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Antimicrobial Activities of *Acacia nilotica*, *Ziziphus Jujube* Linn and *Lawsonia Inermis*

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ABSTRACT

Infectious diseases are important cause of morbidity and mortality due to continuous emergence of microbial resistance to conventional drugs. *Acacia nilotica*, *Ziziphus jujube* Linn and *Lawsonia inermis* are widely used for traditional medicine in Northern Nigeria. However, little is known about the biochemical and microbiological potentials of these indigenous plants. In this study, the plants leaves were screened for phytochemical and *in vitro* antimicrobial potentials using standard methods. Quantitative phytochemical analysis of crude methanolic leave extracts revealed high content of glycoside, tannins and phenols. High levels of saponins and flavonoids were also detected. The extracts exhibited antibacterial effects on *Escherichia coli*, *Pseudomonas flourecense*, *Streptococcus and Staphylococcus aureus*. At 50 mg/ml extract concentration, the zone of inhibition observed was greater than 6mm. This indicates high inhibitory potency of the plants leaves. In comparison to streptomycin sulphate, *A. nilotica* and *L. inermis* had statistically similar (P>0.05) effect on *E. coli* at 50 mg/ml. In general, the inhibitory effect of *A. nilotica* and *L. inermis* were higher than that of *Z. jujube* Linn in all concentrations, except on *E. coli* at 150 mg/ml. Both the extracts and control drug had minimum inhibitory concentration of 10 mg/ml for all the microbes tested except *Streptococcus* (20-25 mg/ml). Furthermore, the average Minimum Bactericidal Concentration was 15 mg/ml except for *Streptococcus* with 20-25 mg/ml. Methanol extracts of *Acacia nilotica*, *Ziziphus jujube* Linn, and *Lawsonia inermis* exhibit antibacterial effect, hence could be used as sources of potent agents against bacterial infection.

Keywords: Antimicrobial, infectious disease, Methanol extract, Phytochemical.

INTRODUCTION

The use of medicinal plants is undoubtedly an art; as old as mankind, employed as a relief for ailments in ancient times and early civilization globally (Srivastava *et al.,* 1996; Mahesh and Satish, 2008). They are also potential sources of pharmacologically active agents useful as drug candidates. Among the approximately estimated 500, 000 plant species, only about 10% are consumed by humans and other animals (Silva and Fernandes, 2010). More so, only few have been investigated phytochemically, and quite a few have been subjected to biological or pharmacological screening (Mahesh and Satish, 2008).

Recently, active compounds from higher plants continuously occupy an important position in modern medicine. Over 130 compounds extracted from higher plants and their synthetically/modified derivatives are currently in use (Newman *et al.,* 2000). Some of these compounds are exploited in orthodox medicine, among which are considered to be potent antimicrobial agents In developing countries however, the continuous emergence of microbes resistant to conventional drugs is a major challenge in the treatment of infectious diseases. This necessitates the search for new potent antimicrobial agents from indigenous plant. This study presents result of an investigation on the antimicrobial activities of *A. nilotica* (Gum arabic), *Z. jujube* Linn (Jujube) and *L. inermis* (Henna).

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Fresh leaves of *A. nilotica*, *Z. jujube* Linn and *L. inermis* were collected from Achida town in Wurno Local Government Area of Sokoto State. The samples were thoroughly washed with distilled water, then air-dried (under shade) for 14 days. The dried leave samples of the plants were pulverized into powder using pestle and mortar. Approximately 100g of each pulverized material was extracted with 95% methanol. The filtrates were evaporated at 45˚C using a rotary evaporator. The resulting residues were store at 4°C until needed.

Qualitative Phytochemical Screening

The methods described by Sofowora (1993), El-Olemyl *et al* (1994) and Harbone (1998) were used for the qualitative phytochemical analyses of the plants extracts.

Quantitative Phytochemical Screening

Each of the residues was reconstituted using distilled water. Quantitative phytochemical screening was employed to determine the concentration of flavonoids (Bohm and Kocopai, 1994), phenols **(**Harbone, 1973), tannins (Swain, 1979), Saponins (Obadoni and Ochuko, 2001) and glycosides **(**Trease and Evans, 1989).

Experimental Design

Collection of Bacteria Isolates

Clinical isolates of gram positive bacteria (*Staphylococcus aureus* and *Streptococcus*) and gram negative bacteria (*Escherichia coli* and *Pseudomonas fluorescense*) were collected from Microbiology Unit of Specialist Hospital, Sokoto. The isolates were further authenticated using microscopic and biochemical techniques. The bacteria cultures were maintained on nutrient broth at 37°C.

Antibacterial Screening

The antibacterial activity of the plant extracts were evaluated using agar well diffusion method described by National Committee for Clinical Laboratory Standards (NCCLS), (1999). Different concentrations (50, 100 and 150 mg/ml) of *A. nilotica*, *Z. jujube* Linn, and *L. inermis* were individually used to screen the organisms for susceptibility. Streptomycin sulphate was used as control drug. The zones of inhibition were measured using transparent meter rule.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the plants leaves extracts was determined using Broth dilution method as described by Wiegand *et al*. (2008). A suspension of the test organisms was diluted in the ratio 1:200 in Mueller Hinton broth. Concentration of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg/ml were used for the test. The least concentration of the samples with no visible growth of the organism was taken as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

A loop of broth was collected from MIC experiment where no growth was observed and inoculated on sterile nutrient agar plates. The plates were incubated at 37ºC for 24 hours. The lowest concentration of the plant extracts that allows less than 0.1% of the original inoculums to survive was taken to be the MBC (NCCLS, 1992).

RESULTS

Preliminary qualitative phytochemical analysis of leaves extracts of *A. nilotica, L. inermis* and *Z. jujube* Linn are presented in Table 1. The results reveal the presence of flavonoids, tannins, saponins, glycosides, steroids, saponin glycosides, terpenes and phenols in all the extracts tested. Volatile oils were detected only in *Z. jujube* Linn extracts.

Table 2 presents quantitative phytochemical constituents of the plants extracts. The results showed that all the plants analysed contain appreciable amount of tannins, saponins, phenols, flavonoid and glycoside in varying concentration. The difference in the concentrations of tannins, saponins, phenols and flavonoid among the three samples were statistically insignificant (P>0.05). However,

glycoside level differ significantly (P<0.05) among plants extract (Table 2). The highest concentration of glycoside was observed in *A. nilotica*, and the least concentration was observed in *Z. jujube* Linn.

The result of antibacterial susceptibility test at 50mg/ml of the extracts is presented in Table 3. It was observed that *A. nilotica* and *L. inermis* extracts exhibited significantly higher (*P*<0.05) antibacterial activity against *S. aureus* and *P. fluorescens* when compared to *Z. jujube* Linn extract. Similarly, the methanol extract of *A. nilotica* had statistically higher (P<0.05) antibacterial activity on *E. coli* compared to *L. inermis* and *Z. jujube* Linn. On the other hand, *L. inermis* extract showed significantly higher activity (P<0.05) on *Streptococcus*, followed by *A. nilotica* then *Z. jujube* Linn extract.

The result of antibacterial screening of the plants extracts at 100mg/ml is presented in Table 4. It was observed that the extract of *A. nilotica* and *L. inermis* exhibited significantly higher (P<0.05) antibacterial activity against *S. aureus* and *P. flourecense* compared to *Z. jujube* Linn extract. The mean zone of inhibition was statistically similar (P>0.05) to that of streptomycin sulphate. The methanol extract of *A. nilotica* exhibited the highest antibacterial activity on *E. coli* compared to *L. inermis* and *Z. jujube* Linn respectively. On the other hand, *L. inermis* extract showed significantly higher activity (P<0.05) on *Streptococcus*, while *Z. jujube* Linn extract had the least activity.

Table 1: Qualitative Phytochemical constituents of *A. nilotica, L. inermis* and *Z. jujube* Linn leave methanol extracts

Phytochemicals	*A. nilotica*	*L. inermis*	*Z. jujube* Linn
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Glycosides	+	+	+
Alkaloids	-	-	-
Cardiac glycosides	-	-	-
Steroids	+	+	+
Saponin glycosides	+	+	+
Balsams	-	-	-
Anthraquinones	+	+	-
Volatile oils	-	-	+
Terpenes	+	+	+
Phenols	+	+	+

+ = Present; – = Not detected

Table 2: Quantitative Phytochemical Composition of Methanol Leaves Extracts of *A. nilotica*, *L. inermis Z. jujube* Linn

Phytochemicals (g/100g)	**Samples**		
	A. nilotica	*L. inermis*	*Z. jujube* Linn
Saponins	0.12 ± 0.20^{a}	0.12 ± 0.12^{a}	0.12 ± 0.20^{a}
Flavonoids	0.41 ± 0.45^{a}	0.38 ± 0.58^{a}	0.37 ± 0.58^{a}
Glycoside	2.70 ± 0.45^{a}	0.87 ± 0.67^{b}	0.09 ± 0.02^{c}
Tannins	1.05 ± 0.06^{a}	1.05 ± 0.45^{a}	1.03 ± 0.04^{a}
Phenols	0.62 ± 0.03^{a}	0.61 ± 0.10^{a}	0.59 ± 0.01^{a}

Mean values having different superscript in a row are significantly different at P<0.05.

Table 3: Antibacterial Activities of Methanol Leaves Extracts of *A. nilotica, L. inermis* and *Z. jujube* Linn (50 mg/ml)

Bacterial *spp.*	**Zones of Inhibition (mm)**				
	A. nilotica	*L. inermis*	*Z. jujube* Linn	Streptomycin Sulphate (100mg/ml)	Distilled Water
S. aureus	30.33±0.58^{a}	30.67±0.58^{a}	15.33±0.58^{b}	30.33±0.58^{a}	0.00
Pseu.	20.67 ±0.58^{a}	20.33±0.58^{a}	7.67±0.58^{b}	25.33±0.58^{c}	0.00
E. coli	30.33 ±0.45^{a}	25.00±0.45^{b}	22.33±1.53^{b}	35.00±0.45^{c}	0.00
Strep.	19.33 ±2.08^{a}	26.00±1.00^{b}	7.67±0.58^{c}	30.00±0.45^{d}	0.00

Mean values having different superscript in a row are significantly different at P<0.05. *Pseu: P. flourecense* and *Strep: Streptococcus.* Size of cup borra = 6mm.

Table 4: Antibacterial Activities of 100 mg/ml of Methanol Leaves Extracts of *A. nilotica, L. inermis* and *Z. jujube* Linn

Bacterial *spp.*	**Zone of Inhibition (mm)**				
	A. nilotica	*L. inermis*	*Z. jujube* Linn	Streptomycin Sulphate (100mg/ml)	Distilled Water
S. aureus	35.33±0.58^{a}	30.67±0.58^{b}	27.33±0.58^{b}	30.33±0.58^{b}	0.00
Pseu.	25.67±2.08^{a}	19.67±0.58^{b}	7.33±0.58^{c}	25.33±0.58^{a}	0.00
E. coli	33.00±0.45^{a}	28.33±0.58^{b}	26.33±1.00^{b}	35.00±0.45^{a}	0.00
Strep.	25.33±0.58^{a}	26.33±1.15^{a}	7.67±0.45^{b}	30.00±0.45^{c}	0.00

Mean values having different superscript in a row are significantly different at P<0.05. *Pseu: P. flourecense* and *Strep: Streptococcus.* Size of cup borra = 6mm.

The effect of 150 mg/ml of the plants extracts is presented in Table 5. It was observed that all the plants extracts analyzed have significantly higher activity (P<0.05) on *S. aureus* compared to streptomycin sulphate. The highest inhibition activity on *E. coli* was exhibited by *Z. jujube* Linn extract, followed by *A. nilotica*, *s*treptomycin sulphate and *L. inermis* respectively. The activity of *s*treptomycin sulphate was statistically similar (P>0.05) to that of *A. nilotica* and *L. inermis* at 150 mg/ml on *Streptococcus*.

The minimum inhibitory concentration (MIC) of the methanol leaves extracts of the plants is presented in the Table 6. It was observed that the MIC of *A. nilotica* is low for all the four bacterial species when compared with the MIC of standard antibiotic, streptomycin sulphate.

Table 5: Antibacterial Activities of 150 mg/ml of Methanol Leaves Extracts of *A. nilotica, L. inermis* and *Z. jujube* Linn

Bacterial *spp.*	**Zone of Inhibition (mm)**				
	A. nilotica	*L. inermis*	*Z. jujube* Linn	Streptomycin Sulphate (100mg/ml)	Distilled Water
S. aureus	39.00±1.00^{a}	37.67±0.58^{ba}	35.67±0.58^{b}	30.33±0.58^{c}	0.00
Pseu.	31.00±1.00^{a}	24.67±0.58^{b}	20.33± 0.58^{c}	25.33± 0.58^{b}	0.00
E. coli	36.00±1.00^{a}	31.33±0.58^{b}	55.00± 1.00^{c}	35.00±0.33^{a}	0.00
Strep.	29.33±0.58^{a}	30.67±1.15^{a}	16.33±0.33^{b}	30.00±0.45^{a}	0.00

Mean value having different superscript letters in rows are significantly different (*P*<0.05). *Pseu: P. flourecense* and *Strep: Streptococcus.* Size of cup borra = 6mm.

Table 6: Minimum Inhibitory Concentration (MIC) of Methanol leaves Extracts of *A. nilotica, L. inermis* and *Z. jujube* Linn on some bacterial strains

Samples	Bacterial *spp.*	Concentration of Extracts (mg/ml)										
		50	45	40	35	30	25	20	15	10	5	MIC
A.nilotica	*S. aureus*	-	-	-	-	-	-	-	-	-	-	5
	Pseu.	-	-	-	-	-	-	-	-	-	+	10
	E. coli	-	-	-	-	-	-	-	-	-	-	5
	Strep.	-	-	-	-	-	-	-	-	+	+	15
L.inermis	*S. aureus*	-	-	-	-	-	-	-	-	-	+	10
	Pseu.	-	-	-	-	-	-	-	-	-	+	10
	E. coli	-	-	-	-	-	-	-	-	-	+	10
	Strep.	-	-	-	-	-	-	-	-	+	+	15
Z.jujube Linn	*S. aureus*	-	-	-	-	-	-	-	-	-	+	10
	Pseu.	-	-	-	-	-	-	-	-	-	+	10
	E. coli	-	-	-	-	-	-	-	-	-	-	5
	Strep.	-	-	-	-	-	-	+	+	+	+	25
Streptomycin	*S. aureus*	-	-	-	-	-	-	-	-	+	+	15
Sulphate	*E. Coli*	-	-	-	-	-	-	-	-	-	+	10
	Pseu.	-	-	-	-	-	-	-	-	-	+	10
	Strep.	-	-	-	-	-	-	-	+	+	+	20

Key: (-) indicate no visible growth of the organisms while sign (+) indicate visible growth of the organisms**.** *Pseu.* = *P. flourecense* and *Strep.*=*Streptococcus.*

The result of minimum bactericidal concentration (MBC) of the plant extracts on different bacteria species is presented in Table 7. With an MBC value of 10 mg/ml, *A. nilotica* exhibited lower MBC for *S. aureus* and *E. coli* when compared with streptomycin sulphate as a standard antibiotic.

DISCUSSION

The result of this study conforms to earlier reports suggesting the presence of flavonoids, tannins, saponins, glycosides, steroids, terpenes and phenols in methanolic leaves extract on *A. nilotica* (Lawaly *et al*., 2017; Mrityunjoy *et al*., 2016; Singh and Thakur, 2016; Raghavendra *et al*., 2006), *L. inermis* (Rao *et al*., 2016; Gull *et al*., 2013; Nayak *et* al., 2007) and *Z. jujube* Linn (Elaloui *et al.* , 2017; Mahajan and Chopda, 2009). In contrast, only *Z. jujube* Linn possessed volatile oils as reported by Kundu *et al*. (1989).

At 50, 100 and 150 mg/ml, the zones of inhibition of all the three plants extract was greater than 6 mm. The activity of *A. nilotica* and *L. inermis* extracts on *E. coli, Streptococcus, S. aureus* and *P. fluorescens* were comparably similar to streptomycin sulphate. The least antibacterial activity was exhibited by *Z. jujube* Linn extract

compared to other extracts investigated. *Z. jujube* Linn extract exhibited greater inhibitory effect on *E. coli* at 150 mg/ml which is greater than other extracts and the control drug. More so, the minimum inhibitory concentration and minimum bactericidal concentration of all the three plant extracts on the tested microbes ranged between 5- 25 mg/ml. In general, the higher antimicrobial activity of *A. nilotica*, *L. inermis* and *Z. jujube* Linn leaves extracts can be attributed to the high presence of glycoside and tannins among other phytochemicals in the plant extracts (Lawaly *et al*., 2017).

Table 7: Minimum Bactericidal Concentration (MBC) of Methanol Leave Extracts of *A. nilotica, L. inermis* and *Z. jujube* Linn on some bacterial strains

Samples	**Bacterial *spp.***	**Concentration of Extracts (mg/ml)**										
		50	45	40	35	30	25	20	15	10	5	MBC
A.nilotica	*S. aureus*	-	-	-	-	-	-	-	-	-	+	10
	Pseu.	-	-	-	-	-	-	-	-	+	+	15
	E. coli	-	-	-	-	-	-	-	-	-	+	10
	Strep.	-	-	-	-	-	-	+	+	+	+	25
L.inermis	*S. aureus*	-	-	-	-	-	-	-	-	+	+	15
	Pseu.	-	-	-	-	-	-	-	+	+	+	20
	E. coli	-	-	-	-	-	-	-	-	+	+	15
	Strep.	-	-	-	-	-	-	-	+	+	+	20
Z. jujube Linn	*S. aureus*	-	-	-	-	-	-	-	-	+	+	15
	Pseu.	-	-	-	-	-	-	-	-	+	+	15
	E. coli	-	-	-	-	-	-	-	-	+	+	15
	Strep.	-	-	-	-	-	-	+	+	+	+	25
Streptomycin	*S. aureus*	-	-	-	-	-	-	-	-	+	+	15
Sulphate	*E. Coli*	-	-	-	-	-	-	-	-	+	+	15
	Pseu	-	-	-	-	-	-	-	-	+	+	15
	Strep	-	-	-	-	-	-	-	+	+	+	20

Key: (-) indicate no visible growth of the organisms while sign (+) indicate visible growth of the organisms**.** *Pseu. = P. flourecense* and *Strep. = Streptococcus.*

The results of this study is in accordance with previous reports suggesting potent antibacterial activity of *A. nilotica* (Ali *et al*., 2017; Singh and Thakur, 2016; Gupta, 2015; Raghavendra *et al*., 2006), *L. inermis* (Chowdhury *et al*., 2014; Gull *et al*., 2013; Musa *et al*., 2011; Akter *et al*., 2010; Babu and Subhasree, 2009; Al-Rubiay *et al*., 2008; Nayak *et* al., 2007) and *Z. jujube* Linn (Elaloui *et al*., 2017; Arifa *et al*., 2016; Naz *et al*., 2013) against both gram negative and gram positive bacteria.

CONCLUSION

The results of this study revealed the presence of saponins, flavonoids, glycoside, tannins and phenols in methanol leaves extract of *A. nilotica*, *L. inermis* and *Z. jujube* Linn. More so, the plants are effective against *S. aureus, Pseu.,E. coli and Strep*. Thus, these plants extracts could be used as sources of agents for the treatment of infections caused by the tested microorganisms.

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