

Phytochemical Screening, Antimicrobial and Anti-inflammatory Activities of Ethanolic Extracts of the Leaf of *Bombax buonopozense* P. Beauv. (Bombacaceae)

Moruf Ademola Yusuf-Babatunde^{1,*}, Lateef Saka Kasim², Thomas Oyeboode Idowu³

¹Department of Pharmacy Technician, Ogun State College of Health Technology, Ilese-Ijebu, Nigeria

²Department of Pharmaceutical and Medicinal Chemistry, Olabisi Onabanjo University, Ago-Iwoye, Nigeria

³Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

Email address:

ogbonronka@gmail.com (Moruf Ademola Yusuf-Babatunde)

*Corresponding author

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Abstract: The plant *Bombax buonopozense* is a large tropical tree that grows up to 40 meters. Scattered in the dry and rain forest, widely distributed in African countries such as Ghana, Nigeria, Sierra Leone, Uganda, Liberia, Gabon, and different parts are used for different ailments. The study targets phytochemical screening, antimicrobial and anti-inflammatory activities of the plant leaf to substantiate some of its ethno-medicinal uses. The phytochemical composition was evaluated using standard procedures and the antimicrobial activity of the extracts using the agar diffusion method on the following clinical isolates: (*Staphylococcus aureus* (ATCC 29213), *Streptococcus faecialis*, *Bacillus subtilis* (ATCC 8263), *Escherichia coli* (ATCC 25922), *Salmonella typhi*, *Pseudomonas aeruginosa* (ATCC 27853), *Candida albican* (ATCC 90029), *Aspergillus niger* and *Phytophthora megakarya*). Anti-inflammatory activity was also evaluated using carrageenan-induced oedema of the rat paw. Phytochemical screening revealed the presence of saponins, tannins, flavonoids, carbohydrates, terpenoids, anthraquinone, steroids, reducing sugar, phenols, alkaloids, and phlobatannins. The leaf extract had strong activity on *Strep. faecalis*, *E. coli*, *P. Aeruginosa* and moderate to little activity on the rest of test organisms. The leaf extract suppressed the onset of inflammatory but did not sustain it till 3rd h compared to reference drug (Diclofenac). The findings indicate leaf extract contain some bioactive compounds which may be used for possible treatment of microbial infections and oxidative stress related diseases which justify its ethno-medicinal uses.

Keywords: *Bombax buonopozense*, Phytochemical, Antimicrobial, Anti-inflammatory

1. Introduction

The functions of plants in maintaining human health is properly documented in Nigeria and other parts of the world. Many indigenous plants are used as spices, food ornamentals or medicinal plants. Many of these plants have bioactive compounds that display physiological activities against bacteria and other microorganisms [1].

B. buonopozense is a large tropical tree that grows to 40 metres (130 feet) in height with large buttress roots that can spread 6 metres (20 feet). The bark is covered with large,

conical spines, particularly when young, but shedding them with age to some degree. The branches are arranged in whorls. The leaves are compound and have 5 to 9 leaflets and 15 to 25 secondary veins which are set on long petioles that typically measure between 22 and 14 cm. The individual leaflets have entire margins and are also quite large, measuring from 8 to 23 cm in length by 3 to 7.5 cm in width. The undersides of the leaflets may be either glabrous (i.e. hairless) or Puberlous (i.e. very finely haired). The buds are conical [2].

Many parts of the plant are eaten as food, used for medicinal purposes, as a source of clothing fiber, as a building material, as cotton wool and as dye. The fruits are eaten by animals. A decoction of the leaves is used to treat feverish conditions, diarrhea, pains and muscle aches. Root decoction is used as antimicrobial and stomach aches [3]. The aim of this study was to investigate the phytochemical, antimicrobial and anti-inflammatory properties of the ethanolic extracts of *B. buonopozense* which have been claimed to be used in ethno- medicine to treat some infection diseases.

2. Materials and Methods

2.1. Plant Collection

The leaves of *B. buonopozense* were harvested in January 2015 in Ilese-Ijebu at an undeveloped land opposite Ogun State College of Health Technology campus. It was authenticated by Mr Ogunowo I. I. of Herbarium unit of Department of Pharmacognosy, Faculty of Pharmacy Obafemi Awolowo University, Ile-Ife and voucher specimen (FPI-2073) deposited at the Herbarium.

2.2. Preparation of Plant Extract

The leaves of *B. buonopozense* were air dried at room temperature and milled. The powdered leaves was macerated with 50% aqueous ethanol at room temperature for 72 hours with constant stirring. The extract was filtered using filter paper and filtrate concentrated to dryness in vacuo on a rotary evaporator.

2.3. Phytochemical Studies

The ethanolic crude extract was put to qualitative phytochemical screening using method of Trease and Evans [4].

2.4. Antimicrobial Susceptibility Testing

The susceptibility testing was carried out using agar well diffusion method of Valgas *et al.* [5]. 1 mL of the standardized 24 hour broth culture of the isolate, adjusted to 0.5 McFarland scale, was mixed with 19 mL of molten Mueller Hinton agar in sterile universal bottle, poured into sterile petri dish and was left to solidify. Using a sterile cork borer of 8mm diameter, equidistant wells were made in the agar. 0.1mL of the 100mg/mL extract was dispensed into the well. Rifampicin (0.025mg/mL) and Flucosazole (0.025mg/mL) were dispensed into two other wells to serve as control. The wells were incubated at 37°C for 24 hours and the zones of inhibition were observed and measured in millimeter (mm) using a standard transparent meter rule.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was carried out using the agar dilution method according to the Clinical Laboratory Standard Institute [6]. Various concentrations (100mg/mL, 90mg/mL, 80mg/mL, 70mg/mL, 60mg/mL, 50mg/mL, 40mg/mL, 30mg/mL, 20mg/mL and 10mg/mL) of the crude extracts were prepared. 1 mL of each concentration is incorporated into 19 mL of Mueller-Hinton agar and poured into sterile petri dish. A 24-hour broth culture of the test organisms, diluted to 0.5 McFarland standard was streaked on the agar plate and incubated at 37°C for 24 hours. 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.

2.6. Anti-inflammatory Activity Testing

The method used was the carrageenan-induced oedema of the rat paw [7]. Male Wistar rats (25) weighing 140-250 g were divided into 5 groups of five animals each. The animals were kept at the animal facility centre of the Faculty of Pharmacy, Olabisi Onabanjo University, Ago-Iwoye, Nigeria. The animals were taken care of according to the standard conditions of humidity, temperature and 12 h light/12 h darkness cycle. The animals were used in accordance with Olabisi Onabanjo University Teaching Hospital Ethical Committee Guide for the care and use of Laboratory. Carrageenan (0.1 ml of 1%, Sigma Chemical Company, USA) was injected into the right hind paw, under the planar aponeurosis (Group 1- carrageenan controls). In a separate group of animals (Group 2), diclofenac (5 mg/kg) was administered orally. The plant extract in three doses 100, 200 and 400 mg/kg were administered orally to the animals of Group 3, 4 and 5, 30 min before carrageenan injection. The hind paw volume was measured with a plethysmometer just before and three hours after carrageenan administration. The difference in left and right paw volume indicated the degree of inflammation. The anti-inflammatory activity of the plant extract was estimated as the degree of oedema inhibition.

3. Results

3.1. Phytochemical Screening

Phytochemical screening of the extract revealed the presence of saponins, tannins, flavonoids, terpenoids, anthraquinone, steroids, phenols and alkaloids, phlobatannins while cardiac glycosides and resins were absent as shown in (Table 1).

Table 1. Phytochemical profile of ethanolic extract of *Bombax buonopozense*.

Phytochemical Tests	Alkaloid	Phenol	Steroids	Resin	Terpenoids	Saponnins	Flavonoid	Anthraquinone	Cardiac glycosides	Phlobatannins	Tannins
Result	++	+++	+++	-	++	++	+++	++	-	++	+++

Key: +++ = highly present, ++ = moderately present, + = slightly present, - = absent.

3.2. Anti-inflammatory Activity of *Bombax buonopozense*

Table 2 shows anti-inflammatory activity screening result of extracts of *Bombax buonopozense*. The aqueous ethanol leaf extracts of *B. buonopozense* seems to suppress the onset of inflammation but did not sustain it till 3rd h when compare to the reference drug (Diclofenac).

Table 2. Anti-inflammatory activity screening result of the extracts of *Bombax buonopozense*.

Treatment	Paw oedema induced control vs drug induced rats (%)		
	1h	2h	3h
Tween 80 (10 mg/kg) (control) Vc	52.62±0.50	61.85±0.55	63.08±0.60
Diclofenac 5 mg/kg Vt	38.84±0.54	18.46±0.82	17.91±0.58
Inhibition of oedema	26.19±0.05	70.17±0.03	71.61±0.03
BBC100 mg/kg Vt	30.84±0.75	55.69±0.45	28.60±0.92
Inhibition of oedema	41.39±0.09	9.96 ±0.12	54.66±0.10
BBC200 mg/kg Vt	23.28±0.25	59.02±0.44	39.02±0.76
Inhibition of oedema	55.76±0.06	4.60 ±0.09	38.14±0.06
BBC400 mg/kg Vt	13.72±1.10	51.98±0.81	39.84±0.94
Inhibition of oedema	73.93±0.06	15.96±0.05	36.84±0.07

Keys

Vc = Percentage difference of increased volume in the control groups

Vt = percentage difference in increase paw volume after the administration of test drugs to rats

BB = *Bombax buonopozense* ethanol crude extract,

Each value is SEM 5 individual operations (appendix 3), % inhibition = $\frac{Vc-Vt}{Vc} \times 100$, P<0.05

Table 3. Antimicrobial activities of ethanolic leaf extract of *B. buonopozense*.

Organism	Crude ethanolic extract (mg/ml) zone of inhibition (mm)*				Rifampicin (5µg) (bacterial positive control)	Flucosazole (5µg) (fungi positive control)	DMSO (negative control)
	100	50	25	12.5			
<i>Staphylococcus aureus</i> (ATCC 29213)	13	8	8	4	23	NA	NA
<i>Streptococcus faecalis</i>	18	10	9	5	20	NA	NA
<i>Bacillus Subtilis</i> ATCC 8263	10	12	10	4	28	NA	NA
<i>Escherchia coli</i> (ATCC 25922)	17	10	8	5	21	NA	NA
<i>Salmonella typhi</i>	10	10	9	4	21	NA	NA
<i>Pseudomonas aeruginosa</i> ATCC 27853	19	12	10	5	22	NA	NA
<i>Candida albican</i> (ATCC90029)	11	6	6	2	NA	21	NA
<i>Aspergillus niger</i>	13	7	5	2	NA	20	NA
<i>Phytophera megakarya</i>	11	9	9	3	NA	19	NA

Keys:

DMSO- Dimethyl sulphur oxide, NA- No activity

The zones of inhibition recorded are less the diameter of the cup, which is 8 mm.

* Represents the mean of three determinations.

3.3. Antimicrobial Activity of the Extract

The table 3 shows antimicrobial results of extracts against the test organisms. Zones of inhibition of growth of isolates are function of relative concentration of extracts. The extract shown strong antibacterial potency against *Step. faecalis* (18

mm), *E. coli* (17 mm), *P. aeruginosa* (19 mm), in comparison to standard drugs Rifampicin with (20 mm), (21 mm), (22 mm), Ofloxacin with (20 mm), (17 mm), (9 mm), antifungi activity against *Aspergillus niger* (13 mm) and, *Phytophera megakarya* (14 mm) in comparison to Fluconazole (22 mm) and (20 mm) respectively.

Table 4. Minimum Inhibitory Concentration (MIC) of ethanolic extract of *B. buonopozense*.

Organisms	<i>Staphy. aureus</i> (ATCC 29213)	<i>Strep. faecalis</i>	<i>B. Subtilis</i> (ATCC 8263)	<i>E. coli</i> (ATCC 25922)	<i>Salmonell a typhi</i>	<i>P. aeruginosa</i> (ATCC 27853)	<i>Candida albicans</i> (90029)	<i>Aspergillus niger</i>	<i>Phytophera megakarya</i>
MIC (mg/ml)	50.0	60.0	60.0	50.0	50.0	50.0	60.0	70.0	50.0

Table 4 shows result of MIC on the test organisms. Lowest MIC of 6.25 mg/mL was effected on *Staphylococcus aureus*. *Streptococcus faecalis*, *Bacillus subtilis*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger* and *Phytophera megakarya*, while MIC of 10.0 mg/mL was effected on *Escherichia coli* and *Pseudomonas aeruginosa*. Distilled water used as respective controls was inactive against the bacteria.

4. Discussion

Phytochemicals present in the ethanolic extract of *Bombax buonopozense* are saponins, tannins, flavonoids, terpenoids, anthraquinone, steroids, phenols and alkaloids, phlobatannins, which are plant secondary metabolites that have been tested to

be responsible for antimicrobial properties of most medicinal plants [8-11]. Saponins present in leaf extract of *B. buonopozense* have been reported to have antimicrobial activity. It had also been reported that saponins alter the permeability of cell walls and this facilitates the entry of toxic materials or leakage of vital constituents from the cell walls [12]. Saponins have been reported to kill protozoans, molluscs and act as antifungal and antiviral agent [13]. Tanins have been reported to have physiological effect like anti-irritant, anti-microbial, anti-parasitic effect. Phytotherapeutically, tannins containing plants are used to treat diarrhea [14]. Flavonoids present in leaf extract of *B. buonopozense* have been known to be synthesized by plant in response to microbial infection [15]. Steroids present in *B. buonopozense* have been reported to exhibit antibacterial activity on some bacterial isolates [16]. It had also been reported that steroids block the development of tumor in colon, breast and prostate glands, the mechanism by which it appear to alter cell membrane transfer in tumor growth and reduce inflammation [17]. Alkaloids, carbohydrates, found in leaf extract of *B. buonopozense* are known to have inhibitory activity against several pathogens therefore its extracts are used traditionally for the treatment of some illnesses [18]. Phenolic compounds found in leaf extracts of *B. buonopozense* are well-documented and have revealed biological and pharmacological properties like antimicrobial, antiviral, antioxidant, anti-inflammatory and cytotoxic activity which could validate the use of the plant in ethnomedicine [19]. Anthraquinones, have been found to exhibit antioxidant, anti-inflammatory and antibacterial activities, etc [20]. Terpenoids, cardiac glycosides, are known to possess anti-inflammatory properties [21].

Anti-inflammatory activity of *B. buonopozense* extracts as shown in Table 2 exhibited inhibitory effect at 1st h in a dose-dependent way which is better than standard drug used, i.e (Diclofenac). However, aqueous ethanol leaf extracts of *B. buonopozense* seem to suppress the onset of inflammation but did not sustain it till 3rd h compare to the reference drug. Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 hours) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (3rd h) is sustained by prostaglandin release and mediated by bradykinin, leukotriens, poly morphonuclear cells and prostaglandins produced by tissue macrophages. Prostaglandin-E2, a powerful vasodilator, synergizes with other inflammatory vasodilators such as histamine and bradykinin contribute to redness and increased blood flow in areas of acute inflammation [22, 23]. Carrageenan-induced paw edema model is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the enzyme, cyclooxygenase involved in prostaglandin synthesis [24]. Therefore at significant level ($P < 0.01$), suppressive activity of aqueous ethanolic leaf extract of *B. buonopozense* showed its potent anti-inflammatory effects at the onset of inflammation.

Results of microbial sensitivity test showed that leaf extract was sensitive to all the test micro-organisms and thus showed the extract has potential antimicrobial agents.

Test organisms used in this study are associated with various forms of human infections. From clinical point of view, *E. coli* is responsible for a number of food related diseases that manifest themselves in the form of diarrhea [25]. *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 [26, 27]. *S. aureus* constitutes a major public health threat, being one of the most common causes of hospital and community acquired infections [28]. The demonstration of activity of extracts of *B. buonopozense* against both gram-negative and gram-positive bacteria is 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. Result of this study is in line with that of the study of Akuodor *et al.* [8] and apart from antimicrobial activities, methanolic extract of *B. buonopozense* was found to possess antidarrhoeal, anticeptic, anti-inflammatory, antipyretic and antimalarial activities [8, 29-31].

5. Conclusion

The findings of this study show the presence of phytochemicals that may be responsible for the antibacterial and antifungi activities of the plant extracts. It can be inferred that the plant extracts showed significant growth inhibiting effects on Gram-positive (*Streptococcus faecalis*,) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *E-coli*). The results present *Bombax buonopozense* extracts as good antibacterial agents to inhibit pathogenic microorganisms. The extracts of *Bombax buonopozense* suppressed the onset of inflammation therefore, it may be used in combination with other anti-inflammatory substances to treat inflammation. The inhibitory effects of the plant extracts justify some of the uses of the extracts of *Bombax buonopozense* leaves in ethno-medicine.

Conflict of Interests

The authors declare that they have no competing interests.

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