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Article

Physicochemical, Phytochemical, and Antibacterial Properties of *Cordia myxa* Bark Used in Darfur for Drinking Water Treatment

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ABSTRACT

The current study aimed to investigate some physicochemical, phytochemical, and antibacterial potential of the bark of *Cordia myxa* that used by local inhabitants in Darfur, for purification of raw water gathered in pools and valleys. According to the data analysis process, the physicochemical analysis showed no effect on water hardness, but the bark of *C. myxa* was rich in potassium (28.17%) and calcium (14.69%). The data analysis also showed good quantities of minerals such as silicon, aluminum, iron, magnesium, sulfur, chloride, bromide, and low quantities of some heavy metals. Some of these minerals are important in purifying water from impurities. In addition, the phytochemical analysis revealed the presence of some interested bioactive compounds, while antibacterial testing exhibited a significant antibacterial activity which could be attributed to the presence of some phytochemical compounds such as terpenoids and coumarins in the *C. myxa* bark. To sum up this section, we can say that, the current study confirms the ethnobotanical use of this plant product in the treatment of water for drinking purposes.

Key words: *Cordia myxa*, Minerals, Total hardness, Terpenoids, Antibacterial.

1. INTRODUCTION

Natural products from plant, marine, and microbial sources have been a major source of primary health-care systems and drug regimens that form the cornerstone of modern pharmaceutical medications. These products considered as the origin for about 50% of modern pharmaceuticals such as quinine, morphine, theophylline, paclitaxel, digoxin, vincristine, doxorubicin, cyclosporine, penicillin G, and Vitamin A and many more [1]. Studies showed that >80% of the population in the developing countries are depending on the traditional medicine and its prescriptions against different ailments, the majority of these populations are in Africa where ethnopharmacology is a fundamental heritage [2]. Literature review also showed that medicinal plants are rich in bio-active and bio-nutrient chemical compounds known as phytochemical compounds or secondary metabolites, some of these compounds have more than one function on both the animal and human body. For example, alkaloids, flavonoids, glycosides, tannins, saponins, phenolics, and terpenoids are among these bioactive compounds [3]. *Cordia myxa* is plant belong to family Boraginaceae; the genus Cordia includes trees and shrubs which are indigenous to tropical regions. It comprises about 300 species, most of them are used in traditional medicine, while, some are used as antimalarial, astringent, cough suppressant, anti-inflammatory, anthelminthic, diuretic, febrifuge, appetite suppressant, in addition to their function in treating lung diseases, leprosy, and urinary tract infections [4]. The tree of *C. myxa* which is widely distributed in northern parts of Darfur, in Sudan is locally known as Mukkait, its fruits are eaten by the local people there after removal of its sour taste with maceration in water several times, its bark is used in removal of impurities from rainwater that accumulated in ponds during autumn seasons, where water stay for months, additionally, domestic animals drink from it. However, local inhabitants believe that bark of *C. myxa* provides them with clean and healthy drinkable water. The aim of this investigation is to determine some physicochemical compounds, phytochemical constituents, and antibacterial properties of *C. myxa* bark to understand its rule in water treatment.

2. EXPERIMENTAL

2.1. Plant Collection and Extraction

About ½ kg of the bark as well as different parts of the targeted plant is used ethnobotanically. Different parts from *C. myxa* tree were collected by local inhabitants from Darfur region, Western Sudan. The parts were then used for authentication of the plant. After that, bark parts (Figure 1) were dried in shade, backed, and transported to our labs at Qassim University, KSA. The dried barks of *C. myxa* were ground to get a fine powder. 50 g of the powder was put inside a well-tighten dark container, mixed with 500 ml methanol (80% v/v) and subjected to continuous shaking for 3 days using a shaker (250 rpm.). Then, filtered twice and the filtrate were evaporated under reduced pressure using rotavapor to get a semi-solid crude, which dried in the incubator at 40°C for up to 48 h.

2.2. Chemical Analysis

First, 0.5 g of the ground bark of *C. myxa* was used to determine the mineral elements such as K, Al, Na, Ca, and Fe using X-ray fluorescence

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Figure 1: Bark of *Cordia myxa* used for the treatment of drinkable water.

(ARL™ QUANT'X EDXRF Spectrometer), as mentioned in Alassane *et al*. [5]. Second, 1 g of the ground *C. myxa* bark was placed in four polyethylene bottles 500 ml. Then, 50 ml of tap water was added to each bottle. After that, the mixtures in the bottles were shaken by a shaker (300 rpm) for 30, 60, 90, and 120 min [6], the suspension was then filtered using a filter paper and the filtrate was used to measure the pH and total dissolved salts through using pH instrument and a direct immersion of electrode in the water. Total Hardness (TH) was also estimated by titration method using 0.01M ethylenediaminetetraacetic acid solution [7].

2.3. Phytochemical Testing

The determination of the presence of various phytochemical constituents in the methanolic extract of *C. myxa* was done by following the method as described by Ghasham *et al*. [8]. The tests used were Wagner's method for alkaloids, Borntrager's method for anthraquinones, Keller-Kiliani method for cardiac glycosides, lead acetate method for flavonoids, ferric chloride method for phenols, HCl method for phlobatannins, Liebermann–Burchard method for steroids, Braemer's method for tannins, Liebermann–Burchard method for terpenoids, acid-alkali method for volatile oils, frothing method for saponins, isoamyl method for leucoanthocyanins, NaOH method for coumarins, NH_4OH-Benzene method for emodins, Liebermann–Burchard method for phytosterols, H_2SO_4-KOH method for quinones, acetic anhydride - H_2SO_4 method for resins, and sodium bicarbonate method for carboxylic acid.

2.4. Antibacterial Testing

This test was used for the screening of the potential antibacterial activity of methanol extract of *C. myxa* bark, following the procedure mentioned in Abdallah [9], with minor modification. Before the experiment, the dried methanol extract was reconstituted in 80% methanol to get 100 mg/ml. A sterile Petri dishes containing an autoclaved nutrient agar (20 ml per dish), were prepared. The diameter of the Petri dish is 90 mm. Four strains of American type culture collections (ATCC) were used in this test (*Bacillus cereus* [BC] ATCC 10876, *Staphylococcus aureus* [SA] ATCC 29213, *Pseudomonas aeruginosa* [PA] ATCC 9027, and *Escherichia coli* [EC] ATCC 25922), which were sub-cultured and a single colony was transferred to a small bottle (size 5 ml) and diluted with sterile normal saline (0.9%) drop by drop to get turbidity equivalent to McFarland standard. The adjusted bacterial suspension was then swapped over Mueller-Hinton Agar plate. Furthermore, 5 wells were punched on Agar plate, 50 µL of reconstituted extract (500 mg/mL) then loaded in 4 wells using Eppendorf pipette, another 50 µL of chloramphenicol (5 mg/mL) was loaded to the fifth well (in the middle of the plate) and served as a positive control.

Table 1: Chemical composition of *Cordia myxa* bark extract.

Compound	m/m%	Element	m/m%*
K_2O	33.94	K	28.17
CaO	20.54	Ca	14.69
SO_3	16.04	SX	6.42
Cl	11.12	Cl	11.12
SiO_2	9.40	Si	4.39
Al_2O_3	3.67	Al	1.94
Fe_2O_3	2.12	Fe	1.49
P_2O_5	1.08	PX	0.47
Br	0.788	Br	0.788
TiO_2	0.477	Ti	0.286
MgO	0.37	Mg	0.22
MnO	0.187	Mn	0.145
ZnO	0.178	Zn	0.143
SrO	0.048	Sr	0.04

*m/m% means: Mass/Mass percent. *C myxa: Cordia myxa*

Table 2: Physicochemical analysis of salty tap water before and after treatment.

Parameters	Tap water without *C. myxa* bark	Tap water with *Cordia myxa* bark (Time intervals)			
		1 h	2 h	3 h	4 h
pH	8.2	5.3	4.9	5.5	4.5
TDS mg/L	360	550	560	550	570
Cond. ms/cm^{-1}	0.73	1.11	1.12	1.11	1.15
TH mg/L	1.7	1.7	1.7	1.7	1.7

*Tap water tested are not used for drinking purposes, ms=micro semen. TDS: Total dissolved salt, Cond.: Conductivity, TH: Total hardness, *C myxa: Cordia myxa*

In addition, 80% of methanol was used in the reconstitution of the plant extract, which has no effect on bacterial growth. The prepared plates containing different bacterial strains were incubated for 24 h at 37°C. Finally, zones of inhibition (ZI) around the wells were measured in millimeters (mm) and the mean of 4 replicates was calculated.

3. RESULTS AND DISCUSSION

Chemical composition analysis result in Table 1 presented that potassium 28.17% and calcium 14.69% were the most abundant minerals present in the bark approximately as obtained in *C. myxa* fruit [10]. Other minerals detected in reasonable amounts were silicon, aluminum, iron, and magnesium, which were approximately 4.39%, 1.94%, 1.49%, and 0.22%, respectively, while, sulfur, chloride, and bromide were 6.42, 11.12, and 0.788%, respectively. A small amount of heavy metals such as zinc and manganese were detected in the bark of the *C. myxa* conversely the fruits which were not detected [11]. These heavy elements may have been absorbed from the soil and the different environment. Potassium and aluminum are important as a constituent of alum is used to coagulate suspended impurities into larger particles, such as algae, protozoa, viruses, bacteria, and some metal ions (such as

Table 3: Phytochemical analysis of methanol extract of *C. Myxa* bark.

Phytochemicals constituents	Results
Alkaloids	-
Anthraquinone	-
Carboxylic acid	+
Cardiac glycosides	+
Coumarins	+
Emodins	-
Flavonoids	-
Leucoanthocyanins	-
Phenols	-
Phlobatannins	-
Phytosterols	+
Quinones	+
Resins	+
Saponins	-
Steroids	-
Tannins	-
Terpenoids	+
Volatile oils	-

+ = Test Positive, - = Test Negative, *C myxa: Cordia myxa*

Table 4: Mean ZI of *C. myxa* extract against tested bacterial strains.

Tested compound	Mean ZI (mm)*			
	Bc	Sa	Ps	Ec
Methanol extract (500 mg/ml)	21.7±1.3	23.2±0.6	26.7±1.7	19.0±0.4
Chloramphenicol (5 mg/ml)	37.0±1.0	31.5±1.5	21.0±1.0	34.0±1.0
Negative control (methanol only)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

*Mean of the extract was taken from four replicates and mean of standard antibiotic was taken from two replicates. Bc: *Bacillus cereus*, Sa: *Staphylococcus aureus*, Pa: *Pseudomonas aeruginosa*, Ec: *Escherichia coli*. *C myxa: Cordia myxa*, ZI: Zone of inhibition

iron and manganese) and the resulting sludge is then removed [12]. Therefore, people in Darfur use the bark of the *C. myxa* in removing the turbidity of water. Table 2 shows the result of physicochemical analysis of tap water after treated with *C. myxa* bark. It also shows that the acidity increases due to the release of sulfur dioxide from the bark to water as well as conductivity and total dissolved salts due to the removal of some elements from the bark to the water. In contrast, the TH did not change, and this confirms its usage in the removal of turbidity.

The results of the phytochemical analysis are represented in Table 3 and showed the presence of carboxylic acid, cardiac glycosides, coumarins, phytosterols, quinones, resins, and terpenoids. Some of these phytochemicals and reported having many bioactive properties for human health; cardiac glycosides have anticancer properties [13]. On the other hand, coumarins have antimicrobial, antioxidant, anti-

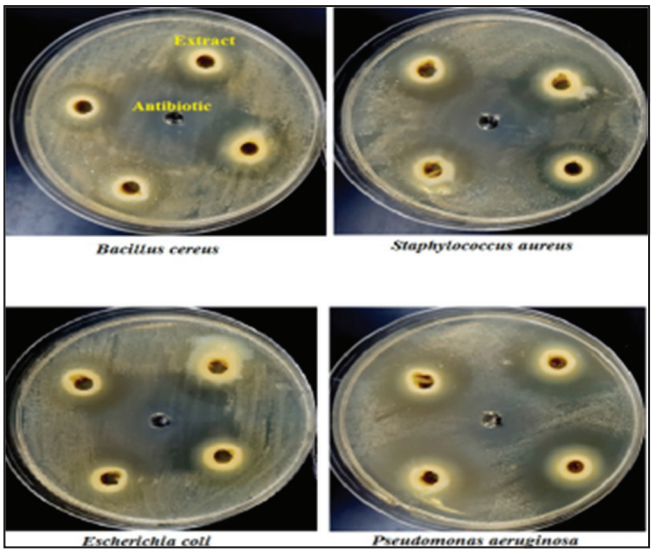

Figure 2: Susceptibility of bacteria to the methanol bark extract of *Cordia myxa.*

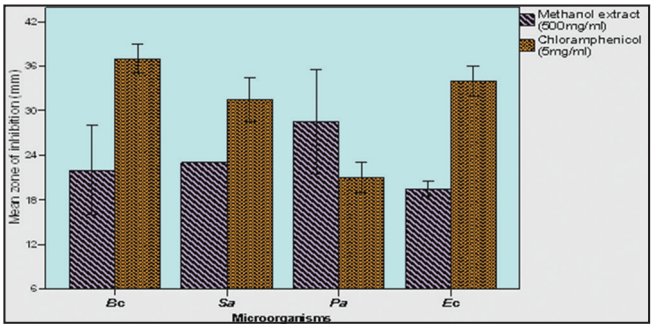

Figure 3: Antibacterial activity of the methanol bark extract of *Cordia myxa.*

allergy, anti-inflammatory, and immunosuppressant activities [14]. Moreover, phytosterols have cholesterol-lowering effect, reduced risk for heart diseases, and cancer protection [15]. In addition, quinones have antioxidant properties and it is currently in clinical trials as a new anticancer agent [16]. Terpenoids, on the other hand, have antibacterial, antifungal, antiviral, antimalarial, anti-inflammatory, and anticancer efficacy as well as some pharmaceuticals derived from terpenoids have been already launched in markets such as Taxol and Artemisinin [17].

The antibacterial screening of the methanol bark extract of *C. myxa* exhibited good antibacterial activity. All tested bacteria were susceptible to the extract (Figure 2). Surprisingly, the most susceptible bacteria were PA, which recorded 26.7±1.7 mm ZI, followed by SA (23.2±0.6 mm ZI), BC (21.7±1.3 mm ZI), and EC (19.0±0.4 mm ZI), respectively (Table 4). Moreover, as shown in Figure 3, the results were considered good antibacterial activity when compared with the wide-spectrum antibiotic tested as a positive control (Chloramphenicol). These remarkable antibacterial activities could be attributed to coumarins and terpenoids detected in the bark of *C. myxa*. In general, little is known about the antibacterial properties of the bark of *C. myxa*. However, bark extract of similar species, *Cordia dichotoma*, represented remarkable antimicrobial activity against EC, and PA, *Staphylococcus pyogenes,* and SA as well as antifungal activity *Aspergillus niger*, *Aspergillus clavatus*, and *Candida albicans* [18]. The results also showed that leaves and mucilage from fruits of *C. myxa* showed effective antibacterial activity against two pathogenic bacterial strains, namely EC and

Klebsiella pneumoniae, which isolated from urine of patients [19]. It is also found that waterborne pathogens considered as a major public health concern worldwide and associated with huge economic loss, bacterial contamination of water particularly coliform bacteria such as EC and PA threaten health and life of people with poor sanitation and hygiene conditions and consume unsafe or raw water [20].

4. CONCLUSION

The present study disclosed that the bark of *C. myxa* could be a typical natural product for raw water treatment, where its richness in minerals resembles – to some extend – the constituents of the alum ($KAl(SO_4)2.12H_2O$). The bark of *C. myxa* also clarifies the raw water from fine suspended materials and the presence of some phytochemical compounds such as terpenoids and coumarins get rids of waterborne pathogens. It provides also other phytochemicals such as carboxylic acid, cardiac glycosides, coumarins, phytosterols, quinones, resins, and terpenoids, which are characterized by some bioactive properties such as antioxidant and antitumor activity. Accordingly, the current investigation scientifically documented the benefit of this plant product to provide clean, healthy drinking water from raw water to indigenous people in Darfur, Sudan.

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