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ANTIMICROBIAL EFFECT OF LEAVE EXTRACT OF *GUIERA SENEGALENSIS* ON AEROBIC BACTERIA ISOLATED FROM DIABETIC FOOT INFECTION IN KHARTOUM STATE, SUDAN

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ABSTRACT

Comparison of in vitro susceptibility of bacteria to commonly used antimicrobial drug and *Guiera senegalensis* which is used as traditional drug in treatment of diabetes mellitus in Sudan was carried out. In this prospective cross-sectional descriptive study, the clinical data and specimens were obtained from 50 patients in three Hospitals in Khartoum state during the period from Mar 2016 to July 2016. The specimens were processed using bacteriology isolation & identification techniques. Antimicrobial susceptibility and antibacterial activity testing were carried out using the disc diffusion and cup plate method. Growth was obtained from all the specimens. *Pseudomonas aeruginosa* (20%) and *Proteus mirabilis was (*17%) while *Proteus vulgaris nd Klebsiella pneumoniae gave* (*15*%) each and *Escherichia coli* (11%), *Klebsiella oxytoca* and *Citrobacter koseri* had (2%) each. On the other hand the gram positive *Staphylococcus aureus* had (15%) while *Enterococcus faecalis* (3%). The antimicrobial susceptibility pattern shows amikacin as effective antibiotic against gram negative bacteria while vancomycin as effective antibiotic against gram positive organism. *Pseudomonas aeruginosa* was highly resistant to most of the antibiotic, seconded by *Proteus mirabilis* then *Proteus vulgaris, Staphylococcus aureus, Klebsiella pneumoniae, Klebsiella oxytoca, Enterococcus faecalis* and *Citrobacter koseri.* The methanol and residual extract of the leave *Guiera senegalensis* showed effective antimicrobial activity against various pathogenic bacteria Screening, proper evaluation, early identification and the proper selection of antibiotics for treatment of the 'at risk foot' is important in management of the Diabetic foot infection.

KEYWORDS: Diabetic Foot, Guiera senegalensis, antimicrobial effect.

INTRODUCTION

Diabetic foot ulcer presents as open sore in about 15% of diabetics. They are commonly located at the bottom of the foot. Most diabetics' hospital admission are results of foot ulcer.[1] Diabetes is a metabolic disorder characterized by hyperglycemia which results from defects in insulin action, secretion or both. Foot ulcers are complications of uncontrolled diabetes. It could also be due to muscle atrophy, foot deformity, peripheral neuropathy and neuropathic fractures.[2] The prevalence of diabetic foot ulcer is about 13% in North America, 3% in Europe, 7.2% in Africa, 18.1% in Khartoum, Sudan, 15% in Tanzania, 13% in Cameroon and 9.5% in Nigeria.[3] These result in two thirds of all non-traumatic amputations. Disease presentations include Charcot joint, ulcerations, fracture or gangrene.[4,5] The bacteriology of diabetic foot infection is highly complicated and mostly polymicrobial. It involves both aerobes and anaerobes. Many researchers have presented a picture of mixed infection with aerobic and anaerobic bacteria.[6] Some of the aerobic bacteria associated with diabetic foot infection include *Staphylococcus aureus*, *S. saprophyticus*, *S. epididermis*, *Streptococcus pyogenes*, *S. mutans*, *Pseudomonas aeruginosa*, Bacillus subtilis, *Proteus* species, *Escherichia coli* and *Klebsiella pneumoniae*. The anaerobic bacteria include *Peptostreptococcus* species, *Anaerobic Streptococci*, *Bacteriodes fragilis* and Clostridium species.[7,8] Pal and Gupta (2016) in their study in Kolkata, India, reported that grade 4 foot ulcers on diabetic patients were commonly infected with mixed variety of bacteria including ESBL Klebsiella species and Methicillin-resistant *Staphylococcus aureus* (MRSA).[9] *Guiera Senigalensis* It plays an essential and multiple role in the balance of the Sahelian and Sudanese ecosystems exploited by man and his animal, of the behavior of cattle, sheep and goats on natural pasture.[10] Effective treatment of diabetes is increasingly dependent on active constituents of *Guiera senigalensis* capable of controlling hyperglycemia as well as is secondary complications.[11] 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 were used for the treatment of infectious diseases in many developing countries.[12] Therefore, in Sudan, with high percentage of multidrug resistant bacteria, we are in urgent need to develop new drug from our traditional medicine. *Guiera senegalensis* may possesses antibacterial activity because it used in rural medical care for treatment of many

infectious and chronic diseases. Thus, this study is aimed to verify the antibacterial activity of this plant against resistant bacteria isolated from diabetic wound infection.

MATERIAL AND METHODS

SPECIMEN AND DATA COLLECTION

This study is descriptive cross-sectional - laboratory based study, carried in Khartoum state in patients with diabetic foot infection attended to Ibrahim Malik Teaching Hospital, Soba University Hospital, Omdurman Teaching Hospital and Bahri Teaching Hospital during the period from March 2016 to July 2016.

Ethical clearance was obtained from International University of Africa.Written informed consent from 50 diabetic patients was obtained, the data were collected according to sex, age, duration and type of diabetes using a structured questionnaire.

Swabs from depth of the lesion of the infected foot were collected in Stewart transport media and delivered within 1-4 hours to the microbiology laboratory at International University of Africa.

BACTERIA IDENTIFICATION AND ANTIMICROBIAL SENSITIVITY TESTING

Specimens were inoculated into blood agar and MaCconkey agar, incubated aerobically at 37°C up to 48 hours. The obtained isolates were purified by streaking on plates containing the appropriate selective and differential culture media, and the purified isolates were identified microscopically using standard methods of identification. Antimicrobial sensitivity testing was done according to the Kirby and Bauer method.

Different discs of the common used antibiotics were placed in the Muller Hinton Agar **(Table 1)**, After 24 hours of incubation on Muller Hinton Agar, the diameter of the zone inhibition was measured and compared with the published tables of the control strains.[13]

IN VITRO TESTING OF EXTRACTS FOR ANTIMICROBIAL ACTIVITY

Extraction *Guiera Senegalensis* (leaves) was carried out according to the method described before.[14] Cup-plate agar diffusion method as described by Kavanagh **(**1972) was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. Aliquot of 1 ml of the standardized bacterial stock suspension (between 108 and 109 CFU/ml) was thoroughly mixed with 100 ml of molten sterile Mueller Hinton agar (HiMedia, India) which was maintained at 45°C. 20 ml aliquots of the inoculated Mueller Hinton agar

were distributed into sterile Petri-dish plates. The agar was left to set and all of these plates 5 cups (8 mm in diameter) were cut using a sterile cork borer and agar discs were removed. Each cups were filled with 0.1 ml of the methanolic extract using an automatic microliter pipette, and thereafter the extract was allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 24 h. Two replicates were carried out for each extract against each of the tested microorganisms. After incubation the diameters of the resulted growth inhibition zones average were measured.[15]

Table 1: Name of Different antibiotic used

Antibiotic name	Abbreviations
Penicillin-G	P
Ampicillin	AMP
Gentamicin	GEN
Erythromycin	E
Tetracycline	TE
Chloramphenicol	C
Cotrimoxazole	CAZ
Vancomycin	VA
Ceftazidime	FC
Amikacin	AM
Ciprofloxacin	CIP
Oxacillin	OX
Piperacillin	PIP
Amoxicillin/clavulanic acid	AMC
Doxycycline	DO
Fusidic acid	FC
Imipenem	IPM
Aztreonam	ATM
Streptomycin	S

RESULTS

Total of 50 diabetic subjects with foot infection were investigated, males were 39(78%) and females were 11(22%). Fifty-four bacterial isolates were obtained from wound swab samples. Gram positive organisms constituted 10 (19%) isolates and the gram negative constituted 44 (81%) isolates, in gram positive *Staphylococcus aureus* (15%) was the predominant isolate also *Enterococcus faecalis* (3%) was isolated, while in gram negative *Pseudomonas aeruginosa* (20%), *Escherichia coli* (11%), *Klebsiella pneumoniae* (15%), *Klebsiella oxytoca* (2%), *Proteus vulgaris* (15%), *Proteus mirabilis* (17%), and were isolated *Citrobacter koseri* (2%) **(Table 2).**

ANTIMICROBIAL SUSCEPTIBILITY TEST RESULTS

The antimicrobial susceptibility pattern showed Vancomycin as an effective antibiotic against gram positive organism. All *Staphylococcus aureus* isolates showed resistances to Oxacillin and Penicillin-G, *Enterococcus faecalis* showed resistance Streptomycin **(Table 3 & 4)**.

Gram negatives isolates showed different sensitivity pattern with isolates of *Citrobacter koseri* are more susceptible to various anti-Gram negative agents, except Ampicillin **(Table 5).**

While Meropenem, Imipenem, Amikacin and Aztreonam as affective antibiotics against *Pseudomonas aeruginosa* **(Table 6)**.

PLANT EXTRACTS SUSCEPTIBILITY

The methanol and residual extract of the leave *Guiera senegalensis* showed effective antimicrobial activity against various pathogenic Bacteria. The result showed that increase in concentration of extracts increase the zone of growth inhibition of the microorganisms **(Table 7).**

Table (2): Bacterial species isolated from patients with diabetic foot infections.

***Bacteria* No. (%)**	
***Escherichia coli* 6 (11)**	***Citrobacter koseri* 1 (2)**
***Klebsiella pneumonia* 8 (15)**	***Pseudomonas aeruginosa* 11 (20)**
***Klebsiella oxytoca* 1 (2)**	***Enterococcus faecalis* 2 (3)**
***Proteus vulgaris* 8 (15)**	***Proteus mirabilis* 9 (17)**
***Staphylococcus aureus* 8 (15)**	**Total 54 (100)**

Table (3): Antimicrobial susceptibility test result of *Staphylococcus aureus.*

	Antibiotics									
	GEN	OX	CIP	VA	P	FC	C	TE	DO	E
S	7%	0%	7%	15%	0%	7%	6%	6%	9%	7%
R	6%	15%	7%	0%	15%	7%	9%	7%	6%	4%
I	2%	0%	1%	0%	0%	1%	0%	2%	0%	4%

Table (4): Antimicrobial susceptibility test result of *Enterococcus faecalis.*

	Antibiotics						
	GEN	CIP	VA	P	TE	S	AM
S	2%	2%	2%	2%	2%	0%	2%
R	0%	0%	0%	0%	0%	2%	0%
I	0%	0%	0%	0%	0%	0%	0%

Table (5): Antimicrobial susceptibility test result of Gram negative bacteria.											
		Antibiotics									
		GEN	CIP	IPM	CAZ	AK	AMC	SXT	TE	AM	DO
Klebsiella pneumonia	S	11%	11%	6%	2%	13%	0%	0%	6%	0%	0%
	R	4%	2%	5%	13%	0%	15%	9%	9%	15%	11%
	I	0%	2%	4%	0%	2%	0%	6%	0%	0%	4%
Klebsiella oxytoca	S	2%	2%	2%	0%	2%	0%	0%	0%	0%	0%
	R	0%	0%	0%	2%	0%	2%	2%	2%	2%	2%
	I	0%	0%	0%	0'%	0%	0%	0%	0%	0%	0%
Proteus vulgaris	S	9%	9%	9%	9%	11%	9%	15%	15%	0%	15%
	R	4%	4%	4%	4%	2%	4%	0%	0%	15%	0%
	I	2%	2%	2%	2%	2%	1%	0%	0%	0%	0%
Proteus mirabilis	S	13%	13%	13%	2%	17%	3%	15%	15%	0%	0%
	R	4%	2%	2%	13%	0%	7%	0%	0%	15%	17%
	I	0%	2%	2%	2%	0%	7%	2%	2%	2%	0%
Escherichia coli	S	9%	9%	9%	11%	9%	11%	9%	9%	0%	9%
	R	2%	2%	2%	2%	0%	0%	2%	2%	11%	2%
	I	0%	0%	0%	0%	2%	0%	0%	0%	0%	0%
Citrobacter koseri	S	*2%*	*2%*	*2%*	*2%*	*2%*	*2%*	*2%*	*2%*	*0%*	*2%*
	R	0%	0%	0%	0%	0%	0%	0%	0%	2%	0%
	I	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%

Table (6): Antimicrobial susceptibility test result of *Pseudomonas aeruginosa.*								
Antibiotics								
	GEN	CIP	IPM	CAZ	AK	MEM	PRL	ATM
S	17%	17%	20%	0%	20%	20%	0%	20%
R	3%	3%	0%	20%	0%	0%	19%	0%
I	0%	0%	0%	0%	0%	0%	1%	0%

Table (7): Antimicrobial screening of different concentration of ethyl acetate of the leave of Guiera senegalensis.																		
Conc. (µg/ml)	Number of (spp S/R)																	
	S. aureus		*E. faecalis*		*K. pneumonia*		*K. oxytoca*		*P. vulgaris*		*P. mirabilis*		*E. Coli*		*C. koseri*		*P. aeruginosa*	
	S/R		*S/R*		S/R		S/R		S/R		S/R		S/R		S/R		S/R	
6	2	6	0	2	2	6	0	1	0	8	0	9	1	5	1	0	0	11
12	2	6	0	2	2	6	0	1	0	8	0	9	1	5	1	0	0	11
25	3	5	1	1	4	4	1	0	0	8	0	9	6	0	1	0	0	11
50	6	2	1	1	5	3	1	0	1	7	0	9	6	0	1	0	0	11
100	6	2	1	1	6	2	1	0	2	6	1	8	6	0	1	0	0	11

DISCUSSION

Diabetic foot infections DFI is a major and increasing problem worldwide, in Sudan. DFI is one of the serious diabetic complications facing surgeons.[16]

Early diagnosis and treatment are essential in preventing DFI complications this is achieved by selection of an appropriate antibiotic therapy.

This study was conducted to determine the relative frequency of bacteria isolated from diabetic foot infection and assess their comparative susceptibility to the commonly used antimicrobial agents. in addition to investigate the in-vitro antibacterial activity of *Guiera senegalensis* on different isolates to overcome the rapidly emergency of resistant to many of commonly used antimicrobial drugs. Our study shows that different isolates of gram positive and gram negative bacteria has been isolated, with Gram negative organisms accounted to greater percentage than gram positive organisms. *Staphylococcus aureus* was the predominant among gram positive isolates while *Pseudomonas aeruginosa* is the most common isolates among gram negatives, this result is in agreement with study was carried in Sudan.[17]

In a previous study from Jaber Abolez Diabetic Center (JADC) Sudan, Staph aureus was isolated in 48.46% of cultures, Pseudomonas aeruginosa (16%) and Kleibsiela (13.85%).[18]

Gram positive cocci was recounted as the most communal pathogenic agent in USA and Europe.[19] Whereas Gram negative bacilli were the most frequently reported pathogenic agents reported from Asian studies[20], two studies carried in India indicated a dominance of Gram-negative.[21,22]

Studies from Malaysia have also reported a predominance of Gram-negative bacteria (52%) in patients with DFI, the most common pathogens isolated being Proteus spp., Klebsiella spp., Pneumoniae, E. coli and Enterobacter cloacae.[23]

Microbial drug resistance is a major public health concern DFI, in this study All *Staphylococcus aureus* isolates showed resistances to Oxacillin and Penicillin-G, Vancomycin is found to be as an effective antibiotic against gram positive organism in our results, which become the drug of choice for *S. aureus* (MRSA).

We found that all gram negative isolates were sensitive to Amikacin, this finding agreement with studies reported that, Amikacin was the most effective treatment for the Gram-negative bacteria we find that Meropenem, Imipenem, Amikacin and Aztreonam are the most affective anti *Pseudomonas* agent.[17,23] The methanol extract of *Guiera senegalensis* was tested against clinical isolates bacteria Gram positive and Gram negative using different concentrations

(100, 50, 25, 12.5 and 6.25 mg/ml). The methanol extract of *Guiera senegalensis* exhibited inhibitory effects against *Staphylococcus aureus, Klebsiella pneumoniae* and *Enterococcus faecalis.* And had no activity against other bacterial tested in the study. These results show that the *Guiera senegalensis* was more effective against *Staphylococcus aureus* while most of the antibiotics were resistant, the results also show that the plant extract used in our study was more effective against *Klebsiella pneumoniae* and *Enterococcus faecalis* though not much as compared to the effect of the tested antibiotic on these bacteria and that the plant extract is more effective against Gram positive than the Gram negative bacteria, these results are compatible with the findings reported by Eidi and others.[24]

CONCLUSION AND RECOMMENDATION

The obtained results indicated that the *Guiera Senegalensis* (leaves) is a good antibacterial therapy in traditional medicine in Sudan and the neighboring countries and this study showed a dominance of gram-negative bacteria among the isolates. The multiple antibiotic resistance of the bacterial isolates calls for the need to monitor resistance. Best practice is to perform antibiotic susceptibility testing before treatment.

Further investigations regarding the mode of action and other related pharmacological studies such as *in vivo* investigation, drug formulation and clinical trials are highly recommended.

Further studies using molecular techniques of bacterial identification and standardized sampling methods, will provide new insights to redirect treatments and might improve clinical outcomes in the future.

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