

Full Length Research Paper

# Evaluation of the antimicrobial activities of two *Ziziphus* species (*Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.) on some microbial pathogens

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The antimicrobial effects of ethanolic extracts of leaves of two species of genus *Ziziphus* were determined against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger* and *Candida albicans*. *S. pyogenes* was the most susceptible followed by *E. coli* while *S. aureus* was the least susceptible. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were 1 mg, 5 mg ml<sup>-1</sup> and 20 mg, 40 mg ml<sup>-1</sup>, respectively. Extracts showed no activity against the fungal isolates - *A. niger* and *C. albicans*. The plants cannot be used in treating any type of fungal infections (dermatophycoses). The phytochemicals identified were cardiac glycosides, polyphenols, saponins and tannins. Extracts from these plants could be useful in the treatment of nosocomial infections, opportunistic infection of the urinary tract (UTI), infantile gastroenteritis, travelers' diarrhea, wound infection, meningitis, and wound infection which are diseases caused by some of these organisms.

**Key words:** Antimicrobial activity, pathogenic microbes, phytochemical constituents, *Ziziphus*.

## INTRODUCTION

The genus *Ziziphus* belongs to the family Rhamnaceae. This genus comprises of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world. *Ziziphus* species can grow either as shrublets, shrubs or trees with thorny branches and are used as a hedge to form defensive fences for animals (Cherry, 1985). Some species, like *Ziziphus mauritiana* Lam. and *Ziziphus spinachristi* (L.) wild occur on nearly every continent. *Z. mauritiana* and *Z. spinachristi* have very nutritious fruits and are usually eaten fresh. The fruits are applied on cuts and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, for dysentery (Adzu et al., 2001). The leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases (Adzu et al., 2001). The seeds are sedative and are taken sometime with buttermilk to halt nausea, vomiting and abdominal pains associated with pregnancy (Kaaria, 1998). The leaves are applied as

poultices and are helpful in liver troubles, asthma and fever (Michel, 2002).

The hepatoprotective activity of ethanol extract of *Ziziphus mauritiana* leaf against CCl<sub>4</sub> - induced liver damage in rats and the anti-diarrhoea activity of the methanol root extract were reported (Dahiru et al., 2005; Dahiru et al., 2006). The antioxidant activity of the aqueous extract of *Ziziphus mauritiana* leaf also reported (Dahiru and Obidoa, 2008). While *Z. spinachristi*, commonly known as Christ's Thorn Jujube is highly respected by the Muslims through the Middle East because it has been widely used as a fruit plant and as a medicinal plant since antiquity and is still in use at present (Dafni et al., 2005). *Z. spinachristi* extract has also been reported to possess protective effect against aflatoxicosis (Abdel-Wahhab et al., 2007) and anti-conceptive properties in the rat and have a calming effect on the central nervous system.

The extract of *Z. spinachristi* found was shown to contain benzoic acid and ceanothic acid, cyclopeptides, as well as saponin glycoside and flavonoids, lipids, protein, free sugar and mucilage (Adzu et al., 2003). Plant materials are cheap and significantly contribute to the improvement

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of human health in terms of cure and prevention of diseases Okoko and Orumbo (2008). Plants have been useful as food and medicine and a few have been studied especially African medicinal plants (Lee et al., 2003; Ogle et al., 2003; Adebooye and Opabode, 2004; Ayodele, 2005). They contain vitamins needed by human body for healthy living (Szeto et al., 2002; Jimoh et al., 2008).

The present research was aimed at investigating the claims of traditional healers from Nupe land on the use of these plants in treating different ailments among their people.

## MATERIALS AND METHODS

### Collection of plant materials

Leaves of the two plants (*Z. mauritiana* Lam. and *Z. spinachristi* L.) were collected in polythene bags from in and around Gbako local government area of Niger State, Nigeria and transported to Federal Polytechnic, Bida and air dried for two weeks in the Microbiology Laboratory. The dried leaf material was then ground into powder using blender (Monlinex 530, 240V) and packed in polythene bags for further use.

### Extraction of active compounds using ethanol as solvent for extraction

Ten grams (10g) of the ground leaf samples were separately soaked in 200 ml of ethanol and allowed to stand for about 72 h for extraction. After the 72 h, it was then filtered using No.1 Whatman filter paper. The filtered samples were sterilized by passing through Millipore filter and later evaporated to dryness (Mann et al., 2008).

### Preparation of test organisms

Clinical specimens of *Staphylococcus aureus*, *E. coli*, *S. pyogenes*, *C. albicans* and *A. niger* were obtained from Federal Medical Centre (FMC), Bida, Niger State. The organisms were sub-cultured on agar slants prior to use. 18 h liquid culture of each of the organisms was used for sensitivity testing.

### Sensitivity testing

Ethanol extract (preparation is shown above) of each plant sample was tested against each of the organisms using agar cup well method as described by Okeke et al. (2001). After making holes with No. 4 cork borer, the surface of the agar was lawned with 18 h culture of the test organism which has been previously standardized to  $10^6$ . Same volume (0.1ml) of different concentrations of the extract (500, 50, 5 and 1 mg) was dropped with the aid of dropper pipette into each well. The plates were incubated at 37°C for 24 h and 72 h at 25°C for bacteria and fungi respectively.

### Control experiment

Standard antibiotics were used as control and the experiment procedure is as described above.

### Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration was determined using tube dilution technique (Give Reference, if any). Varying concentrations of the extracts were prepared and 1 ml introduced into 9 ml of nutrient broth in test tubes. About 0.1 ml of the 18 h culture diluted to  $10^6$  cell  $\text{ml}^{-1}$  was added and incubated accordingly. The least concentration of the extract that did not permit turbidity in the broth was taken as the minimum inhibitory concentration.

### Minimum bactericidal concentration (MBC)

Spread plate technique was employed. A fresh solid medium was inoculated with inoculum from the least concentration that showed no visible growth and incubated for 24 h at 37°C. The lowest concentration in which no growth occurs on the solid medium was accepted as the minimum bactericidal concentration.

### Phytochemical analysis of plant extracts for active components

Phytochemical screening of the extracts was carried out according to the methods described by Trease and Evans (1989) for the detection of active components like saponins, tannins, alkaloids, phlobatanins and glycosides.

- Alkaloids - 1 ml of 1% HCl was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, cooled and filtered. About 2 drops of Mayer's reagent to 1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.

- Tannins - 1 ml of freshly prepared 10% KOH was added to 1 ml of the extract. A dirty white precipitate showed the presence of tannins.

- Glycosides - 10 ml of 50%  $\text{H}_2\text{SO}_4$  was added to 1 ml of the extract and the mixture heated in boiling water for about 15 min. 10 ml of Fehling's solution was then added and the mixture boiled. A brick-red precipitate was confirmatory for the presence of glycosides.

- Saponins - (i) Frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 min. No frothing was observed. (ii) Emulsion test: 5 drops of olive oil was added to 3 ml of the extract in the test tube and vigorously shaken. Absence of stable emulsion formed showed absence of saponins.

- Flavonoids - 1 ml of 10% NaOH was added 3 ml of the extract. There was no yellow colouration which is indicative of the absence of flavonoids.

- Steroids - Salkowski test: 5 drops of concentrated  $\text{H}_2\text{SO}_4$  was added to 1 ml of the extract in a test tube. Red colouration was observed which is indicative for the presence of steroids.

- Phlobatanins - 1 ml of the extract was added to 1% HCl. No red precipitate observed which means negative result.

- Triterpenes - 1 ml of the extract was added to 5 drops of acetic anhydride and a drop of concentrated  $\text{H}_2\text{SO}_4$  added. The mixture was then steamed for 1 h and neutralized with NaOH followed by addition of chloroform. Absence of blue-green colour indicates the absence of triterpenes.

Table 1 shows that *Z. mauritiana* was active against *S. pyogenes* at  $1 \text{ mg ml}^{-1}$ , both plants were active against *E. coli* at  $5 \text{ mg ml}^{-1}$  but active against *S. aureus* only at  $50 \text{ mg ml}^{-1}$ . The two fungal isolates

**Table 1.** Sensitivity analysis showing zones of inhibition (mm) around crude extracts at varying concentrations.

Conc. of extracts (mg/ml)	<i>Ziziphus mauritiana</i>					<i>Ziziphus spinachristi</i>				
	<i>S.a</i>	<i>E.c</i>	<i>S.p</i>	<i>A.n</i>	<i>C.a</i>	<i>S.a</i>	<i>E.c</i>	<i>S.p</i>	<i>A.n</i>	<i>C.a</i>
1	0	0	9 ± 3	0	0	0	0	0	0	0
5	0	9 ± 1	14 ± 3	0	0	0	10 ± 4	13 ± 1	0	0
50	11 ± 2	12 ± 2	16 ± 3	0	0	12 ± 3	14 ± 0	18 ± 0	0	0
500	13 ± 1	16 ± 3	26 ± 2	0	0	15 ± 2	17 ± 1	28 ± 2	0	0

Key: *S.a* = *Staphylococcus aureus*, *E.c* = *Escherichia coli*, *S.p* = *Streptococcus pyogenes*, *A.n* = *Aspergillus niger*, *C.a* = *Candida albicans*.

**Table 2.** Zones of clearing (mm) of susceptibility testing with standard antibiotics.

Organisms	Antibiotics									
	CH	CPX	E	LC	GM	APX	RP	FLX	S	NB
<i>E. coli</i>	9 ± 0.1	8 ± 0.1	9 ± 0.1	0	12 ± 0.1	0	0	9 ± 0.1	8 ± 0.1	12 ± 0.1
<i>S. pyogenes</i>	12 ± 0.1	10 ± 0.1	9 ± 0.1	8 ± 0.1	11 ± 0.1	8 ± 0.1	8 ± 0.1	9 ± 0.1	9 ± 0.1	14 ± 0.1
<i>S. aureus</i>	18 ± 0.1	18 ± 0.1	16 ± 0.1	15 ± 0.1	16 ± 0.1	12 ± 0.1	14 ± 0.1	12 ± 0.1	16 ± 0.1	16 ± 0.1
<i>A. niger</i>	19 ± 0.1	0	0	22 ± 0.1	22 ± 0.1	18 ± 0.1	22 ± 0.1	0	16 ± 0.1	0
<i>C. albicans</i>	0	0	0	0	0	0	0	0	0	0

Key: CH - Chloramphenicol (10 mg), CPX - Ciprofloxacin (10 mg), E - Erythromycin (20 mg), LC - Lincocin (30 mg), GM - Gentamycin (10 mg), APX - Ampiclox (10 mg), RP - Rimbaprim (10 mg), FLX - Floxapin (30 mg), S - Streptomycin (30 mg), NB - Narbactin (10 mg).

**Table 3.** Minimum inhibitory concentration (MIC) of plant extracts against test organisms.

Plant parts	Organisms	Concentration of extract (mg/ml)								
		50	40	30	20	5	1	0	MIC	
<i>Z. mauritiana</i>	<i>E. coli</i>	-	-	-	-	-	-	+	+	5
	<i>S. aureus</i>	-	-	+	+	+	+	+	+	40
	<i>S. pyogenes</i>	-	-	-	-	-	-	-	+	1
<i>Z. spinachristi</i>	<i>E. coli</i>	-	-	-	-	+	+	+	+	20
	<i>S. aureus</i>	-	+	+	+	+	+	+	+	50
	<i>S. pyogenes</i>	-	-	-	-	-	-	+	+	5

Key: + = Growth, - = No growth.

*A. niger* and *C. albicans* were resistant. From Table 2 all the test organisms were susceptible to the antibiotics except *C. albicans* which was resistant. *Z. mauritiana* showed an MIC of 1 mgml<sup>-1</sup> against *S. pyogenes*, 5 mgml<sup>-1</sup> against *E. coli* and 40 mgml<sup>-1</sup> against *S. aureus* while *Z. spinachristi* showed the MIC of 5 mgml<sup>-1</sup> against *S. pyogenes*, 20 mgml<sup>-1</sup> against *E. coli* and 50 mgml<sup>-1</sup> against *S. aureus* in Table 3. *Z. mauritiana* showed an MBC of 20 mgml<sup>-1</sup> against *S. pyogenes*, 30mgml<sup>-1</sup> against *E. coli* and 50 mgml<sup>-1</sup> against *S. aureus* while *Z. spinachristi* showed the MBC of 30mgml<sup>-1</sup> against *S. pyogenes*, 40mgml<sup>-1</sup> against *E. coli* and 50mgml<sup>-1</sup> against *S. aureus* (Table 4). Table 5 showed the presence of five different constituents Cardiac glycosides, polyphenols, Resins, Saponins and Tannins in *Z. mauritiana* but *Z. spinachristi* contains only three of the chemical constituents which include Polyphenols, Saponins and Tannins.

## DISCUSSION

The findings of this research work have shown clearly

that the plants extracts are probably inactive against fungi and may not be useful in treating diseases of fungal origin. The extracts were active against the clinical isolates employed for this analysis. All the plants extracts were active against *S. pyogenes* an indication that the plant can be used to cure acute tonsillitis and sore throat caused by this bacterium. *Z. mauritiana* was active against *E. coli*, *S. pyogenes* and *S. aureus* while *Z. spinachristi* was very active only against *S. pyogenes* but moderately active against the rest test organisms. *Z. mauritiana* showed stronger activity against the organisms compared with *Z. spinachristi*.

The standard antibiotics used as control showed higher activity on the organisms than the extracts (Tables 1 and 2). This is not surprising because standard antibiotics are well refined industrial products so there is no doubt their activity will be more compared to crude extracts. If the

**Table 4.** Minimum bactericidal concentration (MBC) of plant extracts against test organisms.

Plant parts	Organisms	Concentration of extract (mg/ml)							MBC
		50	40	30	20	5	1	0	
<i>Z. mauritiana</i>	<i>E. coli</i>	-	-	-	+	+	+	+	30
	<i>S. aureus</i>	-	+	+	+	+	+	+	50
	<i>S. pyogenes</i>	-	-	-	-	+	+	+	20
<i>Z. spinachristi</i>	<i>E. coli</i>	-	-	+	+	+	+	+	40
	<i>S. aureus</i>	-	+	+	+	+	+	+	50
	<i>S. pyogenes</i>	-	-	-	+	+	+	+	30

Key: + = Growth, - = No growth.

**Table 5.** Results of the phytochemical screening of ethanolic extracts of *Z. mauritiana* and *Z. spinachristi*.

Organic compounds	<i>Z. mauritiana</i>	<i>Z. spinachristi</i>
Alkaloids	-	-
Anthraquinones	-	-
Cardiac glycosides	+	-
Phlobatannins	-	-
Polyphenols	+	+
Resins	+	-
Saponins	+	+
Tannin	+	+

Key: + = present, - = absent.

extracts used in the present work are refined, more and better activity could be observed. The Minimum Inhibitory Concentration of the extracts against the organisms was 1 mgml<sup>-1</sup> against *S. pyogenes* and 5 mgml<sup>-1</sup> against *E. coli* while the Minimum Bactericidal Concentration was 5 and 20 mgml<sup>-1</sup>, respectively against the organisms. Abalaka et al. (2009) had similar results in their experiments involving some of these organisms (Tables 3 and 4). A cidal drug kills pathogens at levels only two or four times the MIC whereas a static drug kills pathogens at much higher concentrations (Prescott et al., 1999). Some of the organic compounds detected in the extracts include tannins, saponins, resins, polyphenols and cardiac glycosides (Table 5). These compounds have variously been reported to have antimicrobial activity and could be the reason for the activities recorded against these test organisms. Plants chemicals are thought to have the potentiality of useful drugs if properly harnessed (Ogbunugafor et al., 2008; Lee, 2006; Lam, 2007).

The plants extracts were found to be inactive against the test organisms even at very high concentration which means they may not be useful against in the treatment of fungal infections such as dermatophycoses. The cell wall components of bacteria are quite different from those of fungi. While the cell wall of bacteria are either made up of acetyl muramic acid (AMA) or acetyl glucose amine (AGA) fungal cell wall is made up of fungal cellulose. This may explain the reason for the differences in their susceptibility to the plants extracts in this experiment.

Going by these results therefore, we would like to state that constituents of these plants extracts may serve as a source of industrial drugs useful in the chemotherapy of some bacterial infections.

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