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Phytochemical screening, antioxidant and *in vitro* anticancer activities of *Bombax buonopozense* stem bark extracts

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ABSTRACT

In recent decades, extensive research has been focused on the screening and investigating the antioxidant and anticancer activities of medicinal plants extracts or isolated natural product-based drugs. Therefore, the present study aimed to evaluate the phytochemical, antioxidant and *in vitro* anticancer activities of *Bombax buonopozense* extracts. Standard methods were used for the identification of alkaloids, tannins, flavonoids, coumarins, saponins, sterol, triterpenes and reducing compounds. Free-radical–scavenging activity was measured by the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical assay, and the cytotoxic potential was evaluated in the P815 murin lymphoblast-like mastocytoma cell line using the MTT assay. Regarding the antioxidant activity, our results showed a powerful *in vitro* free-radical scavenging activity of the ethanolic extract compared to the aqueous extracts, with IC_{50} values of 10 and 220 µg/mL, respectively. In the MTT assay, the ethanolic extract showed a moderate and dose-dependent association growth inhibition of P815 cells in a dose above of 200 µg/mL with IC_{50} of 74 µg/mL compared to cisplatin (IC_{50}= 4 µg/mL). Our findings demonstrate that *Bombax buonopozense* ethanolic extract could be an effective antioxidant and promising antiproliferative agent.

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Bombax buonopozense; phytochemical screening; antioxidant activity; cancer

Introduction

Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for cancer treatment as they are recognized to have less toxic side effects [1,2]. Therefore, herbal remedies have been widely attracting attention as a potential and prospective resource of treatment for several diseases across many civilizations [3,4]. As well known, numerous plant species have long been described to possess pharmacological activities and therapeutic value attributable to their phytochemical constituents such as alkaloids, flavonoids, terpenes, saponins, glycosides, tannins and steroids [5–7]. In recent years, scientists have found that many tropical herbs have been scientifically reported to possess potent anticancer and anti-oxidant activities [8,9].

Bombax buonopozense is an important tropical medicinal tree widely distributed in Africa, with 40 m in height, tall buttresses (6 m), large conical spines, compound leaves with 5–9 leaflets, and branches organized in whorls [10,11]. In Nigeria for example, the floral part of *Bombax buonopozense* is used as a vegetable [12]. Because of its therapeutic properties, the plant has been reported in the literature to possess enormous biological activities. Previous studies have reported its antimicrobial, antidiarrhoeic and antipyretic activities [13–15]. Following the literature search, a rareness of scientific information regarding the biological activities of *Bombax buonopozense* was noted, especially the anticancer activity. Thus, the aims of the present study were to investigate the phytochemical profiles, the antioxidant and the *in vitro* anticancer activities of *Bombax buonopozense* stem bark extract. It is expected that the results established from this study will provide valuable insights into the pharmacological potential of *B. buonopozense*, therefore,

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validating and valorizing the traditional uses of this tropical plant.

Materials and methods

Plant collection

The bark of the stem of *Bombax buonopozense*, harvested in 2018 in Nkolbisson (the central region of Cameroon), was dried at room temperature and then crushed to obtain a fine powder.

Preparation of the extracts

As in numerous herbal preparations, water extraction was carried out by a decoction process [16]. In the present study, the plant material extract was obtained by decoction and maceration using solvents of increasing polarity: hexane, ethyl acetate, ethanol and distilled water. Ten grams of bark powder was introduced into a 250 mL triple-necked flask, 100 mL of distilled water was added. The flask was surmounted by a cooler connected to a tap opened by a pipe. The mixture is maintained at a constant heating temperature for 1 h. After cooling, the mixture is filtered on Whatman paper, the filtrate obtained is displaced in the oven at a temperature of 55 °C, to give a yield of 8%. To do a comparative photochemical analysis of the different chemical groups of the bark powder extract, the maceration method with different polarities of solvents (ethanol, ethyl acetate and hexane) was chosen. In this extraction protocol, 25 g of the bark powder were subjected to maceration with magnetic stirring for 48 h in 125 mL of solvent. The mixture was filtered on a filter paper (Whatman), the filtrate was concentrated and dried with a rotary evaporator. The organic total extract was obtained and give a yield of 18%.

Phytochemical analysis

This qualitative test based on colour reactions and/or precipitation [17], indicates different chemical groups and specific reagents (Table 1).The fluorescence characteristics were observed in visible light and using an ultraviolet (UV) lamp under long UV-light (315 to 400 nm) and short UV-light (100 to 280 nm).

Testing for alkaloids

The characterization of alkaloids was carried out using the Dragendorff's reagent. Each extract (10 mg) was dissolved in 2 mL of hydrochloric acid 5%. After mixing and filtering a few drops of Dragendorff's reagents (Potassium Bismuth Iodide) were added to each. Formation of a red–orange precipitate indicated the presence of alkaloids.

Test for tannins (ferric chloride test)

Briefly, 2 mL of each extract were added to a few drops of ferric chloride aqueous solution 10% *w/v*. The formation of blackish blue or green-blackish colour indicated presence of catechol tannins.

Test for flavonoids

Flavonoids were characterized using Cyanidin reaction. A volume of 2 mL of each plant extract was evaporated to dryness. After cooling, 5 mL of diluted hydrochloric alcohol and a few magnesium turnings were added to each extract residue. Then, a few drops of isoamyl alcohol were added. A pink–orange, purple or red rose indicate the presence of flavonoids.

Test for coumarins

The presence of coumarins was determined using the Bornträger reaction. Spots of each dry extract were applied on the chromatographic plates using thin layer chromatography. Then, the plates were sprayed with 10% of potassium hydroxide and observed on a UV lamp (365 nm). Intense fluorescence indicates the presence of coumarins.

Testing for saponins. The content of saponins was measured by adding 10 mL of the plant extract to a test tube. After shaking vigorously for about 15 s and left to stand for 15 min. The formed foam height was measured. Foam greater than 10 mm in height indicated the presence of saponins (abundant).

Table 1. Specific reactions and phytochemical screening reagents.

Chemical groups	Specific reagents	Characteristic reactions
Alkaloids	Dragendorf (Potassium tetraiodobismuthate)	Orange colouring with appearance precipitate
Tannins	Stiasny reaction (FeCl3)	blue–dark green or black colour
Flavonoids	Cyanidin reaction	orange–pink colouring; purple or red rose
Coumarins	Bornträger reaction-UV	Intense fluorescence
Saponins	Determination of the Foam Index (MI)	Positive test if MI > 1 cm, Intense foam
Sterols and Triterpenes	Libermann-Buchard (acetic anhydride -H_2SO_4)	The appearance at the interface of a purple or purple ring, turning blue to green
Reducing compound	Fehling's reaction	Brick red precipitate

Test for sterols and triterpenoids (Liebermann–Burchard's test)

One milliliter of anhydrous acetic acid and a few drops of concentrated sulfuric acid were added to each extract. After 5 min a blue–green colour middle layer was indicative of sterols, but formation of a purple-colored ring indicated the presence of triterpenoids.

Free reducing sugar (Fehling's test)

About 2 mL of each extract was added into 5 mL mixture of equal volumes of Fehling's solutions A and B and heated in a water bath for 2 min. Formation of a brick-red precipitate was an indication of the presence of reducing sugars.

In vitro anticancer activity

Cytotoxicity was measured using MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-(2H) tetrazolium bromide) assay as described and modified by Tim Mosmann [18]. Briefly, P815 (mouse DBA2 mastocytoma ATCC: TIB64) cancer cells (6.105) were seeded in each well containing 100 µL of the RPMI 1640 medium supplemented with 10% of fetal bovine serum (FBS) and 1% of penicillin and streptomycin in a 96-well plate. After exposure of cells to serial concentrations of tested product for 48 h at 37 °C and 5% CO_2, 20 µL of MTT (5 mg/mL stock solution) were added and the plates were incubated for an additional 4 h in the same conditions, after that the plates were treated with a solution of HCl/isopropanol (24:1) to dissolve the blue intracellular formazan product. One hour later, the plates were read in a MicroELISA reader at dual wavelength of 540–630 nm. Cell lysis (%) was calculated for each concentration as: 100 X (1 – $Abs_{treated}$/Abs_c), where $Abs_{treated}$ and Abs_c are the absorbance readings for the wells with and without product, respectively. All tests and analyses were run in duplicate and mean values recorded. Cisplatin was used as a positive control.

Antioxidant activity: DPPH radical scavenging assay

The 2,2′-diphenyl-1-picrylhydrazyl (DPPH) free radical assay was carried out to measure the free radical scavenging activity. Briefly, DPPH (100 µL, 0.2 mmol/L) solution was added to MeOH solution (200 µL) of the extract or standard compounds at various concentrations. The reaction mixture was shaken vigorously, and the absorbance of remaining DPPH was measured at 517 nm after 30 min in room temperature. Ascorbic acid (Vitamin C) was used as a standard drug. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation:

$$\%\text{Inhibition} = \left[\left(AB - AA\right)/AB\right] \times 100,$$

where AB is the absorption of blank sample, AA is the absorption of test sample.

The percentage of inhibition was calculated and the graph was plotted to determine the IC_{50} value.

Statistical analyses

The results were expressed as mean ± *SD*. The study was analyzed by using Student's *t*-test. The data were presented as mean *SEM* ($n = 3$), and differences were considered statistically significant at a $p < .05$ level.

Results

Phytochemical analysis

The results obtained from the phytochemical assay are given in Table 2. This preliminary phytochemical screening of the stem bark extracts of *Bombax buonopozense* revealed the presence or absence of secondary metabolites. The qualitative analysis showed the presence of alkaloids, terpenes, sterols, flavonoids, tannins and saponins compounds in the various extracts with varying intensities. As shown in Table 2, the majority of these secondary metabolites are present in the ethanolic extract. The presence of flavonoids was noted essentially in the ethanolic extract (Table 2).

Antioxidant activity

The ethanolic extract exhibited a better free-radical-scavenging activity, close to that of vitamin C, compared to the aqueous extract (Figure 1). Overall, the comparison demonstrated that the ethanolic extracts exhibited powerful scavenging effects on DPPH radicals, while the aqueous extract had the lowest, with IC_{50} values of 10 and 220 µg/mL, respectively (Table 3).

In vitro cytotoxic activity

The powerful free-radical-scavenging activity of the ethanolic extract, prompted us to investigate it's *in vitro* cytotoxic potential in a cancer cell line. The

Table 2. Chemical groups in various extracts of stem bark of *Bombax buonopozense*.

Chemical groups	Aqueous extract	Ethanolic extract	Acetate extract	Hexanic extract
Alkaloids	+++	+++	+	–
Tannins	+++	+++	+++	–
Flavonoids	–	+++ (Flavones)	–	–
Coumarins	–	–	–	–
Saponins	+	+	–	–
Sterol and triterpenes	+	+++	+	–
Reducing compound	–	–	–	–

+: Presence.
+++: Intense presence.
–: Absence.

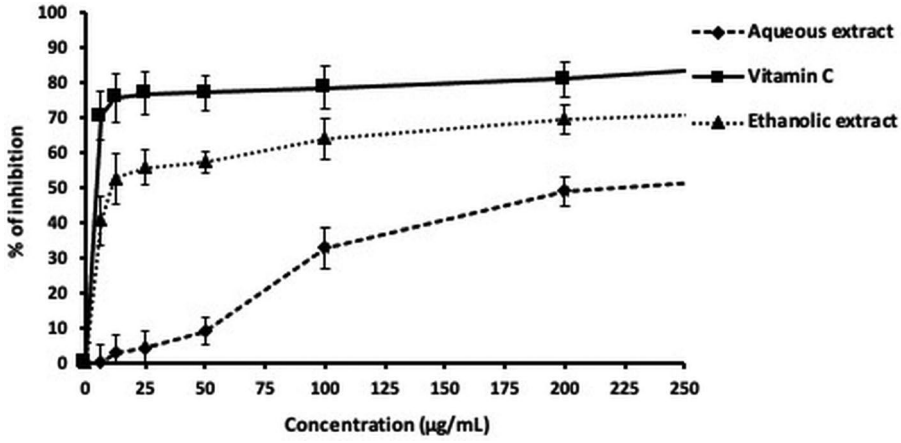

Figure 1. *In vitro* DPPH radical-scavenging potential of aqueous and ethanolic extract of stem bark of *Bombax buonopozense*.

cytotoxic activity of the extract was investigated through 3-(4) 5-Dimethyl-thiazol-Zyl) – 2,5 biphenyl tetrazolium bromide (MTT) assay. The results revealed an increase of cell lysis in a dose-dependent manner. The chemotherapy medication used to treat the P815 cancer cell line in this test is cisplatin; this agent presented powerful IC_{50}= 4 µg/mL (Figure 2); however, the ethanolic extract presented an IC_{50}= 74 µg/mL (Figure 2). As shown in Figure 2, the lysis % showed a dose-dependent association with increasing of ethanolic extract concentration from 1 to 200 µg/mL.

Discussion

The phytochemical screening of *Bombax buonopozense* extracts revealed the presence of various secondary metabolites including alkaloids, tannins, flavonoids, saponins, sterol and triterpenes as shown in Table 2. Similarly, Akuodor et al. have shown that the methanolic extract of *B. buonopozense* obtained by maceration contained tannins, saponins, terpenes, steroids, flavonoids, alkaloids and carbohydrates compounds [10]. Furthermore, a study on the root extract, demonstrated the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids phlo-batannins, anthraquinones and carbohydrates [10]. The phytochemical groups detected are known to have medicinal properties. For example, alkaloids have been reported to exhibit various biological activities like, anti-inflammatory [19], antimalarial [20] and cytotoxic activities [21]. Similarly, tannins, flavonoids and saponins derived from plants are known to possess many biological activities [22–24].

DPPH radical is one of the routinely employed antioxidant assays, which reacts with suitable reducing agents losing color stoichometrically with the number of electrons consumed, which is measured spectrophotometrically at 540 nm. In the present study, both *Bombax buonopozense* extracts exhibited rich scavenging effects on DPPH as illustrated in Figures 1 and 2. However, the ethanolic extract showed stronger free-radical-scavenging activity compared to the aqueous extract, with IC_{50} values of 10 and 220 µg/mL, respectively (Table 3). Stronger radical scavenging effect could be attributed to the presence of flavonoids and higher amount of sterol and terpenoids of the ethanolic extract as shown in Table 2. The higher antioxidant activity of ethanolic extract could be attributed to flavonoid compounds and their hydrogen-donating ability. Studies have concluded

Table 3. IC_{50} values of ethanolic and aqueous extract of stem bark of *Bombax buonopozense* in relation to DPPH inhibition.

Extract type	Aqueous (ETA BbH)	Ethanolic (EXT 18)	Vitamin C
IC_{50} (µg/mL)	220	10	4,5

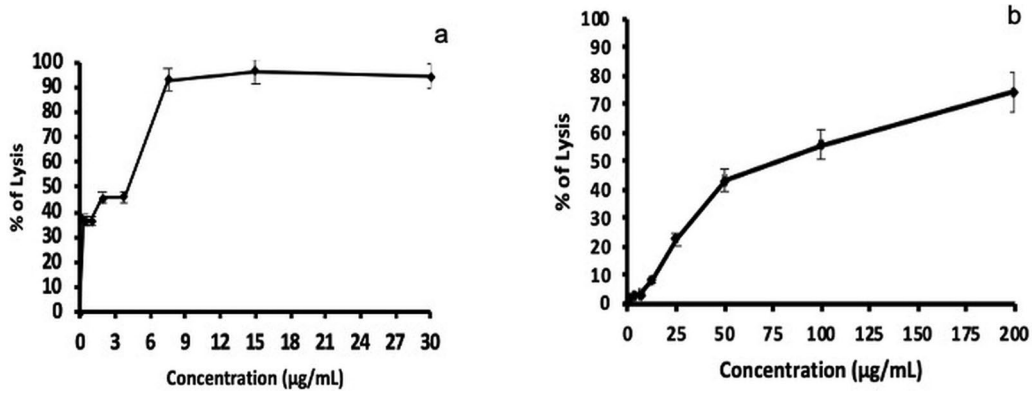

Figure 2. *Cytotoxic* effect of cisplatin (a) and ethanolic extract of stem bark of *Bombax buonopozense* (b) on P815 cancer cell line.

that large plants which contain flavonoid compounds possess antioxidant activity [25–28]. Furthermore, the presence of flavonoids and tannins in the plant extract plays an important role for their antioxidant property. Several plant components like tannins and flavonoids are responsible for showing antioxidant activity.

The important antioxidant activity of the ethanolic extract prompted us to study its *in vitro* cytotoxic capacity. It is well established that flavonoids present in the ethanolic extract are free-radical scavengers and prevent oxidative damage; they have powerful anticancer effect and also protect cells against all stages of carcinogenesis [29–31]. To the best of our knowledge, *B. buonopozense* extracts have not been evaluated for anticancer properties. However, other biological activities of this plant have been studied such as antiulcer [32], antihemorrhoids [33], antidiarrheal [34] and antimalarial [35] activities. The previous study on the anti-ulcer activity of *B. buonopozense* leaf aqueous extract, showed a protective effect in the stomach against the necrotic damage of ethanol at a dose of 400 mg/kg [32]. In the present study, the cytotoxic activity of the ethanolic extract was probably due to its alkaloids, tannins, sterol and triterpenoids contents. There is abundant evidence that a great number of plant extracts, contain such chemical compounds exhibiting anticancer properties [36–41]. Interestingly, studies have reported no acute toxicity of *B. buonopozense*-treated mice at doses ranging from 10 to 5000 mg/kg; no mortality in mice after oral administration of the aqueous extract; and oral LD_{50} greater than 5000 mg/kg [42,43].

Conclusions

This is the first report reporting cytotoxic and antioxidant activities of *Bombax buonopozense* extracts. The ethanolic extract was an effective scavenger and promising cytotoxic agent. The presence of alkaloids, sterol/terpenes and especially flavonoids may explain these biological activities of the ethanolic extract compared to other extracts. Taken together, our results provide the basis to carry out further in-depth research to characterize the compounds and to clarify the molecular mechanisms underlying the observed biological activities.

Author's contributions

Conceptualization, A.Z., M.K, A.H and H.A.M.; Methodology, H.A and M.T., Formal Analysis, M.K, A.H, M.L, H.A.M and A.Z., writing-original draft preparation, A.H and M.T; writing-review and editing A.Z, S.L, S.Z, M.T and H.A.M. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

Data availability statement

All data that support the findings reported in this study are available from the corresponding author (M.T) upon reasonable request.

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