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Comparative Study of the Antibacterial and Cytotoxicity of the Essential Oils from the Leaves, Stem Bark and Roots of *Blighia unijugata* Baker (Sapindaceae)

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ABSTRACT

The antimicrobial activity of the essential oils (EOs) from *Blighia unijugata* Baker (Sapindaceae) was studied. EO was extracted from the leaves (BUL), stem bark (BUB) and roots (BUR) by hydrodistillation. These were all colorless and soluble in water except for BUB. The EOs were active against *Proteus mirabilis*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas mallei*. BUB EO had the highest inhibitory zone (40 mm) against *E. coli*. BUB was also active against all the tested organisms except for *P. fluorescens*. All the EOs exhibited significant inhibitory activity against the pathogenic microorganisms. They all showed potent cytotoxicity with an LC_{50} of 85.20 µg/ml (BUL), 70.50 µg/ml (BUR) and 155.10 µg/ml (BUB), which suggests an ethnomedicinal application of these EOs.

Keywords: brine shrimp, microorganisms, minimum inhibitory concentration, zone of inhibition

INTRODUCTION

The plant kingdom consists of about 400,000 plant species and is a huge reservoir of bioactive molecules, many of which are yet to be exploited for various pharmaceuticals and other industrial applications. Medicinal plants are of great importance to the health of individuals and communities (Hamburger and Hostettmana 1991). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeogu *et al.* 2005).The main advantages of natural bioactive molecules are their mild side effects on the body in comparison to chemically synthesized drugs.

Herbal medicine has long attracted much attention as a means of alternative therapy along with the more orthodox medical system (Normile 2003). Numerous herbal medicines or their standardized extracts have been shown to be safe and effective phototherapeutic agents (Wargovich *et al.* 2001).

Essential oils (EOs) are volatile oils from plants. The screening of EO is of great interest in the discovery of a drug's effectiveness in the treatment of diseases (Atalay *et al.* 2003). *Blighia unijugata* Baker falls into the group of underutilized species of plants, as there is little information on this plant from the literature. It is often planted in towns and villages in Nigeria. The leaf-pulp of *B. unijugata* is used as a rejuvenant and relaxant, it is sedative and its analgesic property is used in treatment of rheumatism (Burkill 2000). We previously reported the antibacterial activity of the ethanolic extracts against pathogenic bacteria which showed significant inhibitory activity (Oderinde *et al.* 2008). In continuation of our work we have decided to investigate the antibacterial activity of the EOs from the leaves, roots, and stem bark of this plant.

MATERIALS AND METHODS

Plant material and extraction

The leaf (mature), stem bark and root (main root) of *B. unijugata* were collected at the University of Ibadan and authenticated at the Botany and Microbiology Department of the Faculty of Science University of Ibadan. Only one tree was sampled. The sample was air dried, ground and set for extraction. Hydrodistillation was used to extract the EOs (Ajayi *et al.* 2008) from the leaves, stem bark and roots of the plant. The plant material was weighed into a round bottom flask and was mixed with water as the extracting solvent in a 3: 1 ratio. The distillate was then transferred into a sample bottle and stored in a refrigerator prior to analysis.

Organisms and media

The organisms employed in this study consisted of Gram-positive (*Enterococcus faecalis*, *Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Pseudomonas mallei*, *Pseudomonas fluorescens*, *Klebsiella pneumonia*, *Escherischia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus mirabilis*) organisms. All organisms were collected from the University College Hospital (UCH), University of Ibadan, Nigeria. The bacterial strains were cultured overnight at 37°C in Muller-Hinton agar (DIFCO, USA).

Antibacterial activity

The agar-well diffusion method was used. Briefly, the bacterial were grown on Muller-Hinton agar medium (pH 7.3). Agar medium were poured into the plates to uniform depth of 5 mm and allowed to solidify. The microbial suspensions at 5×10^6 CFU (colony-forming units)/ml were streaked over the surface of the media using sterile cotton swab to ensure the confluent growth of the organisms. The wells (8 mm in diameter) were cut from the agar and 60 µL of the extracts solution (200 mg/ml) were delivered into them. The plates were incubated at 37°C for 24 h and the growth of inhibition zones were observed and measured. Each test was performed in triplicates and repeated twice. Levofloxacin (Oxoid Ltd., UK) served as standard.

Received: 14 April, 2009. Accepted: 1 July, 2009.

Research Note

Determination of minimum inhibitory concentration

The broth microdilution broth susceptibility assay was used as recommended by NCCLS (National Committee for Clinical Laboratory Standards) for the determination of minimum inhibitory concentration (MIC) (NCCLS 1999). All tests were performed in Muller-Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Geometric dilutions of the extracts were prepared in a 96-well microtiter plate ranging from 0.05 to 200 mg/ml. This also included a growth control (Muller-Hinton broth and Tween 80) and a sterile control (Muller-Hinton broth, Tween 80 and test extract). Test strains were suspended in the Muller-Hinton broth to give a final concentration of 5×10^5CFU/ml. Plates were incubated at 37°C for 24 h. The MIC is defined as the lowest concentration of the extract at which the organism does not demonstrate visible growth. The microorganism growth was indicated by turbidity. Each test was performed in triplicate and repeated twice. Levofloxacin served as positive control.

Hatching of shrimp

Brine shrimp eggs also known as *Artemia salina* (Carolina Biological Supply Co., NC, USA) were hatched in a rectangular glass tank of about 500 cm^3 containing sea water, which was divided into two portions by a perforated net. An air pump was kept at the bottom of the glass tank and a light source was placed outside of the other portion of the tank that attracts the hatched larvae. This was maintained at 37°C for 48 h after which the phototropic nauplii were collected from the light side (Oderinde *et al*. 2008).

Brine shrimp lethality test

This was carried out according to the procedure described by Mayer (1982). Ten shrimp larvae (*Artemia salina*) were transferred to each sample vial and sea water was added up to 5 ml to make final concentrations of 1, 10, 100 and 1000 µg/ml. Survivors were counted under the stereomicroscope after 24 h and the percentage death at each dose and control was determined. Control vials were prepared using DMSO only. The LC_{50} values were determined from the 24 h count using the probit analysis method described by Finny (1971).

Table 1 Characterization of essential oils from *Blighia unijugata*.

Sample	% yield	Colour	Specific gravity	Solubility in water
BUL	0.7112	Colourless	0.8010 ± 0.10 a	Soluble
BUB	0.6110	Colourless	0.7100 ± 0.10 b	Insoluble
BUR	0.4510	Colourless	0.7112 ± 0.00 c	Soluble

Values are mean ± standard deviation of triplicate determinations. Data in a column with different superscript letters are statistically different (P≤ 0.05).

Table 2 Cytotoxicity of the essential oil of *Blighia unijugata* Bak against brine shrimp.

Extract	LC_{50} (µg/ml)
BUL	85.20
BUR	70.50
BUB	155.10

RESULTS AND DISCUSSION

The EOs from the leaves (BUL), stem bark (BUB) and roots (BUR) of *B. unijugata* are characterized in **Table 1**. The EOs were all colorless and were all soluble in water except for BUB. The percentage yield was highest for BUL.

Table 2 shows the results of the brine shrimp lethality test, which is inexpensive (Couladis *et al.* 2001). The EOs from this plant showed different activities against brine shrimps. BUL, BUR and BUB were active against brine shrimps with an LC_{50} of 85.20, 70.50 and 155.10 µg/ml respectively. This cytotoxicity of the EOs against the brine shrimps is an indication of their possibility of being antimicrobial. The LC_{50} obtained in this present work is lower than that obtained in our previous work on the ethanolic extract of this plant (Oderinde *et al.* 2008).

Tables 3 and **4** show the diameter zone of inhibition and MIC of the BUL, BUB and BUR of for each microorganism tested. The EOs from different parts of the plant inhibited the growth of the microorganisms differently. BUB inhibited the growth of all the microorganisms tested except for *P. fluorescens.* It also had the highest inhibition against the growth of *S. typhi*, *P. aeruginosa*, *E. coli*, *K. pneumonia* and *E. faecalis*. The highest inhibition was obtained against *E. coli* (40 mm) and least against *K. pneumonia* (15 mm) when BUR was used. BUL had no activity against the growth of *P. aeruginosa*, *K. pneumonia*, *S. aureus* and *E. faecalis* while BUR had no activity against *S. aureus* and *E. faecalis*. The

Table 3 Antibacterial activity of the essential oils of BUL, BUR and BUB*.

Test organisms	BUL	BUR	BUB
Proteus mirabilis	22.00 ± 0.40 a	35.20 ± 1.50 b	28.20 ± 0.60 c
Salmonella typhi	18.10 ± 0.50 a	25.00 ± 1.20 b	30.10 ± 1.00 c
Pseudomonas aeruginosa	NA	28.00 ± 0.50 a	35.60 ± 0.40 b
Escherichia coli	25.10 ± 0.20 a	34.10 ± 0.50 b	40.30 ± 0.10 c
Klebsiella pneumonia	NA	15.00 ± 1.10 a	38.20 ± 0.10 b
Staphylococcus aureus	NA	NA	21.70 ± 1.10
Pseudomonas fluorescens	30.0 ± 0.00 a	31.20 ± 0.10 b	NA
Bacillus subtilis	30.20 ± 1.00 a	25.50 ± 0.20 b	20.20 ± 0.50 c
Pseudomonas mallei	25.10 ± 0.20 a	18.50 ± 0.20 b	20.30 ± 0.50 c
Enterococcus faecalis	NA	NA	25.10 ± 0.10

Values are mean ± standard deviation of triplicate determinations. Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).
* Diameter of zone of inhibition including well diameter of 8 mm. NA; not active

Table 4 Minimum inhibitory concentration of BUL, BUR, BUB and Levofloxacin.

Test organisms	BUL (mg/ml)	BUR (mg/ml)	BUB (mg/ml)	Levofloxacin (µg/ml)
Proteus mirabilis	2.50 ± 0.2 a	2.00 ± 0.10 b	2.20 ± 0.50c	NA
Salmonella typhi	1.00 ± 0.10 a	1.60 ± 0.40 b	0.60 ± 0.20 c	NA
Pseudomonas aeruginosa	NA	1.00 ± 0.10 a	1.00 ± 0.20b	0.62 ± 0.10
Escherichia coli	1.10 ± 0.20 a	0.70 ± 0.10 b	0.80 ± 0.10 c	0.63 ± 0.10
Klebsiella pneumonia	NA	2.30 ± 0.10 a	2.60 ± 0.10 b	5.00 ± 0.10
Staphylococcus aureus	NA	NA	3.80 ± 0.50	0.37 ± 0.05
Pseudomonas fluorescens	1.00 ± 0.50 a	0.50 ± 0.50 b	NA	0.51 ± 0.20
Bacillus subtilis	1.70 ± 0.10a	3.00 ± 0.10 b	3.50 ± 0.10 c	0.60 ± 0.10
Pseudomonas mallei	2.50 ± 0.10 a	3.00 ± 0.10 b	2.20 ± 0.30 c	0.58 ± 0.02
Enterococcus faecalis	NA	NA	6.80 ± 0.10	10.06 ± 0.10

Values are mean ± standard deviation of triplicate determinations. Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$). NA; not active.

MIC for the EOs ranged from 0.4 to 7.0 mg/ml with BUR having the least value (0.50 mg/ml) against *P. fluorescens* and the highest value being 6.80 mg/ml in *E. faecalis*. The activities of these EOs make them promising antibiotics against pathogenic microorganisms in primary health services and preservatives in the food industries, but which depends on the nature and safety of the composition of these EOs. They also display a broad antimicrobial spectrum.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Chemistry and the Department of Botany and Microbiology, University of Ibadan, for allowing the use of equipments and laboratory.

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