

Research Article

Phytochemical and Antibacterial Screening of the Leaf Extracts of *Bombax buonopozense*

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Abstract

Fresh powdered leaves (200 g) of *Bombax buonopozense* was percolated with 95 % ethanol (1000 cm³) for one week. It was decanted, filtered and evaporated on rota vapor (R110) at 40°C to obtain the crude ethanol extract (F1). It was macerated sequentially with chloroform, ethyl acetate, acetone and water to obtain the chloroform soluble fraction (F2), ethyl acetate soluble fraction (F3), acetone soluble fraction (F4) and water soluble fraction (F5) respectively. All the five fractions were subjected to phytochemical screening to check the presence of some secondary metabolites, also antibacterial screening was carried out against some clinical isolates. Presence of alkaloids, flavonoids, phenolic glycoside, tannins, saponins and anthraquinones were detected in all/ some of the tested fractions. Also a remarkable zones of inhibition on some of the bacterial isolates by some fractions were recorded.

Keywords: Antibacteria; Inhibition; Isolates; Maceration; Phytochemical

Introduction

Bombax buonopozense is a large tropical tree that grows up to 40 metres in height with large buttress roots that can spread 6 metres down [1]. The individual leaf has entire margin and quite large, measuring from 8 to 23 cm in length by 3 to 7.5 cm in width with the under sides of the leaf being conical buds which contains many seeds that are 5 to 6 mm in length, all of which have a cotton-like fibre covering [2]. *B. buonopozense* is called 'Akpu' in Igbo, 'Kurya' in Hausa and 'Ogbolo' in Yoruba. Different parts of the plant are used for different purposes [3]. The edible floral part of the plant is used as vegetable by the inhabitants of North Central Nigeria and is used as medicine due to its nutritive and therapeutic properties [1]. The plant is widely distributed in West African countries such as Ghana [4] and [5], Gambia, Côte d'Ivoire [6], Nigeria [1] and others. Hot decoction of the dried stem bark of the plant is taken orally for the treatment of malaria in Ghana [7]. The bark is used to treat chest pain in Gambia [8]. Decoction of the leaves is used to manage stomach ulcers and burns in Ghana. Aqueous extract of the leaves is claimed to be effective in the treatment of diarrhea and dysentery [9]. The immature fruits are

prepared as an emollient for skin, decoction of the young leaves is used as a warm bath for febrile children. The grounded bark is taken by pregnant women to increase lactation; the extract from the bark is drunk or applied on the head for dizziness. The gum resin from the bark is pulverized, mixed with oil and used to manage skin diseases [10].

The study was set to investigate and identify the phytochemical agents present in the leaf extracts and to carry out the *in vitro* evaluation of the antibacterial activity of the extracts against some selected pathogens.

Materials and Methods

Plant Collection

Fresh leaves of *B. buonopozense* were collected from Dankama Kaita local Government Area, Katsina State. It was identified and authenticated by Dr. Nuhu Yusuf of Plant Sciences Department, Bayero University Kano. It was then air dried before it was ground into powder using mortar and pestle.

Extraction

Two hundred grams (200 g) of the powdered leaves was percolated with 1000 cm³ of absolute ethanol for one week [11].

The extract was decanted, filtered and evaporated using rotary evaporator (R110) at 40°C to obtain the crude ethanol extract which was labeled as F₁ and kept in a refrigerator at 0°C before used.

Maceration of Crude Ethanol Extracts (F₁)

The crude ethanol extract, F₁ was macerated with 400 cm³ of chloroform in parts using 100 cm³ each, four times to get the chloroform soluble fraction (F₂). The insoluble fraction was also macerated with 400 cm³ of ethyl acetate in parts using 100 cm³ each, four times to get ethyl acetate soluble fraction (F₃). The ethyl acetate insoluble residue was also macerated with 400 cm³ of acetone in parts using 100 cm³ each, four times to get the acetone soluble fraction (F₄). The acetone insoluble residue was dissolved in distilled water to give the water soluble fraction (F₅).

Phytochemical Screening

All the fractions (F₁ to F₅) were subjected to phytochemical screening, to determine the types of secondary metabolites present in the different fractions. These include phenolic glycosides, alkaloids, anthraquinones, saponins, tannins and flavonoids.

Test for Alkaloids

Each fraction (0.5g) was dissolved in 5 cm³ of 1 % HCl inside a test tube on a steam bath. The solution was cooled down before it was filtered. An aliquot of the filtrate (1cm³) was treated with 2-3 drops of Dragendoffs reagent. The result was observed and reported.

Test for Flavonoids

Each fraction (0.5g) was dissolved in 2cm³ of 50% methanol by heating inside a test tube. Magnesium metal (10mg) and concentrated hydrochloric acid 50% (5-6 drops) were added (Sofowora, 1993)[12]. The result was observed and reported.

Test for Phenolic Glycosides

2-3 drops of each fraction was added to few drops of sulfuric acid in a boiling tube. The result was observed and reported.

Test for Anthraquinone

Each fraction (2cm³) was boiled with 1cm³ of dilute sulphuric acid for 5min and then filtered while hot. The filtrate was cooled down and shaken with equal volume of dichloromethane. The dichloromethane layer was then separated and shaken with about 3 cm³ of dilute ammonia. The result was observed and reported.

Test for Tannin

Each fraction (2cm³) was treated with 3 drops of 5% FeCl₃[13]. The result was observed and reported.

Test for Saponins

Each fraction (2cm³) was vigorously shaken with distilled water

and allowed to stand for a while [12]. The result was observed and reported.

Antibacterial Bioassay

Preparation of Test Solution and Disc Concentration

The extracts were dissolved using DiMethyl SulphOxide (DMSO). The stock solutions were prepared by dissolving 0.06 mg of each fraction in 1 cm³ of the solvent. For each fraction concentrations of 60 µg/disc, 30 µg/disc, and 15µg/disc were prepared separately in sterilized Bijour bottles containing 50 sterile improvised what man filter paper. The filter papers were saturated (impregnated) with plant extract of desired concentration. The serial dilution was carried out as follows:

Concentration of 60µg/disc: From the stock solution, 0.5 cm³ was taken into a Bijour bottle containing the discs and labeled 60µg/disc.

Concentration of 30µg/disc: 0.5 cm³ of DMSO was added into the remaining 0.5 cm³ of the stock solution making 1 cm³. It was divided into two and half of it (0.5 cm³) was taken into another Bijour bottle containing the discs and was labeled 30µg/disc.

Concentration of 15 µg/disc: to the remaining 0.5 cm³ of 30 µg/disc solution, 0.5 cm³ DMSO was added to make it 1 cm³. It was also divided into two and half (0.5 cm³) of it was taken into another Bijour bottle containing the discs and labeled 15 µg/disc.

Inoculums Standardization

As described by standard sensitivity test National Committee for Clinical Laboratory Standards (NCCLS)[14], loops of the confirmed isolates were introduced in peptone water in separate sterilized bottles and kept overnight in an incubator (37°C). Few colonies of the overnight growth of the isolates to be tested were dispersed in sterile normal saline to form a turbid culture suspension that match 0.5 McFarland turbidity.

Preparation of Media

Nutrient agar (28 g) was dissolved in 1000 cm³ of distilled water, as per manufacturer's instructions. The conical flask containing the nutrient agar solution was autoclaved for 15 minutes at 121°C. 20-25 cm³ of the sterilized nutrient agar was transferred into petri-dishes under septic condition. The petri-dishes were allowed to cool and solidify.

In vitro Antibacterial Susceptibility Test

Antibacterial activities of the extracts were determined using disc diffusion method of Antimicrobial Susceptibility Test (AST) as described by (NCCLS, 2000). Standardized inocula of the isolates were swabbed onto the surface of the solidified and oven-dried nutrient agar in separate petri-dishes under sterilized environment. The four prepared discs of the different concentrations of the extracts were then placed onto the surface of the inoculated media

at intervals in a clockwise direction. The positive control discs were placed at the center of the bacteria inoculated medium. The plates were incubated for 24 hours at 37°C for bacteria cultures, after which the antibacterial activity was observed by measuring the width of the clear zones of inhibition around the discs and the values obtained recorded.

Results and Discussion

The results for extraction using percolation and maceration with different solvents of different polarity were given in (Table 1) below.

Fraction	Texture	Colour	Weight (g)
Crude ethanol extract (F1)	Gummy	Black	37.39
Choloroform fraction (F2)	Gummy	Black	3.07
Ethyl acetate fraction (F3)	Gummy	Black	2.09
Acetone fraction (F4)	Gummy	Dark brown	1.58
Aqueous fraction (F5)	Gummy	Brown	10.8

Table 1: Extraction results with the physical properties of the extracts.

Phytochemical screening was carried out which involves the tests for alkaloids, flavonoids, phenolic glycosides, anthraquinones, tannins and saponins. The crude ethanol extract (F1), acetone fraction (F4) and the aqueous fraction (F5) were found to contain all the tested secondary metabolite. The chloroform fraction (F2) also contains all the tested secondary metabolites except phenolic glycosides. While the ethyl acetate fraction (F3) was found to contains only phenolic glycosides, anthraquinones and alkaloids. All the fractions (F1-F5) were found to form an orange red precipitate/turbidity when treated with Draggendoff’s reagent which indicates the presence of alkaloids [15]. Similarly, all the fractions give a reddish-pink colour when treated with ammonia which confirmed the presence of anthraquinones in all the fractions [12]. Fractions F1, F2, F4 and F5 show the appearance of a red colour when treated with conc. HCl which indicates the presence of flavonoids [12]. Similarly, fractions F1, F2, F4 and F5 gives a dark black coloured

precipitate with ferric chloride which turned green-black to blue-black colouration on dilution which indicates the presence of tannins [13]. Also fractions F1, F2, F4 and F5 shows a persistent frothing when shaken with distilled water which indicates the presence of saponins [12]. Fractions F1, F3, F4 and F5 were found to show a red colouration which disappeared upon addition of distilled water which confirmed the presence of phenolic glycoside. The results were shown in Table 2 below.

The antimicrobial activity of the different fractions of *B. buonopozenes* was investigated against some pathogens. Some fractions were found to possess the bioactivity against some of the tested microorganisms. The tested organisms include *Klebsiella* spp., *Pseudomonas auriginosa*, *Proteus* spp., *E. coli* and *Staphylococcus aureus*. The crude ethanol extract exhibited the highest activity on all the tested pathogens at all concentrations, but with highest inhibition on *E. coli* and *Klebsiella* spp. both at the concentration of 60µgml⁻¹. It was followed by chloroform fraction which showed remarkable zones of inhibition on *E. coli*, *Klebsiella* spp., *Pseudomonas auriginosa* and *Proteus* spp. but with highest inhibition on *E. coli* and *Pseudomonas auriginosa* at concentration of 60µg ml⁻¹. The fraction with least activities was found to be aqueous fraction which exhibited low activity on *Klebsiella* spp., *Pseudomonas auriginosa* and *E. coli* (Table 3).

Secondary Metabolites	F1	F2	F3	F4	F5
Phenolic glycosides	+	-	+	+	+
Anthraquinones	+	+	+	+	+
Flavonoids	+	+	-	+	+
Tannins	+	+	-	+	+
Saponins	+	+	-	+	+
Alkaloids	+	+	+	+	+

Keys: ‘+’ indicate presence, ‘-’ indicate absence.

Table 2: Results of the phytochemical screening.

Zones of Inhibition (mm) at different concentrations in µg/mL																
Pathogens	F ₁			F ₂			F ₃			F ₄			F ₅			AUG (30 µg)
	60	30	15	60	30	15	60	30	15	60	30	15	60	30	15	
<i>Klebsiella</i> spp.	12	10	8	9	8	6	9	7	-	-	-	-	8	7	6	23
<i>Pseudomonas auriginosa</i>	10	9	9	10	9	7	8	7	6	8	6	-	8	7	6	30
<i>Proteus</i> spp.	9	8	7	7	6	-	-	-	-	10	8	7	-	-	-	16
<i>E. coli</i>	13	11	10	12	10	9	9	8	7	10	8	7	9	7	-	33
<i>Staphylococci. aureus</i>	9	7	6	-	-	-	7	6	-	8	6	-	-	-	-	27

Keys: AUG = Augmentin (standard)

Table 3: Antibacterial screening results.

Phytochemical analysis of the plants revealed the presence of alkaloids, phenolic glycosides, anthraquinones, Flavonoids, tannins and saponins. From the results, the leaf extracts of the plant were found to show a significant level antibacterial activity. The revealed secondary metabolites are known to be biologically active and thus aid the antibacterial activities of *B. buonopozense*. Phytochemicals exert antimicrobial activity through different modes of action. For example, tannins act by hydrogen deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells [16]. Alkaloids have been associated with medicinal uses for centuries. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. Flavonoids also exhibit a wide range of biological activities such as antimicrobial, anti-inflammatory, analgesic, hypoglycemic and antioxidant properties [17]. They have the ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy-radicals which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA [18]. Saponins are considered as a key ingredient in Chinese medicine and are responsible for most of the observed biological activity [19]. They are known to produce inhibitory effect on inflammation [20]. Therefore, the presence of these phytochemical in the plant strongly aided the antibacterial activity observed.

Conclusion

Extraction of the leaves of *Bombax buonopozense* was successfully carried out by maceration using solvent of different polarities. Phytochemical screening of the extract revealed the presence of many secondary metabolites which are believed to be responsible for antibacterial activity observed. The results supported the traditional usefulness of *Bombax buonopozense* as a medical remedy for some illnesses.

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