REPORT

Phytochemical screening and antimicrobial activities of red silk cotton tree (*Bombax ceiba* L.)

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Abstract: The present study was conducted to investigate the phytochemical screening and antimicrobial activities of stem bark of *Bombax ceiba* L. The methanol extract was subjected to qualitative phytochemical screening using standard procedures. The results indicated the presence of alkaloids, tannins, glycosides, reducing sugar, saponins, phlobatanins and terpenoids. The antimicrobial activity was measured by disc diffusion method. Data revealed that *Pseudomonas aeruginosa* was inhibited by both methanol and ethanol extracts at the concentration of 2mg disc^{-1} {21.8mm (68.12%) and 21.3mm (66.56%)}. Similarly, methanol extract reduced the growth of *Bacillus subtilis* by 17.1mm (74.34%) at the concentration of 1 mg disc^{-1}. However, ethanol extract showed a good activity of 18mm (121.6%) and 20.6mm (112.5%) against *Xanthomonas maltophilia* at concentrations of 1 and 2 mg disc^{-1}, respectively. Aqueous extract showed 16 mm (53.33% Z.I) against *Escherichia coli* at 2 mg disc^{-1}. *Klebsiella pneumoniae* was found resistant to all of the three extracts, while the growth of *Candida albicans* was inhibited by methanol through 16.5 mm (58.92% Z.I) at 1 mg disc^{-1}. The above study concluded the medicinal potential of *B. ceiba*.

Keywords: Phytochemical screening, antimicrobial activity, *Bombax ceiba*, bacteria, fungi.

INTRODUCTION

The use of medicinal plants for curing wounds is as old as humankind itself (Petrovska, 2012). Medicinal plants are a main source of a variety of drugs as more than 80% of the world's population uses traditional medicine for their primary healthcare needs (Diallo *et al*., 1999). Pathogenic organisms of plants and humans are controlled by different antimicrobial chemicals produced by different medicinal plants (Desalegn, 2014). Plant produce enormous amount of chemical substances which are divided in two main groups, Primary metabolites and secondary metabolites (Agosta, 1996). Important secondary metabolites which are present in medicinal plants are alkaloids, tannins, terpenes, flavonoids, and phenolic compounds (Edeoga *et al*., 2005).

B. ceiba is a fast growing deciduous tree belonging to family Bombacaceae (Rao *et al*., 2014). The family Bombacaceae is comprised of 27 genera and 150 species, out of which 3 genera and 3 species are known for cultivation in Pakistan (Aguoru *et al*., 2015). It has been claimed in Ayurveda that *B. ceiba* possesses proven medicinal properties and Bark gum of *B. ceiba* L. helps in curing of bleeding piles, body burns and blood diseases. Its gum also helps in curing dysentery, influenza, hemoptysis, pulmonary tuberculosis, enteritis, menorrhagia and burning sensation. The paste from the bark is useful for curing chicken pox disease. The roots are sweet, stimulant, cooling, astringent, restorative, demulcent, emetic, alternative, aphrodisiac, and tonic. It is also used for the treatment of wounds. The gum is astringent, stimulant, cooling, aphrodisiac, demulcent and tonic in nature. Flowers are good for skin troubles and piles. Seeds contain chemical compounds which are useful for curing gonorrhea and chronic cystitis. The extract from leaves has unusual antifungal property against ringworm. Boiled extract of young plant roots is useful in urinary infection (Rao *et al*., 2014).

The aims of this study were to evaluate the phytochemical screening, antibacterial and antifungal activities of *B. ceiba* L. against important human pathogens.

MATERIALS AND METHODS

Plant material collection and extraction

Fresh stem bark of *B. ceiba* was collected from Naran gardens on Jamrud Road Peshawar, Pakistan. The plant was identified by a plant taxonomist Prof. Dr. Siraj-ud-Din and a voucher specimen (SS001ICP) has been deposited in the herbarium of the Department of Botany, Islamia College Peshawar. The bark was air dried in shade at room temperature for two months before grinded

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with specialized grinders. Fifteen hundred ml of methanol, ethanol and aqueous was added in three separate beakers, each containing one hundred and fifty grams of bark powdered of *B. ceiba*. Then covered with hard sheet for six days. The mixture was filtered through filter paper. The filtrate was evaporated to concentrate with rotary evaporator model (STRIKE 300) at 50^{o}C with 60 rpm. The filtrate was further concentrated through water bath at 50^{o}C in china dish and the dried extract stored in a bottle at room temperature.

Phytochemical screening

Phytochemical screenings were carried out on the crude methanolic extract for the qualitative determination of phytochemical constituents as described by (Uddin *et al*., 2011; Uddin *et al*., 2012; Rauf *et al*., 2014).

Test for alkaloids

Methanolic extract of (0.2g) was warmed in 2 ml of 2% H_2SO_4 for two min. The reaction mixture was filtered. After filtration few drops of Dragendrof's reagent were added which turned the mixture into orange red precipitation. This indicated the presence of alkaloids.

Test for tannins

Small quantity of extract was mixed with distilled water in test tube and heated on water bath. After heating the mixture was filtered through filter paper. Added few drops of the ferric chloride ($FeCl_3$) to the filtrate, which turned the filtrate into dark green color. This indicated the presence of tannins in the filtrate.

Test for anthraquinone

Methanolic extract (0.5g) was boiled with 10% hydrochloric acid (HCl) for few min and filtered through filter paper and allowed to cool. Equal volume of chloroform ($CHCL_3$) was added to the filtrate with adding few drops of 10% ammonia (NH_3) and heat for few min. Rose-pink color was obtained which showed the presence of anthraquinones.

Test for glycosides

Methanolic extract of (0.6g) was hydrolyzed with HCl and neutralized with NaOH solution and added few drops of Fehling's solutions A and B. The mixture turned into red precipitation which indicated the presence of glycosides.

Test for reducing sugars

Methanolic extract (0.5g) was shaken and mixed well with distilled water and filtered. After filtration added few drops of Fehling's solution A and B and boiled for few min. An orange-red precipitate was formed which indicated the presence of reducing sugar.

Test for saponins

Methanolic extract (0.2g) was shaken with distilled water (5.0ml) and boiled. Small bubbles at the upper area of reaction mixture indicated the presence of saponins.

Test for flavonoids

Methanolic extract (0.2g) was dissolved in the diluted Sodium Hydroxide (NaOH). After dilution added few drops of hydrochloric acid (HCl). The solution turned from yellow to colorless which showed the presence of flavonoids.

Test for phlobatanins

Methanolic extract (0.5g) was dissolved in distilled water and filtered through filter paper. Added few to several drops of 2% hydrochloric acid (HCl) and heated. Red precipitates were acquired which indicated the presence of phlobatanins.

Test for steroids

For steroids test (0.5g) methanolic extract was mixed with (2.0ml) H_2SO_4, and then (2.0ml) acetic anhydride was added which change the color from violet to blue or green. This indicated the presence of steroids in the mixture.

Test for terpenoids

Methanolic extract (0.2g) was stirred with 2ml of chloroform ($CHCL_3$) and then added 3ml concentrated (H_2SO_4) which form a layer. At the interface the reddish brown coloration was formed which showed the positive result of presence of terpenoids.

Test for anthocyanin and betacyanin

Methanolic extract (0.2g) was added with NaOH (1ml, 2 N) and heated for 5 min at 100°C. Bluish green color was observed which showed the presence anthocyanin.

Test for proteins and amino acids

Methanolic extract (0.2g) was dissolved in 0.2% ninhydrin and heated for 5min. The mixture turned into blue color which showed the presence of proteins.

Test for cardiac glycosides

Methanolic extract (0.2g) was mixed with 1.0ml glacial acetic acid and 5% $FeCl_3$. Then add few drops of concentrated H_2SO_4. Greenish blue color of the mixture was observed which showed the presence of cardiac glycosides.

Antimicrobial activity

Culture media

Mueller Hinton agar medium (LAB039, LabM Limited, UK) was used for culturing of all microorganisms including fungus (*Candida albicans*) and Nutrient broth was used for inoculation, shaking, incubation and standardization of these microorganisms (AOAC, 1995; Tassou *et al*., 2000).

Microorganisms Used

Gram -ve (*Pseudomonas aeruginosa*, *Xanthomonas maltophilia*, *Escherichia coli* and *Klebsiella pneumoniae*),

Gram +ve (*Bacillus subtilis*) bacteria and fungus (*Candida albicans*) were obtain from Institute of Biotechnology and genetic Engineering, University of Agriculture, Peshawar, KPK Pakistan.

Disc diffusion susceptibility method

Antimicrobial potentiality of crude extracts of *B. ceiba* bark was evaluated by the paper disc diffusion method (Aida *et al*., 2001). The bacterial cultures were adjusted to 0.5 McFarland turbidity standards which contain bacterial cell density of 10^8/ml and inoculated onto agar plates and incubated at 37°C for 18-24 hrs. Whatman No. 1 Sterile filter paper discs (diameter 6 mm) were impregnated with different plant extracts and antibiotics, and placed with the help of sterile forceps on Mueller Hinton agar and, (for Gram-positive bacteria = Azithromycin 50µg per 6 µl; for Gram negative bacteria = Ciprofloxacin 50µg per 6 µl and for *Candida albicans* = Clotrimazole 50µg per 6 µl) as positive control and DMSO 6 µl (1 mg $disc^{-1}$) and 12 µl (2 mg $disc^{-1}$) as a negative control were also applied on the discs. The plates were then incubated at 37°C for 24 hrs. Antibacterial activity was determined by measurement of zone of inhibition (Z.I) around each paper disc. For each extract three replicate trials were conducted against the tested organism.

STATISTICAL ANALYSIS

Simple statistics was applied. The data recorded in mean values which then converted into percent. The Formulea of calculation in percent is as follows:

$$\text{Percent Inhibition} = \frac{\text{Zone of Inhibition of extract (mm)}}{\text{Zone of Inhibition of Standard (mm)}} \times 100$$

RESULTS

Phytochemical screening

The results indicated that different phytochemicals were detected in methanolic extract of *B. ceiba* L. (stem bark). Tests for alkaloids, tannins, glycosides, reducing sugar, saponins, phlobatanins and terpenoids were positive in methanolic extract, while anthraquinone, flavonoids, steroids, anthocyanin and betacyanin, proteins and amino acids, and cardiac glycosides were not detected. (table 1).

Antimicrobial activity

The present study also conducted to evaluate the antimicrobial activity of crude extracts (methanol, ethanol, and aqueous) of *B. ceiba* against five bacterial strains (Gram positive and Gram negative) and one fungal strain. fig. 1 shows the antimicrobial activities of three extracts of *B. ceiba* L. against *P. aeruginosa*, *Bacillus subtilis*, *Xanthomonas maltophilia*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*. Analysis of the data revealed that methanol and ethanol extracted samples had a profound inhibitory effect against *P. aeruginosa* when compared to the aqueous extracted samples. Methanol and ethanol extracts were more effective against *P. aeruginosa* at both the concentrations when compared to the aqueous extracts, 17.5 mm (62.5% Z.I) and 21.8 mm (68.12% Z.I) by methanol while 18.6 mm (66.42% Z.I) and 21.3 mm (66.56% Z.I) by ethanol at the concentration of 1 and 2mg $disc^{-1}$. The data further reveal that aqueous extract was not so much effective as compared to other extracts and their positive controls and showed 10 mm (35.7% Z.I) and 16.3 mm (50.93% Z.I) inhibition at concentration of 1 and 2 mg $disc^{-1}$.

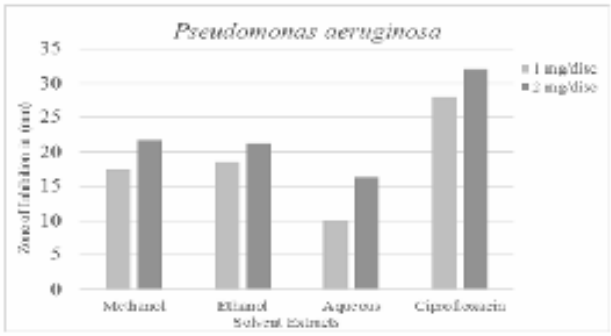

Fig. 1: Antibacterial activity of *B. ceiba* L extracts against *P. aeruginosa*.

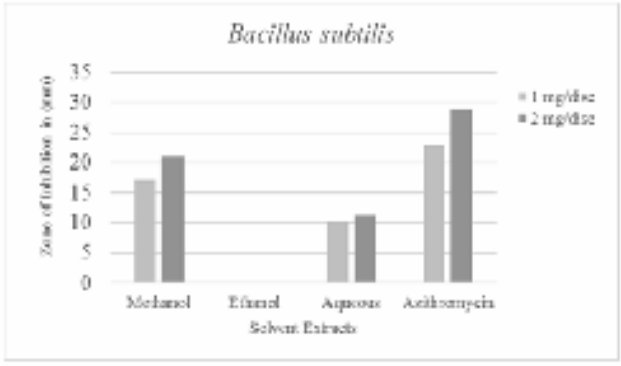

Fig. 2: Antibacterial activity of *B. ceiba* extracts against *Bacillus subtilis*.

Fig. 2 presents data regarding the antibacterial activity of methanol, ethanol, and aqueous extracts of *B. ceiba* against *B. subtilis*. Methanol extract were more effective in reducing the growth of *B. Subtilis* at both concentration showing 17.1mm (74.34% Z.I) at concentration of 1mg $disc^{-1}$ and 21.1mm (72.75% Z.I) at concentration of 2 mg $disc^{-1}$. Ethanol extract had no effect on growth of *B. subtilis* at any concentration. Aqueous extract showed 10.3 mm (44.78% Z.I) at concentration of 1 mg $disc^{-1}$ and 11.3 mm (38.96% Z.I) at concentration of 2 mg $disc^{-1}$.

The antibacterial activities *B. ceiba* extracts against *Xanthomonas maltophilia* is presented in fig. 3. Data revealed that all extracts were effective against *X. maltophilia*. Ethanol extract showed tremendous inhibition of 18mm (121.6% Z.I) at 1mg $disc^{-1}$ and 20.6

mm (112.5% Z.I) at concentration of 2mg disc^{-1} as compared with ciprofloxacin. Methanol and aqueous extracts have also inhibitory effect against *X. maltophilia* and showed 15.1mm (82.51% Z.I) and 12mm (65.57% Z.I) at concentration of 2 mg disc^{-1}, respectively.

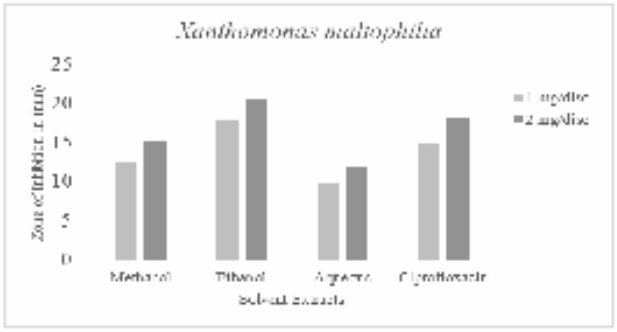

Fig. 3: Antibacterial activity of *B. ceiba* L. extracts against *Xanthomonas maltophilia*.

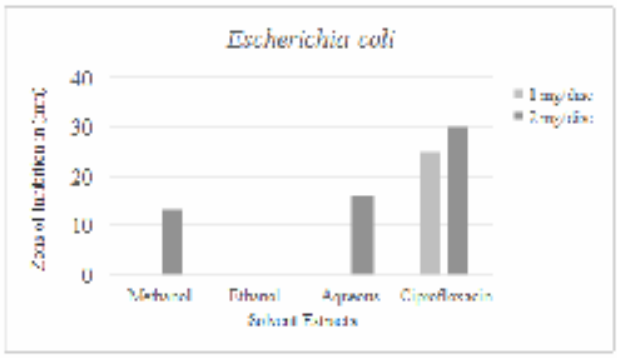

Fig. 4: Antibacterial activity of *B. ceiba* L. extracts against *Escherichia coli*.

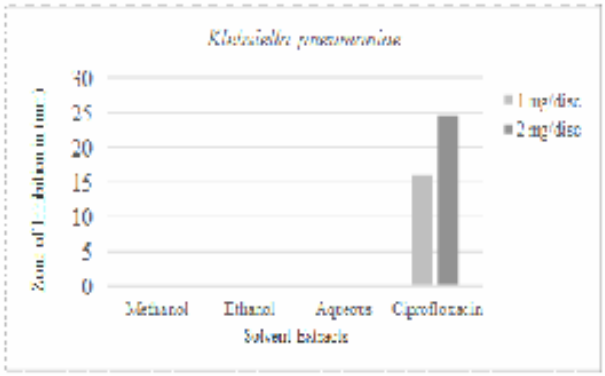

Fig. 5: Antibacterial activity of *B. ceiba* L. extracts against *Klebsiella pneumoniae*.

Data regarding the antibacterial activity of red silk cotton tree against *Escherchia coli* is shown in fig. 4. Aqueous extract showed highest inhibition among all extracts at concentration of 2mg disc^{-1} 16mm (53.33% Z.I). Methanol extract no effect at concentration of 1 mg disc^{-1}, while 13.1mm (43.66% Z.I) at concentration of 2 mg disc^{-1}. Ethanol extract had no effect against *Escherichia coli* at both concentrations of 1 and 2mg disc^{-1}.

Fig. 5 shows data concerning antibacterial activity of *B. ceiba* (stem bark) against *Klebsiella pneumoniae* when applied in different concentrations. The data revealed that *K. pneumoniae* was the most resistant bacteria to all methanol, ethanol and aqueous extracts.

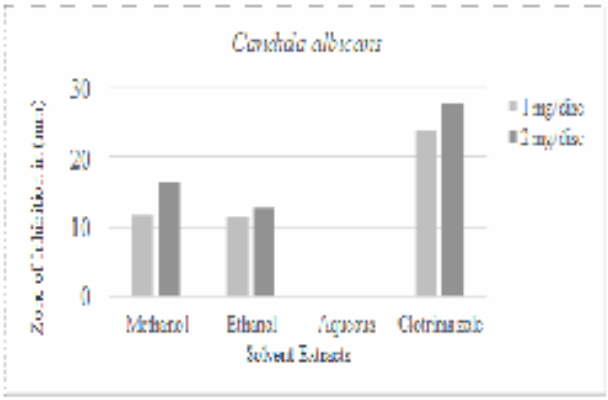

Fig. 6: Antifungal activity of *B. ceiba* L. extracts against *Candida albicans.*

Table 1: Different phytochemicals found in methanolic extract of *B. ceiba* L. (stem bark).

S. No	Phytochemicals	Results
1	Alkaloids	+
2	Tannins	+
3	Anthraquinone	–
4	Glycosides	+
5	Reducing sugar	+
6	Saponins	+
7	Flavonoids	–
8	Phlobatanins	+
9	Steroids	–
10	Terpenoids	+
11	Anthocyanin and betacyanin	–
12	Proteins and amino acids	–
13	Cardiac glycosides	–

The antifungal activity of methanol, ethanol and aqueous extracts of *B. ceiba* against *Candida albicans* is shown in fig. 6. Methanol extract showed 13mm (49.1% Z.I) at concentration of 1mg and 16.5mm (58.92% Z.I) at concentration of 2mg disc^{-1}, respectively. Ethanol showed 11.5 (47.91% Z.I) and 13mm (46.42% Z.I) at concentration of 1 and 2 mg disc^{-1}, respectively. The data further revealed that aqueous extracted sample showed no inhibition of *C. albicana*.

DISCUSSION

Our results showed that different phytochemicals such as alkaloids, tannins, glycosides, reducing sugar, saponins, phlobatanins and terpenoids were found in the stem bark of *B. ceiba*. Gopal, (2014) found saponins, flavonoids, tannins, phenols, sterols and carbohydrates in the stem

bark of *B. ceiba*, while he did not found alkaloids. It is reported that these chemical constituents are responsible for the antimicrobial activity against several human pathogens and therefore could suggest the use traditionally for the treatment of various diseases (Wright and Linda, 2011; Uddin *et al*., 2011).

The antimicrobial activity of different extracts of *B. ceiba* was studied against six different bacterial species and one fungus. Results indicated that all extracts of *B. ceiba* were more effective against all microorganisms except *Klebsiella pneumoniae*. It was also noticed that crude methanolic extract of *B. ceiba* revealed good activity as compared to ethanol and aqueous. Methanol extract inhibited the growth of *P. aeruginosa*, *B. subtilis*, *X. maltophilia* and *C. albicans* at both concentrations. While against *E. coli* methanolic extract had no effect. However, at higher concentration (2mg $disc^{-1}$) the methanolic extract inhibited the growth of *E. coli*. Our results further revealed that methanolic extract does not inhibit the growth of *K. pneumoniae* at any concentration. Gopal, (2014) observed Z.I against *P. aeruginosa* and *B. subtilis* as 9 and 11 mm while he did not observed any inhibition against *E. coli* and *K. pneumoniae*. Similarly, Islam *et al*. (2011) observed Z.I against *P. aeruginosa*, *E. coli* and *C. albicans* as 13, 10 and 10mm at concentration of concentration of 200µg $disc^{-1}$, while he did not observed any inhibition against *B. subtilis.*

The ethanol extracted samples were effective against three tested microorganisms among six. Ethanolic extract showed inhibition against *P. aeruginosa*, *X. maltophilia* and *C. albicans* at both concentrations while against *B. subtilis*, *E. coli* and *K. pneumoniae* the ethanolic extracts showed no inhibition at any concentration. Akerele *et al*. (2007) also reported similar results and found no inhibition against *E. coli* and *K. pneumoniae* while they observed 16 mm Z.I against *B. subtilis* using ethanolic extract of *Sida acuta*. Uddin *et al*. (2010) found no inhibition against *K. pneumoniae* while using ethanolic extract of *Hibiscus rosa-sinensis*.

Aqueous extract of *B. ceiba* showed antimicrobial activity against all bacteria except *K. pneumoniae* and *C. albicans* when measured by disc diffusion method. Aqueous extract showed inhibition against *P. aeruginosa*, *B. subtilis* and *X. maltophilia* at both concentrations while against *E. coli* aqueous extract showed inhibition only at higher concentration 2mg $disc^{-1}$. Kuthar *et al*. (2015) recorded Z.I against *P. aeruginosa*, *B. subtilis*, *E. coli* and *K. pneumoniae* as 23, 21, 28 and 27 at concentration of 100 µg/ml. Nagamani *et al*. (2014) results were contradictory to my results and he did not observed any Z.I against *E. coli* and *B. subtilis* while using aqueous extract of *B. ceiba pentandra* seeds.

CONCLUSION

The phytochemical screening showed the presence of alkaloids, tannins, glycosides, reducing sugar, saponins, phlobatanins and terpenoids, while anthraquinone, flavonoids, steroids, anthocyanin and betacyanin, Proteins and amino acids and cardiac glycosides were absent in methanolic extract of *B. ceiba* (stem bark). Methanol extracted samples showed good activity against *P. aeruginosa* and *C. albicans* but ethanol extract showed tremendous activity against *X. maltophilia*.

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