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Antimicrobial Activity of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Prosopis africana* (Guill., Perrott, & Rich.) (Taub.) Plant Extracts Against Enteric Bacteria

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Abstract

The rise of antibiotic-resistant enteric bacteria poses a significant threat to public health, necessitating the search for alternative antimicrobial agents. Traditional medicinal plants such as Anogeissus leiocarpus and Prosopis africana have long been used in various cultures for their health benefits, including antimicrobial properties. This study aimed to investigate the antimicrobial activity and qualitative phytochemical composition of crude extracts from A. leiocarpus and P. africana against selected enteric bacteria. The phytochemical screening revealed the presence of secondary metabolites including saponins, flavonoids, tannins, alkaloids, and cardiac glycosides in both plants. The aqueous extracts had more abundant secondary metabolites than the methanolic extracts. Antimicrobial testing showed that increasing the concentration of the extracts enhanced their antibacterial effectiveness, with the aqueous extracts displaying higher activity than methanolic ones. The test organisms, including E. coli, K. pneumoniae, P. mirabilis, S. enterica, and Shigella spp., exhibited varying degrees of susceptibility, with a higher sensitivity observed for the aqueous extracts. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were found to be ≥20 mg/mL for both aqueous and methanolic fractions. The findings support the traditional use of A. leiocarpus and P. africana in treating bacterial infections, showcasing their potential as alternative therapeutic agents due to their rich bioactive compounds and significant antibacterial properties. Further research is recommended to explore the mechanisms of action, pharmacokinetics, and safety profiles of these extracts for pharmaceutical applications.

Keywords: Antimicrobial agents, *Anogeissus leiocarpus,* Concentration, Extracts, Enteric Bacteria *Prosopis africana,*

INTRODUCTION

Enteric bacterial infections, caused by pathogens like *Escherichia coli*, *Salmonella*, and *Shigella*, represent a significant global public health issue, especially in developing regions with inadequate sanitation and hygiene (Baker *et al.*, 2016). Rising antimicrobial resistance in these bacteria complicates treatment, emphasizing the urgent need for alternative therapies (Okeke and Edelman, 2018).

Anogeissus leiocarpus (African Birch) and *Prosopis africana* (African Mesquite) are indigenous African plants traditionally used in folk medicine, particularly for gastrointestinal ailments

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like diarrhea and dysentery (Dosumu *et al.,* 2012; Bekoe *et al.,* 2017). Both plants are known to contain a range of bioactive compounds, including flavonoids, tannins, alkaloids, saponins, and phenolic compounds, which exhibit notable antimicrobial properties (Rwang *et al.,* 2016; Ezike *et al.,* 2010).

Considering the need for novel antimicrobial agents, *Anogeissus leiocarpus* and *Prosopis africana* offer promising potential as sustainable, cost-effective sources of bioactive compounds. These plants may provide leads for developing new antimicrobial therapies (Ojo *et al.,* 2018).

This study aimed to bridge existing knowledge gaps by investigating the antimicrobial efficacy and phytochemical profile of crude extracts from *Anogeissus leiocarpus* and *Prosopis africana* against selected enteric bacteria. By identifying the antimicrobial properties of these plants, this research seeks to contribute to alternative therapeutic approaches for managing enteric infections and addressing antimicrobial resistance.

MATERIALS AND METHODS

Ethical Clearance and Consent Form

Ethical clearance for the purpose of this study was obtained from Aminu Kano Teaching Hospital Kano State, before commencement of the study with reference number NHREC/28/01/2020/AKTH/EC/3662

Collection of Samples

The stem bark of *Anogeissus leiocarpus* and *Prosopis africana* were collected from Lere, LGA Kaduna State, and Dutse LGA, Jigawa State respectively. The stem barks, along with their leaves were taken to the herbarium of Department of Biological sciences of Bayero University Kano where they were identified as *Anogeissus leiocarpus* and *Prosopis africana* belonging to the family *Combretaceae* and *Fabaceae,* with accession number BUKHAN 0029 and BUKHAN 0193 respectively.

Preparation of Plant Stem Bark Extracts

The stem bark of *Anogeissus leiocarpus* and *Prosopis africana* were washed with tap water, air-dried for 10 days and grounded into fine powder by using mortar and pestle and kept in sterile bottles according to Mukhtar and Okafor (2002).

Extraction of Plant Stem Bark

One hundred grams (100g) of the powdered plant (i.e. *A. leiocarpus*) was weighed into 2 different 2L capacity bottles and percolated with 1000 ml each of methanol (for 5 days) and water (for 3days) with shaking at regular intervals. The mixture was then filtered through a clean muslin cloth followed by filtration with Whatman No.1 filter paper and the filtrate was allowed to evaporate at ambient temperature. The crude/dried extract was kept under refrigerated condition at 4^0C until required for further use (Betoni *et al.,* 2006). Notably, this procedure was also repeated for *P. africana* stem bark.

Phytochemical Analysis

The extracts were subjected to various phytochemical analyses which identified the chemical constituents present using standard method described by Sofowora (1993). One gram (1 g) of each powdered extract was weighed and dissolved in 10 ml of sterile distilled water and filtered using WhatmanNo. 1 filter paper. One millilitre each of the filtrate of each dissolved extract, was dispensed into various test tubes and used for the following tests: test for

carbohydrates (Molisch's test), test for unsaturated steroid (Liebermann-Bucchard Test), Salkwoski test for unsaturated sterols, test for cardiac glycosides (Keller- Kiliani Test), test for saponin glycosides (Frothing Test), test for tannins (Lead Sub-acetate test), test for Flavonoids (Shinoda's Test), test for alkaloids (Mayer's Test), Dragendoff's Test, Wagner's Test, Test for Free Anthracene Derivatives (Bontrager's Test)

Identification and Characterization of Test Organisms

The clinical test isolates which include; *Escherichia coli, Shigella* spp, *Proteus spp, Salmonella typhi* and *Klebsiella* spp. were obtained from Microbiology Department of Aminu Kano Teaching Hospital (AKTH), Kano and maintained on nutrient agar slants in the refrigerator (4^{o}C) prior to use (Cheesbrough, 2000). The isolates were then taken to the laboratory, Department of Microbiology, Bayero University Kano, and confirmed using the following tests:

Gram staining and microscopy

A smear of the isolate obtained was fixed on a clean, grease-free slide and stained with crystal violet solution (primary dye) for 60 seconds, rinsed with tap water and drained to avoid diluting with the mordant. It was further flooded with Gram's iodine solution (mordant) for 30 seconds and rinsed. Then acetone-alcohol (decolorizer) was applied in drop wise on the tilted slide until all free colours has been removed and subsequently rinsed with tap water. The slide was then flooded with Safranin (secondary dye). The slide was examined under the microscope at ×100 oil immersion objective (Cheesbrough, 2010). Notably, this procedure was repeated for the other isolates.

Biochemical Tests

Biochemical tests were carried out on the bacterial isolates as follows:

Catalase Test

Catalase is an enzyme that catalyzes the decomposition of hydrogen peroxide into oxygen and water. This was done by addition of a drop of the bacterial suspension to a drop of hydrogen peroxide on a clean microscope slide. The appearance of effervescence and bubbling was an indication of a positive reaction. This test was done to identify members of the genus *Staphylococcus* (Cheesbrough, 2010).

Citrate Utilisation

Escherichia coli, Shigella spp, *Proteus spp, Salmonella typhi* and *Klebsiella* spp. all utilizes citrate as their source of carbon. The media was prepared according to manufacturer's instruction by diluting 22.5g of the agar in 1000ml distilled water, boiled and dispensed in bijou bottles in aliquots of 5 mls and sterilized by autoclaving at 121^{o}C for 15minutes, then slopped and the organism was stabbed at the butt of the slant and incubated at 37 °C **(**Cheesbrough, 2010).

Indole Test

This test was carried out by inoculating the organism in sterile tryptone water and incubated at 37^{o}C for 48 h. Thereafter, 0.5ml Kovac's reagent was added and shaken well and observed immediately for colour change.(Cheesbrough, 2010).

Urea agar test

Briefly, colonies were picked from using a straight inoculating wire and inoculated into separated urea agar slopes. The urea agar was incubated at 37 ^{o}C for 5 hours. Urease positive microbes turned the inoculated slopes pink whereas in the case of the urease negative microbes, the urea agar plates remained colourless or yellow (Chessbrough, 2010).

Triple Sugar Iron (TSI) agar test

TSI is a composite medium for the differentiation of Enterobacteriaceae according to their ability to ferment lactose, sucrose and glucose, and to produce hydrogen sulphide (Chessbrough, 2010). It contains phenol red which is a pH indicator. Below pH 6.8 it turns yellow and above 8.2 it turns red. Not only does this medium perform most of the functions of Kligler Iron Agar but, in addition, its sucrose content permits the recognition and exclusion of sucrose-fermenting species (Chessbrough, 2000). A straight inoculation rod was sterilized by flaming. The top of a well isolated colony was touched and used to streak the slope, with the butt stabbed and incubated at 37 ^{o}C overnight. Failure to turn the butt yellow indicates that no fermentation has occurred, and that the bacterium is an obligate aerobe.

Antibacterial Susceptibility Test

Nutrient Agar (Titan Biotech Ltd. Bhiwadi- 301 019, Rajasthan, India.) was used for the antibacterial susceptibility testing. It was prepared according to manufacturer's instructions by suspending 28g of medium in 1000ml distilled water, sterilized at 121^{o}C, and cooled to room temperature prior to dispensing in Petri dishes.

Preparation of Extract Concentration

This was carried out according to the method described by Srinivasan *et al.,* (2009). Stock solution of the plant extracts were prepared by adding 0.4g of each crude plant extract in 2ml of 10% dimethylsulphuroxide (DMSO). From each of the stock solutions, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml concentrations were prepared using dilution method. These concentrations were labelled and kept in bijou bottles for subsequent use.

Preparation of Turbidity Standard

McFarland standards are used as a reference to adjust the turbidity of microbial suspension so that the number of bacteria will be within a given range. Firstly, BaCl2 (1%w/v) and H2S04 (1% v/v) were prepared by dissolving 1g of BaCl2 in 100ml of sterile distilled water and 1ml of concentrated H2S04 in 99ml of sterile distilled water respectively to serve as stock solutions for the preparation of the McFarland standard. From the stock solutions, 0.5McFarland scale was prepared by adding 9.95ml of (1%v/v) H2S04 to 0.05ml of (1% w/v) BaSO4 whose density is equivalent to 1.5×10^{8} CFU/ml approximate cell density of bacteria. The barium sulphate suspension in 6ml aliquots were transferred in to screw-cap tubes, tightly sealed, and stored at room temperature in order to prevent loss by evaporation. This was subsequently used for comparison with the turbidity of the bacterial inoculum (Cheesbrough, 2010).

Standardization of Bacterial Inocula.

For inocula standardization, the density of each isolated bacterial culture was adjusted equal to that of 0.5 McFarland standards (1.5×10^{8} CFU/ml) by suspending 2 or 3 colonies of each bacterial culture into 2ml of sterile physiological saline as a suspending medium. The physiological saline was prepared by dissolving 8.5g of NaCl2 in 1L of distilled water before sterilizing (Cheesbrough, 2010).

Antimicrobial activities of the extracts

The antimicrobial activities of *A. leiocarpus* and *P. africana* crude extract and fractions (Aqueous and Ethanolic) against the test organisms was evaluated using agar well diffusion method of susceptibility test (Srinivasan *et. al.,* 2009). Nutrient agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Wells of 6mm size were made with sterile cork borer into the inoculated agar plates. Using micropipette, 0.1ml volume of the various

concentrations; 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml each of the crude extracts and fractions were dispensed into wells of inoculated plates. The prepared plates were then left at room temperature for10minutes, allowing the diffusion of the extracts into the incubation at 37 ^{o}C for 24 hours. The diameter of inhibition zones (DIZ) was measured and expressed in millimeters after incubation. The mean values of the diameter of inhibition zones were calculated to the nearest whole number. DMSO was used as negative control. Commercially available standard antibiotic, ciprofloxacin (5µg) was used as positive control parallel with the extracts. For the antibiotic inhibition zone was interpreted in accordance with Clinical Laboratory Standards Institute (CLSI), (2011) interpretation guidelines.

Determination of Minimum Inhibitory Concentration (MIC)

Extracts that exhibited activities against the test organisms were further assayed for their minimum inhibitory concentrations (MIC). The broth dilution method was employed using Nutrient broth as described by Andrews (2001). Dilutions of each reconstituted extract was made to obtain the following concentrations; 35, 30,25 and 20mg/ml. Each extract concentration was then inoculated into tubes containing 100µl of active inoculum of standardized bacterial isolates and incubated for 24 hours at 37^{o}C. The MIC was determined as the lowest concentration of the extract that inhibited the organism and results were observed in the form of turbidity.

Determination of Minimum Bactericidal Concentration (MBC)

Minimum Bacteriocidal Concentration (MBC) for each extract was determined from the MIC tube that showed no visible bacterial growth by sub-culturing a loopful from each tube on to nutrient agar plate and incubated at 37^{o}C for 24h. The lowest concentration of each extract that yielded no growth was recorded as the MBC (Andrews, 2001).

Solvent Fractionation of Crude Plant Extracts

The active crude extract (stem bark) of *A. leiocarpus* and *P. africana* was fractionated in accordance with the procedures of Venskuttn *et al.* (2009). The extraction solvents were methanol, and water (polar). The procedure was carried out in a separating funnel in which fractions obtained were evaporated to dryness on a water bath to remove the solvent.

Data Analysis

The data generated are presented in Tables and Charts and were analysed statistically using the Statistical Package and Service Solution Package (SPSS) version 18. Analysis of Variance (ANOVA) was used to compare means of the plant extracts at different concentrations, the standard strain, and the positive control antibiotics if there is any statistically significant difference in the diameter of zones of inhibition.

RESULTS

Phytochemical Properties of *Anogeissus leiocarpus* and *Prosopis africana* Crude Extracts

The results of the phytochemical properties of crude stem bark extracts of *Anogeissus leiocarpus* and *Prosopis africana* are shown in Table 1. Upon conducting quantitative and qualitative phytochemical assessments on fractions of the plant extracts, the qualitative analyses revealed the presence of various secondary metabolites in the aqueous extracts. These include saponins, anthraquinone, flavonoid, cardiac glycosides, steroids, terpenoids, tannins and alkaloids; saponin, flavonoid, cardiac glycosides, tannins and alkaloids were present in all the stem bark extracts. Anthraquinone was only present on the aqueous extract of *Anogeissus leiocarpus*, while steroids were present in PAW, PAM and ALM. Nonetheless, terpenoids were present only in PAW, PAM and ALW respectively (Table 1).

Table 1: Phytochemical Properties of *Anogeissus leiocarpus* and *Prosopis Africana* Crude Extracts

Properties	PAW	PAM	ALW	ALM
Saponin	+	+	+	+
Anthraquinone	-	–	+	–
Flavonoid	+	+	+	+
Cardiac Glycoside	+	+	+	+
Steroids	+	+	-	+
Terpenoids	+	+	+	-
Tannins	+	+	+	+
Alkaloids	+	+	+	+

Keyword: ALW - *Anogeissus leiocarpus* water, ALM - *Anogeissus leiocarpus* methanol, PAW - *Prosopis africana* water, *PAM - Prosopis africana* methanol, + = Present and - = Absent

Antibacterial Activities of Aqueous and Methanolic Stem Bark Extracts of *Anogeissus leiocarpus* on the Diameter Zone of Inhibition of Test Isolates

The results of the antibacterial activities of aqueous and methanolic stem bark extracts of *Anogeissus leiocarpus* against diameter zone of inhibition of test isolates is shown in Table 2. The effect of ALW and ALM on the diameter zone of inhibition of *E. coli* ranged from 9±2.53 (25 mg/ml) to 19±0.87 mm (200 mg/ml) and 8±1.02 (25 mg/ml) to 19±0.87 mm (200 mg/ml) with significant ($p<0.05$) difference. *Klebsiella pneumoniae* was found to have diameter zone of inhibition ranging from 7±3.03 to 27±3.47 mm (200 mg/ml) and 10±3.74 to 31±4.51 mm (200 mg/ml) with significant ($p<0.05$) in ALW and ALM respectively. *Proteus mirabilis* was also found to diameter zone of inhibition ranging from 9±0.52 to 23±1.49 mm (200 mg/ml) and 10±2.04 to 25±3.67 mm (200 mg/ml) with significant ($p<0.05$) difference in ALW and ALM respectively. Moreso, *Salmonella enterica* was found to range from 13±0.64 to 23±1.82 mm (200 mg/ml) and 12±0.88 to 23±0.93 mm (200 mg/ml) with significant difference in ALW and ALM sequentially. In that light, *Shigella spp* was also found to have diameter zone of inhibition ranging from 10±1.55 to 32±3.59 mm (200 mg/ml) and 10±1.29 to 27±3.54 mm (200 mg/ml) with significant ($p<0.05$) difference in ALW and ALM respectively. Notably, the observed diameter zone of inhibition increased with increase in the stem bark aqueous and methanolic extracts of *Anogeissus leiocarpus* in all the experimented enteric bacteria.

Table 2: Antibacterial Activities of Aqueous and Methanolic Stem Bark Extracts

Isolate	Extract concentration (mg/ml)	Diameter zone of inhibition (mm)	
		ALW	ALM
Escherichia coli strain LAW4	200	19±0.87	19±0.87
	100	17±3.01	14±2.88
	50	11±0.68	11±0.68
	25	9±2.53	8±1.02
	Ciprofloxacin 5ug/ml	12±3.48	0±0.00
p-value (0.05)		0.00	0.00
Klebsiella pneumoniae	200	27±3.47	31±4.51
	100	24±2.06	26±3.47
	50	19±1.08	20±1.81
	25	14±0.99	15±1.06
	Ciprofloxacin 5ug/ml	7±3.03	10±3.74
p-value (0.05)		0.00	0.00
Proteus mirabilis strain SA Ant1	200	23±1.49	25±3.67
	100	20±2.21	23±3.29
	50	18±1.77	17±0.69
	25	14±2.09	12±3.88
	Ciprofloxacin 5ug/ml	9±0.52	10±2.04
p-value (0.05)		0.00	0.00
Salmonella enterica subsp. Enteric serovar Typhi Str ST4	200	23±1.82	23±0.93
	100	18±2.38	21±4.02
	50	13±1.66	17±2.75
	25	12±1.29	13±0.62
	Ciprofloxacin 5ug/ml	13±0.64	12±0.88
p-value (0.05)		0.00	0.00
Shigella sp. M29	200	32±3.59	27±3.54
	100	23±2.04	25±3.07
	50	20±1.02	20±2.76
	25	13±2.60	14±0.85
	Ciprofloxacin 5ug/ml	10±1.55	10±1.29
p-value (0.05)		0.00	0.00

Keyword: ALW - *Anogeissus leiocarpus* water, ALM - *Anogeissus leiocarpus* methanol, PAW - *Prosopis africana* water and PAM - *Prosopis africana* methanol

Antibacterial Activities of Aqueous and Methanolic Stem Bark Extracts of Prosopis Africana on Diameter Zone of Inhibition of Test Isolates

The results of the antibacterial activities of aqueous and methanolic stem bark extracts of *Prosopis africana* against diameter zone of inhibition of test isolates is shown in Table 3. The effect of PAW and PAM on the diameter zone of inhibition of *E. coli* was found to be highest with mean value of 14±2.33 mm (200 mg/ml) and 11±1.85 mm (200 mg/ml) with significant ($p<0.05$) difference respectively. *Klebsiella pneumoniae* was observed to have diameter zone of inhibition with mean value of 20±2.81 mm (200 mg/ml) and 9±1.24 (25 mg/ml) to 15±1.09 mm (200 mg/ml) with significant ($p<0.05$) difference in PAW and PAM respectively. *Proteus mirabilis* was also observed to have diameter zone of inhibition with mean value of 16±2.09 mm (200 mg/ml) and 10±1.66 (control) to 16±0.71 mm (200 mg/ml) with significant difference in PAW and PAM sequentially. Moreso, *Salmonella enterica* was found to be highest with mean value of 19±2.36 mm (200 mg/ml) and 10±2.11 (control) to 16±1.29 mm (200 mg/ml) with significant ($p<0.05$) difference in PAW and PAM respectively. *Shigella sp.* was found to have diameter zone of inhibition ranging from 0±0.00 (control) to 16±1.09 mm (200 mg/ml) with significant ($p<0.05$) difference for both PAW and PAM respectively. Notably, it was observed

that PAW extracts recorded slightly higher diameter zone of inhibition when compared to PAM extracts. Also as earlier observed in *Anogeissus leiocarpus* extracts, the diameter zone of inhibition increased with increase in concentration.

Table 3: Antibacterial Activities of Aqueous and Methanolic Stem Bark Extracts of *Prosopis Africana* on Diameter Zone of Inhibition of Test Isolates

Isolate	Extract concentration (mg/ml)	Diameter zones of inhibition (mm)	
		PAW	PAM
Escherichia coli strain LAW4	200	14±2.33	11±1.85
	100	13±1.94	10±2.06
	50	11±0.47	8±0.86
	25	10±0.38	7±1.07
	Ciprofloxacin 5ug/ml	0±0.00	0±0.00
p-value (0.05)		0.00	0.00
Klebsiella pneumoniae	200	20±2.81	15±1.09
	100	18±5.08	11±2.88
	50	15±3.17	10±0.46
	25	12±2.02	9±1.24
	Ciprofloxacin 5ug/ml	0±0.00	10±3.86
p-value (0.05)		0.00	0.00
Proteus mirabilis strain SA Ant1	200	16±2.09	16±0.71
	100	14±0.78	15±0.28
	50	14±3.25	14±1.07
	25	12±1.05	12±2.01
	Ciprofloxacin 5ug/ml	0±0.00	10±1.66
p-value (0.05)		0.00	0.00
Salmonella enterica subsp. Enteric serovar Typhi Str ST4	200	19±2.36	16±1.29
	100	17±1.27	14±2.38
	50	15±2.04	12±3.07
	25	12±1.61	10±2.11
	Ciprofloxacin 5ug/ml	0±0.00	10±1.04
p-value (0.05)		0.00	0.00
Shigella sp. M29	200	16±1.09	16±1.92
	100	17±2.27	17±2.63
	50	15±0.33	15±1.27
	25	12±3.05	12±0.88
	Ciprofloxacin 5ug/ml	0±0.00	0±0.00
p-value (0.05)		0.00	0.00

Keyword: ALW - *Anogeissus leiocarpus* water, ALM - *Anogeissus leiocarpus* methanol, PAW - *Prosopis africana* water and PAM - *Prosopis africana* methanol

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Aqueous and Methanolic Extracts of *Anogeissus leiocarpus* and *Prosopis africana* and Bacterial Isolates

The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and methanolic extracts of *Anogeissus leiocarpus* and *Prosopis africana* on bacterial isolates is shown in Table 4. The MIC of ALW and ALM were found to range from 20±5.16 (*P. mirabilis*) to 303.08 (*E. coli, K. pneumoniae* and *S. Enterica*) and 25±3.77 (*K. pneumoniae*) to 35±2.03 (*Shigella spp.*) with significant ($p<0.05$) difference respectively, after 24 hours of incubation. However, after a follow-up assay, the MBC of ALW were found to be 30±1.29 for all the tested isolates with no significant difference while ALM ranged from 25±2.77 (*K. pneumoniae*) to 35±3.33 (*P. mirabilis* and *Shigella spp.*) with significant ($p<0.05$). Nonetheless, the MIC of PAW and PAM were found to range from 20±2.37 (*Shigella spp.* and *P. mirabilis*) to 35±0.77 (*E. coli*) and 25±2.86 (*E. coli* and *Shigella spp*) to 35±3.41 (*P. mirabilis*) with significant ($p<0.05$) difference respectively, after 24 hours of incubation. Nonetheless, after a follow-up assay, the MBC of PAW and PAM were found to range from 20±2.86 (*K. pneumoniae*)

to 35±3.18 (*E. coli* and *S. enterica*) and 25±3.77 (*Shigella spp*) to 35±2.80 (*P. mirabilis* and *S. enterica*) with significant difference respectively.

Table 4 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Aqueous and Methanolic Extracts of *Anogeissus leiocarpus* and *Prosopis africana* an Bacterial Isolates

Bacterial isolates	MIC_ALW	MIC_ALM	MBC_ALW	MBC_ALM	MIC_PAW	MIC_PAM	MBC_PAW	MBC_PAM
Escherichia coli strain LAW4	30±3.08	30±4.24	30±1.29	30±4.62	35±0.77	25±2.86	35±3.18	30±1.65
Klebsiella pneumoniae	30±3.08	25±3.77	30±1.29	25±2.77	20±2.37	30±2.69	20±2.86	30±1.65
Proteus mirabilis strain SA Ant1	20±5.16	35±2.03	**	35±3.33	25±1.17	35±3.41	**	35±2.80
Salmonella enterica subsp. Enteric serovar Typhi Str ST4	30±3.08	30±4.24	30±1.29	30±4.62	25±1.17	30±2.69	35±3.18	35±2.80
Shigella sp. M29	25±3.55	35±2.03	**	35±3.33	20±2.37	25±2.86	**	25±3.77
p-value (0.05)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Keyword: MIC – Minimum Inhibitory Concentration, MBC – Minimum Bactericidal Concentration, ** - Greater Than 35mg/ml, ALW - *Anogeissus leiocarpus* aqueous, ALM - *Anogeissus leiocarpus* methanol, PAW – *Prosopis africana* aqueous and PAM - *Prosopis africana* methanol

DISCUSSION

The saponin and flavonoid, cardiac glycosides, tannins and alkaloids were present in all the stem bark extracts of PAW, PAM, ALW and ALM. This is in conformity with the findings of Baburo *et al.* (2019), Rufai *et al.* (2021) and Muhammad *et al.* (2022) in a similar study. The difference in the concentration of these phytochemicals when compared to those in literature might be due to geographical variations, nutrients, sunlight, irrigation, time of collection, age of plant among others (Rufai *et al.*, 2021). More so, the aqueous extract had more secondary metabolites in both *Anogeissus leiocarpus* and *Prosopis africana*. This may be due to the fact that water dissolves most of the substances than any other liquid. However, both water and methanol extracts contain more secondary metabolites which may be related to the polarity of both solvent and the constituents of the extracts. The findings of this study indicated that water extracted more components compared to methanol, which had the lowest percentage of extract. This result may be due to the polarity of the components, making them more soluble in the more polar solvent (water) than in the less polar solvent (methanol).

This solubility difference could be responsible for the variation in the physical properties of the extracts. These results are consistent with the findings of Barku and Abban (2013), who reported that *A. leiocarpus* extracts contain a range of secondary metabolites, including tannins, saponins, flavonoids, steroids, amino acids, and reducing sugars. Although carbonyls were reported in their study and were not detected in the present study, similar findings have been reported by Mann *et al*. (2010) and Kabore *et al.* (2010). Their research revealed the presence of alkaloids, glycosides, phenols, steroids, tannins, saponins, flavonoids, and anthraquinones. Notably, some of these metabolites have been associated with the antimicrobial activity observed in certain ethno-medicinal plants, as noted by Singh and Bhat (2003).

The presence of flavonoids in the aqueous stem bark extract of *A. leiocarpus* could account for its use as an anti-inflammatory agent (Ekwueme *et al.*, 2015). It also means that the plant could be used to prevent damage caused by free radicals in the body (Dweck and Mitchell, 2002). Oxidative stress induced by ethanol was suppressed in the treatment group which proves the radical scavenging activity of the extract. Flavonoids exhibit dramatic effects on immune and inflammatory cells; these can be either immunosuppressant or immune stimulatory (Huang

et al., 2010). These biological active compounds also known as secondary metabolites constitute an important source of antimicrobials and many pharmaceutical drugs. These metabolites also help in the antimicrobial activities of the plant through different mechanisms. The antibacterial effectiveness of the extracts was found to increase with higher extract concentrations. This observation aligns with the findings of Banso *et al.* (1999) and Mann *et al.* (2008), who also noted that increased concentrations of antimicrobial substances resulted in significant growth inhibition. The varying zones of inhibition produced by the test organisms demonstrated their susceptibility to the plant extracts, differing based on both the organism and the plant extract. Prescott (2002) noted that an agent's effect varies with the target species. Hugo and Russell (1998) further explained that the zone of inhibition's size (diameter) depends on the initial population density of the organism, their growth rate, and the antimicrobial agent's diffusion rate, accounting for the observed differences. The stem bark extract and the combination of both extracts showed antibacterial activity against the test organisms, supporting their traditional medicinal use in Niger state, likely due to the presence of active compounds. Secondary plant metabolites are vital sources of microbiocides, pesticides, and numerous pharmaceuticals (Ibrahim *et al.,* 1997; Kolapo *et al.,* 2007). The aqueous and methanolic extracts of the plant parts demonstrated antibacterial activity against pathogenic organisms, suggesting potential therapeutic applications. Further research is recommended to isolate the active antibacterial agents and conduct toxicological studies on the plant parts.

The antibacterial activity of the plant extracts revealed that the test organisms were more sensitive to the aqueous extracts, with some sensitivity also noted towards the methanol extract fraction of the stem bark of *A. leiocarpus* and *P. africana.* This may be due to the fact that these organisms are highly resistant, and there is limited literature showing the antibacterial activity of these extracts against multidrug-resistant, Extended Spectrum Beta-Lactamases (ESBLs) producing Gram-negative enterobacteriaceae. The sensitivity of ESBL-producing bacteria to the aqueous and methanolic extracts of *A. leiocarpus* and *P. africana* varies among different organisms. These findings align with Barku and Abban (2013), who observed that the zones of inhibition produced by test organisms indicated their susceptibility to the extracts, with variation seen among different organisms. The extracts showed greater activity on Gram-negative bacterial strains such as *Klebsiella pneumoniae* and *E. coli* than on Gram-positive strains. The antimicrobial properties observed in this study may be due to secondary metabolites like alkaloids and tannins, whose antimicrobial properties are well-documented (Tschehe, 1971). The present study's results are also consistent with Ikhram *et al.* (2015), who found that all tested organisms, including *E. coli*, *Proteus mirabilis, Klebsiella pneumoniae*, *Shigella spp* and *Salmonella enterica* (Gram-negative enterobacteria), were sensitive to the extracts and fractions of *A. leiocarpus P. africana*. Therefore, this study demonstrates the antimicrobial properties of the stem bark of *A. leiocarpus* and *P. africana*, which may be valuable for future ethnomedicinal and pharmacological research.

The MIC and MBC for the aqueous and methanolic stem bark extracts of *Anogeissus leiocarpus* and *Prosopis africana* were both found to be higher than the earlier reported values by Mann (2012) but at the same time lower than the report of Muhammad *et al.,* (2022) of 50 – 100mg/ml in a similar study. These might be unconnected to the presence of the active phytochemical compounds Umar *et al.* (2015). The inhibition of the microbes by these secondary metabolites indicated their potentials in the treatment of diseases caused by the organisms. The aqueous and methanolic stem bark extracts had bactericidal effect ranging from 20 – 35 mg/ml for all the tested clinical isolates especially *E. coli* and *S. typhi*, indicating relative effects of concentrations, and consequently suggesting the higher concentrations than the selected

range for *E. coli* and *S. typhi*. The activity against *Escherichia coli* is in line with the findings of Sore *et al*., (2012), Mann *et al*., (2008); Elegami, (2002) and Adigun *et al*., (2000). This supports the use of the plant to treat *Escherichia coli* related infectious wounds and diarrhea in ethno-medicine. Activity of the plant extract against *Salmonella typhi* validates the use of the plant for the treatment of headache and fever in trado-medicine. The plant is also active against *Klebsiella pneumonia* and this buttresses the plant's use to treat upper respiratory tract infections (e.g. cough, asthma, bronchitis and pneumonia) traditionally Usman *et al.* (2020).

The results of the present investigation also demonstrated the antimicrobial potentials of the crude aqueous and methanolic extracts of both *A. leiocarpus* and *P. africana*. The two extracts from these plants possess significant in vitro antimicrobial activities against some of the bacteria implicated in the pathogenesis of human infections. Some infections such as: respiratory tract inflammations caused by *Salmonella* spp, are often difficult to combat, but the growth of these organisms was greatly inhibited by extracts from both plants. While *E. coli* incriminated as the causative agent of gastro-intestinal and also causes infections in the lungs especially in immunodeficient patients Black (1996) was also susceptible to both extracts from this study. It is worthy to note that the antimicrobial activity found in *Anogeissus* species in the Sudan has been attributed to 3, 3, 4' –tri-O-methylflavellagic acid extracted from the bark Almagboul *et al.* (1988). However, the activities of the stem bark extract of *A. leiocarpus* and *P. africana* against *E. coli, Klepsiellia spp, Proteus spp, Salmonella spp.* and *Shigella spp.* have been reported by different researchers (Mann *et al.* 2008; Rufai *et al.* 2021 and Muhammad *et al.* 2022). It is a common practice among the traditional healers in Kano, Kaduna and Niger State to prepare an infusion of *A. leiocarpus* and *P. africana* separately to relieve upper respiratory tract infections, fever, cough, TB and stomach pains. The susceptibility of these microbes to the extracts of these plants may be a pointer to their potentials as drugs that can be used against these organisms.

CONCLUSION

The aqueous and methanolic stem bark extracts of *A. leiocarpus* and *P. africana* obtained from Lere LGA, Kaduna State and Dutse LGA, Jigawa State, Nigeria were found to possess antibacterial activity against *S. typhi*, *E. coli*, *P. mirabilis*, *K. pneumoniae* and *Shigella*. spp from Microbiology Laboratory, Aminu Kano Teaching Hospital, Nigeria. This study has justified the use of *A. leiocarpus* and *P. africana* in the treatment of some bacterial diseases in folkloric herbal medicine. Notably, both plant extracts exhibited significant antimicrobial effects against tested bacteria, suggesting their potential as therapeutic agents. These plants are rich in bioactive compounds such as flavonoids, tannins, alkaloids, saponins, and phenolics, which contribute to their antimicrobial properties.

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