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The Antibacterial Activity of *Borreria verticillata*

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With 3 tables

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ABSTRACT

The *in-vitro* antibacterial activity of the crude extract of the leaf of *Borreria verticillata* was tested on *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 6570) and some bacteria isolated from clinical specimens, namely, *Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis* and *Staphylococcus epidermidis*, by a broth dilution method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using three different media, viz., nutrient broth/agar, glucose mineral salts broth/agar and Mueller-Hinton broth/agar. The extract showed antibacterial activity against all the organisms. In all the media used, the extract was most effective against *S. aureus* (MIC: 2 - 8 mg/ml) followed by *S. epidermidis* (MIC: 5 - 15 mg/ml), *B. subtilis* (MIC: 6 - 17 mg/ml), *B. cereus* (MIC: 10 - 20 mg/ml), *E coli* (MIC: 12 - 22 mg/ml), *Proteus mirabilis* (MIC: 14 - 26 mg/ml) and *K. pneumoniae* (16 - 28 mg/ml) while *Pseudomonas aeruginosa* (MIC: 18 - 30 mg/ml) was the least sensitive. The MBC values showed a similar pattern with a two-fold increase over the MIC. There appears to be some corroboration between the results obtained from this study and its usage by herbal healers. However, further studies need to be carried out in order to determine the efficacy of this plant product in the treatment of human infections as well as the active compenents.

Key words: *Borreria verticillata,* antibacterial, herb, extract
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Introduction

In most developing countries, the use of indigenous, natural drugs is a common practice because life-saving synthetic drugs are beyond the reach of the poor people. In countries such as China, India and others, it is not only the unavailability or inaccessibility of modern pharmaceuticals that drives people to traditional remedies, but, more importantly, the existence of a medical system enshrined within their customs [Ndamba *et al.,* 1994].

Some of these herbal remedies are used in the treatment of a wide variety of infectious diseases. One of the earliest records of the use of herbal medicine is that of Chaul Moogra oil from *Hydnocarpus gaerth* used for the treatment of leprosy [Le Strange, 1977].

Now, there has been an upsurge in the interest in herbal remedies in several parts of the world. Many of these herbal remedies have found their ways into orthodox medical practice [Okunzua, 1973]. In Zimbabwe, about eight

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different plant materials have been identified and used in the treatment of urinary schistosomiasis [Ndamba *et al.*, 1994]. In Nigeria, some of the plant materials used in the treatment of ailments include, the Ogbolo roots (*Cissus populared*) used by the Yoruba of Western Nigeria to improve low sperm count (oligospermia) and lack of sperm (azospermia) [Kafaru, 1995], seeds of *Ricinus communis* used as contraceptives [Sofowora, 1984], while *Grewia mollis* is used for the treatment of typhoid and paratyphoid fevers by natives of central (middlebelt) Nigeria [Joseph, 1998]. In Plateau State of Nigeria, herbalists use *B. verticillata* for the treatment and control of various skin infections, such as eczema and rashes. Some antibacterial constituents that can be found in this plant are indol alkaloids as well as irodiod compounds.

This study was, therefore, undertaken to determine the antibacterial activity of the leaves of *B. verticillata* against some selected bacteria commonly associated with contamination of wounds.

Materials and Methods

The plant material

Borreria verticillata is a dicotyledonous plant, which has a wide distribution in Nigeria. It consists mainly of trees and shrubs. The leaves are opposite, whole and entire [Benjamin, 1979]. The plant is known by different names in various parts of Nigeria. Among the Hausas, it is called Nyenyere. The Yorubas call it Irawo ile, while the people of Edo State call it, Akhevemose.

The herb was obtained from Dr. O. Azija, the ethnopharmacologist in the Department of Pharmacology and Clinical Pharmacy, University of Jos, Jos, Nigeria and identified by Professor S. W. H. Hussaini of the Department of Botany of the University of Jos.

Test bacteria

The test organisms were clinical isolates from the Jos University Teaching Hospital (JUTH), Jos, Nigeria. They include, *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus cereus, Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermidis. Eschericia coli* (NCTC 10418) and *Staphylococcus aureus* (NCTC 6570) were included as controls. Each of these organisms were subcultured onto nutrient broth, glucose mineral salts broth and Mueller-Hinton broth.

Preparation of ethanol extract of leaves of Borreria verticillata

Fresh leaves of *B. verticillata* were collected and sun-dried for several days. The leaves were then pounded using a mortar and pestle to give a fine powder. Fifty grams of this powder was weighed and extracted with 95% ethanol in a ratio of 1:3 of powdered drug to ethanol (i.e., 50 g of powder to 150 ml of 95% ethanol). The extraction was done by gentle but continuous agitation of the mixture for 3 hr. The mixture was then filtered and the filtrate then evaporated to dryness in an evaporating dish on a water bath at a temperature of 70 °C.

Culture media used

Mueller-Hinton, glucose mineral salts and nutrient broth/agar media at pH 7.5 were used as culture media. The broths were used for the minimum inhibitory concentration (MIC) determination, while the agar media were used for the minimum bactericidal concentration (MBC) determination. The media were prepared and sterilized as instructed by the manufacturers. About 25 ml of molten agar was then poured into 90 mm diameter sterile Petri-dish to give a depth of 4 mm.

Standard drug for comparison

Gentamycin® (Lek Pharmaceuticals and Chemical Company, Ljubljana, Slovenia) was used as the standard drug for comparative purposes with the extract.

Stock concentrations of standard drug and crude extract

Stock concentrations of the standard drug and crude extract were prepared in sterile distilled water to give a concentration of 1000 μg/ml for gentamycin and 1000 mg/ml for the extract. The solutions were sterilized by passing them through a sterile millipore filter.

Test for antibacterial activity

Determination of the minimum inhibitory concentration (MIC)

The tube dilution method described by Scott [1989] was used. Briefly, 3 sets of 9 tubes labeled 1 - 9 were selected. Each set contained 5 ml of double strength of either nutrient broth, glucose mineral salts broth or Mueller-Hinton broth. 5 ml of the crude extract or gentamycin in the desired concentration was introduced into tube 1 of each set and mixed thoroughly. 5 ml of the content was transferred into tube 2. Tube 2 was mixed thoroughly, and

5 ml of the content transferred into tube 3. The procedure was repeated for the remaining tubes up to tube 8. 5 ml were discarded from tube 8 with tube 9 containing no drug. To all the tubes (1 - 9), drops (0.02 ml) of 24-hr broth cultures of the test organisms diluted to yield 2.5×10^5 cfu/ml were then added. Suspensions used as inocula were compared with a standardized barium sulphate suspension according to the method of Vandepitte *et al.* [1991]. The tubes were then incubated at 37°C for 24 hr after which they were examined for microbial growth. The MIC of a drug/crude extract is the smallest concentration of such drug/crude extract that is capable of inhibiting the growth of a specific inoculum of the test organism.

The determination of the minimum bactericidal concentration (MBC)

The minimum bactericidal concentrations (MBCs) were determined by first selecting tubes that showed no growth during MIC determination. One loopful from each of these tubes was subcultured over the surface of extract/drug-free nutrient agar, glucose mineral salts agar and Mueller-Hinton agar in Petri-dishes and incubated for a further 24 hr at 37°C. The lowest concentration at which no growth was observed on the agar was noted as the MBC.

Results

The susceptibility of 8 selected bacteria and 2 standard strains to the crude extract of *B. verticillata* using nutrient medium (agar or broth) is presented in Table 1.

Table 1. Susceptibility of some selected Gram-positive and Gram-negative bacteria to the crude extract of the leaf of *B. verticillata* using nutrient medium

Organisms	MIC (mg/ml)	MBC (mg/ml)	Gentamycin (μg/ml)	
			MIC	MBC
Pseudomonas aeruginosa	18	36	28	56
Klebsiella pneumoniae	16	32	26	52
Proteus mirabilis	14	28	24	48
Escherichia coli (NCTC 10418)	13	26	22	44
Escherichia coli	12	24	20	40
Bacillus cereus	10	20	19	38
Bacillus subtilis	6	12	18	36
Staphylococcus epidermidis	5	10	12	24
Staphylococcus aureus (NCTC 6570)	4	8	10	20
Staphylococcus aureus	2	4	8	16

The minimum inhibitory concentration shows that the growth of *Staphylococcus aureus* was the most inhibited followed by *Staphylococcus epidermidis, Bacillus subtilis, Bacillus cereus, Escherichia coli, Proteus mirabilis* and *Klebsiella pneumoniae*, while *Pseudomonas aeruginosa* was the least susceptible. The susceptibility profile of the standard strains of *Staphylococcus aureus* (NCTC 6570) and *Escherichia coli* (NCTC 10418) were similar to those of the clinical strains. The minimum bactericidal concentration (MBC) pattern of activity was similar to that of the MIC. Its bactericidal effect was most pronounced for *Staphylococcus aureus* and least for *Pseudomonas aeruginosa*. In general, the Gram-positive organisms were more sensitive than the Gram-negative bacteria. There was a two-fold increase of the MBC over the MIC in all the bacteria tested.

The susceptibility of the test organisms to the extract using glucose mineral salts medium is presented in Table 2. The MIC and MBC patterns were similar to those obtained using the nutrient medium. The MICs and MBCs were, however, slightly higher when the glucose mineral salts medium was used. The MIC and MBC values for the standard drug (gentamycin) were also higher.

Table 3 shows the susceptibility of the organisms to the crude extract using the Mueller-Hinton medium. The MIC and MBC patterns were again similar to those obtained when nutrient medium was used. The MIC and MBC values were, however, higher than those obtained using nutrient medium and glucose mineral salts medium. The MICs and MBCs for gentamycin were comparable to those obtained with the glucose mineral salts medium.

Generally, the MIC and MBC values varied with the type of media used (Tables 1, 2 and 3). The values

obtained with nutrient medium were lower followed by the glucose mineral salts medium and finally, the Mueller-Hinton medium.

Table 2. Susceptibility of some selected Gram-positive and Gram-negative bacteria to the crude extract of the leaf of *B. verticillata* using glucose mineral salts medium

Organisms	MIC (mg/ml)	MBC (mg/ml)	Gentamycin (μg/ml)	
			MIC	MBC
Pseudomonas aeruginosa	24	48	46	92
Klebsiella pneumoniae	20	40	40	80
Proteus mirabilis	18	36	38	76
Escherichia coli (NCTC 10418)	16	32	34	68
Escherichia coli	14	28	32	64
Bacillus cereus	12	24	30	60
Bacillus subtilis	10	20	28	56
Staphylococcus epidermidis	8	16	24	48
Staphylococcus aureus (NCTC 6570)	7	14	22	44
Staphylococcus aureus	6	12	20	40

Table 3. Susceptibility of some selected Gram-positive and Gram-negative bacteria to the crude extract of the leaf of *B. verticillata* using Mueller-Hinton medium

Organisms	MIC (mg/ml)	MBC (mg/ml)	Gentamycin (μg/ml)	
			MIC	MBC
Pseudomonas aeruginosa	30	60	40	80
Klebsiella pneumoniae	28	56	38	76
Proteus mirabilis	26	52	37	74
Escherichia coli (NCTC 10418)	24	48	36	72
Escherichia coli	22	44	34	68
Bacillus cereus	20	40	32	64
Bacillus subtilis	17	34	31	62
Staphylococcus epidermidis	15	30	29	58
Staphylococcus aureus (NCTC 6570)	14	28	26	52
Staphylococcus aureus	8	16	20	40

Discussion

The findings from this study clearly demonstrated the appreciable antibacterial activity of the plant extract of *B. verticillata* compared to gentamycin, suggesting the potential use of the plant extract for further development. However, further clinical trials need to be carried out in order to determine the efficacy of this plant product in treating infections caused by susceptible organisms in human populations.

Irrespective of the type of medium used (nutrient, glucose mineral salts or Mueller-Hinton media), *Staphylococcus aureus* was observed to be most susceptible to the extract while *Pseudomonas aeruginosa* was the least susceptible to the crude extract. It is noteworthy to emphasize that herbal healers in Plateau State, Nigeria, use the leaves of this plant to treat various types of skin infections. On the other hand, *S. aureus, Proteus* spp. and *P. aeruginosa* have been documented to play significant roles in wound infections [Egah, 1996]. The *in-vitro* sensitivity of *S. aureus* to the crude extract in this study corroborates its use by the herbal healers.

It is not surprising that *P. aeruginosa* was the least susceptible to the crude extract since it is generally known

that this bacterium possesses great resistance properties against many antimicrobial agents presently in use [Ogunshola *et al.*, 1997].

Results from this study also indicate that the spore-producing bacteria (*Bacillus cereus* and *Bacillus substilis*) were the least sensitive to the extract among the Gram-positive bacteria. Similar studies using crude extracts of *Parkia filicoidea* have shown a similar pattern [Olukemi *et al.*, 1997]. It is well documented that spoe-forming bacteria are generally more resistant to many antimicrobial agents [Montefiore *et al.*, 1989].

The Gram-positive bacteria were more sensitive than the Gram-negative ones to the plant extract. The observed difference could be attributed to the differences in their cell wall structures and composition. The active ingredient(s) may be affecting certain cell constituents present in Gram-positive but not the Gram-negative organisms, or that the more complex cell wall in Gram-negative bacteria does not permit easy transportation of the extract into the cell walls [Murray *et al.*, 1998]. However, additional studies are needed in order to substantiate these assertions.

Our findings also indicate that the MIC and MBC values varied with the type of medium used. This observed variation is in agreement with findings from other workers where the MIC and MBC values varied with the type of media used [Hugo and Russel, 1983].

This study, therefore, has revealed that the leaf of *Borreria verticillata* has useful antibacterial properties which tend to support its traditional usage. Further work is on to purify the active ingredient and to determine its mode of action.

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References

Benjamin, T. V. (1979). Investigation of ***Borreria verticillata*** an anti-eczematic plant of Nigeria. *Qrt. J. Crude Res.* **17:** 135-140.

Egah, D. Z. (1996). Microbial flora of wounds and their antibiogram in Jos. Unpublished M. Sc. Dissertation, University of Jos, Jos, Nigeria. pp. 1-26.

Hugo, W. B. and Russel, A. D. (1983). *Pharmaceutical Microbiology*, 4th ed. Blackwell Scientific Publications, Oxford, England. pp. 122-139.

Joseph, A. E. (1998). Studies on *in-vitro* effects of *Grewia mollis* extracts on *Salmonella typhii* and *Salmonella paratyphii*. *Book of Abstracts of the 22nd Annual Scientific Conference of Medical Laboratory Scientists of Nigeria*, August 25 - 29, 1998, University of Jos, Jos, Nigeria. pp. 24.

Kafaru, E. O. (1995). Medicinal effects of *Cissus populareae* on low sperm count. *Book of Abstract XXII of Annual Conference of West African Society of Pharmacology*, October 18 - 21, 1995, University of Jos, Jos, Nigeria. pp. 47.

Le Strange, R. (1977). *A History of Hernal Plants*, 4th ed. Angus and Robertson Ltd., London. pp. 50-55.

Montefiore, D., Rotimi, V. O. and Adeyemi-Doro, F. A. B. (1989). The problem of bacterial resistance to antibiotics among strains isolated from hospital patients in Lagos and Ibadan, Nigeria. *J. Antimicrobiol. Chemotherap.* **23:** 641-651.

Murray, P. R., Rosenthal, K. S., Kobayashi, G. S., and Pfaller, M. A. (1998). *Medical Microbiology*, 3rd ed. Mosby Publishers, Missouri, USA. pp. 10-21.

Ndamba, J., Nyazema, N., Makaza, N., Anderson, C. and Kaondera, K. C. (1994). Traditional herbal remedies used for the treatment of urinary schistosomiasis in Zimbabwe. *J. Ethnopharmacol.* **42:** 125-132.

Okunzua, G. O. (1973). Leaves are man's servants for all purposes. In: *Traditional Therapy: A Critical Appraisal.* Lagos University Press, Lagos, Nigeria. pp. 24-28.

Ogunshola, F. T., Kesah, C. N. and Odugbemi, T. (1997). Antimicrobial resistance in Nigeria. An overview. *Nig. Qrt. J. Hosp. Med.* **7(1):** 57-61.

Olukemi, M. A., Kandakai-Olukemi, Y. T. and Bello, C. S. S. (1997). Antibacterial activity of the stem bark of *Parkia filicoidea. J. Pharm. Res. Dev.* **2(1):** 64-66.

Scott, A. C. (1989). Laboratory control of antimicrobial therapy. In: *Medical Microbiology* (Collee, J. G., Duguid, J. P., Fraser, A. G. and Marmion, B. P., eds.), Churchill Livingstone, New York. pp. 9-180.

Sofowora, A. (1984). *Traditional Medicine: Practice in West Africa,* 2nd ed. Ibadan University Press, Ibadan, Nigeria. pp. 3-26.

Vandepitte, J., Engbaek, K., Piot, P. and Iteuk, C. C. (1991). *Basic Laboratory Procedures in Clinical Bacteriology.* World Health Organization, Geneva. pp. 85.