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Phytochemical screening and antioxidant evaluation by DPPH of *Capparis cartilaginea* Decne leaves

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Abstract
Capparis cartilaginea Decne (Capparaceae) leaves are used in traditional medicine of Yemen for treatment of various illness. Phytochemical data about this plant has been not found before this study. The aim of this study was to determine the chemical composition and to evaluate the antioxidant activity of the these leaves. Phytochemicals such as carbohydrates, saponins, polyphenols, flavonoids, tannins, triterpenes, sterols, amino acid and protein were found. The flavonoid and saponin content were 5.1% and 1.8% respectively. Thin layer chromatography of different extracts was performed. The antioxidant activity was evaluated by dot-blot and DPPH staining. Methanol and water extract showed high antioxidant activity when compared with standard quercetin at similar concentrations. In methanol extract seven antioxidant constituents were found and three flavonoids including rutin were detected. These results validate the exploitation of the studied medicinal plant, for further isolation of chemical constituents and their application in medicine.

Keywords: *Capparis cartilaginea*, leaves, phytochemical, antioxidant

Introduction
Medicinal plants are of great value in the field of treatment and prevention of diseases. Plant derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins and amino acids have received considerable attention [1] in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [2]. A large number of medicinal plants and their purified constituents have been shown to exhibit antioxidant activity [3, 4]. Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to human against infection and degeneration diseases [5]. Currently, the possible toxicity of synthetic antioxidants has been criticized and interest in natural antioxidant especially of plant origin, has greatly increased [6]. Herbal medicine represents one of the most important fields of traditional medicine in Yemen especially in rural areas. Thus, phytotherapy is practiced by a large proportion of Yemen population for the treatment of several physical, physiological, mental and social ailments [7]. The leaves of *Capparis cartilaginea* Decne (Capparaceae) are used to treat itching, shortness of breath, head cold, for tumors [8], for wounds and boil [9]. The leaves are boiled for external application on painful knees [10]. Some studies showed that the leaves of *Capparis cartilaginea* Decne growing in Yemen has antimicrobial activity [11, 12], but data about its chemical composition has been not found before this study. Therefore the leaves were selected for phytochemical analysis with standard methods described in standard texts and to evaluate antioxidant activity by dot-blot assay and TLC analysis of antioxidant constituents. The data obtained from this research was analysed by using appropriate references and statistical test where ever necessary.

Materials and methods
Collection and identification of plant material
The fresh leaves of *Capparis cartilaginea* Decne (Capparaceae) were collected in September 2015 from Zarah - Abyan, Republic of Yemen, dried in the shaded area and then manually grinded and stored at room temperature for further analysis. The plant sample was identified by a taxonomist, Professor Abdul Nasser Algifri, of the department of Biology, of University of Aden, Yemen.

Preparation of the extracts

The shade dried leaves of studied plant were coarsely powdered and subjected to extraction by maceration method. Leaves powder (50 g) was macerated with petroleum ether. The extraction was continued till the defatting of the material had taken place. The petroleum ether extract was filtered and evaporated to dryness. The dried defatted marc of plant material was then macerated with methanol for 48 hrs. The methanol extract was filtered and evaporated to dryness. Lastly, the dried marc was subjected to hot water maceration. The maceration was continued for a period of 28 hrs. The aqueous extract was filtered and evaporated to dryness. The percentage yield of petroleum ether, methanol and water extracts were 1.50%, 4.60% and 15.37% respectively. The dried crude extracts were stored in air tight bottle at 4 °C for farther study.

Qualitative phytochemical analysis

Phytochemical screening was conducted to detect various classes of chemical constituents in the extracts of studied leaves, using the procedures described previously for qualitative phytochemical screening [13, 14, 15, 16].

Quantitative phytochemical studies

Determination of Flavonoids

The flavonoid content of the leaves was determined by gravimetric method [17]. A weighed sample 5g was hydrolyzed by boiling in 100mls of 10% (v/v) sulfuric acid for about 35 mins. The hydrolysate was filtered to recover the extract (filtrate). The filtrate was treated with 50 ml of ethyl acetate to obtained two layers. In the upper layer (ethyl acetate layer) flavonoids were precipitated and recovered by filtration using a weighed filter paper after drying in the oven at 100 ^{0}C for 30 mins, it was cooled in a desiccators and reweighed. The difference in weighed gave the weighed of flavonoids which was expressed as a percentage of the weighed of sample analysed. The measure was performed in triplicate.

$$\% \text{ Flavonoid} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

Where:
W1 = Weight of empty filter paper;
W2 = Weight of paper + flavonoid precipitate

Determination of Saponins

The saponins were isolated from leaves. The first step of isolation was the extraction with petrol ether and chloroform, than the degreased vegetal material was extracted with methanol. The methanol extract was concentrated and the triterpenic saponins were precipitated from this extract with acetone [18, 19]. The isolated saponins were evaporated to dryness in a pre-weighed evaporating dish. It was dried at 60 ^{0}C in the oven and reweighed. The experiment was repeated two more times to get an average.

$$\% \text{ Saponins} = \frac{W2 - W1}{\text{Weight of sample}} \times 100$$

Where:
W1 = Weight of evaporating dish;
W2 = Weight of dish + sample

Thin layer chromatography

Thin Layer Chromatography (silica gel G 60 F254 TLC plates of layer thickness 0.2mm, Allugram- Germany) of prepared extracts was performed to determine the number of spots and Rf values [20, 21]. Various solvent systems were tested to obtain best results. TLC plates were first viewed in day light then in UV chamber and Rf of all were calculated.

Antioxidant studies

Rapid screening of antioxidant by dot-blot and DPPH staining

Each diluted sample (methanol extract, water extract and quercetin) was carefully loaded onto a 20 cm × 20 cm TLC layer (silica gel G 60 F254) and allowed to dry (3 min). Drops of each sample were loaded in order of decreasing concentration (2, 1, 0.5, 0.25 and 0.125 mg/ mL) along the row. The staining of the silica plate was based on the procedure. The sheet bearing the dry spots was placed upside down for 10 s in a 0.05% 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution, then the excess of solution was removed with a tissue paper and finally the layer was dried. Stained silica layer revealed a purple background with yellow spots at the location where radical-scavenger capacity presented. The intensity of the yellow color depends upon the amount and nature of radical scavenger present in the sample [22].

TLC analysis for antioxidant constituents

About 2 micg of methanol extract was loaded on TLC plate (20 cm X 20 cm). The plates were developed in solvent system BAW (4:1:5) to separate different constituents and various spraying reagents were used. The antioxidant constituents were analyzed by DPPH technique [23, 24]. For this one plate sprayed with 0.05% of DPPH solution in methanol and incubated for 10 min at room temperature. The active antioxidant constituents of the extract were detected in sunlight as yellow spots produced via reduction of DPPH by resolved bands against purple back ground on the TLC plate. Second developed plate, after air-dried, the spots were visualized by spraying with Aluminum chloride (5% in methanol) and the third developed plate was sprayed with ferric chloride (2% in methanol). Chromatograms were evaluated under UV light and in sunlight before and after derivatization with reagents. The colour of the spots was noted and Rf values were calculated [20, 21].

Statistical analysis

Analysis of variance of data was evaluated by Student's t test P-values less than 0.05 was considered to be statistically significant.

Results and discussion

Qualitative phytochemical analysis

Phytochemical screening of the studied extracts was represented in Table 1 and in Figures 1- 4. Phytochemicals like carbohydrates, saponins, polyphenols, flavonoids, tannins, triterpenes, sterols, amino acid and protein were presented, while alkaloids were not present. These secondary metabolites of plants serve as medicine in the treatment of various infectious diseases [25].

Table 1: Results of phytochemical screenings of successive extracts of the leaves of *Capparis cartilaginea* Decne

Phytochemical Screening		Petroleum Ether extract	Methanol extract	Water extract
Alkaloids	Wagner's test	-	-	-
	Mayer's test	-	-	-
	Dragendorff's reagent	-	-	-
Polyphenols	Ferric chloride test	-	++	-
Flavonoids	Shinoda test	-	++	++
	NaOH Test	-	++	+
	Lead acetate test	-	++	++
	Aluminium solution test	-	++	++
Saponins	Foam test	-	++	++++
	Haemolysis test	-	++	++
Sterols/ Triterpenes	Salkowski test	++	+++	+++
	Liebermann-Burchard test	++	+++	+++
Carbohydrates	Molisch's test	+	++	++
	Fehling's test	+	++	++
Tannins	Ferric chloride test	-	++	-
	Gelatin test	-	-	-
Amino acid/ Protein	Ninhydrin test	-	+	+++

+++ = Most intense, ++ = moderately intense, + = Least intense, - = absent.

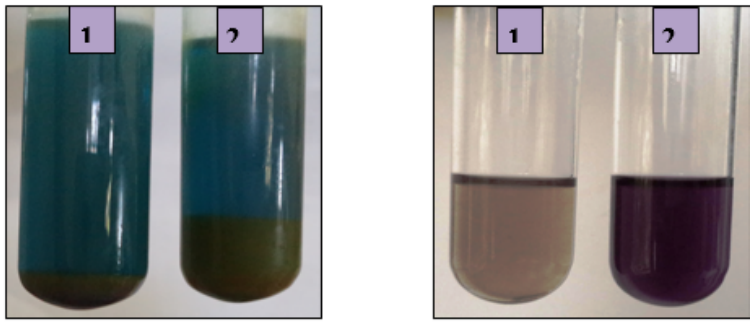

Fig 1: Test tubes with fehling's test for identification of carbohydrates (A), ninhydrin test for identification of amino acid/ protein (B) in methanol (1) and water (2) extracts.

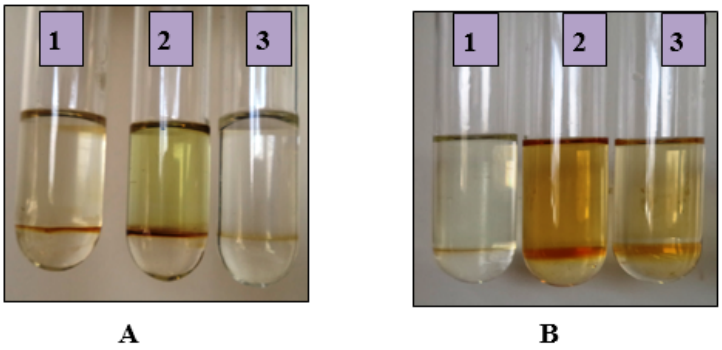

Fig 2: Test tubes with Salkowski (A), Liebermann-Burchard (B) reactions for the identification of the triterpenes/sterols in petroleum ether (1), methanol (2) and water (3) extracts.

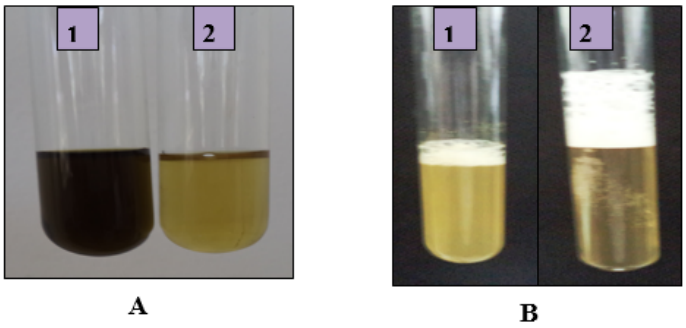

Fig 3: Test tubes with ferric chloride test for the identification of polyphenols (A) and foam test (B) for the identification of saponins in methanol (1) and water (2) extracts.

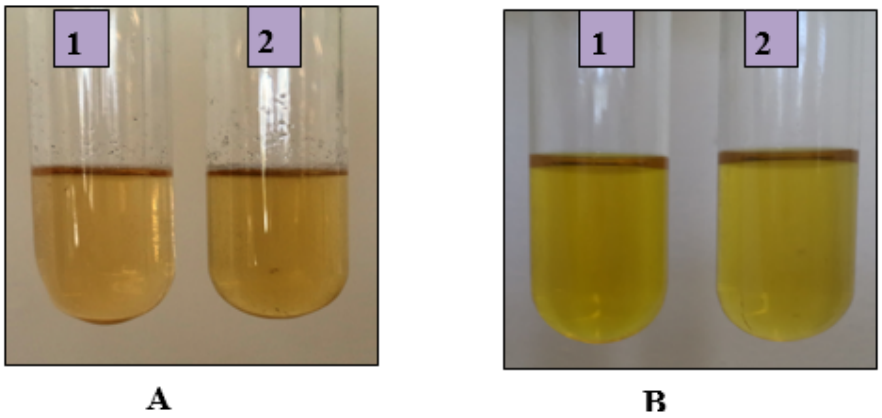

Fig 4: Test tubes with Shenoda reaction (A) and dilute sodium hydroxide 10% reaction (B) for the identification of flavonoids in methanol (1) and water (2) extracts.

Quantitative phytochemical studies

Determination of Flavonoids

The percentage concentration of flavonoids in the leaves of this plant was determined gravimetric method and the result was 5.1±0.03%. The measure was performed in triplicate. The result indicate that the leaves of this plant have high amount of flavonoids, confirm their medicinal value.

Determination of Saponins

Saponin content of the sample was determined by double solvent extraction gravimetric method. The total saponin content of plant drug was 1.8 ±0.03%.

Thin layer chromatography

Thin layer chromatography is the simplest of all the widely used chromatographic methods to perform. TLC is the most versatile and flexible chromatographic method for separation of all types of organic and inorganic molecules [26]. TLC of petroleum ether and methanol extract was performed to separate and determine Rf values. Various solvent systems were tested to obtain best results. The best solvent system for petroleum ether extract was Toleune-Ethyl acetate (93:7), where detected 5 spots (Fig. 5). The best solvent system for methanol extract was Butanol –Acetic acid –Water (4:1:5), where detected 9 spots (Fig. 6, 7). Photos of the plates were taken in UV chambers and *Rf* values of developed spots of different extracts were calculated as well as the colour of spots were observed, which are mentioned in Table 2.

Fig. 5: TLC plate under UV 254 nm.

Fig. 6: TLC plate under UV 254 nm.

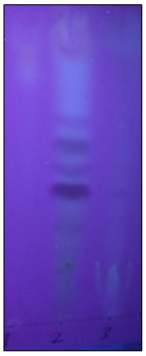

Fig. 7: TLC plate under UV 365 nm.

Table 2: Observations of thin layer chromatographic studies of *Capparis cartilaginea* Decne leaves.

Extracts	Mobile phase	No. of spots	Rf values	Colour of the spot at 254 nm	Colour of the spot at 365 nm
Petroleum Ether	Toleune-Ethyl acetate (93:7)	5	0.15	Not visible	Pink
			0.39	Dark spot	Pink
			0.47	Dark spot	Not visible
			0.52	Dark spot	Pink
			0.58	Dark spot	Not visible
Methanol	Butanol –Acetic acid –Water (4:1:5)	9	0.33	Dark spot	Light purple
			0.40	Dark spot	Deep purple
			0.42	Not visible	Blue
			0.45	Not visible	Purple
			0.47	Dark spot	Blue
			0.52	Not visible	Purple
			0.55	Dark spot	Blue
			0.60	Dark spot	Purple
			0.64	Not visible	Blue

Antioxidant studies

Rapid screening of antioxidant by dot-blot and DPPH staining

A great number of TLC techniques have been developed and successfully applied for qualitative and quantitative analysis of antioxidants [27, 28], and the stable free radical (DPPH) was often used as a derivatization reagent for this purpose [29]. The antioxidant potential activity of methanol and water extracts was determined via eye-detected semi-quantitatively via a rapid DPPH staining-TLC technique. Each diluted samples were applied as a dot on a TLC layer that was then stained with DPPH solution. Quercetin was used as a reference compound. This method was depend up on the inhibition of the accumulated of oxidized products and the generation of free radicals was inhibited via the addition of antioxidant and masking of the free radicals [22, 30]. The results of dot-blot assay showed yellow coloured spot when stained with DPPH solution (Fig. 8). All dots at concentration of 2.0 mg /ml, 1.0 mg/ml 0.5 mg/ml and 0.25 mg/ml showed higher scavenging activity, exception dots 0.25 mg/ml, 0.125 mg/ml (water extract) and 0.125 (methanol extract) showed weak scavenging activity.

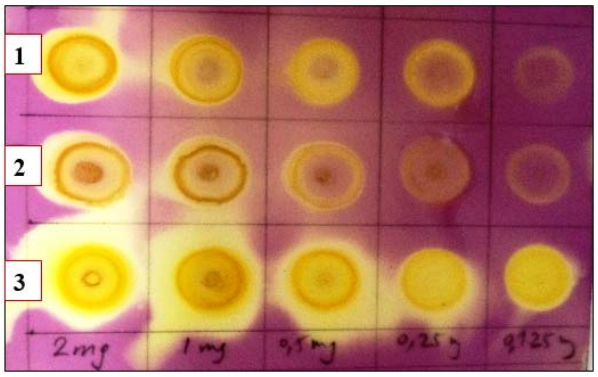

Fig. 8: Dot blot assay of the methanol (1), water extracts (2) and quercetin (3) on a silica sheet stained with a DPPH solution

TLC analysis for antioxidant constituents

Antioxidant constituents of studied leaves were analysis by TLC. Phenolic compounds such as phenolic acid, flavonoid and tannin are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity [31, 32, 33]. Methanol extract was subjected to TLC. The plates were developed in BAW (4:1:5). First developed plate sprayed with 0.05% DPPH reagent to give antioxidant constituents. Second plate sprayed with ferric chloride and the third plate sprayed with aluminum chloride to identify the respective compounds. Plate sprayed with 0.05% DPPH reagent showed the present of 7 spots, with yellow colour corresponding with antioxidant behavior. The active antioxidant constituents of the extract were detected in sunlight as yellow spots produced via reduction of DPPH by resolved bands against purple back ground on the TLC plate [24, 34]. In second plate were detected 6 spots, represented polyphenolic compounds and in third plate were detected 3 spots (flavonoid compounds). Photos of the plates were taken in UV chambers before derivatization with reagents (Fig. 9 a, b), in daily light after derivatization with 0.05% DPPH (Fig. 10), with ferric chloride (Fig. 11) and with aluminum chloride (Fig. 12) reagents, then Rf values were calculated as well as the colour of spots were observed (Table 3). Rutin was used as a reference compound (Rf = 0.52).

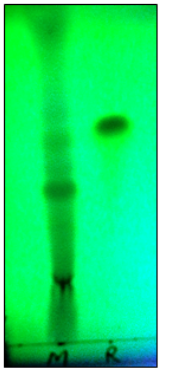

Fig. 9 a: TLC plate under UV 254 nm.

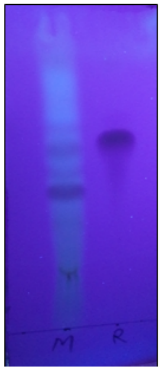

Fig. 9 b: TLC plate under UV 365 nm.

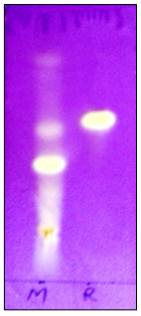

Fig. 10: TLC plate with DPPH.

Fig. 11: TLC plate with $FeCl_3$ reagent.

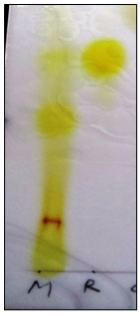

Fig. 12: TLC plate with Al Cl_3 reagent.

Table 3: Analyses of TLC plates of phytochemical constituents of methanol extract of *Capparis cartilaginea* leaves and rutin developed in BAW (4:1:5).

Extract/ Compound	Rf	Colour of the spot before derivatization		Colour of the spot in daily light after derivatization with			Assigned substance
		At 254 nm	At 365 nm	ferric chloride reagent	Aluminum chloride reagent	0.05% DPPH reagent	
Methanol extract	0.26	Not visible	Not visible	Brown	Not visible	Light yellow	Phenolic compound
	0.33	Dark spot	Blue	Light brown	Yellow	Yellow	Flavonoid
	0.40	Dark spot	Deep blue	Deep brown	Deep yellow	Deep yellow	Flavonoid
	0.42	Not visible	Light blue	Not visible	Not visible	Not visible	Phenolic compound
	0.47	Dark spot	Light blue	Brown	Not visible	Not visible	Phenolic compound
	0.52	Dark spot	Blue	Brown	Yellow	Deep yellow	Rutin

	0.55	Not visible	Light blue	Light brown	Not visible	Not visible	Phenolic compound
	0.60	Dark spot	Light blue	Not visible	Not visible	Very light yellow	Phenolic compound
	0.69	Not visible	Light blue	Not visible	Not visible	Very light yellow	Phenolic compound
	0.78	Not visible	Not visible	Not visible	Not visible	Light yellow	Phenolic compound
Rutin	0.52	Dark blue	Deep blue	Deep brown	Deep yellow	Light yellow	Flavonoid

Conclusions

The present study indicates that the leaves of *Capparis cartilaginea* Decne might serve as source for pharmaceutical drug. Preliminary phytochemical analysis showed presence of phytochemicals such as carbohydrates, saponins, polyphenols, flavonoids, tannins, triterpenes, sterols, amino acid and protein; these compounds are known to have curative activity against several pathogens and therefore can be suggested for the treatment of different diseases. The percentage concentration of flavonoids (5.1%) and Saponin content (1.8%) are presented in significant amount in the leaves and may be responsible for their valuable pharmacological activities. The result of antioxidant evaluation by DPPH method showed that the methanol and water extract exhibited antioxidant activity by dot-blot and DPPH staining. Also the result of TLC analysis of methanol extract showed presence of seven antioxidant constituents related to phenolic compounds, three of them were flavonoids including rutin. Phytochemical composition, antioxidant evaluation by dot-blot and TLC analysis of antioxidant constituents of the leaves of *Capparis cartilaginea* Decne growing in Zarah - Abyan, Republic of Yemen have been determined and reported for the first time. All the scientific data produced from this study will be useful to support the traditional use of this plant. However, a more extensive study is necessary to isolate and identify the chemical constituents. Further work therefore needs to be carried out on the pharmacological activity and toxicological analysis.

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