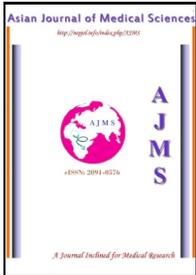


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## Phytochemical and antimicrobial properties of the methanolic extracts of *Bombax buonopozense* leaf and root

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### Abstract

**Objective:** The leaf and root of *Bombax buonopozense* which have some ethnomedicinal applications were subjected to phytochemical screening and antimicrobial activity against some disease causing microorganisms.

**Material & Methods:** The phytochemical composition was evaluated using standard procedures. Susceptibility of these clinical isolates (*Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus spp.* and *Escherichia coli*) to the extracts was determined using the agar diffusion method.

**Results:** Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phlobatannins, anthraquinones and carbohydrates (mostly in root). The root extract demonstrated antibacterial activity against all the organisms tested, while the leaf extract had activity on *S. aureus* and *B. subtilis* only.

**Conclusion:** The findings indicate that the root extract contain the most active components which may be used to source antibiotic substances for possible treatment of bacterial infections.

**Key Words:** *Bombax buonopozense*; phytochemical; antimicrobial agent; medicinal plant

### 1. Introduction

The search for newer sources of antimicrobial agents is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs.<sup>1</sup> Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths every day.<sup>2</sup> Natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents. There is growing interest in plants with antimicrobial activity. Researchers are increasingly becoming involved in the screening of such plants with the aim of establishing their potential antimicrobial effects and

identifying the compounds responsible for their antimicrobial properties<sup>3,4</sup>.

*Bombax buonopozense* P. beauv (*Bombacaceae*) is a large tropical tree that grows to 40 metres in height with large buttress roots that can spread 6 metres. The bark is covered in large, conical spines, especially when young, but shedding them with age to some degree. The branches are arranged in whorls; the leaves are compound and have 5-9 leaflets and 5-25 secondary veins. The individual leaflets have entire margins and are also large. The undersides of the leaflet may be glabrous or puberulous.<sup>5</sup> It is found widely distributed in Africa from Ghana to Sierra Leone, Uganda and Gabon. Common vernacular names include: Vabga (Dagbani language in Ghana), Kurya (Hausa language in Northern Nigeria). Many parts of the plant are used for medicinal purposes, as food, as a source of clothing fibre, as a

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building material, as cotton wool and as dye. The fruits are eaten by animals such as water chevrotain.<sup>6</sup> A decoction of the leaves is used for feverish conditions, diarrhea, pains and muscle aches. Root decoction is used as antimicrobial and stomach aches. The aim of the present study was to investigate the phytochemical and antimicrobial properties of the leaf and root extracts of *B. buonopozense* which have been claimed to possess some ethnomedicinal uses. The study will make for more economic and optimal use of the plant in alternative medicine.

## 2. Material and Methods

### 2.1. Plant collection

The leaves and roots of *B. buonopozense* were collected from Chaza Village, Suleja, Niger State, Nigeria in April 2009. The plant was identified and authenticated by Mrs Grace Ugbabe, a taxonomist in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (No.6402) was deposited at the herbarium of the Institute.

### 2.2. Preparation of plant extract

The fresh leaves and roots of *B. buonopozense* were air-dried at room temperature and pulverized into a dry powder, and macerated with 70% methanol in water for 72h with constant shaking. The resultant mixture was filtered using whatman (No 1) filter paper and the filtrate concentrated to dryness using water bath to give a yield of 7.7% (w/w). The extracts were reconstituted in normal saline at appropriate concentration for the various experiments conducted.

### 2.3. Phytochemical studies

The extracts were subjected to qualitative phytochemical screening according to standard methods.<sup>7, 8</sup>

### 2.4. Test Organisms

Clinical isolates of *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Proteus spp.* and *Escherichia coli* obtained from the Microbiology Department, Federal Medical Centre, Owerri, Nigeria were used for this study. The organisms were subcultured in Nutrient broth at 37°C for 6 h prior to antimicrobial testing.

### 2.5. Antimicrobial Screening

The agar diffusion technique as described by Osadebe and Ukwueze<sup>9</sup> was used to determine the antimicrobial

activity of the plant extracts. Broth cultures of the test isolates (0.1 ml) containing  $1.0 \times 10^6$  cfu/ml of organism was introduced into a sterile Petri dish and 15 ml of Mueller Hinton agar were added. It was properly mixed and allowed to solidify. Holes were bored in the plates, using a standard sterile cork borer of 6 mm diameters and equal volumes of the plant extracts were transferred into the well respectively. The experiments were carried out in duplicate. The plates were allowed to stand for 1 h for pre diffusion of the extracts to occur<sup>10</sup> and incubated at 37°C for 24 h. At the end of the incubation, inhibition zones formed on the medium were evaluated.

### 2.6. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by previously described methods.<sup>4</sup> Nutrient agar solution was prepared according to the manufacturer's standard of 28g /100 ml. The agar was allowed to set and the solution properly mixed with molten double strength nutrient agar in Petri dishes and allowed to solidify for 1 h. Different dilutions of the extracts were prepared to give final concentration in the range of 5, 25, 50, 75, 100, 125, 150, 175, 200 mg/ml. Each plate was divided into eight equal sections and labeled appropriately. The 6 mm in diameter filter paper disc were carefully placed in the plate, 0.2 ml of each bacterial suspension was taken and transferred into the paper disc on the agar plates. They were incubated at 37°C for 24 h. The plates were then examined for the presence or absence of growth. The lowest concentration that inhibited growth was taken as the minimum inhibitory concentration of the respective extracts.

## 3. Results

### 3.1. Phytochemical Screening

Phytochemical analysis of the leaf and root of *Bombax buonopozense* extracts gave positive reaction for each of the following secondary metabolites: alkaloids, flavonoids, tannins, saponins, terpenoids, steroids phlobatannins, anthraquinones and carbohydrates (Table-1)

Table-1: Phytochemical profile of the leaf and root extracts of *B. buonopozense*

Phytoconstituet	Leaf	Root
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Terpenoids	+	+
Steroids	+	+
Anthraquinones	-	+
Phlobatannins	-	+
Carbohydrates	+	+

Table-2: Antimicrobial activities of leaf and root extracts of *B. buonopozense*

Organism	Extract(mg/ml) zone of inhibition (mm)									
	50		100		150		200		250	
	LE	RE	LE	RE	LE	RE	LE	RE	LE	RE
<i>S. aureus</i>	0	14	12	17	14	20	18	24	20	30
<i>B. subtilis</i>	0	16	14	18	16	20	20	22	24	28
<i>K. pneumonia</i>	0	12	0	16	0	18	0	20	0	28
<i>Proteus spp.</i>	0	14	0	16	0	18	0	20	0	20
<i>E. coli</i>	0	12	0	14	0	16	0	20	0	20

LE= leaf extract; RE= root extract

### 3.2. Antimicrobial activity of the extracts

The results of the antimicrobial activity of the extracts against the test organisms are shown in table-2. The zones of the growth of the isolates are a function of the relative antimicrobial activity of the extracts. Root extract shown more activity against the organisms *S. aureus* (30 mm), *B. subtilis* (28 mm), *K. pneumonia* (28 mm), *Proteus spp.* (20 mm) and *E. coli* (20mm).

Table-3: Minimum Inhibitory Concentration (MIC) of leaf and root extracts of *B. buonopozense*

Organism	MIC(mg/ml)	
	LE	RE
<i>S. aureus</i>	150	100
<i>B. subtilis</i>	150	100
<i>K. pneumonia</i>	—	150
<i>Proteus spp.</i>	—	150
<i>E. coli</i>	—	150

-- = No MIC values, LE=leaf extract, RE= root extract.

While the leaf exhibited activity against *S. aureus* (20 mm), *B. subtilis* (24 mm), and did not show activity against other organisms. Table-3 shows the results of MIC determination on the test organisms. The lowest MIC of 100mg/ml was demonstrated against *S. aureus* and *B. subtilis*, while the values of 150-200mg/ml were demonstrated against the rest of the test organisms. Distilled water used as respective controls were inactive against the bacteria.

### 4. Discussion

The phytochemical constituents identified in the leaf and root extracts of *Bombax buonopozense* such as flavonoids, alkaloids, tannins and terpenes, which are the plant secondary metabolites, have been established to be frequently responsible for antimicrobial properties of most medicinal plants.<sup>11-14</sup> Hence, the observed antimicrobial activity of the leaf and root extracts against the test micro-organisms may be due to the presence of the phytochemical components.

The findings demonstrated that the root extract was sensitive to all the test micro-organisms, and thus showed that the extract contained potential

antimicrobial agents. On the overall, the results of the sensitivity test showed that leaf extract contained less potential antimicrobial agents against all the test micro-organisms when compared with the root extract. Since the leaves which are more readily accessible than the roots also contain active agents against some of the tested micro-organisms, they could be used to treat some microbial diseases in the absence of the root of this plant.

The test organisms used in this study are associated with various forms of human infections. From clinical point of view, *Klebsiella pneumonia* is the most important member of the *Klebsilla* genus of *Enterobacteriaceae* and it is emerging as an important cause of neonatal nosocomial infection.<sup>15</sup> *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitated and immunodeficient patients.<sup>16</sup> *E. coli* is also responsible for a number of food related diseases that manifest themselves in the form of diarrhea.<sup>17</sup> *Proteus spp.* causes wound infections and urinary tract infection in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores.<sup>18,19</sup> *S. aureus* constitutes a major public health threat, being one of the most common causes of hospital and community acquired infections.<sup>20</sup> The demonstration of activity against both gram-negative and gram-positive bacteria are an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against both clinical and laboratory isolates is also an indication that it can be used against drug resistant microorganisms prevalent in hospital environment. Several studies have been conducted in the past that focus on the antimicrobial properties of herbs, spices and their derivatives such as extracts and decoctions.<sup>21-23</sup>

Apart from antimicrobial activities, these plant extracts are also exploited for therapeutic purpose to cure several disorders. The methanolic leaf extract of *Bombax buonopozense* was found to possess anti-diarrhoeal, antinociceptive, anti-inflammatory, antipyretic and antimalarial activities.<sup>24-26</sup>

The results obtained in the present study justify the use of the extracts of *Bombax buonopozense* leaves and roots in traditional medicine for the treatment of microbial disease, especially those caused by *S. aureus*, *B. subtilis*, *K. pneumoniae*, *Proteus spp.* and *E. coli*. The

bioassay-guided fractionation and further characterization of the active constituents responsible for the antimicrobial potential of this medicinally important plant is under way in our laboratory.

**Declaration of conflict of interest:** The authors declare that there was no conflict of interest

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#### **5. References**

1. Latha SP, Kannabiran K. Antimicrobial activity and phytochemicals of *Solanum trinobatum* Linn. *Afr. J. Biotechnol.* 2006; 5(23): 2402-4.
2. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J. Ethnopharm* 2001; 74: 87-91. [http://dx.doi.org/10.1016/S0378-8741\(00\)00335-4](http://dx.doi.org/10.1016/S0378-8741(00)00335-4)
3. Abinu I, Adenipeku T, Adelowotan T, Ogunsanya T, Odugbemi T, Evaluation of the Antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. *Afr. J. Trad. Cam* 2007; 4: 185-90.
4. Ndukwe IG, Amupitan JO, Isah Y, Adegoke KS. Phytochemical and antimicrobial screening of the crude extract from the root, stem bark and leaves of *Vitellaria paradoxa*. *Afri. J. Biotechnol.* 2007;6 (16): 1905-1909.
5. Beentje H, Sara S. Plant systemic and phytogeography for the understanding of African Biodiversity. *Systemics and Geography of plants.* 2001, 71: 284-6.
6. Dubost G. Comparison of diets of frugivorous forest mammals of Gabon. *J. Mammalogy* 1984; 65: 298-316. <http://dx.doi.org/10.2307/1381169>
7. Trease GE, Evans WC. *Pharmacognosy* 13th ed. Bailliere Tindall, London. 1989, pp 176-80.
8. Sofowora A. *Medicinal plants and Traditional Medicine in Africa.* Spectrum books, Ibadan 1993, pp.150.
9. Osadebe PO, Ukwueze SE. A comparative study of the phytochemical and antimicrobial properties of the Eastern Nigerian species of African Mistletoe (*Loranthus nucrantus*) sourced from different host trees. *J. Biol. Res. Biotechnol.* 2004, 2 (1): 18-23.
10. Esimone CO, Adikwu MU, Okonta JM. Preliminary antimicrobial screening of ethanolic extract from the Lichen *usnea subfloridans* (L). *J. Pharm. Res. Dev.* 1998, 3 (2): 99-101.
11. Cowan MM. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 1999; 12: 564-82 PMID:10515903 PMCID:88925
12. Esimone CO, Ebebe IM, Chah KE, Onyeka CG. Comparative antimicrobial effects of *Psidium guajava*. *Journal of Trop. Med. Plants.* 2003; 4: 185-9.
13. Adejumobi AJ, Ogundiya MO, kolapo KA, Okunade MB. Phytochemical composition and in vitro antimicrobial activity of *Anogeissus leiocarpus* on some common oral pathogens. *Journal of Med. Plants Res.* 2008; 2: 193-196.
14. El astal ZY, Aera A, Aam A. Antimicrobial activity of some medicinal plant extracts in Palestiine. *Pak. J. Med. Sci.* 2005; 21(2):187.
15. Gupta P, Murali MV, Faridi MMA, Kaul PB, Ramachandran VC, Talwa V. Clinical profile of *Klebsiella septicaemia* in neonates. *Indian J. Paediatr.* 1993; 60: 565-72. <http://dx.doi.org/10.1007/BF02751435>
16. Black JG. *Microbiology: Principles and applications.* Practice Hall, NewYork. p. 260.
17. Adams MR, Moss M. *The Royal Society of Chemistry, Cambridge. Food Microbiology.* 1999, pp. 181-186.
18. Cheesbrough M. *Medical Laboratory Manual for Tropical Countries.* Microbiology, Linacre House, Jordan Hill Oxford 2000, pp 260
19. Parekh J, Chanda S. In vitro screening of antibacterial activity of aqueous and alcoholic extracts of various Indian Plants species against selected pathogens from Enterbacteriaceae. *Afr. J. Microbiol. Res.* 2007;1(6): 92-99.
20. Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol* 2000; 88: 308-16. <http://dx.doi.org/10.1046/j.1365-2672.2000.00969.x> PMID:10736000
21. Hsieh PC, Mau JL, Huang SH. Antimicrobial effect of various combinations of plants extracts. *Food Micro-*

- biol. 2001; 18: 35-43. <http://dx.doi.org/10.1006/fmic.2000.0376>
22. Alma MH, Mavi A, Yildirim A, Digrak M, Hirata T. Screening chemical composition and in vitro antioxidants and antimicrobial activities of the essential oils from the *Origanum syriacum* L. growing in Turkey. *Biol. Pharm. Bull.* 2003, 26: 1735-39. <http://dx.doi.org/10.1248/bpb.26.1725>
23. Aires-de-Sousa M, Conceicao T. de. Lencatre H. Unusually high prevalence of Nosocomial panton-Valentine Leukocidin-positive staphylococcus aureus isolates in Cape Verde Island. *J. Clin. Microbiol.* 2006, 3790-3. <http://dx.doi.org/10.1128/JCM.01192-06> PMID:17021112 PMCID:1594790
24. Akuodor GC, Muazzam I, Idris-Usman M, Megwas UA, Akpan JL, Chilaka KC, Okoroafor DO, Osunkwo UA. Evaluation of the antidiarrhoeal activity of methanol leaf extract of *Bombax buonopozense*. *Ibnosina J. Med. Biomed. Sci.* 2011a; 3(1); 15-20.
25. Akuodor GC, Idris-Usman M, Ibrahim JA, Chilaka KC, Akpan JL, Dzarma S, Muazzam I and Osunkwo UA. Anti-nociceptic, anti-inflammatory and antipyretic effects of the methanolic extract of *Bombax buonopozense* leaves in rats and mice. *Afr. J. Biotech.* 2011b; 10(16): 3191-6.
26. Akuodor GC, Mbah CC, Megwas UA, Ikoru NC, Akpan JL, Okwuosa BO, Osunkwo UA. In vivo antimalarial activity of methanolic leaf extract of *Bombax buonopozense* in mice infected with *plasmodium berghei*. *Int. J. Biol. Chem. Sci.* 2011c; 5(5): 1790-6.