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Research Article
Antimicrobial Activity of Some Sudanese Medicinal Plants Against its Pollutant Isolated Bacteria

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Abstract

Background and Objective: Medicinal plants have been known to cure many diseases due to the presence of active bio constituents, the goal of this study to detect and evaluate the effectiveness of antimicrobial activity of some Sudanese medicinal plants. **Materials and Methods:** Methanolic extracts of the following types of local medicinal plants *Artemisia herba-alba*, *Cyperus rotundus*, *Cymbopogon schoenanthus* sb sp. *Proximus*, *Trigonella foenum-graecum*, *Nigella sativa*, *Hyphaene thebaica*, *Nauclea latifolia* and *Ziziphus spina-christi* were tested against eight types of bacteria that were isolated from the same plants as their pollutants. Cup-plate method was used and then the inhibition zone of each plant was measured and compared with the inhibitory zone of some antibiotics used for the same isolates. **Results:** Methanol extracts of some plant species showed antibacterial activity against most of the studied bacterial species. *Cymbopogon schoenanthus* ssp. *Proximus* showed antibacterial activity against most isolated bacterial species in comparison with commercial antibiotics used. **Conclusion:** According to the findings of this research, several medicinal plants have efficacy and effect on certain types of bacteria and are superior or comparable to certain types of antibiotics. When compared to the other medicinal plants used in this study*, Cymbopogon schoenanthus* sbsp. *proximus* has the highest activity on most types of Gram-positive and Gram-negative bacteria, as well as the superiority of some antibiotics.

Key words: Medicinal plants, methanolic extraction, pollutants, antimicrobial sensitivity, Methanol extracts, inhibition zone

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of medicinal plants is growing all over the world[1]. Despite modern advances in synthetic chemistry, the most effective drugs available were derived directly or indirectly from the plant kingdom. Indigenous communities had long used plant extracts to treat illness and most of these extracts had demonstrated efficacy, with new bioactive compounds being extracted and screened yearly[2]. These extracts have also been shown to be effective sources of therapeutic agents for the treatment of infectious diseases. Sudan has amassed a vast body of empirical knowledge about the use of medicinal plants in the treatment of various diseases throughout its long history[3]. The greater search for therapeutics derived from plant species is clarified by the emergence of diseases that go untreated, as well as the advancement of scientific knowledge about medicinal herbs as important alternative treatments. As a result, the safety and quality of herbal products are also very important[4]. Herbs can be contaminated with microbes from the soil, air and water, which can present possibly harmful microbes to humans. The presence of a microbial contaminant in non-sterile medicinal plant products can decrease or even inactivate the therapeutic effect of the medicinal plants, potentially harming patients who use the plants. The national and global markets for traditional medicines are rapidly expanding and worldwide sales of herbal products are generating significant economic gains[5,6].

The local markets in Khartoum State, Sudan, is an important part of the people's lives. Medicinal plant traders in this market sell large amounts of medicinal plants to people seeking their assistance. They primarily sell the various plant substances in dried form.

MATERIALS AND METHODS

Study area: The study was carried out at the Microbiology lab, College of Science and Technology, Sudan from June, 2019-October, 2020.

Plant materials: Eight medicinal plants, namely, *Artemisia herba alba* (aerial parts), *Cyperus rotundus* (Roots), *Cymbopogon schoenanthus* subsp. *proximus* (aerial parts), *Trigonella foenum-graecum* (Seeds), *Nigella sativa* (Seeds), *Hyphaene thebaica* (Fruits), *Nauclea latifolia* (Fruits) and *Ziziphus spina-christi* (Fruits) were purchased from markets (Fig. 1a-c). The plants were identified in the Botany Department, Faculty of Science and Technology, Omdurman Islamic University.

Evaluation of the microbial quality of plant: Standard plate count method was performed by a pour plate procedure standard method[7]. The test is used for quantitative evaluation of mesophilic bacteria and fungi that grow aerobically.

Isolation and identification of the Isolates: The isolation techniques were described by Duhan *et al.*[8]. The Vitek2 Compact System was designed for bacterial and yeast identification, as well as susceptibility testing of clinically significant bacteria.

Preparation of the crude methanolic plant extracts: Two hundred grams of each plant was macerated separately with 100 mL of 100% methanol (MeOH) overnight. The mother liquor was filtered and evaporated to dryness. The dry crude extract was sterilized. All extracts were stored dry in sterilized containers at room temperature until used for antibacterial testing[9].

Fig. 1(a-c): Images of some medicinal plants
(a) *Trigonella foenum-graecum*, (b) Nauclea latifolia and (c) Artemisia herba-alba

Antimicrobial testing for bacteria and fungi: The antimicrobial activity was determined using the well agar diffusion method[10]. Each test organism's inoculum size was adjusted to a suspension of 10^8 cells. Wells (10 mm in diameter) were bored in the agar with a sterile borer and the agar discs were removed. A total of 0.1 mL aliquots of the reconstituted extract were placed in a well with a pipette and the plates were held at room temperature for 2 hrs to allow the extracts to diffuse in the agar. For 24 hrs, the plates were incubated at 37°C. Following incubation, the diameters of the zones of inhibition were measured to the nearest millimeter[11].

The mean inhibition zone diameter was used to assess the strains' sensitivity to extractives from the plant species studied (MIZD) As: sensitive: >18 mm, intermediate:14-18 mm and resistant: <14 mm. The following multidisc (from Axiom laboratories) for antimicrobial susceptibility testing were used: Ampicillin/sulbactam (AS) 20 mcg, Co-Trimoxazole (Ba) 25 mcg, Cefotaxime (CF) 30 mcg, Piperacillin/Tazobactam (TZP) 100/10 mcg, Chloramphenicol (CH) 30 mcg, Ciprofloxacin (CP) 5 mcg, Ceftizoxime (CI) 30 mcg, Tetracycline (TE) 30 mcg, Ofloxacin (OF) 5 mcg, Gentamicin (GM) 10 mcg, Amikacin (AK) 30 mcg and Gatifloxacin (GF) 5 mcg for Gram-Negative Isolates. Ampicillin/sulbactam (AS) 20 mcg, Co-Trimoxazole (Ba) 25 mcg, Cephalexin (PR) 30 mcg, Tetracycline (TE) 30 mcg, Cefotaxime (CF) 30 mcg, Ciprofloxacin (CP) 5 mcg, Levofloxacin (LE) 5 mcg, Linezolid (LZ) 30 mcg, Cloxacillin (CX) 5 mcg, Roxithromycin (RF) 15 mcg, Lincomycin (LM) 15 mcg and Gentamicin (GM) 10 mcg were used for Gram-Positive Isolates. New Delhi 1100055 and methanol were used as a control.

RESULTS

Isolation and identification of isolates from some medicinal plants: As shown in Table 1, a total of eight bacteria strains was isolated from eight different medicinal plant materials. As a morphology test, bacteria colonies were identified based on their form margin, appearance, pigment, texture, colour, size and chemically properties (Fig. 2). The isolates were identified as: *Sphingomonas paucimobilis*, *Enterococcus cecorum*, *Pantoea* spp, *Klebsiella pneumonia* subsp *ozaenae* (Fig. 2a), *Kocuria rosea* (Fig. 2b), *Aeromonas salmonicida, Leuconostoc pseudomesenteroides* and *Staphylococcus lentus* (Fig. 2c).

Antimicrobial activity of medicinal plants against the bacterial isolates: Methanol extracts of some plant species showed antibacterial activity against most of the studied bacterial species (Table 2). *Cymbopogon schoenanthus* ssp.

Table 1: Types of bacteria isolated from selected medicinal plants

Plants	Bacterial isolates
Artemisia herba alba	*Sphingomonas paucimobilis*
Cyperus rotundus	*Sphingomonas paucimobilis, Enterococcus cecorum*
Cymbopogon schoenanthus sbsp. *proximus*	*Sphingomonas paucimobilis, Enterococcus cecorum*
	Pantoea spp., *Klebsiella pneumonia* subsp *ozaenae, Kocuria rosea*
Trigonella foenum-graecum	*Sphingomonas paucimobilis, Aeromonas salmonicida*
Nigella sativa	*Sphingomonas paucimobilis*
Hyphaene thebaica	*Sphingomonas paucimobilis, Aeromonas salmonicida*
Nauclea latifolia	*Sphingomonas paucimobilis, Leuconostoc pseudomesenteroides*
Ziziphus spina-christi	*Sphingomonas paucimobilis, Aeromonas salmonicida*
	Leuconostoc pseudomesenteroides, Staphylococcus lentus

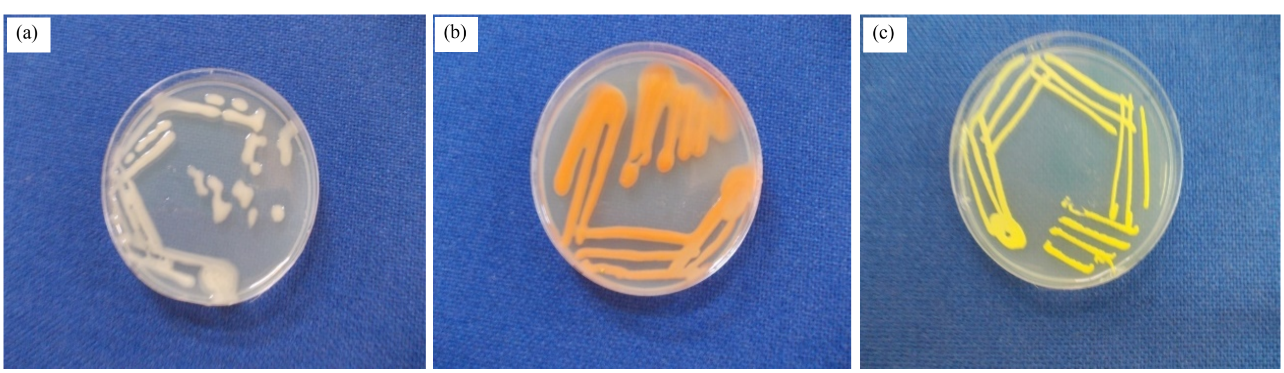

Fig. 2(a-c): Images of some bacterial isolates
(a) *Klebsiella pneumonia* sp. *ozaenae*, (b) *Kocuria rosea* and (c) *Staphylococcus lentus*

Table 2: Effect of plant extracts on the isolates and measuring of the inhibition zone

Plant extracts	Part of plant	Susceptible isolates	Inhibition zone (mm)
Artemisia herba-alba	Aerial	*Sphingomonas paucimobilis*	18 mm[S]
Cyperus rotundus	Root	*Enterococcus cecorum*	20 mm[S]
		Sphingomonas paucimobilis	19 mm[S]
Cymbopogon schoenanthus sbsp. *Proximus*	Aerial	*Pantoea* spp.	28 mm[S]
		Enterococcus cecorum	23 mm[S]
		Kocuria rosea	16 mm[I]
		Sphingomonas paucimobilis	15 mm[I]
		Klebsiella pneumonia subsp *ozaenae*	15 mm [I]
Trigonella foenum-graecum	Seed	*Aeromonas salmonicida*	20 mm [S]
		Sphingomonas paucimobilis	11 mm [R]
Nigella sativa	Seed	*Sphingomonas paucimobilis*	14 mm [I]
Hyphaene thebaica	Fruit	*Aeromonas salmonicida*	25 mm [S]
		Sphingomonas paucimobilis	19 mm [S]
Nauclea latifolia	Fruit	*Leuconostoc pseudomesenteroides*	22 mm [S]
		Sphingomonas paucimobilis	15 mm [I]
Ziziphus spina-christi	Leaf	*Sphingomonas paucimobilis*	15 mm [I]
		Aeromonas salmonicida	15 mm [I]
		Leuconostoc pseudomesenteroides	15 mm [I]
		Staphylococcus lentus	15 mm [I]

S: Sensitive, I: Intermediate, R: Resistant

Table 3: Antibacterial activity of antibiotics against gram-positive bacteria

Antibiotics	Concentration (mg^{-1})	Bacterial isolates and inhibition zones (mm)			
		S. le	*k. ro*	*E. ce*	*L. ps*
AS	20	10	20	25	12
BA	25	13	20	20	5
PR	30	7	25	20	8
TE	30	12	25	20	7
CF	30	9	-	20	8
CP	5	7	25	20	7
LE	5	8	20	20	5
LZ	30	12	20	20	10
CX	5	5	20	20	10
RF	15	10	15	-	-
LM	2	5	-	10	3
GM	10	8	15	-	5

Bacterial Isolates: *S. le*: *Staphylococcus lentus*, *L. ps*: *Leuconostoc pseudomesenteroides*, *E. ce*: *Enterococcus cecorum*, *K. ro*: *Kocuria rosea*

Proximus showed antibacterial activity against most isolated bacterial species. Seeds extracts *of Trigonella foenum-graecum* (Fig. 1a) was found effective against one tested organism*, Aeromonas salmonicida*. Fruits extract of *Nauclea latifolia* (Fig. 1b) showed high antibacterial activity against *Leuconostoc pseudomesenteroides* and moderately activity against *Sphingomonas paucimobilis*. Arial parts of *Artemisia herba-alba* (Fig. 1c) showed promising results against *Sphingomonas paucimobilis*. Fruits extract of *Hyphaene thebaica* showed high antibacterial activity against *Aeromonas salmonicida* and moderately activity against *Sphingomonas paucimobilis*. Extract of *Cyperus rotundus* roots was showed good result against *Enterococcus cecoru*m and *Sphingomonas paucimobilis*. Some medicinal plants showed excellent efficacy against isolated microbes compared to antibiotics (Table 3-6).

Antibiotics: Multi-disk for antimicrobial susceptibility testing for gram-positive isolates: Ampicillin/sulbactam AS 20 mcg, Co-Trimoxazole BA 25 mcg, Cephalexin PR30 mcg, Tetracycline TE 30 mcg, Cefotaxime CF 30 mcg, Ciprofloxacin CP 5 mcg Levofloxacin LE 5 mcg, Linezolid LZ 30 mcg, Cloxacillin CX 5 mcg, Roxithromycin RF 15 mcg, Lincomycin LM 15 mcg, Gentamicin GM 10 mcg.

Antibiotics: Multi-disk for antimicrobial susceptibility testing for gram-negative isolates: Ampicillin/sulbactam (AS) 20 mcg, Co-Trimoxazole (BA) 25 mcg, Cefotaxime CF 30 mcg, Piperacillin/Tazobactam TZP 100/10 mcg, Chloramphenicol CH 30 mcg, Ciprofloxacin CP 5 mcg, Ceftizoxime CI 30 mcg, Tetracycline TE 30 mcg, Ofloxacin OF 5 mcg, Gentamicin GM 10 mcg, Amikacin AK 30 mcg, Gatifloxacin GF 5 mcg.

Table 4: Antibacterial activity of antibiotics against gram-negative bacteria

		Bacterial isolates			
Antibiotics	Concentration (mg^{-1})	*S. pa*	*A. sa*	*P. spp*	*K. pn*
AS	20	18	19	25	20
BA	25	17	20	20	20
CF	30	7	20	25	20
TZP	100/10	23	24	20	20
CH	30	10	8	25	20
CP	5	7	20	20	20
CI	30	-	13	20	-
TE	30	15	19	25	-
OF	5	17	18	25	20
GM	10	15	20	20	20
AK	30	7	18	20	20
GF	5	17	8	15	-

Bacterial isolates: *S. pa*: *Sphingomonas paucimobilis*, *A. sa*: *Aeromonas salmonicida*, *P. spp*: *Pantoea* spp., *K. pn*: *Klebsiella pneumonia* subsp *ozaenae*, gatifloxacin GF 5 mcg

Table 5: Comparison of the effectiveness of some antibiotic discs and plant extracts on G+ve isolates with the same concentration

	Inhibition zone due to the plant's effectivity (mm)								Inhibition zone due to the discs effectivity (mm)		
Isolates	*A. he*	*C. ro*	*C. sc*	*T. fo*	*N. sa*	*H. th*	*N. la*	*Z. sp*	CP	LE	CX
E. ce	-	20	23	-	-	-	-	-	20	20	20
K. ro	-	-	16	-	-	-	-	-	25	20	20
L. ps	-	-	-	-	-	15	22	15	7	5	10
S. le	-	-	-	-	-	-	-	15	7	8	5

Medicinal plants: *A. he*: *Artemisia herba-alba*, *C. ro*: *Cyperus rotundus*, *C. sc*: *Cymbopogon schoenanthus* sbsp. *Proximus*, *T. fo*: *Trigonella foenum-graecum*, *N. sa*: *Nigella sativa*, *H. th*: *Hyphaene thebaica*, *N. la*: *Nauclea latifolia*, *Z. sp*: *Ziziphus spina-christi*. Bacterial isolates: *E. ce*: *Enterococcus cecorum*, *K. ro*: *Kocuria rosea*, *L. ps*: *Leuconostoc pseudomesenteroides*, *S. le*: *Staphylococcus lentus*

Table 6: Comparison of the effectiveness of some antibiotic discs and plant extracts on G-ve isolates with the same concentration

	Inhibition zone due to the plant's effectivity (mm)								Inhibition zone due to the discs effectivity (mm)		
Isolates	*A. he*	*C. ro*	*C. sc*	*T. fo*	*N. sa*	*H. th*	*N. la*	*Z. sp*	CP	OF	GF
S. pa	18	19	15	11	14	19	15	15	7	17	17
P. spp	-	-	28	-	-	-	-	-	20	25	15
K. pn	-	-	15	-	-	-	-	-	20	20	-
A. sa	12	-	-	20	-	25	-	15	20	18	8

Medicinal plants: *A. he*: *Artemisia herba-alba*, *C. ro*: *Cyperus rotundus*, *C. sc*: *Cymbopogon schoenanthus* sbsp. *Proximus*, *T. fo*: *Trigonella foenum-graecum*, *N. sa*: *Nigella sativa*, *H. th*: *Hyphaene thebaica*, *N. la*: *Nauclea latifolia*, *Z. sp*: *Ziziphus spina-christi*. Bacterial isolates: *S. pa*: *Sphingomonas paucimobilis*, *P. spp*: *Pantoea* spp., *K. pn*: *Klebsiella pneumonia* subsp *ozaenae*, *A. sa*: *Aeromonas salmonicida*

DISCUSSION

In the current study, eight different types of medicinal plants were used. A group of eight microbes that are contaminating these plants were isolated. One of the major shortfalls of herbal preparation in developing economies is the unhygienic conditions under which they are delivered[12]. It was discovered in the current study that herbal remedies were not sterile. This could be due to a lack of proper handling and marketing services in the markets visited, causing the natural products to fail to withstand pollution. Contamination may be even worse where dispensing hygiene is poor[13]. The most common source of plant infection was contact with polluted water, but recent studies have shown that some bacterial strains can also attack and replicate inside plant tissue[14].

In this study, compounds were extracted from different parts of these medicinal plants such as roots, fruits, seeds, aerials and leaves. These extracts were used against the types of microbes isolated from the plants themselves and then the effectiveness and sensitivity of these compounds in killing the microbes were measured. Concerning plants, it was found that *Cymbopogon schoenanthus* sbsp. *Proximus* is more effective against isolated microbes when extracting from the aerials. As for the microbes, *Sphingomonas paucimobilis* bacteria is more sensitive to more than one type of medicinal plants such as *Cyperus rotundus* and *Cymbopogon schoenanthus*. *Proximus* and *Hyphaene thebaica*, however these findings consistent with previous data exhibited the *C. schoenanthus* essential oil was found to have antibacterial activity against five of the ten investigated bacterial pathogens. Using the well-diffusion approach, El-Kamali *et al.*[15] discovered that the essential oil of *C. nervatus* demonstrated antibacterial action against all microorganisms tested such as *S. aureus*, *Bacillus subtilis*, *E. coli, P. aeruginosa*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, excepting for Salmonella typhi. The greatest inhibiting impact was shown against *S. dysenteriae* and *K. pneumoniae*. Furthermore, ethanol and chloroform extracts of *C. schoenanthus* obtained in Salboukh, Saudi Arabia, were found to have antibacterial activity against *S. aureus*. The MICs of both extracts for *S. aureus* were greater than what Lahlou[16] reported. This difference could be attributed to the process of essential oil extraction. Significant discrepancies in results for the same plant species are fairly uncommon. These variances could be attributable to a variety of factors, including the type of essential oil extraction, meteorological, seasonal and geographical conditions and harvest timing. As a result, it is critical to standardize extraction processes and identify all circumstances that may affect extraction[17].

In this research, we found that most types of medicinal plants used in this study affected 4 types of Gram-positive isolated bacteria. *Cymbopogon schoenanthus* sbsp. *Proximus*, *Cyperus rotundus* and *Nauclea latifolia* plants had the highest inhibitory zone compared to commercial antibiotics used such as Ciprofloxacin, Levofloxacin, Cloxacillin. *Hyphaene thebaica* and *Ziziphus spina-christi* have an intermediate inhibitory effect than the antibiotics used. Regarding the Gram-negative bacteria isolated, *Cymbopogon schoenanthus* sbsp. *Proximus* and *Hyphaene thebaica* showed high efficacy compared to the antibiotics used. The previous study reported that *Cyperus rotundus* extract possesses an antimicrobial effect and inhibits the growth of ampicillin-resistant *Staphylococcus aureus* and thus may be considered as an effective agent in treating patients infected with ampicillin-resistant strains of *Staphylococcus aureus*[18,19]. Another study displayed that the crude extract and fractions of whole fruit of *Nauclea latifolia* exhibited a broad-spectrum antibacterial activity and the rate of killing of the extract appears to be time and concentration-dependent. This activity showed the plant's potentials in developing therapeutic agents for the treatment of bacterial infections[20]. *Hyphaene thebaica* fruit possessed antimicrobial activities against the tested organisms, *Staphylococcus aureus* DQ269498.1, *Listeria monocytogenes* GE305625.1, *Escherichia coli* CP009273.1, and *Salmonella typhi* GU390666. Therefore, Toxicity and clinical investigations are advised for potential medication development[21,22]. The antimicrobial activity of *Ziziphus spina-christi* extracts was evaluated against four standard bacteria species (Gram-positive; *Bacillus subtilis*, *Staphylococcus aureus*) and (Gram-negative; *Escherichia coli*, *Pseudomonas aeruginosa*). Antimicrobial studies revealed that the methanolic extract reduced the growth of all bacteria and most extracts shown numerous points of antimicrobial activity[23].

In general, *Cymbopogon schoenanthus* sbsp. *proximus* is the most effective plant against most types of bacteria isolated, whether Gram-negative or Gram-positive and is superior to most of the antibiotics used in the study.

CONCLUSION

From this study, it can be concluded that some medicinal plants have efficacy and effect on some types of bacteria and are superior or similar to some types of antibiotics. It has been found that *Cymbopogon schoenanthus* sbsp. *Proximus* has the highest activity on most types of Gram-positive and Gram-negative bacteria compared to the rest of the other medicinal plants used in this study and the superiority of some antibiotics.

SIGNIFICANCE STATEMENT

This study discovered that some medicinal plants, such as *Cymbopogon schoenanthus* sbsp, have efficacy and effect on specific types of bacteria and are superior or similar to various forms of antibiotics. When compared to the other medicinal plants included in this study, *Proximus* had the highest activity against most types of Gram-positive and Gram-negative bacteria. This research will assist the researcher in determining the critical area of medicinal plants and their advantage over some antibiotics.

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