl of MHB broth alone was used to confirm the sterility of the broth. The microtiter plates were incubated for 24 h at 35 °C. The MIC value was recorded as the mean concentration of triplicates. The antibacterial activity was categorized as strong if MIC was less than 1.00 mg/ml, moderate if MIC was between 1.00 and 4.9 mg/ml and weak if MIC was more than 5.00 mg/ml [31].

Statistical analysis

Results were expressed as mean±Standard deviation (SD) of the three triplicates. The data were analyzed using Statistically Package for Social Sciences (SPSS) version 11.5. Categorical variables were represented by frequencies and percentages. The paired T-test was used to test the significance of the differences between each two groups. The significance level was set at 0.05.

RESULTS

Phytochemical screening and thin layer chromatography

The chemical tests used for identifying the active constituents showed the presence of various bioactive components. Compounds, such as alkaloids, carbohydrates, phytosterol, phenolic compounds and tannins, were found in the leaves, twigs and stem of *C. cartilaginea*. However, fixed oils, fats and glycosides were found only in the leaves and stem extracts. The TLC showed one spot of alkaloid in each extract (table 1). A number of spots corresponding to the bitter principles in the leaves and stems extracts were higher than that of those of the twig extract. Phenolic compounds, such as flavonoids and coumarins were found in the three extracts, however, saponins and anthraglycosides were absent. The solvent systems, the Rf values and the identified active constituents for each extract are summarized in table 1.

Essential oil

C. cartilaginea leaves yielded a final 0.35% pale yellowish oil. Table 2 shows the percentage of each component in the essential oil of *C. cartilaginea* (the components are arranged in order of elution on RTx-5 column). Three compounds were identified in the leaves of *C. cartilaginea* representing 99.64% of the oil. The oil composed mainly of isopropyl isothiocyanate (69.41%), butane, 1-isothiocyanate (26.97%) and isobutyl isothiocyanate (3.26%).

Acute toxicity

The examined doses (500, 1000, 2000, 3000, and 5000 mg/kg b.w.) of *C. cartilaginea* did not produce any notable acute toxic effects. No diarrhea, hematuria, restlessness, uncoordinated muscle movements, respiratory distress or death in all groups of rats during 48 h.

Anti-inflammatory activity

The leaves extract at a dose of 200 and 400 mg/kg significantly (p = 0.004 and 007, respectively) inhibited paw edema at the 3rd h following inflammation induction compared with the control animals which received distilled water with percentage inhibition of 49.14% and 54.03%, respectively (table 3). This effect was similar to the effect induced by diclofenac sodium (positive control) 20 mg/kg (58.87%). There was no significant difference between the two doses (200 and 400 mg/kg) of the extracts and diclofenac.

The antioxidant activity

All extracts showed antioxidant activities except the essential oil. The highest activity was obtained from the leaves with IC_{50} value of 91.8 µg/ml followed by the twigs and stem extracts with IC_{50} values of 502 and 584 µg/ml, respectively (fig. 1).

Table 1: TLC investigation of methanol extracts of a different part of *Cappariscartilaginea*

No.	Constituent	Solvent system	Part	No. spots	Rf values
1	Anthraglycoside	EtOAc: MeOH: water (100:13.5:10)	Leaves	-	-
			Stem	-	-
			Twig	-	-
2	Bitter principles	EtOAc: MeOH: water (100:13.5:10)	Leaves	4	0.11 0.16 0.43 0.58
			Stem	5	0.08 0.28 0.43 0.45 0.54
			Twig	2	0.11 0.15
3	Alkaloid	EtOAc: MeOH: water (100:13.5:10)	Leaves	1	0.02
			Stem	1	0.03
			Twig	1	0.05
4	Flavonoid	EtOAc: formic a.: glacial acetic a.: water (100:11:11:26)	Leaves	2	0.22 0.3
			Stem	2	0.22 0.3
			Twig	1	0.3
5	Saponins	$CHCl_3$:glacial acetic a.: MeOH: water (64:32:12:8)	Leaves	-	-
			Stem	-	-
			Twig	-	-
6	Coumarins	Diethyl ether: toluene (1:1)	Leaves	3	0.15 0.24 0.3
			Stem	3	0.15 0.24 0.3
			Twigs	3	0.15 0.24 0.3

Where, TLC-thin layer chromatography, EtOAc-ethyl acetate, MeOH-methanol, $CHCl_3$-chloroform, a.-acid, Rf-retention factor,--absent

Table 2: Percentage composition of the leaves oil of capparis cartilaginea on RTx-5 column

NO.	Components	Retention indices			
		*Percentage (%)	RI_x	RI[32]	ID methods
1	Isopropyl isothiocyanate	69.41	833	837	i, ii
2	Butane,1-isothiocyanate	26.97	928	920	i, ii
3	Isobutyl isothiocyanate	3.26	949	-	ii
	Total	99.64			

*Percentage were expressed as peak area normalization on column RTx-5 without correction factor. ID methods-identification methods, i-retention index, ii-mass spectrum. RIx-retention index measured relative to n-alkanes on Rtx-5 column, RI-retention index on a DB-5 column obtained in literature.

Table 3: Effect of the oral doses (200 mg/kg and 400 mg/kg) of *Capparis cartilaginea* leaves extract and diclofenac on formalin-induced paw edema in rat

Time (h)	Distilled H_2O	Extract (mg/kg)		Diclofenac (20 mg/kg)
		200	400	
0	3.58±0.2	3.58±0.2	3.75±0.3	4.02±0.04
1	5.08±0.2	4.92±0.5 (11.11)	5.00±0.4 (20.0)	5.27±0.4 (16.67)
2	5.58±0.5	5.00±0.3 (29.17)	5.08±1.5 (33.33)	5.15±0.3 (43.33)
3	5.65±0.05	4.63±0.4* (49.14)	4.70±1.4* (54.03)	4.87±0.2* (58.87)
4	5.03±0.7	4.42±0.4 (42.53)	4.40±0.3 (55.17)	4.73±0.2 (50.57)

Values are expressed as mean±SD, n=6, *P<0.01 when compare samples with the control group (Distilled H_2O), the Percentage inhibition of the formalin-induced inflammation (edema) produced by test extract and Diclofenac are indicated in parenthesis.

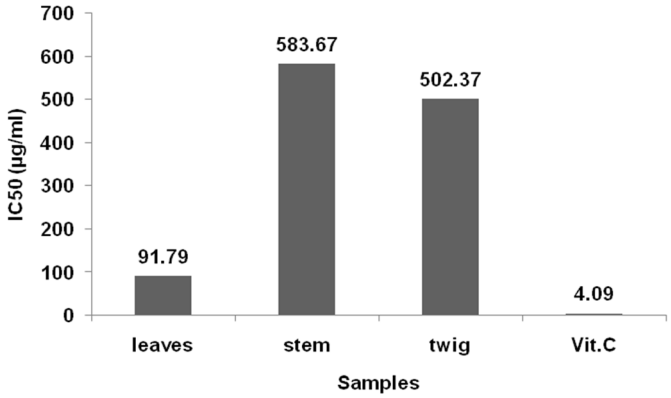

Fig. 1: IC_{50} values (µg/ml) of the methanol extracts of leaves, stem and twigs of *Capparis cartilaginea* and vitamin C (vit. C)

Table 4: IC_{50} values (µg/ml) for cell growth inhibition of the methanol extracts of *Capparis cartilaginea* against MCF-7, A-549 and HTC-116 cell lines

Samples	MCF-7	A-549	HTC-116
Leaves	602.5	>1000	>1000
Stem	407	240	871
Twig	380	57.5	656
Doxorubicin	1.2	0.9	1.24

Where, MCF-7-an adenocarcinoma cell line, A-549-a human lung cancer cell line, HCT-116-a human colon adenocarcinoma.

Table 5: The minimum inhibitory concentration (MICs) (mg/ml) of *Capparis cartilaginea* extracts

Plant sample	*S. aureus*	*E. coli*	*P. aeruginosa*
Leaves	5±0.0	>5±0.0	>5±0.0
Twig	>5±0.0	>5±0.0	>5±0.0
Stem	5±0.0	>5±0.0	>5±0.0
Essential oil	>5±0.0	>5±0.0	>5±0.0
Ceftriaxon	nd	<0.15±0.0	<0.15±0.0
Amikacin	<0.15±0.0	nd	nd

Values of inhibition zone are obtained as mean±SD. Each value is the mean of three triplicate. *S. aureus-Staphylococcus aureus, E. coli-Esherichia coli, P. Erogenous-Pseudomonas aeruginosa.* nd-not determined.

The cytotoxic activity

The results showed that the twigs extract possessed the highest cytotoxic activity against A-549 cell line with an IC_{50} of 57.5 µg/ml, followed by the stem extract (IC_{50} of 240 µg/ml) (table 4).

The antibacterial activity

The leaves and stem extracts showed weak antibacterial activity against *S. aureus* with MIC values of 5.0 mg/ml, whereas other extracts of different parts as well as essential oil of *C. cartilaginea* showed no activity against *E. coli* and *P. aeruginosa* (table 5).

DISCUSSION

In this study, the phytochemical screening of various parts of *C. cartilaginea* as well as the biological activities of leaves extracts, including antibacterial, antioxidant, cytotoxicity, acute toxicity and anti-inflammatory activities were evaluated. Phytochemical results indicated the presence of alkaloids, carbohydrates, protein, coumarin, phytosterols, bitter principles, phenols and tannins and the absence of saponins and anthraglycosides in the *C. cartilaginea* extracts. This study is in agreement with a recent study carried out in Abyan, Yemen [10] except that alkaloids were absent and saponins were found in the leaves extract in their study. These differences may be ascribed to the environmental factors, such as altitude, temperature, illumination, precipitation, humidity, soils and locations [33].

Our findings show that isothiocyanates, including isopropyl isothiocyanate, butane, 1-isothiocyanate and isobutyl isothiocyanate, represented 99.64% of the essential oil of *C. cartilaginea* leaves. A previous study has isolated isothiocyanate components from the leaves of *C. cartilaginea* which is obtained from Egypt [3]. In fact, isothiocyanate compounds found to be common in *C. cartilaginea* as other species of *Capparis* [3, 32]. In addition, the essential oil of *C. cartilaginea* showed weak antibacterial and antioxidant activities. These results could be responsible for the antagonistic effects of other compounds or the presence of other non-active compounds [34]. Volatile sulfur-containing compounds, such as isothiocyanate have showed antioxidant, cytotoxic effects and apoptosis induction [35] and cancer chemoprevention properties [36, 37]. Individual components of the essential oil need to be further investigated for their biological activities.

Notably, the leaves extract of *C. cartilaginea* did not show toxicological or behavioral changes in the rats even at the highest dose used (5000 mg/kg). Doses of 200 and 400 mg/kg were selected in this study to determine the anti-inflammatory effect of the plant. The anti-inflammatory effect of leaves extracts was assessed using the formalin-induced inflammation method (2%). Formalin has been used as an inflammatory inducer to evaluate the anti-inflammatory effect of plant extracts in several previous investigations [24, 38, 39]. Intraplantar formalin injection in rats produces a biphasic response where the neurogenic pain is induced in the first phase, while inflammatory processes are induced in the second phase [40]. Various mediators are produced in inflammation, including prostaglandins, serotonin, histamine, bradykinin and cytokines [41]. Interestingly, the leaves extract showed significant inhibition of edema during the second phase of the edema process. The anti-inflammatory effect of the extract was not significantly different from that of diclofenac, a

prostaglandin synthesis inhibitor, indicating a possible similar action. However, the mechanism of anti-inflammatory action of *C. cartilaginea* needs to be elucidated. The presence of alkaloids, flavonoids, coumarins, phenolic and tannin compounds in the leaves extract may contribute to its anti-inflammatory activity [42].

The extracts of *C. cartilaginea* showed antioxidant activity which was in agreement with previous studies [9,10]. Plant phenolic compounds of secondary metabolites, such as simple phenolics, phenylpropanoids, coumarins, lignans, quinones, tannins and flavonoids are known as antioxidants [43, 44]. It is well known that free radicals contribute to various human disorders, such as atherosclerosis, arthritis, gastritis and cancer [45] which play an important role in the pathogenesis of inflammation as well [46]. Therefore, *C. cartilaginea* leaves could have a role in preventing these disorders due to their antioxidant activity.

In the present study, the extract of twigs showed high cytotoxic activity against lung cell lines (A-549) followed by the stem extract. However, a previous study has reported no cytotoxic effect (>50 µg/ml) of leaves extract of *C. cartilaginea* on lung (A-427), urinary bladder (5637) and breast (MCF-7) cell lines [9]. Another study has also reported that the methanol extract of a flowering branch of the species exhibited no cytotoxic effect (>50 µg/ml) against cancer cells of MCF-7, human hepatocellular carcinoma (HepG-2), mouse fibrosarcoma (WEHI-164), A549 and Madin-Darby bovine kidney (MDBK) [14, 38]. These contradictory findings may be due to the fact that in the current study, the lowest IC_{50} value was 57.5 µg/ml while in the previous studies, 50 µg/ml was used as a maximum concentration.

The current study leaves and twigs extracts showed weak antibacterial activity against *S. aureus* and no effect against *E. coli* and *P. aeruginosa*. These results were in consistence with those reported by Mothana *et al.* [9]. However, a recent study indicated that the extract of the aerial parts of *C. cartilaginea* showed antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* with MIC values of 13.69, 62.5 and 52.08 µg/ml, respectively [17]. Quantity and quality of chemical components of plants may be responsible for the different activities of the plant extracts [47].

CONCLUSION

Three isothiocyanates, isopropyl isothiocyanate, butane,1-isothiocyanate and isobutyl isothiocyanate, represented the major components of the essential oil of *C. cartilaginea* leaves. The methanol leaves extract of *C. cartilaginea* has been shown to have antioxidant effects and significant anti-inflammatory effect without causing toxicity upon oral administration. Therefore, the leaves extract is a potential source of bioactive compounds that could have a role in anti-inflammation. The twigs extract of the species also possesses a potential cytotoxic effect on human lung cell line suggesting the presence of selective cytotoxic compounds.

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AUTHORS CONTRIBUTIONS

Dr. Bushra Abdulkarim Moharram and Dr. Hassan M. AL-Mahbashi conceived, designed and performed the experiments.

Faten Ali Aqlan helped in the experiment part**.**

Dr. Bushra Abdulkarim Moharram and Dr. Riyadh Saifali analyzed the data and wrote the paper. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interests.

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