



Received on 05 May 2019; received in revised form, 24 August 2019; accepted, 01 September 2019; published 01 February 2020

VARIOUS MEDICINAL PLANTS: A PROMISING TREATMENT FOR MULTIDRUG-RESISTANT BACTERIA ISOLATED FROM WOUND INFECTION

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Keywords:

Wound infection,
Multidrug-Resistant bacteria,
Medicinal plants, Sudan

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ABSTRACT: Bacteria have proved remarkably developing resistant to a known antibiotic; however, Medicinal plants have manifested notably useful for the control of bacterial infections. This study aimed to isolate and identify the multidrug-resistant bacteria from wound infection, then examine the antibacterial activity of different concentration of the ethanolic extracts of *Cymbopogon proximus*, *Azadirachta indica*, and *Grewia senegalensis* plants against it. One hundred specimens collected from wound infection, isolation and identification done according to standard methods. Antibiotics susceptibility test performed using the Kirby-Bauer disc diffusion method for: Cotrimoxazole (25 µg) Ciprofloxacin (5 µg), Gentamicin (10 µg), Imipenem (10 µg), Erythromycin (15 µg), Tetracycline (30 µg) Methacillin (5 µg), Ceftazidime (30 µg). 94 isolates showed bacterial growth, 40 (42.6%) isolates were Gram-positive bacteria (*Staphylococcus aureus*) and 54 sample Gram-negative bacteria; 15 (27.8%) *Pseudomonas aeruginosa*, 13 (24.1%) *Proteus* spp, 12 (22.2%) *Escherichia coli*, 10 (18.5%) *Klebsiella pneumoniae*, 4 (7.4%) *Citrobacter freundii*. Ethanolic extracts from Sudanese medicinal plants namely *Cymbopogon proximus*, *Azadirachta indica*, and *Grewia senegalensis*, screened for their antimicrobial properties against isolated multidrug-resistant bacteria at different concentrations; 100, 50, 25, 12.5 and 6.25 mg/ml using agar disc diffusion and agar well diffusion method. The result revealed that all plants extracts were active against most of the resistant bacteria with MIC ranges from 50-6.25 mg/ml. In conclusion, the number of multidrug-resistant bacteria in wound infection was high, and the tested plant's species have a promising level of activity against multidrug-resistant bacteria. Therefore, manufacture these extracts at pharmacological form such as topical ointment should also be considered.

INTRODUCTION: The term multidrug-resistant's (MDR) applies to the bacterium that is simultaneously resistant to some antimicrobials belonging to different chemical classes ¹⁻³.

Furthermore, Antibiotic resistance is a global challenge that impacts all pharmaceutically used antibiotic.

In recent years' pharmaceutical companies have almost stopped producing new antibiotics which have led researchers to look for alternative antimicrobial. Herbs widely use for the treatment of infectious diseases in many developing countries ⁴. Therefore, in Sudan, with a high percentage of multidrug-resistant bacteria, we in urgent need to develop a new drug from our traditional medicine.

<p>QUICK RESPONSE CODE</p>	<p>DOI: 10.13040/IJPSR.0975-8232.11(2).839-43</p> <hr/> <p>This article can be accessed online on www.ijpsr.com</p> <hr/> <p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(2).839-43</p>
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A wide range of medicinal plant parts use for extract as unprocessed drugs, and they possess various medicinal properties. The secondary metabolism of the plant was found to be a source of various phytochemicals that could directly be used as intermediates for the production of new drugs⁵⁻⁸.

Nosocomial infection is one of the critical reasons why wound healing may still leading to increased risk of patient morbidity and mortality⁹⁻¹¹. These infections often occur following a break-in healthy skin integrity from either trauma or skin disease; the vast majority of these infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*^{1, 11}. The problem of microbial resistance is growing, and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, action must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to understand the genetic mechanism of resistance better, and to continue studies to develop new drugs, either synthetic or natural^{12, 13}.

Three Sudanese medicinal plants, namely *Cymbopogon proximus*, *Azadirachta indica*, and *Grewia senegalensis* may possess antibacterial activity because it used traditionally for the treatment of many infectious and chronic diseases. Consequently, this study aimed to: firstly, verify the antibacterial activity of that plant against multidrug-resistant bacteria isolated from wound infection. Secondly, to isolate and identify the pathogenic bacteria from wound infection, thirdly, evaluate *in-vitro* susceptibility pattern of bacteria isolated from wound specimen against multidrug, besides, to determine the prevalence of resistant bacteria in wound infection. Fourthly, compare between the antibacterial activity of selected plants extracts on Multidrug-Resistant (MDR) pathogenic bacteria with determining the minimum inhibitory concentration (MIC) of these plants extracts and compare their activity with the standard used antibacterial agent in Sudan.

MATERIALS AND METHODS:

The Study Area, Collection, and Identification of Samples: This study was a descriptive and cross-sectional study carried out in Omdurman Military Hospital and Jabir Abulaiz Hospital in

Khartoum State, Sudan. During the period from February to July 2018, which studied the occurrence of patients admitted with a symptom of chronic diseases of wound infections in 100 patients, samples collected under the aseptic condition, then cultured on Blood agar and McConkey's agar. Then Inoculated plates were incubated both aerobically and facultative an aerobically at 37 °C for 18-24 h¹⁴. Isolation and identification were done according to gram stain and biochemical tests^{15, 16}.

Antimicrobial Susceptibility Test: Susceptibility testing performed on all bacterial isolates using the Kirby-Bauer disc diffusion method using: Cotrimoxazole (25 µg) Ciprofloxacin (5 µg), Gentamicin (10 µg), Imipenem (10 µg), Erythromycin (15 µg), Tetracycline (30 µg) Methacillin (5 µg), Ceftazidime (30 µg). This method performed on Mueller-Hinton agar (Himedia, India) following the performance standards for antimicrobial susceptibility testing recommended by the Clinical and Laboratory Standards Institute guidelines (CLSI) 2017.

Antimicrobial Activity of Selected Medicinal Plants: Ethanolic extracts of *Grewia senegalensis* whole plant, *Azadirachta indica* leaves and *Cymbopogon proximus* whole plant obtained from medicinal and aromatic plant and Traditional Medicine Research Institute, National Center for Research, Khartoum, Sudan.

Plants extracts diluted into different concentration as follows: 100, 50, 25 and 12.5 mg/ml. The new subculture of the selected multidrug-resistant bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Citrobacter* spp) standard bacteria used as a control. Bacteria Isolated from the clinical sample inoculated into 3.0 ml of sterile normal saline. Inoculum density compared with McFarland stander solution using agar disc diffusion assay. Minimum Inhibition Concentration (MICs) determined by broth media using the tube method incubated overnight at 37 °C.

Data Analysis: The data analysed by a statistical package for social sciences (SPSS) software programme version 20. (SPSS Inc., Chicago, IL).

Chi-square test was used to correlate between antimicrobial growth inhibition zone and various medicinal plants, a P-value of <0.05 was considered significant.

RESULTS: Out of the 94 positive culture for bacterial growth, 40 were Gram-positive (42.6%), and 54 were Gram-negative (57.4%). Identify and isolated bacteria showed: *Staphylococcus aureus* was most abundant 40 (42.6%), 22 of *S. aureus* was found resistant to methicillin and considered as MRSA, while 54 Gram-negative follow by *P.*

aeruginosa 15 (27.7%), *Proteus mirabilis* 8 (14.8%), *Proteus vulgaris* 5 (9.2%), *E. coli* 12 (22.2%), *K. pneumonia* 10 (18.5%) and *Citrobacter freundii* 4 (7.4%).

Only multi-drug resistant isolated bacteria; which showed resistant to three antibiotic or more tested for their sensitivity to selected medicinal plants extracts. These include 22 *Staphylococcus aureus*, 11 *Pseudomonas aeruginosa*, 5 *Proteus mirabilis*, 5 *Escherichia coli*, and 5 *Klebsiella pneumonia* as shown in **Table 1**.

TABLE 1: ANTIMICROBIAL ACTIVITIES OF SELECTED ANTIBIOTICS AGAINST CLINICAL STRAINS ISOLATED FROM WOUND INFECTIONS

Isolated bacteria	No. of isolates showing resistance to an antibiotic									
	Methicillin	Penicillin	Ceftazidime	Imipenem	Erythromycin	Tetracycline	Gentamycin	Coltrimoxazole	Ciprofloxacin	Total
<i>Staphylococcus aureus</i>	22	24	25	-	24	-	-	18	1	40
<i>Pseudomonas aeruginosa</i>	-	-	-	0	-	8	6	8	5	15
<i>Proteus mirabilis</i>	-	-	-	0	-	5	1	5	2	5
<i>Proteus vulgaris</i>	-	-	8	0	-	7	0	4	3	8
<i>Escherichia coli</i>	-	-	12	0	12	10	0	9	7	12
<i>Klebsiella pneumonia</i>	-	-	-	0	9	7	1	5	3	10
<i>Citrobacter freundii</i>	-	-	-	0	-	4	0	2	2	4

The Activity Ethanolic Extracts of Tested Plants on Wound Isolates:

***Cymbopogon proximus*:** The antimicrobial test showed the strong inhibited against that growth of multidrug-resistant bacteria studies, which showed activity in all tested concentrations. The *Cymbopogon proximus* was effective against 17 *Staphylococcus aureus*, 9 *Pseudomonas aeruginosa*, 3 *Proteus mirabilis*, 3 *Escherichia coli* and 5 *Klebsiella pneumonia*.

At the highest concentration applied of 100 mg/ml were exhibited large inhibition zone against *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia* 22 mm in diameter at

100mg/ml, followed by *Staphylococcus aureus*, *Escherichia coli* 21mm in diameter at 100 mg/ml. The MIC values were as follows: 12.5 mg/ml for *Staphylococcus aureus* and *Proteus mirabilis*, 6.25 mg/ml for *Pseudomonas aeruginosa*, 25 mg/ml for *Escherichia coli* and *Klebsiella pneumonia* ethanol extract of *Cymbopogon proximus*.

***Azadirachta indica*:** The results showed that extract of neem possesses antimicrobial activity against all tested multidrug-resistant bacteria and the MIC value was as follows: 12.5 mg/ml for *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella pneumonia*, 25 mg/ml for *Pseudomonas aeruginosa* and *Escherichia coli*.

TABLE 2: ANTIMICROBIAL ACTIVITIES OF ETHANOLIC EXTRACTS OF CYMBOPOGON PROXIMUS, AZADIRACHTA INDICA AND GREWIA SENEGALENSIS AGAINST MDR ISOLATED BACTERIA

Tested bacteria and number	Mean inhibition zone in mm at 100mg/ml			P. value
	<i>C. proximus</i>	<i>A. indica</i>	<i>G. senegalensis</i>	
<i>S. aureus</i> (22)	18.72	17.63	12.4	0.006
<i>P. aeruginosa</i> (11)	18.27	16.64	15.64	0.055
<i>P. mirabilis</i> (5)	18.80	14.60	13.60	0.935
<i>E. coli</i> (5)	17.40	16.00	13.8	0.799
<i>K. pneumonia</i> (5)	19.00	14.46	13.40	0.938

Grewia senegalensis: The antimicrobial effect of ethanolic extract of *Grewia senegalensis* was shown to have activity against multidrug-resistant bacteria studies and showed activity only in concentration 100 mg/ml **Table 2**.

DISCUSSION: Multidrug resistance bacteria characterized by the different patterns of resistance found in healthcare-associated, antimicrobial-resistant bacteria was defined as acquired non-susceptibility to at least three or more antimicrobial, novel development combination therapies for the treatment of MDR bacteria^{17, 18}. This study showed a high number of multidrug resistance in isolated bacteria; gram-negative bacteria, in particular, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* and *Staphylococcus aureus* were most abundant which agreed with previous studies^{19, 20}.

Several drugs of plant, mineral and animal origin described in the Ayurveda for their wound healing properties²¹. The current study was carried out to screen the antibacterial activity of *Cymbopogon proximus*, *Azadirachta indica* and *Grewia senegalensis*, ethanolic extract against wound infection bacterial isolates *S. aureus*, *P. aeruginosa*, *Proteus* spp, *E. coli*, and *K. pneumonia*. The results showed the high activity of ethanolic extract of *Cymbopogon proximus*; these results ultimately agreed with that obtained by²². The *Azadirachta indica* exhibited high antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from some diabetes mellitus patients.

It agreed with reported by^{23, 24}. Moreover, several compounds derived from plants revealed significant activity against gram-positive bacteria only²⁵. The result agreed with Samy and Selim, 2011, who reported the activity component of *C. proximus* to consider as strongly inhibition bacteria growth: piperitone, elemol, α -eudesmol, β -eudesmol, and limonene²².

The activity of *A. indica* and *G. senegalensis* might be due to the secondary metabolites, as it is reported the active antimicrobial compound for *A. indica* and *G. senegalensis* leaves alkaloids, glycosides, flavonoids, saponins, tannins and phenolic compound²⁴.

CONCLUSION: Ethanolic extracts of *Cymbopogon proximus*, *Azadirachta indica*, and *Grewia senegalensis* possessed antibacterial activity against same multidrug-resistant bacteria; which justify their use in Sudanese traditional medicine as wounds healer and for treatment of various infection caused by resistant pathogenic bacteria. The ethanolic extract showed high activity against gram-positive than gram-negative. The efficiency of the antibacterial of ethanolic extract was found to increase by increasing concentration. Based on this study, we recommended that; isolation and purification of the active ingredient in these plants extracts responsible for the antibacterial activity should be done, determination of the toxicity of the active ingredient and Manufacture these extracts at pharmacological form such as topical ointment should be considered. Decrease the antimicrobial-resistant phenomena by increase the patient's awareness about the impact of improper using of antibiotics.

ACKNOWLEDGEMENT: The author is thankful to the Department of Medical Microbiology, Faculty of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum, Sudan, for their generous support. The author is also thankful to researcher Mr. Yahya Suliman, Department of Taxonomy and photochemistry, Medicinal and Aromatic Plant Research Institute, National Center for Research, for authentication of the plant. The authors are thankful to the Deanship of Scientific Research, Prince Sattam bin Abdulaziz University.

CONFLICTS OF INTEREST: The author declares no conflict of interest.

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How to cite this article:

Moglad EHO, Boon SKA and Ali HTO: Various medicinal plants: a promising treatment for multidrug-resistant bacteria isolated from wound infection. *Int J Pharm Sci & Res* 2020; 11(2): 839-43. doi: 10.13040/IJPSR.0975-8232.11(2).839-43.

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