Special Conference Edition, April, 2022
http://dx.doi.org/10.4314/bajopas.v13i1.47S

***Bayero Journal of Pure and Applied Sciences, 13(1): 305 -* 312**
ISSN 2006 – 6996

DETERMINATION OF ANTIBACTERIAL ACTIVITY OF FIVE MEDICINAL PLANTS TRADITIONALLY USED FOR THE TREATMENT OF DIABETIC FOOT INFECTION IN KANO STATE, NIGERIA

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ABSTRACT
The use of traditional medicine in various therapies by the indigenous population cannot be over emphasized. Traditional healers in many countries including Nigeria use different species of plants as usual sources of medicine. Diabetic foot ulcers are the fastest growing complication of diabetes mellitus. The disease is highly susceptible to infection leading to tissue destruction and amputation. The aim of the current study was to evaluate the antibacterial activity of Guiera senegalensis, Ficus glumosa, Acacia nilotica, Anogeissus leiocarpus and Balanite aegyptiaca against some bacterial isolates from diabetic foot ulcer. Ethanol and aqueous extracts of the selected plants material were prepared by maceration and decoction methods respectively. Determination of the antibacterial activity was carried out by agar well diffusion method. Minimum inhibitory and minimum bactericidal concentrations were also evaluated. Maximum extraction yield was obtained from the ethanol Pod extract of Acacia nilotica (33%). While Anogeisus leiocarpus ethanol stem bark extract exhibited the least extraction yield (6.89%). All the studied plant materials showed a potential antibacterial activity by growth inhibition of one or more tested organisms. Highest zone of inhibition of 23.0 ± 0.00mm was obtained in the aqueous extract of A. nilotica on Citrobacter specie with no significant difference with the control (P<0.05). The MIC and MBC values ranged from 1mg/ml – 8mg/ml. The studied plants possessed appreciable antibacterial activity against the tested bacterial isolates from diabetic foot ulcer. Hence these plants can be considered as a potential lead for the development of new drug for the treatment of diabetic foot infection.
Keywords: diabetic foot infection, bacterial isolates, antibacterial activity, Guiera senegalensis, Ficus glumosa, Acacia nilotica, Anogeissus leiocarpus, Balanite aegyptiaca.

INTRODUCTION

Diabetic foot ulcers (DFUs) are the fastest growing chronic complication of diabetes mellitus, with more than 400 million people diagnosed globally. Fifteen percent of all diabetics patients develop a foot ulcer at some point in their lives which is highly susceptible to infections and that spreads rapidly, leading to overwhelming tissue destruction and subsequent amputation (Lipsky *et al.*, 2004).Wound infections are a factor in the delay in the healing process, and, if they are not treated properly, they could lead to systemic compromises (Manuel *et al.*, 2019). According to a study published by the Eurodiale study group approximately 58% of DFU patients will become clinically infected. Diabetic foot infections are usually polymicrobial, caused by aerobic gram positive cocci like *Staphylococcus aureus*, gram negative bacilli (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), and anaerobes. Proper management of these infections needs appropriate antibiotic selection (Saseedharan *et al.*, 2018). However, the clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens, strains with reduced susceptibility as well as, undesirable side effects of certain antibiotics.

Traditional medicine involving medicinal plants had been in used for many centuries due to their availability, accessibility and minimal side effect.

The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Ellof,1998). *Invitro* studies by many researchers had proven the antibacterial efficacy of many medicinal plants against bacterial isolates from diabetic foot ulcer. Therefore, there is need to study additional medicinal plants in order to develop new antibiotics for diabetic foot infection.

Ficus glumosa Delile commonly known as the fig tree or "African rock fig"is indigenous to the tropical and subtropical Africa, including Nigeria, with a few species extending into the semi-warm temperate zones in South West Asia and the Mediterranean region (Umar *et al*., 2013).It is used in pharmacopeia of Cameroon, Senegal, and East Africa for the treatment of edema, hemorrhoid, cardiovascular diseases, especially hypertension and the decoction of the leaves are used for the treatment of skin diseases and diabetes (Ntchapda, *et al*., 2014). In Nigeria the stem is used for the treatment of diabetic foot ulcer (Buhari *et a*l., 2021).The methanolic extract of the stem bark of *F. glumosa* has demonstrated in vivo anti-diabetic, in vitro antioxidant (Nana *et al*., 2012) and antimicrobial activities (Kwazo *et al*., 2014).

Anogeissus leiocarpus (DC.) Guill. & Perr *(*Combretaceae) also known as African birch is a tall evergreen tree native to savannah of tropical Africa. The leaves of *A. leiocarpus* are used in the treatment of skin diseases, fever, diarrhoea, malaria, stomach infections(Chaabi *et al*., 2008) and wound healing (Inngjerdinge*et al*., 2004). The stem bark is used in the treatment of cough (Sani and Aliyu, 2011a), Diabetes (Salihu *et al*., 2015), wound healing (Adetutu *et al*., 2012) and diabetic foot ulcer (Buhari *et al*., 2021). Biological activities of the plant includes; antibacterial (Sani and Aliyu, 2011b), wound healing effect (Victor *et al*., 2013) antioxidant (Eltayeb *et al*., 2016) and Leishmanicidal activity (Shuaibu, 2008)

Balanites aegyptiaca (L.) Delile also known as desert date is native to dry land areas of Africa and South Asia. In Nigeria the leaves are used in the treatment of stomachache (Kankara *et al*., 2015), the fruit and decoction of the bark are taken to lower blood glucose level (Salihu *et al*., 2015). The stem bark is used for diabetic foot ulcer (Buhari *et al*., 2021). Pharmacological properties of the plant includes; antioxidant, antibacterial (Kahsay *et al*., 2014), antidiabetic, anti-inflammatory, antiviral, analgesic and wound healing activity (Chothani and Vaghasiya, 2011).

Acacia nilotica (L.) Delile commonly known as gum arabic tree, Babul or Egyptian thorn is a species of Acacia native to Africa and the Indian subcontinent. The pod is traditionally used in Nigeria for wound healing (Kankara *et al*., 2015), pile (Ali *et al*., 2017), diabetic foot ulcer (Buhari *et a*l., 2021). The decoction of the stem bark is used for diabetes (Abubakar *et al*., 2017). Several studies have reveal the antibacterial, antioxidant, antimutagenic, antidiabetic, analgesic (Jame, 2018) and wound healing effect (Kankara *et al*., 2017) of the plant.

Guiera senegalensis J.F.Gmel. *(*Combretaceae) also known as Senegal guiera is widely distributed in the savannah region of west and central Africa, Nigeria, Senegal, Gambia, Mali, Niger (Denou *et al*., 2016). In Nigeria *G. senegalensis* leaves are used for diarrhea (Kankara *et al*., 2015), chiken pox (Ohemu *et al*., 2014), Diabetes (Salihu*et al*., 2015), diabetic foot ulcer (Buhari *et al*., 2021). Pharmacological evidence shows the antibacterial (Mamman and Isa, 2013; Ali, 2020), antioxidant (Mariod *et al*., 2006) activities of the plant.

MATERIALS AND METHODS

Authentication of the study plant materials

The selected plants materials were obtained from their natural sources and were authenticated in the Herbarium of Department of Plant Biology, Bayero University Kano.

Extraction of plant materials

Ethanol extraction; The collected plant materials were washed under running water and shade dried at room temperature for two weeks. The dried parts were then ground in to powder. Fifty grams (50g) of each powdered plant materials was macerated with 500ml of ethanol for 72hrs. Thereafter, it was filtered through 8 layers of muslin cloth and then re-filtered through whatman No.1filter paper. The filtrate was then concentrated in a rotary evaporator at 40°C and dried at room temperature yielding corresponding extracts. The yield of each extract was calculated using the following formula: % yield = [We/Wp] × 100. Where; 'We' indicates weight of extract and 'Wp' indicates weight of powder

Aqueous decoction extraction; The aqueous extract was prepared by decoction method to mimic the mode of extraction by the herbalists. Fifty grams (50g) of each powdered part was mixed with 500ml of distilled water and boiled for 15minutes on a hot plate (Kenyanga *et al*., 2018). After cooling and filtration in 8 layer muslin cloth and Whatmann No.1 filter paper, the filtrate was concentrated and the yield of the extract was also determine.

Antibacterial activity determination

Selected bacterial isolates

The bacterial organisms used in this study were clinical isolates from diabetic foot infection and these includes; *Klebsiella Pneumoniae*, *Proteus* specie, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Citrobacter* specie and *Escherichea coli*.

Preparation of stock solution

Eight milligram (8mg) of the ethanol and aqueous extracts were dissolved in 1ml of DMSO and distilled water respectively to obtain a concentration of 8mg/ml ready for the analysis

Agar well diffusion method

Agar well diffusion method was employed for the determination of the antibacterial activity of the selected plant materials. Standardized inoculum (10^8 CFU/mL) test bacterium turbidity equivalent to 0.5 Mcfarland standard was spread on sterile Muller Hilton agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0mm was used to bore wells in the agar plates. Subsequently, a 100µl volume of the extracts (8mg/ml) was introduced in to the wells of the Mueller-Hinton agar culture using Pasteure pipette (Sani *et al*., 2021). Ciprofloxacine (30µg) was used as a positive control.

Minimum inhibitory concentration (MIC) method

Equal volume of the extract and Mueller-Hinton broth i.e 2ml each were dispensed into sterilized test tubes to obtain concentrations of 8mg/ml to 0.5mg/ml. The organism (0.1ml of standardized inoculum) was inoculated into each tube containing the broth and the extract. The tubes were incubated at 37˚C for 24hrs. At the end of the incubation period the tubes were examined for the presence or absence of colony growth using turbidity as a criterion. The lowest concentration in the series without visible sign of growth was considered to be the MIC (Nuhu and Odinaka, 2017).

Minimum bactericidal concentration (MBC) method

The MBC was determined by first selecting the tubes or the least concentrations of extracts that showed no turbidity during the MIC determination. One loopful from each of these tubes was sub-cultured onto the surface of the extract free Mueller-Hinton agar and incubated at 37˚C for 24hrs. The lowest concentration at which no growth was observed on the agar was noted as the MBC (Lar *et al.* 2011).

Data analysis

The data was analyzed using descriptive statistics such as frequency and percentage. One-way analysis of variance (ANOVA) and Turkey tests were carried out to determine significant group differences ($p < 0.05$) between means by using SPSS statistical software package (SPSS, version 20.0).

RESULTS

Yields of the selected plants extracts

The voucher number of the selected plants materials and yield of the plants extracts are shown in Table 1. Maximum extraction efficiency was obtained from the ethanol pod extract of *Acacia nilotica* with 35.16% followed by *Ficus glumosa* ethanol stem extract (28.9). *Anogeissus leucarpus* ethanol extract exhibited the lowest extraction yield of 13.8%.

Table 1: Percentage yields of the selected plants extract.

Plant sample	Familly	Plant part	Voucher number	Extract	Weight (g)	Yield (% w/w)
Ficus glumosa	Moraceae	Stem	BUKHAN186	Ethanol	14.45	28.9
				Aqueous	12.32	24.64
Anogeissus leiocarpus	Combretaceae	Stem	BUKHAN29	Ethanol	6.89	13.8
				Aqueous	12.57	25.14
Balanites aegyptiaca	Zyophylaceae	Stem	BUKHAN359	Ethanol	11.41	22.82
				Aqueous	7.56	15.12
Acacia nilotica	Fabaceae	Pod	BUKHAN186	Ethanol	16.5	33.0
				Aqueous	14.21	28.4
Guiera senegalensis	Combretaceae	Leaves	BUKHAN32	Ethanol	9.21	18.42
				Aqueous	7.07	14.1

Antibacterial activity of the selected plants extract against bacterial pathogens from diabetic foot ulcer

Table 2 presents the antibacterial activity of the ethanol extracts of the selected plants materials against the tested organisms.

It was observed that *F.glumosa* had activity against *Citrobacter* specie, *S. aureus*, *E.coli* and *P. vulgaris* with highest zone of inhibition of 20.5±0.71mm on *Citrobacter* specie which is significantly different with the control (P<0.05). *A. leucarpus* exhibited activity against all the tested organisms with highest zone of inhibition of 19.0±0.00mm against *Citrobacter* specie and least zone of inhibition of 12.5±0.71mm on *E.coli*. Similarly *B. aegyptiaca* showed activity against all the isolates with greater activity on *Citrobacter* specie (17.5±0.7 mm 1) and less activity of 9.5±0.71mm against *P.a eruginosa*. In addition, in *A. nilotica*, activity was observed against all the tested organisms with highest zone of inhibition of 20.0±0.71mm which is significantly different with the control. Least zone of inhibition was observed on *P. vulgaris*. *G. senegalensis* also exhibited activity against *Citrobacter* specie, *S. aureus*, *K. pneumoniae* and *E.coli* with highest zone of inhibition of 18.5±0.71mm on *Citrobacter* specie. Least zone of inhibition of 13.0±0.00mm was observed on *K. pneumoniae*.

Table 3 showed the antibacterial activity of aqueous extract of the selected plants material against the tested bacterial isolates. It was observed that the inhibition zone diameter of the standard drug was significantly higher than the plants extract against all the isolates except in *A. nilotica* extract (23.0±0.00mm) against *Citrobacter* specie where there was no significant difference with the control (P<0.005). In *F. glumosa*, activity was observed only against *S.aureus* (11.0±0.00mm) and *E.coli* (10.5±0.00mm). Aqueous extract of *A. leucarpus* exhibited activity against *Citrobacter* specie, *S. aureus* and *P. aeruginosa*, but no activity was observed on *K. pneumonie*, *E.coli* and *P.vulgaris*. However, the extract of *B. aegyptiaca* did not show activity against the isolates. In addition, the extract of *G.senegalensis* showed activity against *S.aureus*, *K. pneumoniae* and *P. aeruginosa*. But no activity was observed against *Citrobacter* specie, *E. coli* and *P. vulgaris*

Figure 1 demonstrated MIC and MBC values of ethanol extract of the selected plant materials. MIC was carried on the extract that showed activity by agar well diffusion method. Lowest MIC value of 1mg/ml was observed on *F. glumosa* against *Citrobacter* specie, *A. leucarpus* against *E. coli*, *B. aegyptiaca* against *E.coli*, *A. nilotiica* against *P. vulgaris*, *Citrobacter* specie and *S.aureus.* Similarly, lowest MBC value of 1mg/ml was observed in *A. leucarpus* extract against *E.coli*, *F. glumosa* against *Citrobacter s*pecie, *A. nilotica* extract against *P. vulgaris* and *B. aegyptiaca* against *E.coli.*

Figure 2 presented the MIC and MBC values of aqueous extract of the selected plants extract against the tested isolates. Lowest MIC and MBC value of 1mg/ml was observed in *A.nilotica* extract against *Citrobacter* specie.

Table 2: Antibacterial activity of ethanol extract of the selected plants against some of the isolated bacterial pathogens from diabetic foot ulcer

Plant extract	**Test organism/ zone of inhibition (mm)**					
	Citrobacter specie	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Escherichea coli*	*Proteus vulgaris*
F.glumosa	20.5±0.71^{b}	11.5±0.71^{d}	0.0±0.00^{e}	0.0±0.00^{e}	13.5±0.00^{c}	16.0±0.00^{b}
A.leucarpus	19.0±0.00^{b}	16.5±0.71^{b}	16.5±0.71^{b}	13.5±0.71^{c}	12.5±0.71^{c}	14.0±0.00^{c}
B.aegyptiaca	17.5±0.71^{c}	15.0±0.00^{c}	14.5±0.00^{c}	9.5±0.71^{d}	11±0.00^{d}	10.0±0.00^{d}
A.nilotica	20.0±0.71^{b}	15.0±0.71^{c}	13.0±0.00^{d}	17.0±0.00^{b}	13.0±0.00^{c}	12.0±0.00^{e}
G.senegalensis	18.5±0.71^{c}	16±0.00^{b}	13±.0.00^{d}	0.0±0.00^{e}	17.5±0.71^{b}	0.0±0.00^{f}
Ciprofloxacin 30µg/ml	24.25±0.35^{a}	23.5±0.71^{a}	23.0±0.00^{a}	21.0±0.00^{a}	22.5±2.12^{a}	22.0±1.41^{a}

Data given are means of two replicates±standard deviation. Along the columns, different superscript letters indicate statistical significant differences in the turkey test, with (P< 0.05).

Table 3: Antibacterial activity of aqueous extract of the selected plants against some of the isolated bacterial pathogens from diabetic foot ulcer

Plant extract	**Test organism/ zone of inhibition (mm)**					
	Citrobacter specie	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Escherichea coli*	*Proteus vulgaris*
F.glumosa	0.0±0.00^{c}	11.0±0.00^{d}	0.0±0.00^{d}	0.0±0.00^{e}	10.5±0.71^{c}	0.0±0.00^{c}
A.leucarpus	17.5±1.41^{b}	17.5±3.54^{c}	0.0±0.00^{d}	15.5±0.71^{b}	0.0±0.00^{d}	0.0±0.00^{c}
B.aegyptiaca	0.0±0.00^{c}	0.0±0.00^{e}	0.0±0.00^{d}	0.0±0.00^{e}	0.0±0.00^{d}	0.0±0.00^{c}
A.nilotica	23.0±0.00^{a}	22.0±2.83^{b}	13.0±0.00^{c}	11.0±0.00^{c}	11.5±0.71^{b}	13.0±0.00^{b}
G.senegalensis	0.0±0.00^{c}	10.0±1.41^{d}	16.5±0.71^{b}	8.5±0.71^{d}	0.0±0.00^{d}	0.0±0.00^{c}
Ciprofloxacin 30µg/ml	24.25±0.35^{a}	23.5±0.71^{a}	23.0±0.00^{a}	21.0±0.00^{a}	22.5±2.12^{a}	22.0±1.41^{a}

Data given are means of two replicates±standard deviation. Along the columns, different superscript letters indicate statistical significant differences in the turkey test, with (P< 0.05).

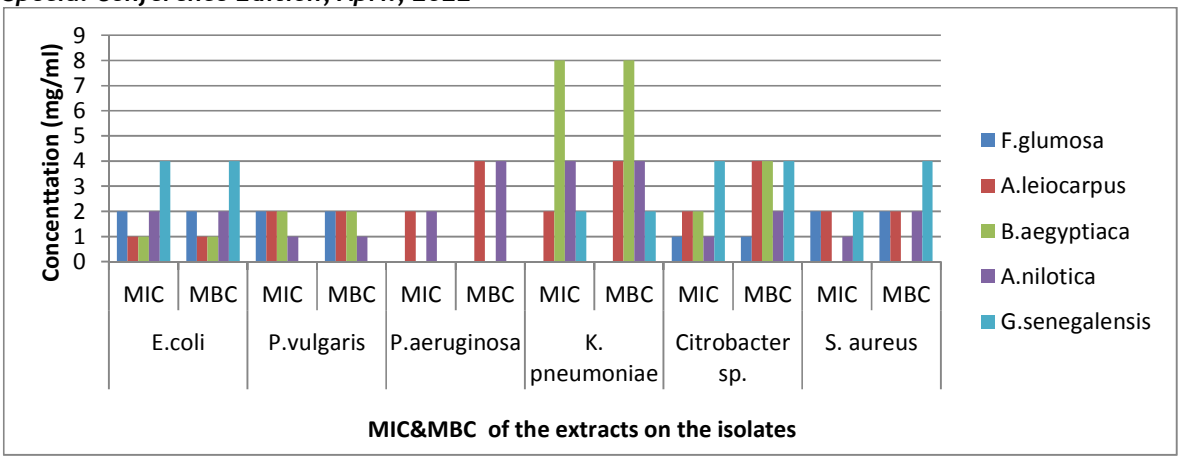

Figure 1: Minimum inhibitory concentration and minimum bactericidal concentration of ethanol extracts of the selected plants extract on the tested bacterial isolates from diabetic foot ulcer

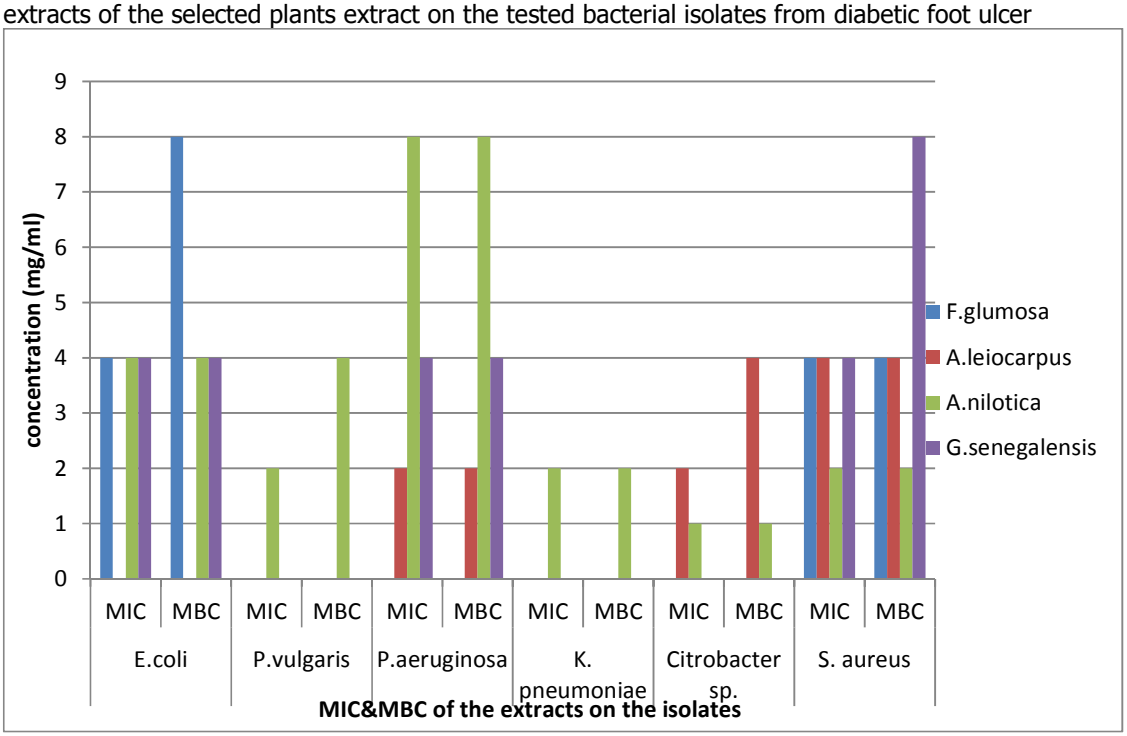

Figure 2: Minimum inhibitory concentration and minimum bactericidal concentration of aqueous extracts of some selected plants extract on the tested bacterial isolates from diabetic foot ulcer

DISCUSSION

In the present study, some of the ethanol extracts showed better yield than aqueous decoction. This may be because some of the phytoconstituents in the aqueous decoction are denatured due to the application of heat. Our findings are in contrary to the reports of Abkhoo and Jahani (2017), where aqueous decoction showed better yield than the ethanol extract. Although, they mentioned that greater efficiency in solution extraction is not related to higher antibacterial activity. Pinelo *et al.* (2004) suggested that the chemical properties of the solvent and method of extraction show distinct behavior.

The results of antibacterial activity revealed that the selected plant extracts showed antibacterial activity against the isolates However, the activity of all the plant extracts was lower than that of the positive control (ciprofloxacin) except in aqueous extract of *A. nilotica* where there was no significant difference. But this suggests that higher concentrations of the extract could produce comparable antibacterial result.

Moreover, it could be observed from the study that the ethanol extracts showed more activity against the isolates than the aqueous decoction extracts.

The less activity of the aqueous extracts against the isolates is in agreement with the work of Busani *et al*. (2012) who reported that the aqueous extract of plants generally exhibited little or no antibacterial activity. In addition, the fact that ethanol extract produced higher activity against *Citrobacter* specie suggested that the extracts may be effective against diabetic foot infections cause by *Citrobacter* specie.

The major goal of the antimicrobial screening of medicinal plants is to find substances with novel mechanism of actions against emerging resistant strains of pathogenic bacteria. The antibacterial activity of *F. glumosa* against *E. coli* was in line with the study of Usman *et al*. (2017) who reported the antibacterial activity of ethanol extract of the plant against *E. coli* with zone of inhibition of 10.00mm at a concentration of 80mg/ml. the activity of ethanol extract of *A. leucarpus* corroborate with the result of Mann *et al*. (2008) who showed that the ethanol extract of *A.leiocarpus* exhibited antibacterial activity against *S. aureous* and *E. coli* isolates from infected wound. Moreover, the activity of *G. senegalensis* is in consistence with the report of Mamman and Isa (2013) which showed the antibacterial activity of the aqueous and ethanol extract of *G. senegalensis* against *S. aureus*, *E. coli* and *K. pneumonia*. In addition, the activity of *A. nilotica* against the tested isolates correlate with the findings of Naser *et al*.(2018) where aqueous stem bark extract of *A. nilotica* inhibit the growth of *P. aeruginosa*, *Klebsiella* specie, *S. aureus*, *E. coli* and *Proteous* specie isolated from diabetic wound. A study by Gmaraldeen *et al*. (2016) also shows the antibacterial activity of *Acacia nilotica* methanol pod extract against *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Anani *et al*. (2015) reported the inhibitory effect of hydroethanol extract of the bark of *B. aegyftiaca* against multidrug resistance *P. aeruginosa* and *S. aureus*. In addition, Fahmi and Albegadir (2016) also reported the antibacterial activity of methanol bark extract of *B. aegyptiaca* against *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

Minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanol and the aqueous extracts of each plant that showed activity by agar well diffusion method were determined. Determination of the MIC and MBC was performed in order to evaluate the efficacy of the antimicrobial agent. In this study the both the MIC and MBC values obtained were between the range of 1-8mg/ml. It was reported that low MIC and MBC values indicate high antibacterial activity. According to Agyare *et al.* (2014) extract exhibiting antibacterial activity where MIC values is below 8mg/ml possess very effective antibacterial activity.

CONCLUSION

The studied plants extracts exhibited appreciable antibacterial activity against the tested bacterial isolates from diabetic foot infection. Hence these plants can be considered as a potential lead for the development of new drug for the treatment of diabetic foot infection. Further studies need to be carried out to screen and identify the biological active compounds present in the investigated plants extract.

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