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Antibacterial Activity of Ethanolic Extract of Whole Fruit of *Lagenaria breviflora* Roberts

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Abstract: A large proportion of the population in Africa still relies on the use of herbal remedies, which have been claimed to produce beneficial responses. *Lagenaria breviflora* is one of those numerous plants used as antibacterial and antiviral herbal remedies in local communities in Nigeria. In this study, the efficacy of ethanolic extract of whole fruit of *Lagenaria breviflora* against common bacteria species such as *B. subtilis*, *S. aureus, S. gallinarium, P. aeruginosa, Klebsiella* sp., *Proteus* sp. and *E. coli* was investigated. Antibacterial activity was determined by agar-well diffusion method and expressed as the average diameter of the zone of inhibition of bacterial growth around the wells. The effect of the extract was compared with that of the two standard antibiotics (ofloxacin and erythromycin) used. The difference of the means was considered significant at $p<0.05$ using Student t-test. The extract potently inhibited the growth of all the bacterial colonies studied. This inhibitory effect was also dose dependent. The antibacterial effect of *L. breviflora* has a broad spectrum activity because it inhibited the growth of colonies of Gram positive bacteria (*B. subtilis* and *S. aureus*) and that of Gram negative bacteria (*S. gallinarium*, *P. aeruginosa*, *Klebsiella*, *Proteus* and *E. coli*). Compared with standard antibiotics, the extract had moderate activity. While ofloxacin was observed to be significantly ($p<0.05$) more potent than the extract for all the bacteria species studied, the extract was itself more potent than erythromycin on all the bacteria species; *P. aeruginosa, S. aureus* and *Proteus sp* were not even sensitive to erythromycin at all in this study. The degree of inhibition by the plant extract varies from one bacteria colony to the other. The bacteria colonies were susceptible to the antibacterial activity of the extract of *L. breviflora* in the following descending order; *B. subtilis*> *S. gallinarium*> *S. aureus*> *P. aeruginosa*> *Klebsiella*=*Proteus*> *E. coli* Findings from this study show that the ethnomedical use of the plant as antibacterial remedy is well placed and further effort is warranted; in order to isolate and elucidate the active principles in the plant with the view of deriving the potential therapeutic benefits inherent in *L. breviflora*.

Key words: Ethanolic extract, fruits, *Lagenaria breviflora*, antibacterial effect, population, becteria

INTRODUCTION

Lagenaria breviflora (Robert) is a seasonal creeping plant of cucurbitaceae family (Gourd family) found in several African countries (Germplasm Resources Information Network, 2001).The fruit (bulb) of the plant is used in herbal medicine. The fruit is green with white poker dots and it is oval in shape. The size of the fruit varies from about 15 to 25 cm in diameter. The use of the leaves and stem for medicinal purpose is not known (Elujoba and Hymete, 1986). *L. breviflora* is used by trado-mediciners and nursing mothers for treatment of measles, enteritis and as wound antiseptics (e.g., umbilical incision wound) where as livestock owners use it for treatment of Newcastle disease in poultry in many parts of South-Western Nigeria.

There are other plants of cucurbitaceae family that have been described as very useful to the rural dwellers in Africa. Some members of this family are edible, others are medicinal (*Lagenaria siceraria)* (Morimoto *et al.*, 2005) while others are put into both uses (*Telfaria occidentalis*) (Emudianughe and Aderibigbe, 2001). The hypoglycaemic and haemopoietic effects of *Telfaria occidentalis* was confirmed by Emudianugbe and Aderibigbe (2001), respectively.

Lagenaria siceraria (Latin Lauki) is otherwise known as bottle gourd. These gourds contain moderate amounts

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of Vitamins C and B complex and a few proteins. Their high water content is reputed to have cooling and lubricating effect (Morimoto *et al.*, 2005). Curcumin is a principle found in *Curcuma longa* and is reported to exert anti-inflammatory action by inhibiting a number of molecules involved in inflammatory processes. Studies have described a number of such molecules as phospholipases, lipoxygenase, cyclo-oxygenase 2 and tumour necrosis factor etc to mention a few (Chainani, 2003).

Laboratory evidences have been provided for anti-fertility effect of *Lagenaria breviflora* (Elujoba *et al.*, 1985) and its local use for treatment of schistosomiasis (Ajayi *et al.*, 2002). However, there is virtually no reported study on the often claimed antibacterial properties of *Lagenaria breviflora*. This study therefore aims at evaluating the antibacterial activity of the extract of the fruit of *L. breviflora* in order to verify its widespread ethnomedicinal application as antimicrobial.

MATERIALS AND METHODS

Extraction of the fruit: Fresh fruits of Lagenaria breviflora were obtained from local markets, in Ibadan, Oyo State Nigeria. The fruits were washed, cut and weighed. They were tied up in small quantities in sieves and placed in plastic containers. Sufficient ethanol covering each portion was poured into each container. The fruit was left in ethanol for 3-4 days after which the ethanol was drained off and replaced with fresh ethanol. This procedure continued until the fruit was no longer extracting, which took an average of 1week. The filterate was kept in plastic containers and refrigerated at 20°C.

Separation of the extract: The filterate was concentrated by removal of ethanol and subsequent solidification of the fruit extract. The extract was clarified by filtration through celite on water pump which was then concentrated *in vacuo* using a rotation evaporator at low temperatures. The ethanol remaining in the extract was finally removed by placing small volumes in porcelain dishes in the oven set at low temperature of 40°C. The extract came as a semi-solid greenish-brown paste. A stock solution of the extract was prepared by dissolving 100 gm of the extract in 100 mL of distilled water.

Purification of bacteria colonies: A total of seven bacteria species were used in this study. Two gram-positive (*Staphylococcus aureus and Bacillus subtilis*) and five gram-negative bacteria (*Salmonella gallinarum*, *Escherichia coli*, *Pseudomonas aeruginosa*, Klebsiella sp. and *Proteus* sp.) isolated in the laboratories of Departments of Medical Microbiology, University College Hospital (UCH) and Veterinary Microbiology, University of Ibadan, Ibadan were used for the antibacterial study. Samples of each bacterium were collected on slopes and sub-cultured on Mackonkey agar and subsequently on Nutrient agar for 24 h at 37°C. The characteristic morphology, colour and odour of the colony growths were verified. Gram staining was done in order to characterize each colony isolated and Catalase test was conducted additionally for *S. aureus*.

Determination of Minimum Inhibitory Concentration (MIC): Several dilutions of the stock extract solution were prepared between 50 to 1600 mg mL^{-1}. (i.e., 50, 100, 150, 200, 250, 300, 350, 400, 600, 800, 1000, 1200, 1400 and 1600 mg mL^{-1}) in order to determine the MIC. Each of this concentration was applied individually on each bacteria culture plate after which the zone of inhibition was measured 24 48 and 72 h post-application.

Evaluation of antibacterial activity of extract of *Lagenaria breviflora*: Pure colonies of these bacteria were inoculated into sterile peptone water and incubated for 24 h at 37°. Muller-Hilton agar was prepared and plated after which the plates were incubated for 24 h at 37°C. Three plates were prepared for each bacteria species.

The labelled sterile plates were flooded with the broth culture and incubated for 1 h at 37°C. One hole each was punched on the points designated 1, 2, 3, 4 and 5 on the plates after which the plates were incubated for another 1 h.

Application of extract, ofloxacin and erythromycin on bacterial culture: Three concentrations of the extract; 200, 400 and 800 mg mL^{-1}: Were used as test while two standard antibiotics; ofloxacin and erythromycin were applied at their reported MIC of 30 μg mL^{-1} (Petri, 2006) and 5 μg mL^{-1} (Chambers, 2006), respectively as positive control. The three concentrations of the extract were added into the punched holes (i.e., 1, 2 and 3) and antibiotic-impregnated discs were placed on points labelled 4 (ofloxacin) and 5 (erythromycin). All the plates were then returned into the incubator and the zone of inhibition was measured 24, 48 and 72 h post-application of the extract and antibiotics.

Statistical analysis: The zone of inhibition was measured as mean + S.E.M for each group. The difference of the means were considered significant at $p<0.05$ using Student t-test (Steel and Torrie, 1996).

RESULTS

The effects of the extract, ofloxacin and erythromycin on *S. gallinarium*: The ethanolic extract of *L. breviflora* inhibited the growth of *S. gallinarium* colony. The zone of inhibition was dose dependent but the degree of inhibition recorded for each concentration of the extract declined with days. Comparatively the degree of inhibition for the extract (800 mg mL^{-1}) was significantly ($p<0.05$) lower than the inhibition recorded for ofloxacin and erythromycin for the 3 days consecutively (Table 1).

The effects of the extract, ofloxacin and erythromycin on *B. subtilis*: *L. breviflora* also inhibited the growth of *B. subtilis* colony. The clear zone of inhibition was directly proportional to the dose administered. The area of inhibition produced by 800 mg mL^{-1} of the extract was significantly ($p<0.05$) larger than the zone of inhibition caused by 200 mg mL^{-1} of the extract, but the zone of inhibition observed for each concentration of the extract per day was almost consistent for the three days. Comparatively the degree of inhibition caused by the extract (800 mg mL^{-1}) was significantly ($p<0.05$) lower than the inhibition recorded for ofloxacin but was however significantly ($p<0.05$) higher than that of erythromycin recorded for each day (Table 2).

The effects of the extract, ofloxacin and erythromycin on *P. aeruginosa:* *L. breviflora* inhibited the growth of *P. aeruginosa*. This effect varied proportionally to the dose of extract administered on the culture plate. The zone of inhibition produced by each dose remained the same for the three days. The bacteria colony was ofloxacin-sensitive but not erythromycin-sensitive and the zone of inhibition produced by ofloxacin was significantly ($p<0.05$) wider than that of the well treated with 800 mmg mL^{-1} of extract solution (Table 3).

The effects of the extract, ofloxacin and erythromycin on *S. aureus*: Ethanolic extract of *L. breviflora* inhibited the growth of *S. aureus.* The zone of inhibition increased dose dependently for each day of the study. The inhibition produced by the 800 mg mL^{-1} of the extract was significantly ($p<0.05$) higher than inhibition produced by that of 200 mg mL^{-1} of the extract for the three days consecutively. Generally, zones of inhibition observed for the different doses of extract and ofloxacin had decreased by the 3rd day. Erythromycin produced no effect on the bacteria colony while the inhibitory effect of ofloxacin was significantly ($p<0.05$) higher than that of the extract (Table 4).

The effects of the extract, ofloxacin and erythromycin on *E. coli*: Ethanolic extract of *L. breviflora* inhibited the growth of *E. coli.* The degree of inhibition was directlyproportional to the does administered. The inhibition produced by the 800 mg mL^{-1} of the extract was significantly ($p<0.05$) higher than inhibition produced by 200 mg mL^{-1} of the extract, but the zone of inhibition observed did not change for each concentration of the extract for the 3 days of the study. Comparatively the degree of inhibition for the extract (800 mg mL^{-1}) was slightly lower ($p>0.05$) than the inhibition recorded for ofloxacin and was significantly ($p<0.05$) higher than that of erythromycin 3 days consecutively (Table 5).

The effects of the extract, ofloxacin and erythromycin on *Proteus* sp.: Ethanolic extract of *L. breviflora* inhibited the growth of *Proteus* sp. The zone of inhibition was also directly proportional to the dose of extract administered. The inhibition produced by the 800 mg mL^{-1} of the extract was significantly ($p<0.05$) higher than inhibition produced by 200 mg mL^{-1} of the extract, but the zone of inhibition observed for each dose remain constant for the three days. Comparatively the degree of inhibition for the extract (800 mg mL^{-1}) was slightly lower than the inhibition recorded for ofloxacin while erythromycin did not produced any inhibitory effect on the bacterial colony for any day of the study (Table 6).

The effects of the extract, ofloxacin and erythromycin on *Klebsiella* sp.: Ethanolic extract of *L. breviflora* inhibited the growth of *Klebsiella* sp. The zone of inhibition was directly proportional to the dose of extract administered. The inhibition produced by the 800 mg mL^{-1} of the extract was significantly ($p<0.05$) higher than inhibition produced by 200 mg mL^{-1} of the extract, but the zone of inhibition observed for each dose was almost consistent for the 3 days. Comparatively the degree of inhibition for the extract was significantly ($p<0.05$) lower than the inhibition recorded for ofloxacin and was significantly ($p<0.05$) higher than that of erythromycin for the 1st day and non-significantly ($p>0.05$) for the 2nd and 3rd day (Table 7).

Table1: Mean (±S.E.M) zone of inhibition measured on cultured plate of *S. gallinarium*

	Extract (200 mg mL^{-1}) (n = 3)	Extract (400 mg mL^{-1}) (n = 3)	Extract (800 mg mL^{-1}) (n = 3)	Ofloxacin (30 mg mL^{-1}) (n = 3)	Erythromycin (5 mg mL^{-1}) (n = 3)
Day 1	0.9+± 0.06^{ab}	0.9±0.00^{cd}	1.1±0.04^{aef}	2.4±0.06^{bceg}	0.5±0.06^{dfg}
Day 2	0.7±0.03^{ab}	0.8±0.03^{cd}	0.8±0.03^{ef}	2.3±0.08^{aceg}	0.4±0.08^{bdfg}
Day 3	0.5±0.03^{a}	0.7±0.03^{b}	0.8±0.03^{c}	2.3±0.06^{abcd}	0.4±0.03^{d}

Means with the same superscripts are statistically significant at p=0.05 on the same row

Table 2: Mean (±S.E.M) zone of inhibition measured on cultured plate of *B. subtilis*

	Extract (200 mg mL^{-1}) (n = 3)	Extract (400 mg mL^{-1}) (n = 3)	Extract (800 mg mL^{-1}) (n = 3)	Ofloxacin (30 mg mL^{-1}) (n = 3)	Erythromycin (5 mg mL^{-1}) (n = 3)
Day 1	1.3±0.03^{abcd}	1.4±0.04^{aefg}	1.6±0.00^{behi}	3.2±0.04^{cfhj}	0.5±0.07^{dgij}
Day2	1.2±0.03^{ab}	1.3±0.05^{cde}	1.6±0.05^{afg}	3.2±0.06^{bdfh}	0.4±0.03^{cegh}
Day3	1.2±0.03^{ab}	1.4±0.05^{acde}	1.6±0.03^{bcfh}	3.1±0.05^{dfg}	0.4±0.03^{egh}

Means with the same superscripts are statistically significant at p = 0.05 on the same row

Table 3: Mean (±S.E.M) zone of inhibition measured on cultured plate of *P. aeruginosa*

	Extract (200 mg mL^{-1}) (n = 3)	Extract (400 mg mL^{-1}) (n = 3)	Extract (800 mg mL^{-1}) (n = 3)	Ofloxacin (30 mg mL^{-1}) (n = 3)	Erythromycin (5 mg mL^{-1}) (n = 3)
Day 1	0.2±0.04^{abc}	0.5±0.04^{ade}	0.7±0.05^{bdf}	1.1±0.03^{cef}	0.0±0.0
Day 2	0.1±0.04^{abc}	0.4±0.04^{ade}	0.7±0.05^{bdf}	0.9±0.03^{cef}	0.0±0.0
Day 3	0.1±0.04^{abc}	0.4±0.04^{adef}	0.7±0.05^{bdg}	0.9±0.03^{cef}	0.0±0.0

Means with the same superscripts are statistically significant at p = 0.05 on the same row

Table 4: Mean (±S.E.M) zone of inhibition measured on cultured plate of *S. aureus*

	Extract (200 mg mL^{-1}) (n = 3)	Extract (400 mg mL^{-1}) (n = 3)	Extract (800 mg mL^{-1}) (n = 3)	Ofloxacin (30 mg mL^{-1}) (n = 3)	Erythromycin (5 mg mL^{-1}) (n = 3)
Day 1	0.2±0.04^{abc}	0.6±0.05^{ad}	0.8±0.05^{be}	1.4±0.05^{cde}	0.0±0.00
Day 2	0.2±0.03^{abc}	0.5±0.05^{ad}	0.7±0.03^{be}	1.2±0.08^{cde}	0.0±0.00
Day 3	0.0±0.00^{abc}	0.4±0.06^{ad}	0.6±0.03^{be}	1.2±0.05^{cde}	0.0±0.00

Means with the same superscripts are statistically significant at p = 0.05 on the same row

Table 5: Mean (±S.E.M) zone of inhibition measured on cultured plate of *E. coli*

	Extract (200 mg mL^{-1}) (n = 3)	Extract (400 mg mL^{-1}) (n = 3)	Extract (800 mg mL^{-1}) (n = 3)	Ofloxacin (30 mg mL^{-1}) (n = 3)	Erythromycin (5 mg mL^{-1}) (n = 3)
Day 1	0.1±0.00^{ab}	0.2±0.00^{c}	0.4±0.00^{ad}	0.5±0.04^{bce}	0.2±0.01^{de}
Day 2	0.1±0.00^{ab}	0.2±0.00^{c}	0.4±0.00^{ad}	0.6±0.04^{bce}	0.2±0.01^{de}
Day 3	0.1±0.00^{ab}	0.2±0.00^{c}	0.4±0.00^{ad}	0.6±0.04^{bce}	0.2±0.01^{de}

Means with the same superscripts are statistically significant at p = 0.05 on the same row

Table 6: Mean (±S.E.M) zone of inhibition measured on cultured plate of *Proteus* sp

	Extract (200 mg mL^{-1}) (n = 3)	Extract (400 mg mL^{-1}) (n = 3)	Extract (800 mg mL^{-1}) (n = 3)	Ofloxacin (30 mg mL^{-1}) (n = 3)	Erythromycin (5 mg mL^{-1}) (n = 3)
Day 1	0.2±0.00^{a}	0.3±0.00^{b}	0.4±0.00^{c}	3.2±0.00^{abc}	0.0±0.00
Day 2	0.2±0.00^{a}	0.3±0.00^{b}	0.4±0.00^{c}	3.2±0.00^{abc}	0.0±0.00
Day 3	0.2±0.00^{a}	0.3±0.00^{b}	0.4±0.00^{c}	3.0±0.00^{abc}	0.0±0.00

Means with the same superscripts are statistically significant at p = 0.05 on the same row

Table 7: Mean (±S.E.M) zone of inhibition measured on cultured plate of *Klebsiella* sp.

	Extract (200 mg mL^{-1}) (n = 3)	Extract (400 mg mL^{-1}) (n = 3)	Extract (800 mg mL^{-1}) (n = 3)	Ofloxacin (30 mg mL^{-1}) (n = 3)	Erythromycin (5 mg mL^{-1}) (n = 3)
Day 1	0.2±0.03^{abc}	0.3±0.07^{adef}	0.4±0.09^{bdgh}	2.7±0.05^{cegi}	0.2±0.05^{fhi}
Day 2	0.1±0.03^{a}	0.2±0.03^{b}	0.3±0.03^{c}	2.5±0.06^{abcd}	0.2±0.03^{d}
Day 3	0.1±0.03^{a}	0.2±0.03^{b}	0.3±0.03^{c}	2.3±0.05^{abcd}	0.1±0.03^{d}

Means with the same superscripts are statistically significant at p = 0.05 on the same row

Comparative bacterial sensitivity to extract of *L. breviflora*: Using the zone of inhibition as an index of sensitivity of bacterial colonies to the antibacterial effect of *L. breviflora*, the sensitivity of the bacterial colonies was observed in descending order as follows; *B. subtilis*> *S. gallinarium*> *S. aureus*> *P. aeruginosa*> Klebsiella=proteus> *E. coli.*

DISCUSSION

In this study, the ethanolic extract of *L breviflora* exhibited antibacterial activity which was measured by degree of inhibition of growth of bacterial colonies on culture plates. All the bacteria under study showed sensitivity to the antibacterial effect of ethanolic extract of

L breviflora. The antibacterial effect of *L. breviflora* has a broad spectrum activity because it inhibited the growth of colonies of Gram positive bacteria (subtilis and Aureus) and that of Gram negative bacteria such as *S. gallinarium*, *P. aeruginosa*, *klebsiella*, *proteus* and *E. coli*. Similar positive findings have been reported on antibacterial activities of medicinal plants in Palestine (Ali-Shtayeh *et al*., 1998), Jordan, North America, Brazil and South Africa (Mathabe *et al*, 2006) to mention a few. The degree of antibacterial effect of *L. breviflora* was found to be dose dependent. The degree of inhibition elicited by 800 mg mL^{-1} was higher than that of 400 mg mL^{-1} of the extract and significantly higher than that of 200 mg mL^{-1} of the extract consistently for the three test days for each bacterial colony studied. It was discovered that the zones of inhibition produced by each concentration of the extract was maintained for the three days of study for five of the seven bacterial colonies tested. This development indicates that the antibacterial effect produced by a single administration of the extract persisted for long enough and was not easily overcome by the bacterial colonies. This vividly underscores the potentials of herbal remedies which have been reported to produce beneficial responses (Olia *et al*., 2001). In actual fact, findings have shown that some of these antibacterial plants principles exhibited rapidly achieved bactericidal activity against Methicillin-Resistant Staphylococcus Aureus (MRSA), Vancomycin-resistant and other known resistant bacteria strains (Shen *et al*., 2002).

Comparison analysis in this study showed that ofloxacin consistently had higher potency than 800 mg mL^{-1} of the plant extract. However, the bacterial inhibition elicited by the plant extract was higher than that of erythromycin for all the bacteria studied. In fact, *P. aeruginosa*, *S. aureus* and *Proteus* sp. were not sensitive to erythromycin where as the growth of these three bacteria were inhibited by the extract. It is believed that isolation, characterization and purification of the antibacterial principle present in the plant will significantly improve the potency of *L. breviflora*.

Though all the 7 bacteria studied were sensitive to the extract of *L. breviflora*, the degree of response varied. Using the zone of inhibition, the following relationship was observed in the descending order: *B. subtilis*> *S. gallinarium*> *S. aureus*> *P. aeruginosa*> *klebsiella*=*proteus*> *E. coli*. This means that *B. subtilis* was the most susceptible while *E. coli* was the least susceptible bacteria colony to the extract. It is not yet clear what is the mechanism of action of the antibacterial activity of the extract; it is therefore difficult to speculate what could be the factor responsible for this differential response. It is however suffice to mention for now that the effect of the extract is broad spectrum. Further study is therefore strongly indicated. It is important to continue to investigate our plant kingdom, especially the world tropical reserves as an alternative for finding new and or better drugs. It should therefore be essential to follow-up this type of investigation to isolate and elucidate the active antibacterial principles of this bioactive plant.

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