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Phytochemical analysis and anti-microbial activity of aqueous extract of flowers of *Bombax ceiba* L.

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Abstract

Phytochemicals are naturally occurring compounds which is responsible for providing protection to plants against pathogenic attack. *Bombax ceiba* L. belongs to the family Bombacaceae. It is commonly known as the Red-silk cotton tree, Semal, Semul, etc. It is a multipurpose tropical tree providing food, fodder, and timber. This plant also possesses medicinal value. Phytochemical analysis of theaqueous extract of flowers was done to test the presence of phenols, tannins, carbohydrates, etc. Different phytochemicals play different roles. Antimicrobial activity of flower was done in aqueous extract. The extracts inhibit the growth of microbes.

Key words : *Bombax ceiba* L., phytochemicals, Bombacaceae, multipurpose.

Phytochemicals are biologically active chemical compounds occurring naturally in plants. They protect the plants against pathogenic attackand diseases, in addition to these they provide colour, aroma and flavour to plants[12,21].

Bombax ceiba L. is a member of family Bombacaceae. In different parts of the world it has different names; Red silk-cotton tree, Silk-cotton tree or Kapok. In India, it has different names in different languages; Semul or Semal, Kantakdurma, Salmali[15]. The name *Bombax ceiba* L. is derived from two languages; 'Bombyx' meaning silky hair surrounding the seeds; and *ceiba* is a vernacular name from America[17]. The tree is found in almost all parts of the world India, Pakistan, Sri Lanka, Bangladesh, Myanmar, Malaysia, Sumatra, Australia, Thailand[1,3,24].

The tree is about 20-40 m tall. It has a tall, straight, rigid trunk. It is deciduous in winter. The leaves are palmate, entire, with about 6-7 leaflets radiating from a central point, each leaflet is lanceolate in shape. Flowers

bloom from January to March. Flowers are bright-red or crimson-red in colour, large, pentamerous, filaments are stiff. Flowers are stiff and ornithophilus because of the presence of nectar with cup-shaped, thick and fleshy sepals. Fruiting is from April to June. Seeds are numerous, ovoid, black or grey coloured, and covered with white silky cotton hairs[8,14,19].

Its each and every part has medicinal properties and are used for medicine since ancient times. In Ayurveda it is explained that the plant has stimulant, astringent, hemostatic, aphrodisiac, diuretic, antidiarrheal , cardiotonic, antidysenteric, properties[11,16,17,21]. It has anti-diabetic, anti-oxidant, anti-angiogenic, anti-bacterial, anti-pyretic, analgesic activity[9,11]. Flower has a bitter action, acrid, cooling, dry, anti-inflammatory action. It removes bile, and shows good response in leucorrhoea[24].

The anti-microbial activity of flowers of *Bombax ceiba* L. was tested against some pathogenic microbes like *Streptococcus mutans* (gram-positive bacteria), *Bacillus subtilis* (gram-positive bacteria), *Lactobacillus acidophilus* (Gram-positive bacteria), *Enterococcus faecalis* (Gram-positive bacteria), *Pseudomonas areuginosa* (Gram-negative bacteria), and *Candida albicans* (fungus).

Fresh flowers of *Bombax ceiba* L. were collected from Ranchi (23°18'N and 85°17'E), Jharkhand, in the month of January. Cold extraction method was employed for the extract preparation. Dust and dirt were washed off in running tap water. After that the flowers were sun dried for 15-20 days. Dried flowers were then ground into fine powder. In 1:10 (w/v) ratio, powdered flowers (10 gm) were 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.

Phytochemical screening:- For Phytochemical screening, the powder of dried flowers was dissolved in 250 ml distilled water. After 24 hrs the mixture was filtered using Whatsman filter paper No. 1, obtained filtrate was then used for qualitative analysis of different phytochemicals present in the flowers of *Bombax ceiba* L. [5,6,12].

Alkaloids : In 1 ml of aqueous extract 2 ml of Dragendroff's was added. On addition of Dragendroff'sto the extract it gives turbid orange colour, which is an indication of Alkaloids.

Saponins : Two 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 H_2SO_4 was added carefully. Upper layer turned red and

H_2SO_4 layer showed yellow coloured with green fluroscence, 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 H_2SO_4 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 H_2SO_4 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.

Antimicrobial activity : Antimicrobial activity of flowers of *Bombax ceiba* L. was done by both disc-diffusion method as well as well-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. 200µl 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, and for well-diffusion method wells were made with sterilized cork-borer; then samples of varied concentrations were loaded. After that the plates were placed in incubator at 37°Cfor 18-20 hours[4,7].

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.

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

Phytochemical screening: Qualitative phytochemical analysis of flowers of *Bombax ceiba* L. showed that alkaloids, trepenoids, steroids, carbohydrate, glycosides, anthocynaide, phlobatannins, phenol, tannin, saponin, flavonoids (Table-1) were present in the flowers.

Antimicrobial activity :

The current study has shown that anti-microbial activity against of flowers of *Bombax ceiba* L. against some microbial strains. The anti-microbial action was done in aqueous extract, both by disc-diffusio n method and well-diffusion method.

Table-1. Result of Phytochemicals in Flowers 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

Table-2. Anti-microbial activity of Flowers of *Bombax ceiba* L. in Aqueous Extract by D-diffusion Method

Microbial Strains	Zone of Inibition						
	Aqueous Extract					H_2O	Tetracyclin
	2µl	4µl	6µl	8µl	10µl	2µl	2µl
Lactobacillus acidophilus	_	7.33±0.38	8.33±0.69	11.33±0.19	12±0.33	_	16±0.58
Streptococcus mutans	_	7±0.33	9±0.33	9.67±0.19	11±0.33	_	14.67±0.84
Enterococcus faecalis	6.67±0.19	7.67±0.38	8.33±0.19	9.67±0.19	11±0.33	_	17.67±0.19
Pseudomonas aeruginosa	_	_	7±0.33	9±0.33	9.33±0.38	_	13.67±0.51
Bacillus subtilis	7±0.33	7.67±0.19	10.67±0.19	12±0.33	12.33±0.19	_	15.33±0.84
Candida albicans	6.67±0.19	7±0.33	8.67±0.19	12±0.33	14±0.33	_	14.33±0.69

Table-3. Anti-microbial activity of Flowers of *Bombax ceiba* L. in Aqueous Extract by Well-diffusion Method

Microbial Strains	Zone of Inhibition					
	Aqueous Extract				H_2O	Tetracyclin
	5µl	10µl	15µl	20µl	5µl	5µl
Lactobacillus acidophilus	_	_	_	_	_	15±0.33
Streptococcus mutans	14.33±0.19	17±0.33	17.33±0.19	31±0.58	_	30.67±0.69
Enterococcus faecalis	6.67±0.19	7±0.33	7.67±0.19	9.67±0.19	_	26.67±0.51
Pseudomonas aeruginosa	_	_	_	_	_	21.33±0.51
Bacillus subtilis	7±0.33	7.67±0.19	9.67±0.19	15.67±0.19	_	26±0.33
Candida albicans	7.67±0.51	11±0.58	14.67±0.19	17±0.33	_	20.67±0.19

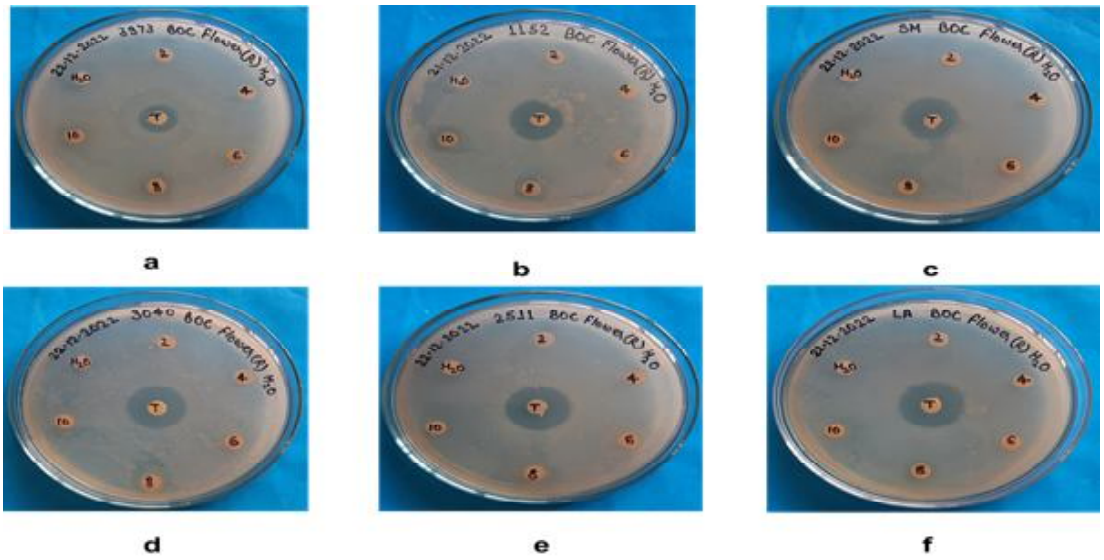

Fig. 1- (a-f) Antimicrobial activity of flowers of *Bombax ceiba* L. by disc-diffusion method:
(a) *Pseudomonas aeruginosa*, (b) *Candida albicans*, (c) *Streptococcus mutans*,
(d) *Enterococcus faecalis*, (e) *Bacillus subtilis*,
(f) *Lactobacillus acidophilus*

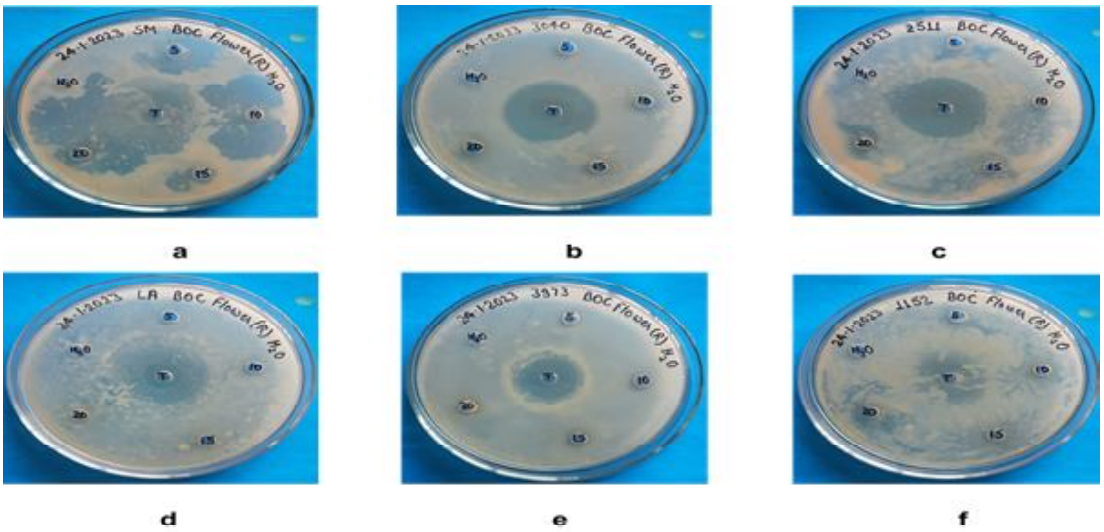

Fig. 2- (a-f) Antimicrobial activity of flowers of *Bombax ceiba* L. by well-diffusion method:
(a*) Streptococcus mutans*, (b) *Enterococcus faecalis*, (c) *Bacillus subtilis*,
(d) *Lactobacillus acidophilus*, (e) *Pseudomonas aeruginosa*,
(f) *Candida albicans*

All the phytochemicals were present in the flowers of *Bombax ceiba* L. which are helpful in protecting the plant against pathogenic attack. In anti-microbial activity, maximum zone of inhibition was observed against *Candida albicans* at 10µl in disc-diffusion method; and in well-diffusion method maximum was observed against *Streptococcus mutans*at 20µl.

From present study it is concluded that flowers of *Bombax ceiba*L. shows antimicrobial property against *Lactobacillus acidophilus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Candida albicans*. The aqueous extract inhibit the growth of said microbes. In disc-diffusion method small concentration was used because of its low absorbing capacity, but in well-diffusion method high concentration was used to test antimicrobial activity. Well-diffusion method showed good result as compared to disc-diffusion method. The phytochemicals present present in the flowers of *Bombax ceiba* L. helps to work against microbes.

The plant *Bombax ceiba* L. shows antimicrobial activity against microbes. In previous studies, *Lactobacillus acidophilus* and *Candida albicans*showed satisfactory results for antimicrobial activity[22].

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