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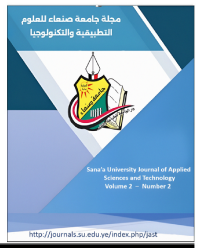

Antioxidant and antimicrobial of three extracts of *Caralluma deflersiana* Laver

Bushra M. al-attab[1] , Maher A. Almaqtari[1] and Ahmed Y. Mubarak[2†]

[1]**Chemistry Department, Faculty of Science, Sana'a University, Yemen,**
[2]**Chemistry Department, Faculty of Applied Sciences & Humanities, Amran University, Yemen.**

[†]Corresponding author: Ahmed.Y.mub@gmail.com

Abstract

This study focused on one traditional herb used in Yemen, *Caralluma deflersiana*. The antioxidant activity of the extracts was examined using the bipyridine-ferric reducing antioxidant capacity assay, as well as the antibacterial activity against *S. aureus*, *P. aeruginosa*, E. coli, and *C. albicans* microbial strains using the agar diffusion method. The highest antioxidant activity was observed for the ethyl acetate extract. 370 μg ASA/mg ascorbic equivalent μg ASA/mg ($p< 0.05$). Acetone and chloroform extracts showed decreased activity and 340 and 305 ascorbic equivalents μg ASA/mg, respectively. The three extracts inhibited the growth of the three bacterial and one fungal species, with MIC values ranging from 55.0-80.7 μg/ml. The selected extracts may be developed to explore new pharmacological possibilities for their application.

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1. INTRODUCTION

Biologically active compounds have grown in popularity in recent years owing to their potential to promote good health and prevent various diseases and disorders. Plants are an important source of bioactive chemicals. Plants have become accessible targets for isolating such chemicals because of the rapidly expanding demand for bioactive goods [1]. Caralluma R.Br belongs to the Asclepiadaceae family, Asclepiadodideae subfamily which comprises approximately 200 genera and 2500 species [2]. Caralluma plants are typically succulent perennial herbs that lack leaves [3]. Almost all *Caralluma* species have high levels of bioactive components, such as flavonoids [4] and pregnane glycosides [5, 6], or their esters [7, 8] and acylated and non-acylated polyoxypregnane glycosides [9], which play an important role in anticancer [10], anti-inflammatory [11], antinociceptive [7], antioxidant, and hypolipidemic actions [12]. In addition to these benefits, the majority of *carallumas* are used to prevent diabetes, obesity, skin damage, skin infections, ulcers, and as antidotes [13, 14]. *C. deflersiana* displays strong antioxidant activity and inhibits bacterial and fungal growth [15]. *Caralluma deflersiana* grows in Yemen [16, 17], and there are few studies on its biological activity and phytochemical composition [18]. Therefore, the current study examined the antioxidant and antibacterial activities of the *C. deflersiana* extracts and its fractions against some harmful microorganisms. This study will be useful in illustrates that *Caralluma* species in Yemen are a prospective source of various chemicals that may be utilized as antioxidants and antibacterial agents. Traditional uses of *Caralluma deflersiana* in gastric ulcers [18]. This study aimed to determine the antioxidant and antimicrobial activities of *C. deflersiana* using three extraction solvents (ethyl acetate, acetone, and chloroform).

2. MATERIALS AND METHODS

2.1. Plant material

Whole parts of ***Caralluma deflersiana Laver*** *C. deflersiana* were obtained in June 2022 from Amran Governorate, Yemen, and dried in shade. Botanical identification was performed by Dr. Hassan M. Ibrahim, head of the herbarium , the herbarium number was 732 Bio-

logical Science Department, Faculty of Science, Sana'a University.

2.2. Procedure for Plant Extract

Fresh leveas picked and cleaned with tap water, then deionized water, before being dried the shade. The plant raw material thoroughly monitored for any fungal growth or rotten smell and the material dried and make to powder form by using electric blender for obtaining a uniform size of 80 meshes. 10 g of *C. deflersiana* was extracted by soaking it in 100 ml of chloroform, acetone, and ethyl acetate with 24 h of agitation. The extracts were filtered using Whatman filter paper No.1, and the solvents were evaporated using a rotary evaporator at 40°C. Dry matter was collected and yields were determined. The dry materials were stored in Eppendorf tubes under dark conditions at a temperature of 4°C for further analysis. Dry extracts were dissolved in the same solvent as the samples used for analysis.

2.3. Antioxidant Activity

2.3.1. Ascorbic acid (Antioxidant Activity)

Ascorbic acid was estimated by the oxidation-reduction reaction using [19]. Bipyridine- Ferric Reducing of Antioxidant Capacity assay Preparation of solutions: $FeCl_3$ (0.01 M) was dissolved in 0.068 g in 25 mL deionized water. Bipyridine (0.1%) was dissolved in 0.025 g of 25 mL HCl (20 mM). Ascorbic acid (0.1 g/L) was dissolved 0.0025 g in 25 mL of deionized water. Acetate buffer was then added. Three grams of Sodium acetate trihydrate were weighed, and 15 mL of glacial acetic acid was added, and the solution pH was adjusted to 4 to a final volume of 1 L using deionized water.

2.3.2. Calculations

The standard curve was drawn at A535nmVs ascorbic concentration, and the equation for the best-fit line was obtained. The sample absorbance was used to obtain the value of ascorbic acid equivalent in g/L.

2.4. Disc diffusion assay of standard antibiotic disc

Standard antibiotic discs of penicillin. Vancomycin and Ampicillin were used as the controls. The antibiotic discs were placed on plates that were swabbed by a particular bacterial culture on an agar medium. After incubation for 24 h, the diameters of the growth inhibition zones were measured in millimeters.

2.5. Antimicrobial activity

S. aureus, P. aeruginosa, E. coli and *C. albicans* were used in the antimicrobial assay. Organisms were obtained from the bacteriological laboratory of the National Center for Public Health Laboratories. Yemen's capital, Sana'a. The extracts were dissolved in Dimethyl Sulfoxide (DMSO) to a concentration of 4 mg/ml with a stock solution and stored in a refrigerator until use [20]. Three different dilutions of the extract (600 μg, 400 μg, and 200 μg) were prepared and tested on sterile Muller Hinton agar plates according to the manufacturer's instructions. Then that, 0.1 ml of each stock solution of the test isolates was spread-plated in separate plates of the prepared Muller Hinton agar, and five wells were created in each of the inoculated plates using a sterile cork borer (6.00 mm in diameter). To serve as a negative control, 0.1 ml of sterile DMS was added to the central well of each plate, while 0.1 ml of each of the three extracts was added. Each test organism was replicated in triplicate on the plates and incubated at 37^0C for 24 h, except for *C. albicans* which was incubated for 48 h. Millimeters of the zones of inhibition formed by various doses of the extract were measured for each of the test isolates, and the triplicate data generated for each concentration were transformed to means and standard error of mean via the GraphPad Instant statistical program [21].

2.6. Determination of Minimum Inhibitory Concentration (MIC)

The antimicrobial activities of the three extracts were measured using the agar dilution method. A stock solution of the extract at a concentration of 2 g/20 ml was prepared by dissolving 2 g of the extract in 20 ml Dimethyl Sulfoxide (DMSO). Serial double dilutions of the stock solution were made into four test tubes containing 7 ml of Dimethyl Sulfoxide (DMSO) by transferring 7 ml of the stock to the first tube and then 7 ml from the first tube, concentrating as follows (50, 25, 12.5, and 6.25 ml). Six Muller–Hinton agar plates were used for each sample. The diluted extracts were aseptically applied to appropriate plates using a micropipette. For 24 h, six sets of plates for each organism were incubated at 37 °C. The minimum inhibitory concentration (MIC) of the extract is approximately 99 percent [21].

3. RESULTS

3.1. Antioxidant Activity

The ethyl acetate extract exhibited the highest Total Antioxidant Activity. 370 Ascorbic equivalent μg ASA/mg ($p < 0.05$). Acetone and chloroform extracts showed a decrease in the activity of 340 and 305 ascorbic equivalents μg ASA/mg, respectively (Table 1). The total antioxidant capacity of the acetone extract was significantly higher than that of the chloroform extract ($P < 0.05$).

Table 1. Antioxidant activity of *C. deflersiana* three extracts

Concentration µg/ml	Ascorbic acid							*Caralluma deflersiana*		
								Et.AOc	Acetone	Chloroform
	0.2	0.4	0.6	0.8	1	2	4	1.142	1.321	1.112
λ=535nm	0.019	0.042	0.068	0.089	0.101	0.223	0.440	0.131	0.129	0.112

3.2. Disc diffusion Assay of standard Antibiotics

Using the disc method, the effectiveness of the three antibiotics was determined against three bacteria and one fungus; ampicillin showed the highest zone of inhibition (22 mm) against *Staphylococcus aureus*, *penicillin* showed the minimum zone of inhibition against *P.aerugnosa* (5 mm) not aginst *E. coli* (9 mm) and all antibiotics showed a low zone of inhibition against *C. albicans* (Table 2).

Table 2. Showing results for zone of inhibition **(Antibiotics)**

S No.	Bacterial strain	zone of inhibition in diameter (mm) of control		
		penicillin	Ampicillin	Vancomycin
1	*S. aureus*	6 mm	22 mm	4 mm
2	*P. aeruginosa*	5 mm	18 mm	12 mm
3	*E. coli*	9 mm	15 mm	20 mm
4	Fungi *C. albicans*	3 mm	11 mm	5 mm

3.3. Antimicrobial activity test

The inhibition zones of the three extracts (chloroform, acetone, and ethyl acetate) from *C. deflersiana* were determined using the agar-disk diffusion method, as shown in Table 3. The three extracts showed antibacterial activities against *S. aureus*, *P. aeruginosa*, *E. coli*, and *C. albicans*. *C. deflersiana* extracts (chloroform, acetone, and ethyl acetate) are displayed in (Table 3) which expressed variable degrees of growth inhibition of the three bacterial and one fungal strains with MIC values ranging from 55.0 to 80.7 μg/ml.

Table 3. Zone Inhibition in mm of the selected microorganisms against extracts of *C. deflersiana* (600,400, and 200 μg/ml by ethyl acetate, acetone and chloroform.

Inhibition zone diameter (mm)				MIC 200 µg/ml
Test organsim	Extract of C. deflersiana µg/ml			
Antibacterial	Ethyl acetate	Acetone	Chloroform	
E. coli	15 mm	13.0 mm	10.1 mm	80.7
S. aureus	14 mm	12.0 mm	10.0 mm	67.3
P. aeruginosa	13.8 mm	11.3 mm	9.0 mm	62.0
Antifungal C. albicans	10.2 mm	6.0 mm	7.0 mm	55.0

4. DISCUSSION

The medical and pharmacological activities of therapeutic plants frequently depend on the presence of bio-active molecules (secondary metabolites) [22]. Plants also provide a valuable supply of potentially beneficial striders for the development of novel chemotherapeutic agents. The chosen plant was based on its traditional application as a folk medicinal herb for the treatment of many different types of diseases in several countries, including Yemen [16]. Spectrophotometric investigation of three extracts of *C. deflersiana* showed high antioxidant activity against the ethyl acetate extract (370 μg ASA/mg), followed by acetone and chloroform extracts (340 and 305 μg ASA/mg). The antioxidant properties of this plant extract are due to the presence of phenolic compounds that contribute to its antioxidant activity of the plant extract [23]. In the present study, the antimicrobial activity was examined against three bacteria and one fungus. Our findings indicated a difference in *C. deflersiana* antibacterial activity of *C. deflersiana*. Various solvents have been employed to extract various phytoconstituents based on their solubility or polarity in the solvent [24], which explains the different results of the antibacterial activity of the extracts using different solvents.

5. CONCLUSION

The findings of this study revealed that the *Caralluma deflersiana* plant extract had antioxidant properties. Therefore, it may be recommended for use in the development of novel drugs in the healthcare industry. Further research on *C. deflersiana* is required to determine the pharmacokinetic characteristics of this plant.

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