

Full Length Research Paper

Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*

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The aqueous and organic leaves extracts of *Balanites aegyptiaca* and *Moringa oleifera* traditionally used for the treatment of infectious disease were tested for their activity against *Salmonella typhi* isolated from blood clot culture using the disc diffusion method. Extracts of *B. aegyptiaca* demonstrated higher activity (16 mm zone of inhibition) than those of *Moringa oleifera* (8 mm zone of inhibition) at 100 mg/ml. Of the three solvents used, ethanolic extracts of both plants demonstrated the highest activity, while the aqueous extracts showed the least activity at 100 mg/ml. The activities of these plant extracts were comparable to those of antibiotics, ciprofloxacin, cotrimoxazole and chloramphenicol, commonly used for treating typhoid fever. The antibacterial activity appears to increase when extracts of the two plants were used in combination at 100 mg/ml each (18 mm zone of inhibition). Preliminary phytochemical screening showed that both plant extracts contains saponins, tannins and phenols while only *M. oleifera* possesses alkaloids and *B. aegyptiaca* possesses anthraquinones. The antibacterial activities of the extracts on *S. typhi* was reasonably stable when treated at 4, 30, 60 and 100°C for 1 h, however it reduces significantly when the pH was altered towards alkalinity.

Key words: *Balanites aegyptiaca*, *Moringa oleifera*, *Salmonella typhi*, antimicrobial activity, minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Typhoid fever is a global bacterial infection caused by the bacterium *Salmonella typhi*. The disease is transmitted by water, milk, food, fruits and vegetables contaminated with the bacterium. It is also transmitted by healthy carriers and contaminated food handlers. The bacilli may be carried mechanically from feces to food by flies. Reptiles such as snakes, turtles, lizards and common domestic pets have been associated with transmission of *Salmonella* spp. (Birgitta et al., 2005). The World Health Organization estimated an annual rate of 12.6 million typhoid fever infections with nearly 600,000 deaths every year (WHO, 2003). In Africa, poor hygienic conditions and inadequate water supply and poverty is further aggravating an increase in cases of typhoid infections and acute gastroenteritis/diarrhea due to non typhoidal salmonellosis (John et al., 2003). In recent years there

has been a rapid rise in multidrug resistance by *S. typhi* all over the world (Chin et al., 2002; Benoit et al., 2003; Abdullah et al., 2005). In Nigeria, the Federal Government, concerned by the rapid increase and threat of typhoid fever, embarked on the distribution of antityphoid vaccines for vaccination of its population (Punch, 2003). Because of the increase in cases of non-responsive treatment of typhoid infections by conventional antityphoid agents, herbs are gaining popularity among both rural and urban dwellers for the treatment of the disease. Besides, the medicinal plants are said to have minor side effects compared to the chemical agents (Maghrani et al., 2005).

In the present scenario of emergence of multidrug resistance to human pathogenic infections including typhoid, it has become very necessary to search for new antimicrobial substances from other sources such as plants. This work was therefore carried out in order to investigate the effect of *Balanites aegyptiaca* and *Moringa oleifera* leaf extracts on *S. typhi*.

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MATERIALS AND METHODS

Plant samples

Fresh leaves of *B. aegyptiaca* and *M. oleifera* were collected from the wild in Yola North Local Government Area of Adamawa state, Nigeria and were identified and authenticated by Mr. Bristone Basiri of the Biological Sciences Department, Federal University of Technology, Yola. Adamawa State, Nigeria.

Preparation of crude and organic extracts

This was carried out as described by Predrag et al. (2005) with slight modifications. After collection, the leave materials were shade dried at room temperature (32 - 35°C) to constant weight over a period of 5 days. 50 g of each of the plant parts were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender (National MX 795). The powder was transferred into closed containers. Each of the powdered shade-dried plant material was extracted with water, acetone and ethanol. 25 g of each powdered sample was mixed in a conical flask with 100 ml of deionised distilled water or organic solvent, plugged, then shaken at 120 rpm for 30 min and allowed to sediment at room temperature for 72 h with manual agitation of the flask using a sterile glass rod after every 24 h. After 72 h, each of the extracts was filtered rapidly through four layers of gauge and then by a more delicate filtration through Whatman No 1 filters paper. The resulting filtrates were then concentrated in a rotary evaporator and subsequently lyophilized to dryness. The yield of extracts for the leaves of *B. aegyptiaca* was 22% (w/w) and 28% (w/w) for *M. oleifera*.

Test organisms

S. typhi was isolated from clinical samples of clotted blood obtained from the Federal Medical Center, Yola, Adamawa State, Nigeria. The isolates were screened for β -lactamase production using standard procedures (Livermore and Brown, 2001). β -Lactamase positive isolates were confirm using standard biochemical procedures and serology and were maintained on MacConkey agar at 4°C to be used for this work (WHO, 2003).

Preliminary phytochemical studies

Preliminary phytochemical studies to determine the presence of alkaloids, anthraquinones, saponons, tannins, and phlobatamins were carried out using the method described by Odebiyi and Sofowora (1999).

Determination of antimicrobial activity

Antibacterial activity of the aqueous and organic extracts of the plant sample was evaluated by the paper disc diffusion method (Aida et al., 2001). The bacterial cultures were adjusted to 0.5 McFarland turbidimetric standard and inoculated onto Nutrient agar (oxid) plates (diameter: 15 cm) and incubated at 37°C for 18 h. Sterile filter paper discs (diameter 6 mm) impregnated with 100 μ l of extract dilutions reconstituted in minimum amount of solvent at concentrations of 20 to 100 mg/ml were applied over the culture plates. Paper discs impregnated with 20 μ l of a solution of 10 mg/ml of ciprofloxacin, chloramphenicol and cotrimoxazole as standard antimicrobials were used for comparison. Filter paper discs dipped into sterile distilled water and allowed to dry were used as control. The plates were then incubated at 37°C for 24 h. Antibacterial acti-

vity was determined by measurement of zone of inhibition around each paper disc. For each extract three replicate trials were conducted against the test organism.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) of the extracts was estimated for the test organisms in triplicates. To 0.5 ml of varying concentrations of the extracts (20.0, 18.0, 15.0, 10.0, 8.0, 5.0, 1.0 and 0.5 mg/ml), 2 ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidimetric standard was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin, chloramphenicol and cotrimoxazole). A tube containing nutrient broth only was seeded with the test organism as described above to serve as control. All the broth cultures were then incubated at 37°C for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity.

To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any visible sign of growth and inoculated on sterile nutrient agar by streaking. Nutrient agar plates were streaked with the test organisms only to serve as control. The plates were then incubated at 37°C for 24 h. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration.

Effect of temperature and pH on antimicrobial activity of extracts

Five milliliters of 60 mg/ml of acetone extracts were constituted in test tubes and treated at 4 °C in the refrigerator and 30, 60 and 100°C in a water bath for 1 h and tested for antimicrobial activity. To determine the effect of pH, acetone extracts were treated at pH ranges of 3 to 10 using 1 N HCl and 1 N NaOH solutions respectively in series of test tubes for 1 h and then tested for antibacterial activity.

Combined effect of the extracts of the plants on the test organism

Equal amounts (100 mg/ml) of the ethanol extracts of the plants were mixed and tested for antibacterial activity.

RESULTS AND DISCUSSION

Results of phytochemical analysis demonstrated the presence of the common phytoconstituents saponins, tannins and phenols in both the plant extracts, while alkaloids were only seen in *M. oleifera* and anthrax-quinones only in *B. aegyptiaca* (Table 1). The presence of these constituents has been reported to account for the exertion of antimicrobial activity by plants (Clark, 1981; Gonzalel and Mather, 1982; Lutterodt et al., 1999; Pretorius and Watt, 2001).

Results of the antibacterial activity of the ethanol extracts of the plant materials shows that *B. aegyptiaca* demonstrated higher activity (16 mm zone of inhibition) against the test organism compared to that of *M. oleifera* (8 mm zone of inhibition) at 100 g/ml (Table 2). The re-

Table 1. Preliminary phytochemical analysis of leaves of *Balanites aegyptiaca* and *Moringa oleifera*.

Plant material	Phytoconstituents present
<i>Balanite aegyptiaca</i>	Saponnins, tannins, phenols, anthraquinones
<i>Moringa oleifera</i>	Alkaloids, saponins, tannins, phenols

Table 2. Antimicrobial activities of leaf extracts of *Balanite aegyptiaca* and *Moringa oleifera*.

Plant Sample	Solvent	Zone of inhibition (mm)					
		100 mg/ml	80 mg/ml	60 mg/ml	40 mg/ml	20 mg/ml	10 mg/ml
<i>Balanite aegyptiaca</i>	AE	14	10	6	3.5	2	1
	EE	16	14	8	6	4	2.5
	WE	9	6	3	2	-	-
<i>Moringa oleifera</i>	AE	7	6	4	1.5	-	-
	EE	8	7	5	2.5	1	-
	WE	4	3	1	-	-	-
Combined extract	EE	18	16	12	8	4	2
Antibiotics	Cp	NA	NA	NA	NA	NA	10
	Ct	NA	NA	NA	NA	NA	8
	Ch	NA	NA	NA	NA	NA	5

WE, water extract; AE, acetone extract; EE, ethanol extract; -, no measurable zone; Cp, ciprofloxacin; Ct, cotrimoxazole; Ch, chloramphenicol; and NA, not applicable.

Table 3. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of ethanolic extracts of *Balanite aegyptiaca* and *Moringa oleifera*

Plant extract	MIC (mg/ml)	MBC mg/ml)
<i>Balanites aegyptiaca</i>	6.5	5
<i>Moringa oleifera</i>	8	8.5

sults also showed that the organic extracts (acetone and ethanol) had higher activity compared to the aqueous extracts. It has been reported that different solvents have different extraction capacities and different spectrum of solubility for the phytoconstituents (Majorie, 1999; Srinivasan et al., 2001). The activities of the plant extracts were also comparable to those of the antibiotics, ciprofloxacin, cotrimoxazole and chloramphenicol. At 100 mg/ml the activity of ethanolic extracts of *B. aegyptiaca* (16 mm zone of inhibition) was higher than that of ciprofloxacin (10 mg/ml, 10 mm zone of inhibition) and the rest of the antibiotics. This is an indication that the plant extracts when purified will be very potent antityphoid agents. The result of the investigation also revealed that when equal concentration of the plant extracts were mixed and their combined effect tested against the test organism showed an increase in activity. At 100 mg/ml concentration, the zone of inhibition of *B. aegyptiaca* and *M. oleifera* extracts were 16 and 8 mm,

respectively, but in combination, a zone of inhibition of 18 mm was obtained. This may suggest synergistic action of the two plant materials and it further gives scientific bases for the use of concoctions traditionally in the treatment of ailments. Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) showed that the MIC and MBC of the two plant extracts are higher than those of antibiotics. This is so because the antibiotics are in a refined state while the plant extracts are still in a crude state (Table 3).

The effect of temperature on the antibacterial activity of ethanol extracts of *B. aegyptiaca* and *M. oleifera* showed that at various temperature ranges of 4, 30, 60 and 100°C, the antibacterial activity remained relatively unaffected (Figure 1), but it reduces when the pH was adjusted from acidity towards alkalinity (pH 8 – 10) (Figure 2). The result may be an indication that the phytoconstituents of these plants are heat stable but are labile in alkaline conditions. The high temperature resistance may explain why the traditional usage of these plants by boiling at very high temperatures for a longer period of time still achieves the desired effect of cure to local ailments that are being treated with them. The effect of alkaline pH on the activity of the plant extracts may be one of the reasons why the efficacy of the plants in its traditional usage takes a very longer duration, possibly because of gradual denaturation of the phytoconstituents by the addition of potash practiced commonly among the

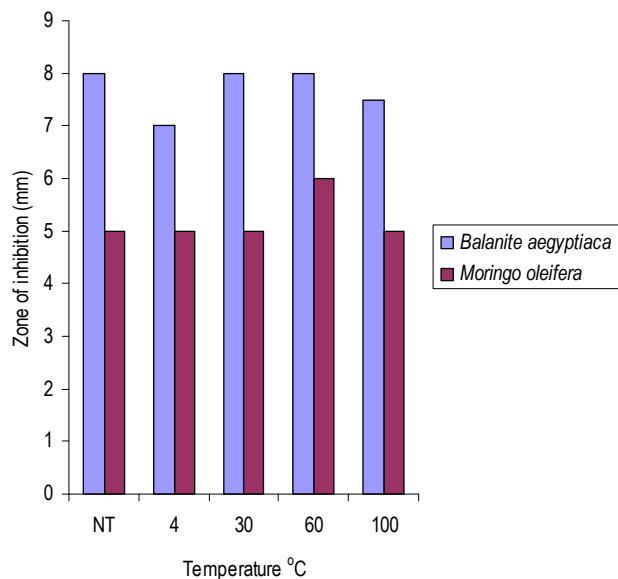


Figure 1. Effect of temperature on antimicrobial activity of *Balanite aegyptiaca* and *Moringa oleifera*. NT, Not treated and kept at room temperature.

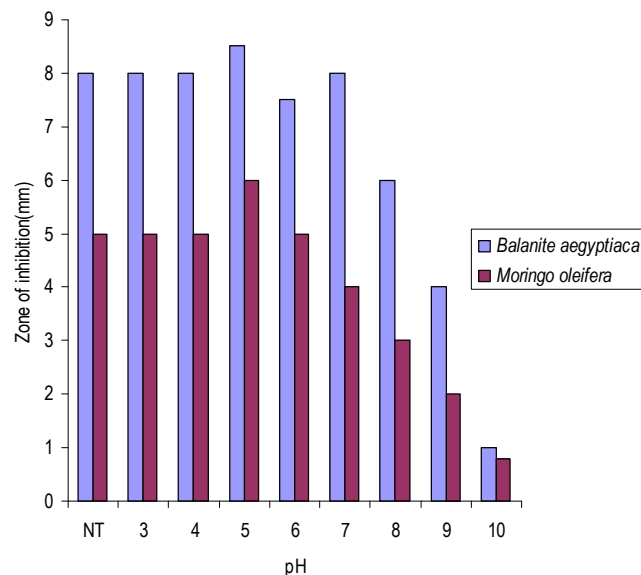


Figure 2. Effect of pH on antimicrobial activity of *Balanite aegyptiaca* and *Moringa oleifera*. NT, Not treated and the original pH value of extracts of *B. aegyptiaca* and *M. oleifera* were 4.5 and 4.8, respectively.

herbalists during the preparation of these plants.

Conclusion

The demonstration of antityphoid activity by *B. aegyptiaca* and *M. oleifera* is indeed a promising development that will help to discover new chemical classes of antibiotics that could serve for treating the infection that otherwise has become highly refractive to most of the conventional antibiotics used for its treatment. The fact that the plants are very common makes it a cheaper alternative for drug development for human consumption provided toxicological investigations and further purification is carried out.

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