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Nutritional Elements, Antibacterial Activity and Cytotoxicity of the Leaf, Root and Stem Bark of *Blighia unijugata* Baker (*Sapindaceae*)

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ABSTRACT

A comparative study was carried out on the mineral nutrient, cytotoxicity and antibacterial activity of the ethanol extracts of the leaf (BUL), root (BUR) and stem bark (BUB) of *Blighia unijugata Baker* (*Sapindaceae*). The phytochemical test showed the presence of steroid, saponin and tannin in all the extracts. A total of ten metals, six trace metals (Fe, Mn, Cu, Pb, Cd and Zn) and four macro nutrients (Na, K, Mg and Ca) were determined using Atomic Absorption Spectrophotometry. The concentration of the macronutrients was the highest with Mg being the highest (21.01 ± 1.21 ppm) in the BUR and the Na being the lowest (517.01 ± 0.50 ppm) in BUB. The concentration of the trace metal also differs with Mn being the highest (104.35 ± 0.11 ppm) in BUR and Pb being the lowest (0.36 ± 0.01 ppm) in the BUB. Mg also had the highest transfer factor (0.9501) in BUL. The antibacterial activity of these extracts against pathogenic bacterial showed significant inhibitory activity. BUL was active against all the tested bacterial strains. Both BUR and BUB did not show any activity against the growth of *Klebsiella pneumoniae.* BUR has no activity against *Enterococcus faecalis* as BUB was not also active against *Salmonella typhi* and *Pseudomonas aeruginosa.* All the extracts had high sensitivity to *Staphylococcus aureus*. The ethanol extracts of BUF and BUR showed potent cytotoxicity with LC_{50} of 196.50 and 269.05µg/ml, respectively when tested against Brine shrimp larvae, which supports the ethnomedical claims for the plant.

Keywords: antibacterial activity, brine shrimp, cytotoxicity, mineral elements, phytochemical screening

INTRODUCTION

Many diseases are known to be treated with herbal remedies throughout the history of mankind. Today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria 1991). Higher plants have been shown to be a potential source for the new anti-microbial agents (Mitscher *et al*. 1987). The screening of plant extracts have been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases. A number of the antimicrobial activities of some medicinal plant extracts have been reported but the vast majority has not been adequately evaluated. This is also particularly valid for the Nigerian flora, which has one of the most extensive floras in continental Africa. *Blighia unijugata Baker* (*Sapindaceae*) falls into this group of under utilized species of plants, as there is little information on them from the literature.

B. unijugata 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. The leaf has been used to treat fever and small-pox (Burkill 2000).

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). However, their bioactive constituents and their corresponding remedial mechanisms are still not completely understood. To date, it is generally accepted that multiple constituents are responsible for the therapeutic effect of some herbs; therefore the evaluation of the quality of herbal products is obviously not a straight forward task.

A number of minerals are required by human body in order to maintain good health. Some of these essential minerals are accumulated in different parts of plants as it accumulates minerals essential for growth from the environment. It has been reported that trace metals can be detected in plants and foodstuffs. Recently, plant species have been identified that contain nutrients displaying new beneficial medicinal or therapeutic properties (Almoaruf *et al*. 2003). The aim of this study is to quantify the nutritional elements and to look for the antibacterial activity of the leaf, root, and stem bark of *B. unijugata.*

MATERIALS AND METHODS

Plant material and extraction

The leaf, stem bark and 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 in December, 2007. The different organs (leaf, root and the stem bark) were separately air dried, powdered and extracted successively with hexane (96%) and ethanol (98%) in a Soxhlet extractor for 18-20 h. The extracts were concentrated under reduced pressure and preserved in the refrigerator at 4°C. All the chemicals used were of analytical grade and were obtained from Sigma-Aldrich Chemical Co., Steinheim, Germany.

Mineral determination

Metals determined were lead, cadmium, copper, zinc, iron, magnesium, calcium, sodium, potassium and manganese. This was achieved by digesting 0.8 g each of the different organs powders and ethanol extracts separately using 5 ml (2:1) of 69.40% w/w nitric acid and 90.00% (w/w) perchloric acid (Oderinde and Ajayi

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Table 1 Phytochemical screening of the ethanol extract of BUL, BUR and BUB.

Sample	Steroid	Flavonoid	Glycoside	Reducing sugar	Saponin	Tannin	Phlobatinin	Anthraquinone
BUL	+	+	+	-	+	+	-	-
BUR	+	-	+	-	+	+	-	-
BUB	+	-	-	-	+	+	-	-

Each value is the mean of three replicates.
- Not present; + Present

2000). These metals were analyzed by Atomic Absorption Spectrophotometry (Perkin-Elmer, GMBH, Ueberlingen, Germany).

Phytochemical test

The different extracts were tested for the presence of chemical constituents using standard methods (Harborne 1984; Trease and Evans 1986).

Microorganisms and media

The microorganisms employed in this study consist of Gram positive and Gram negative bacteria. All microorganisms were collected from the University College Hospital (UCH), University of Ibadan, Nigeria. The bacterial strains were cultured overnight at 37°C in Mueller-Hinton agar (DIFCO, USA).

Antibacterial activity

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/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 observed growth inhibition zones were measured (Kuronayagi *et al*. 1999). Each test was performed in three replicates and repeated twice. Levofloxacin served as standard.

Determination of minimum inhibitory concentration (MIC)

Broth microdilution susceptibility assay was used as recommended by NCCLS (National committee for Clinical Laboratory Standards) for the determination of MIC (NCCLS 1997). 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 includes a growth control (Muller-Hinton broth and Tween 80) and a sterile control (Muller-Hinton broth, Tween 80 and tested extract). Bacteria strains were suspended in the Muller-Hinton broth to give a final concentration of $5x10^5$CFU¥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 three replicates and repeated twice. Levofloxacin served as positive control.

Hatching of shrimp

Brine shrimp (*Artemia salina*) eggs (Carolina Biological Supply Company, NC, USA) were hatched with the help of a rectangular glass tank containing sea water, which was divided into two compartments by a perforated net. 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 (larvae) were collected from the light side.

Brine shrimp lethality test

This was carried out according to the procedure described by Mayer (Mayer 1982). Ten shrimp larvae ("*Artemia salina*", a species of aquatic Crustacea) 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).

RESULTS AND DISCUSSION

Steroids, saponins and tannins were found in all the extracts while reducing sugars, phlobatinnins and anthraquinones were absent as shown in **Table 1**. Flavonoids were only found in the leaf extract while glycoside was present in the leaf and root extracts only. The presence of these compounds indicates the possibility of these extract being used as antimicrobial agents (Rauha *et al*. 2000).

A total of ten elements (Na, Mg, K, Ca, Fe, Mn, Zn, Cu, Cd, and Pb) were determined in the leaf (BUL), root (BUR) and stem bark (BUB) powders and also in the ethanol extracts of these plant parts by Atomic Absorption Spectrophotometry (AAS). These metals were determined in both the powders and the ethanol extracts so as to quantify these metals if present in the ethanol extracts. This will also help in evaluating the extracts nutritionally if they will have application in traditional herbal medicine. **Table 2** shows the mean concentration of the various metals in the leaf, root and stem bark while **Table 3** shows those of the ethanol extracts. This study shows that the leaf, root and stem bark of *B. unijugata* and their respective ethanol extracts are rich in Na, K, Mg, Zn and Ca. These metals are known to play vital roles in metabolic activities in both plants and animals

Table 2 Result of the metal contents from the analyzed samples of BUL, BUR and BUB Powder (ppm).

Metals	BUL	BUR	BUB
Na	602.10 ± 0.20^a	811.00 ± 0.01^b	517.01 ± 0.50^c
K	10200 ± 0.21^a	18100 ± 0.61^b	11010 ± 0.67^c
Fe	120.21 ± 0.04^a	98.71 ± 0.12^b	100.22 ± 0.21^c
Mg	16000 ± 1.10^a	21011 ± 1.21^b	14210 ± 0.71^c
Ca	3200 ± 0.67^a	5351 ± 0.81^b	2555 ± 0.76^c
Cu	3.17 ± 0.01^a	5.02 ± 0.72^b	5.21 ± 0.81^c
Pb	2.10 ± 0.01^a	1.21 ± 0.05^b	0.36 ± 0.01^c
Cd	1.11 ± 0.01^a	0.75 ± 0.01^b	0.45 ± 0.04^b
Zn	36.81 ± 0.20^a	33.37 ± 0.11^b	31.81 ± 0.01^c
Mn	72.50 ± 0.07^a	104.35 ± 0.52^b	57.00 ± 0.03^c

Average concentration ± standard deviation of triplicate determination (ppm) (mg/kg) of dry matter.

Table 3 Result of the metal contents from the analyzed ethanol extracts of BUL, BUR and BUB Powder (ppm).

Metals	BUL	BUR	BUB
Na	211.10 ± 0.01^a	321.01 ± 0.50^b	231.00 ± 0.06^c
K	7110 ± 0.50^a	10000 ± 0.01^b	5011 ± 0.03^c
Fe	95.61 ± 0.21^a	75.81 ± 0.01^b	45.11 ± 0.26^c
Mg	15201 ± 0.61^a	11000 ± 0.35^b	12111 ± 0.22^c
Ca	11001 ± 0.72^a	3211 ± 0.33^b	1955 ± 0.11^c
Cu	0.71 ± 0.11^a	1.22 ± 0.51^b	0.81 ± 0.41^c
Pb	0.31 ± 0.31^a	0.16 ± 0.11^b	0.05 ± 0.01^c
Cd	0.05 ± 0.13^a	0.02 ± 0.22^a	0.26 ± 0.10^a
Zn	5.61 ± 0.31^a	20.77 ± 0.10^b	16.77 ± 0.10^c
Mn	65.01 ± 1.01^a	89.22 ± 0.52^b	32.01 ± 0.62^c

Average concentration ± standard deviation of triplicate determination (ppm) (mg/kg) of dry matter.

(Oderinde and Ajayi 1998). The concentrations of these metals were significantly different among the ethanol extracts of the organs (leaf, root and stem bark) of the plant studied except for Cd where there was no significant difference among these studied organs as shown in **Table 3**. For the powdered samples there was a significant difference among the organs except for Cd where there is no significant difference between BUR and BUB.

Zn, which functions in nucleic acid synthesis in animals (Bowen 1966), has the highest concentration in the leaf while Cu was highest in the root. The concentrations of these metals are within the tolerable level. The tolerable range of elements in agricultural products has been reported as 4-15 and 15-200 mg/l for Cu and Zn, respectively (Allaway 1968). The mean concentration of Fe ranged from 98-121 mg/l while Mn ranged from 57-72.50 mg/l. Fe plays an important role in blood cell chemistry since it is an important part of the haemoglobin. The level of Fe in these analysed samples showed that they can serve as good source of Fe especially in anaemic patients. Mn, which is an essential component of co-enzymes important in growth and photosynthesis, was also found in appreciable concentration and within the tolerable limit (Badeae *et al*. 1999). The concentration of Pb was found to be low. The permissible limit for medicinal plants based on Acceptable Daily Intake is 10 mg/l. Na is know to play a crucial role in conduction of nerve impulse while the role of Ca in the structure and metabolism of bones, blood clotting and muscle contraction can not be overemphasized. These two essential metals including K and Mg are high in these plant parts and their extracts.

The medicinal value of some plant species used in homoeopathic system of medicine has been traced for the presence of Ca, Cr, Cu, Fe, Mg, Na, K and Zn in plants (Perman *et al*. 1993). Mg and Zn have important role in the metabolism of cholesterol as well as heart diseases. The presence of Mn may be correlated with therapeutic properties against diabetic and cardiovascular diseases (Schwart 1975). Deficiency or excess of Cu, Mn, Zn, Ca, Mg and K may cause a number of disorders. They also take part in neurochemical transmission and also as a co-factor for various enzymes and in variety of different metabolic processes.

The transfer factor (FT) is an indicator of the leaching of metal from the parts of the plant studied to the ethanol extract (Cui *et al*. 2004). When the FT = 1, it shows that the leaching of the metal into the extract is very high which also indicates the affinity of the metals for the ethanol extract and also the probability of the metal to form a complex in the ethanol extract. The transfer factor (TF) of these metals is presented in **Table 4**. In all the samples analyzed Cd is the lowest in BUR (0.0267) while Mg is the highest in BUL (0.9501). This shows that the migration of Cd to the extract is not high indicating some level of safety from the toxicity of this heavy metal when the ethanol extract is used directly in traditional herbal medicine. On the other hand Mg is known to be essential; the TF is almost 1 indicating the availability of this metal in the extract. This extract can be taking as a good source of Mg if required in traditional herbal medicine.

The results of the present study showed a high level of macro-elements accumulation in different plant parts. It is important to note that the desirable benefit for human health depends on obtaining the correct amount of supplement in the right form and at the right time.

The brine shrimp lethality assay is a bench top inexpensive test, primarily aimed at screening crude extracts of plant materials in the laboratories (Couladis *et al*. 2001). In the present study of the ethanol extracts from the different parts of *B. unijugata* extracts from the leaf (BUL) and root (BUR) were found to be active against brine shrimps with LC_{50}: 196.50 and 269.05 µg/ml, respectively while the stem bark (BUB) had LC_{50} value greater than 1000 µg/ml as shown in **Table 5**. The stem bark (BUB) was considered to be inactive based on the fact that crude extracts with LC_{50} values less than 1000 µg/ml are taken to be significant in brine shrimp lethality assay (Meyer *et al*. 1982)

Table 4 Transfer factor (FT) of the movement of metals from the raw samples to the ethanol extracts.

Metals	BUL	BUR	BUB
Na	0.3506	0.3958	0.4468
K	0.6971	0.5525	0.4551
Fe	0.7954	0.7601	0.4501
Mg	0.9501	0.5235	0.8523
Ca	0.3438	0.6000	0.7652
Cu	0.2240	0.2410	0.1555
Pb	0.1476	0.1322	0.1389
Cd	0.0450	0.0267	0.5778
Zn	0.1524	0.6224	0.5980
Mn	0.8967	0.8550	0.5616

Transfer factor (TF) = $\frac{\text{Concentration of metal in the ethanol extract}}{\text{Concentration of metal in the sample}}$

Table 5 Cytotoxicity of *Blighia unijugata* Bak ethanol extracts against brine shrimp.

Extract	LC_{50} (µg/ml)
BUL	196.50
BUR	269.05
BUB	>1000

Each value is the mean of three replicates.

Table 6 Antibacterial activity of the ethanol extracts of BUL, BUR and BUB

Microorganisms tested	BUL [a]	BUR [a]	BUB [a]
Proteus mirabilis	28.00 ± 3.00^{a}	25.00 ± 1.20^{b}	22.40 ± 0.20^{c}
Salmonella typhi	25.20 ± 0.30^{a}	20.30 ± 0.10^{b}	NA
Pseudomonas aeruginosa	24.10 ± 1.00^{a}	15.00 ± 1.00^{b}	NA
Escherischia coli	29.02 ± 2.00^{a}	24.30 ± 0.10^{b}	20.20 ± 0.31^{c}
Klebsiella pneumonia	20.22 ± 0.50	NA	NA
Staphylococcus aureus	31.32 ± 0.85^{a}	18.00 ± 0.30^{b}	23.11 ± 2.20^{c}
Pseudomonas fluorescens	26.30 ± 2.50^{a}	18.25 ± 0.11^{b}	20.00 ± 3.70^{c}
Bacillus subtilis	24.41 ± 1.25^{a}	15.40 ± 0.00^{b}	12.04 ± 0.81^{c}
Pseudomonas mallei	29.20 ± 3.20^{a}	20.11 ± 3.70^{b}	30.10 ± 0.41^{c}
Enterococcus faecalis	23.10 ± 1.00^{a}	NA	18.22 ± 0.40^{b}

[a]Each value is the mean of three replicates.
[a] Zone of inhibition including well diameter of 8 mm.
NA: not active

The results of the antimicrobial activity of the tested samples are shown in **Tables 6** and **7**. The ethanol extract of the leaf showed inhibition against all the microorganisms tested. Both the extracts from the stem bark and root did not have activity against the growth of *Klebsiella pneumoniae*. The stem bark was not active against *Salmonella typhi* and *Pseudomonans aeruginosa* just as the root extract was not also active against the growth of *Enterococcus faecalis.* The data obtained indicated that *Staphylococcus aureus* was the most sensitive microorganism tested with the largest inhibition zone (31 mm) and *Bacillus subtilis* exhibited the smallest inhibition zone (12 mm). There was a significant difference among the ethanol extracts. *Salmonella typhi* had the lowest MIC (0.91 mg/ml) and *Proteus mirabilis* had the highest MIC (11.2 mg/ml). There was no significant difference among the studied ethanol extracts except in the case of *P. fluorescens*, *S. aureus* and *E. coli*. The extract from the leaf displayed a broad antimicrobial spectrum than extract from the root and the stem bark. The inhibition zone exhibited by the leaf extract is higher than others except for the action of the stem bark extract on *Pseudomonas mallei* as shown in **Table 6**. The root and stem bark show activity against *Bacillus subtilis* (5.91 and 4.88 mg/ml, respectively). The activity of these extracts against this microorganism makes them promising antimicrobial for food preservation.

The antimicrobial activities of these extracts could be attributed to the presence of the metabolites in them especially the tannins, glycosides, anthraquinone and flavonoids (Chung *et al*. 1998). Further work could be carried out on the isolation of the compounds present in the extracts in order to know the precise compound responsible for the ac-

Table 7 Minimum inhibitory concentration (mg/mL) of BUL, BUR, BUB ethanol extracts and Levofloxacin.

Microorganisms	BUL (mg/mL)	BUR (mg/mL)	BUB (mg/mL)	Levofloxacin (µg/mL)
Proteus mirabilis	10.81 ± 4.50^{a}	10.75 ± 1.00^{a}	11.21 ± 0.20^{a}	NA
Salmonella typhi	1.31 ± 0.40^{a}	0.91 ± 0.00^{a}	NA	NA
Pseudomonas aeruginosa	4.85 ± 5.00^{a}	6.22 ± 0.01^{b}	NA	0.62
Escherischia coli	3.10 ± 8.20^{a}	5.80 ± 0.10^{b}	6.13 ± 1.01^{c}	0.63
Klebsiella pneumonia	15.21 ± 1.00^{a}	NA	NA	5.00
Staphylococcus aureus	0.93 ± 0.34^{a}	2.31 ± 0.02^{b}	1.62 ± 0.22^{c}	0.37
Pseudomonas fluorescens	2.17 ± 0.30^{a}	5.91 ± 0.30^{b}	4.88 ± 0.80^{c}	0.51
Bacillus subtilis	3.76 ± 0.10^{a}	3.01 ± 0.10^{a}	4.11 ± 1.30^{a}	0.60
Pseudomonas mallei	3.51 ± 0.20^{a}	3.82 ± 0.40^{a}	1.21 ± 0.60^{b}	0.58
Enterococcus faecalis	13.11 ± 0.20^{a}	NA	18.61 ± 1.21^{b}	10.06

. Each value is the mean of three replicates.
NA: not active.

tivity. It is even possible that the active compound if isolated and tested could be a better antibacterial agent than that of the standard (Levofloxacin).

CONCLUSION

The mineral nutrient, cytotoxicity and antibacterial activity of the ethanolic extracts of the leaf (BUL), root (BUR) and stem bark (BUB) of *Blighia unijugata* were evaluated including the mineral evaluation of the powdered form of theses plant parts. Our results suggest that *B. unijugata* could be explored for possible antimicrobial and cytotoxic agents. The mineral analysis also shows that this plant can serve as a good source of minerals nutrient. However, more studies should be carried out on the isolation of the bioactive constituents.

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