

Phytochemical screening and antimicrobial activity of bark of *Bombax ceiba* L.

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Abstract

Bombax ceiba L. is the member of family Bombacaceae. It is commonly known as Salmali, Ruka-Pusha, Semul or Semal, etc. It is a fast-growing tree because of which, is suitable for avenue plantation. Spines are found on the trunk of young tree for providing protection. The tree is about 20-40 m tall. Flowers are red-coloured and large-sized, and therefore it is known as Red-Silk cotton tree. Qualitative phytochemical screening of aqueous extract of bark shows the presence of alkaloids, steroids, terpenoids, phlobatannins, carbohydrates etc. The phytochemicals present in the plant provide protection, strength to plants. The anti-microbial activity was done in different solvents. The maximum zone of inhibition of 26.67 mm was observed against *Bacillus subtilis* of DMSO (Dimethyl Sulfoxide) extract. The minimum zone of inhibition of 7 mm was observed against *Streptococcus mutans* of ethanol extract, and *Bacillus subtilis* of aqueous extract.

Keywords: *Bombax ceiba* L.; Phytochemical; Anti-microbial; DMSO

1. Introduction

Every plant has medicinal value, and *Bombax ceiba* L. is one of them. It has a great medicinal property and it's each part is used in treatment of different diseases. *Bombax ceiba* L. commonly known as Red-Silk cotton tree is a member of family Bombacaceae (Choudhary et.al., 2012, Antil, 2013)^[1,2]. The tree is about 20-40 m tall. It is a fast-growing, light demander tree. It has tall, straight rigid trunk. The leaves are palmate, compound, entire, digitate, glabrous with about 5-6 leaflets radiating from the tip of petiole. Flowering is between January to March, and fruiting from April to June. The main characteristics of flowers of *Bombax ceiba* L. are large-sized and red coloured. They grow in bunches and are generally thick and fleshy. Spines were found on the trunk of tree. Fruit of this plant is capsule and seeds are numerous, round or ovoid, black-coloured, covered with white silky cotton hairs^[3,4,5,6] (Raut et.al., 2017; Kulkarni et.al., 2018).

It is widely distributed in South-east Asia and India^[7]. In India, it is known by different names like, Salmali, Ruka- Pusha, Kankardurma, Semul or Semal, etc. . *Bombax ceiba* L. serves various purposes of everyday life like providing food, fodder, fuel and fibre. Pillows are made from the cotton hairs of *Bombax ceiba* L and matchsticks, coffins, packing cases, etc. are made from its wood. The tree shows rapid growth and hence, is best for agro- forestry.^[8,9] The plant has stimulant, astringent and diuretic property. It has anti-inflammatory, anti- diabetic, anti-oxidant and also anti-microbial activity.^[10,11]

2. Materials and Methods

Fresh bark with spine of *Bombax ceiba* L. were collected from Ranchi (23°18'N and 85°17'E), Jharkhand, in the month of March. Cold extraction method was employed for the extract preparation. Dust and dirt were washed off in running tap water. After that the bark with spine was sun dried for 15-20 days. Dried bark was then ground into fine powder. In

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1:10 (w/v) ratio, powdered bark along with spine (10 gm) was mixed with methanol (100 ml). After that the mixture was put in shaker incubator for 5 days, after that the mixture was filtered and kept for drying. It took about 8-10 days for drying of the filtrate. Prepared extract was then dissolved in different solvents.

2.1. Phytochemical Screening

For Phytochemical screening, the powder of dried bark was dissolved in 250 ml distilled water. After 24 hrs the mixture was filtered using Whatman filter paper No. 1, obtained filtrate was then used for qualitative analysis of different phytochemicals present in the flowers of *Bombax ceiba* L. (Kamble et. al., 2017; Bhandary et. al., 2012; Chauhan et. al., 2017)^{12,13,14}.

- Alkaloids: In 1 ml of aqueous extract 2 ml of Dragendroff's was added. On addition of Dragendroff's to the extract it gives turbid orange colour, which is an indication of Alkaloids.
- Saponins: 2 ml of extract taken in test-tube was shaken vigorously. Development of foam on the surface lasts for about 6-7 minutes, which is an indication of Saponin.
- Carbohydrates: In 1 ml extract 3 ml Benedict's reagent was added, which gives bluish-green colour, which is an indication of Carbohydrate.
- Flavonoids: In 1 ml extract, 3 ml of lead acetate (10%) was added, brown-coloured precipitate was formed which is an indication of Flavonoids.
- Steroids: In 2 ml extract 3 ml chloroform and 3 ml concentrated H₂SO₄ was added carefully. Upper layer turned red and H₂SO₄ layer showed yellow coloured with green fluorescence, which is an indication of Steroids.
- Phenols: In 2 ml extract 0.5 ml Ferric Chloride (5%) was added, solution changed to brownish-green colour, which is an indication of Phenols.
- Terpenoids: In 2 ml extract 2 ml chloroform and 3 ml concentrated H₂SO₄ was carefully added along the boundary of test-tube. There is appearance of reddish brown colour at the boundary, which is an indication of Terpenoids.
- Glycosides: In 5 ml extract 2 ml glacial acetic acid was added and then mixed with 1-2 drops of 5% ferric chloride. After that 1-2 drops of concentrated H₂SO₄ was added, brown ring forms at the interface, indicating the presence of Glycosides.
- Tannins: In 2 ml extract 0.5 ml Ferric Chloride (5%) was added, solution changed to brownish-green colour, which is an indication of Tannins.
- Anthocyanide: In 1 ml extract 5 ml dilute HCl was added. Appearance of pale pink colour is an indication of Anthocyanide.
- Phlobatannins: In 1 ml extract 1 ml dilute HCl was added which gives dark red precipitate. This indicates the presence of Phlobatannins.

2.2. Antimicrobial activity

Antimicrobial activity of bark of *Bombax ceiba* L. was done by disc-diffusion method. For antibacterial activity Nutrient agar media; and for antifungal activity Potato Dextrose Agar media was poured in sterilized petri plates inside laminar air flow and the media was left to solidify. 100µl of microbial inoculum was inoculated and spread on the media and the inoculum was left for drying. After that sterilized paper disc were placed on the inoculated media for disc-diffusion method; then samples of varied concentrations were loaded. After that the plates were placed in incubator at 37°C for 18-20 hours (Bauer et.al., 1966; Nagamani et.al., 2015; Masood et.al., 2017)^{15,16,17}.

2.3. Microorganisms Used

For the antimicrobial activity, *Bacillus subtilis* (2511 MCC), *Pseudomonas aeruginosa* (3973 MCC), *Enterococcus faecalis* (3040 MCC), *Streptococcus mutans* (SM MCC), *Lactobacillus acidophilus* (LA MCC), and *Candida albicans* (1152 MCC) microorganisms were used.

2.4. Statistical analysis

The experiments were done in triplicate and mean and standard deviation (SD) was calculated.

3. Result and Discussion

3.1. Phytochemical screening

Qualitative phytochemical screening of bark of *Bombax ceiba* L. showed the presence of alkaloids, phenol, tannin, saponin, flavonoid, terpenoid, steroid, carbohydrates, glycoside, anthocyanide, phlobatannin in aqueous extract.

Table 1 Phytochemical Analysis of Bark of *Bombax ceiba* L.

Phytochemical	Observation	Inference
Alkaloids	Turbid Orange colour	Present
Phenol	Brownish-green colour	Present
Tannin	Brownish-green colour	Present
Saponin	Development of foam lasts for about 6-7 minutes	Present
Flavonoid	Brown precipitate formed	Present
Terpenoids	Appearance of red colour at the interface	Present
Steroids	Red-colour formed at upper layer	Present
Carbohydrates	Bluish-green colour	Present
Glycosides	Brown ring formed at the interface	Present
Anthocyanide	Pale-pink colour	Present
Phlobatannins	Dark-red precipitate	Present

3.2. Antimicrobial activity

The antimicrobial activity of bark of *Bombax ceiba* L. was done in different solvents like DMSO, Ethanol, Aqueous by disc-diffusion method in some microbial strains to check their activity.

Table 2 Anti-microbial Activity of Bark in DMSO Extract

Microbial Strains	Zone of Inhibition						
	DMSO Extract					DMSO	Tetracycline
	2µl	4 µl	6µl	8µl	10µl	2µl	2µl
<i>Lactobacillus acidophilus</i>	7.67±0.19	10.33±0.19	11.67±0.38	14.33±0.19	15±0.33	0±0	20.67±0.19
<i>Streptococcus mutans</i>	9.33±0.19	11±0.33	12.33±0.19	13.67±0.38	17.67±0.19	0±0	20.67±0.19
<i>Enterococcus faecalis</i>	10±0.33	10.67±0.19	14.33±0.19	14.67±0.51	20±0.67	0±0	22.33±0.38
<i>Pseudomonas aeruginosa</i>	11.33±0.19	12.33±0.38	14.67±0.38	14.67±0.19	18±0.33	0±0	13.67±0.19
<i>Bacillus subtilis</i>	7.67±0.19	9.67±0.19	15±0.33	25.67±0.69	26.67±0.19	0±0	18.67±0.19

<i>Candida albicans</i>	8.33±0.19	11.33±0.19	12±0.33	15±0.33	16.67±0.19	0±0	16.67±0.38
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Table 3 Anti-microbial Activity of Bark in Ethanol Extract

Microbial Strains	Zone of Inhibition						
	Ethanol Extract					Ethanol	Tetracycline
	2µl	4 µl	6µl	8µl	10µl	2µl	2µl
<i>Lactobacillus acidophilus</i>	7.33±0.19	14±0.33	18.33±0.38	20.33±0.51	22.67±0.38	0±0	18.33±0.19
<i>Streptococcus mutans</i>	7±0.33	14.67±0.19	18±0.33	20.33±0.19	21.67±0.19	0±0	20.67±0.38
<i>Enterococcus faecalis</i>	9±0.33	14.33±0.69	16±0.33	17.33±0.19	20.67±0.77	0±0	28±0.67
<i>Pseudomonas aeruginosa</i>	9.33±0.19	15.67±0.19	16.33±0.38	17.67±0.19	20.67±0.38	0±0	13.67±0.51
<i>Bacillus subtilis</i>	8.33±0.69	10.67±0.38	16.33±0.38	17±0.33	19.67±0.19	0±0	22±0.67
<i>Candida albicans</i>	7.67±0.19	11±0.33	15.33±0.19	16±0.33	19.67±0.19	0±0	17.67±0.19

Table 4 Anti-microbial Activity of Bark in Aqueous Extract

Microbial Strains	Zone of Inhibition						
	Aqueous Extract					H ₂ O	Tetracycline
	2µl	4 µl	6µl	8µl	10µl	2µl	2µl
<i>Lactobacillus acidophilus</i>	0±0	8±0.33	9.33±0.19	9.67±0.19	12.33±0.19	0±0	17.67±0.19
<i>Streptococcus mutans</i>	7.67±0.19	9±0.33	9.33±0.19	14.67±0.19	16±0.33	0±0	20.33±0.19
<i>Enterococcus faecalis</i>	0±0	7.67±0.19	11±0.33	11.67±0.19	12.67±0.19	0±0	17.33±0.38
<i>Pseudomonas aeruginosa</i>	8.33±0.19	9.67±0.19	12±0.33	14.33±0.19	16±0.33	0±0	12.67±0.19
<i>Bacillus subtilis</i>	7±0.33	7.67±0.19	9.33±0.19	12±0.33	14.67±0.19	0±0	18.33±0.19
<i>Candida albicans</i>	8.33±0.19	9.67±0.19	10.33±0.19	11±0.33	13.33±0.19	0±0	17.67±0.19

4. Conclusion

In the phytochemical screening of bark, all the phytochemicals were present which provide protection to plants. Anti-microbial activity was done against some microbes in different solvents. Maximum zone of inhibition of 26.67 mm was observed against *Bacillus subtilis* in DMSO extract, and minimum of 7 mm was observed against *Streptococcus mutans* and *Bacillus subtilis* in ethanol and aqueous extract respectively. No zone of inhibition was observed in *Lactobacillus acidophilus* and *Enterococcus faecalis* in the aqueous extract.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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