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Research Article

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EVALUATION OF PHYTOCHEMICAL PARAMETERS, ANTIBACTERIAL AND ANTIHELMINTHIC ACTIVITY OF LEAVES AND BARK EXTRACTS OF PLANT BOMBAX CEIBA

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ABSTRACT
Bombax ceiba, commonly known as the red silk-cotton tree, is a large, briefly deciduous tree occurring in warm monsoon forests in southern Asia. Various phytochemical constituents present in different plant parts has been already reported earlier to possess many biological activities, here in this work extraction was performed by mixture of solvent (distilled water: ethanol) as well as the individual solvents (water) and (ethanol). The phytochemical screening of the leaves and barks was found to exhibit the presence of carbohydrates, glycoside, saponin, phytosterol phenol, tannins, flavonoid, proteins and diterpenes and negative result for alkaloids in all the solvent extracts. The ethanolic extract of leaves was subjected to antihelmenthic activity and the result was found to be moderate.

INTRODUCTION

Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agent, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds [1]. *Bombax ceiba,* commonly known as the red silk-cotton tree, is a large, briefly deciduous tree occurring in warm monsoon forests in southern Asia. Easily one of the world's most spectacular flowering trees, it is famous for its large, showy, six-inch flowers with thick, waxy, red petals that densely clothe leafless branch tips in late winter and early spring [2]. *Bombax ceiba* D.C. Family (Bombacaceae) is a tall tree buttressed at the base, widely distributed throughout India, and Ceylon, Malaya, up to 1500 m. Many parts of the plant

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(Root, stem bark, gum, leaf, flower, fruit, seed and heartwood) are mainly used by various tribal communities and forest dwellers for the treatment of wide variety of ailments [3].

Various activities have been reported in almost all parts of *Bombax ceiba*, some of these include hypertensive, antioxidant, hypoglycemic, antipyretic and hepatoprotective. It is used in tradition system of medicine and exhibits diuretic, dysenteric, emetic, diarrhoeal, Wounds, Acne, skin blemish and pigmentation, cold and cough [4]. Quercetin, one of the most important flavonoids is active against various cardio vascular diseases, cancer, tuberculosis, neurological diseases, cataract etc [5].

Though various researches has already been performed on *Bombax ceiba* starting from traditional one to scientific based but still lots of experiments are still under investigation regarding new chemical constituents and their application. So, here one attempt has been made based on those literatures to modify previous research or to develop a new scheme by performing further extraction with suitable solvents or mixture of solvents which may hope to yield expected or new biological activities so far a new protocol can be designed by this research which should meet all the ideal natures of research like consumption of time and resources.

MATERIALS AND METHODS

Plant collection and authentication

The fresh plant parts were collected from the open field located near Dharan-4, LaxmiChowk and the plant was authenticated by Professor Sasinath Jha, Head of Department of Botany, Post Graduate Campus, TU, Biratnagar, Nepal.

Worm Collection and Authentication

The earthworm's adult *Pheritima posthuma* were collected from pond area of Tarahara, Sunsari district and washed with normal saline to remove all fecal matter and was used for the anthelmintic study. The worm was authenticated by zoologist Ganesh Tamang, Sunsari Technical College.

Pharmacognostical studies

The pharmacognostical study of *Bombax ceiba* was performed by naked eye to detect the specific features of the plant, which helps in easy identification and avoidance of adulteration and contamination due to misidentification of plant species.

Macroscopical Analysis

Macroscopical examination was carried out with the naked eye, which gave details concerning the plant aspect, general appearance, colour and odor.

Loss on drying

About 1g of the powdered crude drug was accurately weighed in a tared dish and dried in an oven at 100-105^{o}c.It was cooled in a dessicator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

$$\text{Loss on drying} = \frac{\text{Weight of empty desiccators} + \text{Sample weight} - \text{Weight after drying}}{\text{Sample weight}}$$

Thin layer chromatography

Thin layer chromatography of different extracts was carried out by preparing glass plates using Silica gel slurry (stationary phase). The plates were then dried in hot air oven and a drop of each extract of leaves, roots and rhizomes were placed in different plates and the plates were kept in beaker containing the appropriate solvents(mobile phase) and followed by calculation of R_f value.

Extraction

The plant materials (leaves and bark) after collection was washed with distilled water to remove all fibrous and soil debris and then solar dried for 3 days and leaves were grinded to powder and were cut into pieces and shade dried for one day. Dried sample was crushed into powder by electric blender (electric grinder) and the coarse powder was passed through 40 mess sieve and the fine powder was subjected to Soxhletation by using different polarity solvents. The extract were dried to remove almost all the moisture and solvents and thus the final products were kept in air tight containers and thus stored at 4°C at refrigerator for further study.

Phytochemical screening

The phytochemical screening was performed to identify the main groups of chemical constituents present in different extracts of *Bombax ceiba* by their colour reactions with different reagents. Each extracts was treated to alkaloids (Mayer's test, Wager's test and Hager test), carbohydrates (Molish's test, Benedicts test, Fehlings test), Glycosides,

Saponins, Phytosterols, Phenols, Tannins, Flavonoids, Proteins and Dietpenens tests.

Selection of standard antibiotics

Microorganisms were obtained from Microbiology Laboratory of Sunsari Technical College. Gentamycin was purchased from local market in Dharan (Cipla, Mumbai).The purity of the antibiotic was 99.9% pure.

Dilution and inoculums preparation

The leaf and bark extracts of the plant; was dissolved in sterile distilled water to obtain concentration of 800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml, 25µg/ml respectively. *Staphylococcus aureus* and *Shigella dysentriae* were prepared in nutrient broth medium and at 30°C for 24 h and the stock culture was maintained at 4°C and sub-cultured as needed. Gentamycin 100µg/ml concentration was used for the standard drugs.

Procedure for performing disc diffusion test

Nutrient Agar media was prepared (in accordance with *M.K Lalitha* 2004 with slight modifications) by dissolving 28 gm of media in distilled water by heating in a steam bath the agar was melted out and sterilized by autoclave at 121°C temperature and 15 lb/inch^2 pressure for 15 minutes (final pH 7 at 25°C). The prepared media was sterilized in an autoclave at 121°C for 15 minutes. In this technique, petri dishes of agar are prepared by pouring melted Agar media previously inoculated with selected microorganism. After the solidification of agar cups, they were bored with the help of borer and were filled with solutions of suitable concentrations of sample and standard respectively and were inoculated at 37ºC for 24 hours. The sterile discs were loaded with different concentrations of about 800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml and 50µg/ml, 25µg/ml of plant extract and antibiotic gentamycin into each separate disc of about 100µl. The discs were placed on the medium suitably apart and the plate were incubated at 5˚C for 1 hour to allow good diffusion and then transferred to an incubator at 37˚C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader.

Antihelmintic activity of ethanolic extracts of leaves

Observations were made from the time taken to paralysis and till death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Procedure for antihelmintic activity

Anthelmintic activity of the plant materials was evaluated by exposing the adult *Pheritima posthuma to* different plant extracts. Five Petridishes were used i.e. three for extracts of different concentration to be tested, one for standard (mebendazole) and one for normal as control. Observations were made on the basis of motility/survival of worms. The anthelmintic activity was performed according to the method with slight modifications [6].

On adult Indian earthworm *Pheritima posthuma*as, it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Fifteen groups of approximately equal sized Indian earthworms consisting of Five earthworm of approximately equal size (3-5 cm in length and 0.1-0.2cm in width) Indian earthworms were released into 30 ml each extract of three concentration (15, 25 and 50 mg/ml) prepared in distilled water. These three concentration (15, 25 and 50) mg/ml of each extracts were subjected to anthelmintic activity. Mebendazole of concentration 10 mg/ml has been used as standard concentration dissolve in 6% DMSO solution, distilled water served as negative control.

bservations were made on the basis of time taken for paralysis and death of individual worm. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was confirmed when the worms lost their motility followed by fading away of their body colors.

RESULT AND DISCUSSION

PHARMACOGNOSTICAL STUDIES

Macroscopical studies:

General appearance:

Leaves: palmately compound, 15-30 cm long, petiole 4-8 inches long; leaflets 3-7, lanceolate, acuminate and entirely glabrous, leaflet stalks 0.6-1.6 inches long.

Bark:1.8 -2.5 cm thick, young trees: smooth and greenish gray, older trees: rough, checked, with irregular vertical fissures.

Organoleptic properties

Colour: Leaves are green in colour and barks are gray-white to silver-gray, greenish gray or brown.

***Odor*:** Characteristics

Taste: Slightly bitter

Table 1: Phytochemical tests of leaves

Tests	Aqueous	Ethanol	d/w: ethanol
Alkaloids			
Mayer's test	-	-	-
Wager's test	-	-	-
Hager test	-	-	-
Dragendroff	-	-	-
Carbohydrates			
Molish's test	-	+	+
Benedicts test	-	-	-
Fehlings test	+	+	+
Glycosides			
Borntrager test	+	-	+
Legal	+	+	+
Saponins			
Froath test	+	+	+
Foam test	+	+	+
Phytosterols			
Salkowski's test	+	+	+
Phenols			
Ferric chloride test	+	+	+
Tannins			
Gelatin test	+	_	_
Flavonoids			
Alkaline reagent test	+	_	_
Lead acetate test	+	+	+
Proteins			
Xanthoprotic test	+	+	+
Ninhydrin test	_	+	_
Diterpenens			
Copper acetate test	+	+	+

The preliminary phytochemical screening of the leaves and barks was found to exhibit the positive tests for carbohydrates, glycoside, saponin, phytosterol phenol, tannins, flavonoid, proteins and diterpenes and negative result for alkaloids in all the solvent extracts. Presence of glycosides, saponins, flavonoids, tannins phenolic compounds, proteins, phytosterols, carbohydrates, gums and mucilages, have great potential as antimicrobial activity and can be used in the treatment of infectious diseases caused by resistant microorganisms [7].

Table 2: Phytochemical tests of barks

Tests	Aqueous	Ethanol	d/w ethanol
Alkaloids			
Mayer's test	-	-	-
Wager's test	-	-	-
Hager test	-	-	-
Dragendroff	-	-	-
Carbohydrates			
Molish's test	+	+	-
Benedicts test	+	+	+
Fehlings test	+	-	+
Glycosides			
Borntrager test	-	+	+
Legal	+	+	+
Saponins			
Froath test	+	+	+
Foam test	+	+	+
Phytosterols			
Salkowski's test	+	+	+
Phenols			
Ferric chloride test	+	+	+
Tannins			
Gelatin test	+	+	+
Flavonoids			
Alkaline reagent test	+	-	-
Lead acetate test	-	-	-
Proteins			
Xanthoprotic test	+	+	+
Ninhydrin test	-	-	-
Diterpenens			
Copper acetate test	+	+	+

Table 3: % Loss on Drying

Extract	Leaves	Barks
% LOD	12	14

Table 4: R_f value of leaves extracts:

Solvents	R_f (Leaves)	R_f (Bark extracts)
Distilled water	0.68	0.53
Ethanol	0.7	0.85
d/w : ethanol	0.73	0.81

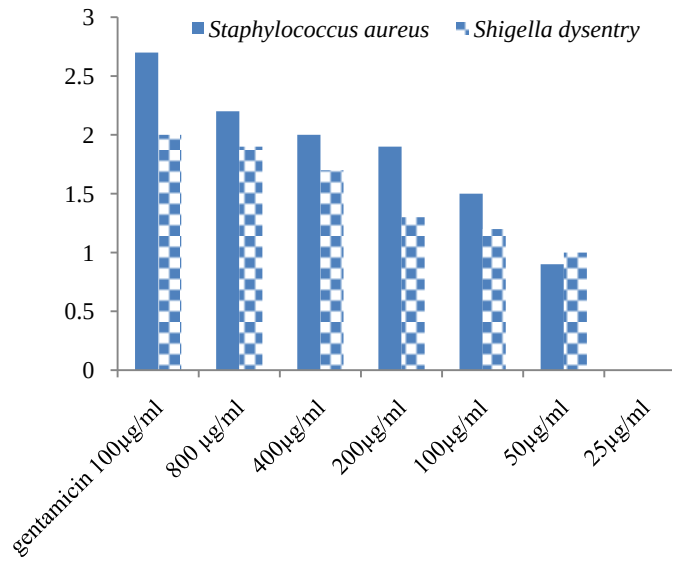

Figure 1: zone of inhibition (mm) of aqueous Leaves extract in different concentrations Vs standard

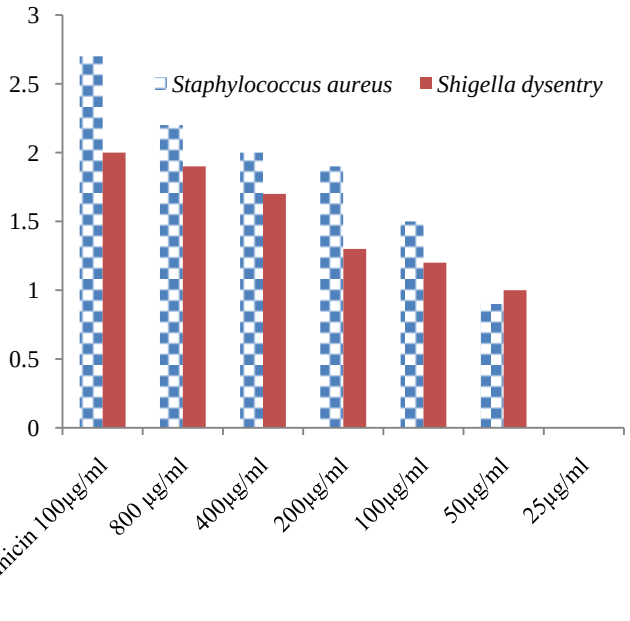

Figure 2: zone of inhibition(mm) of aqueous bark extract in different concentrations Vs standard

Here the study revealed that *Bombax ceiba* is active against both gram positive (*Staphylococcus aureus*) and gram negative bacteria *Shigella dysentariae*. all the extracts exhibited moderate activities in different dilutions against both the strains of bacteria. Phytoconstituents such as saponins, phenolic compounds has been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections [8].

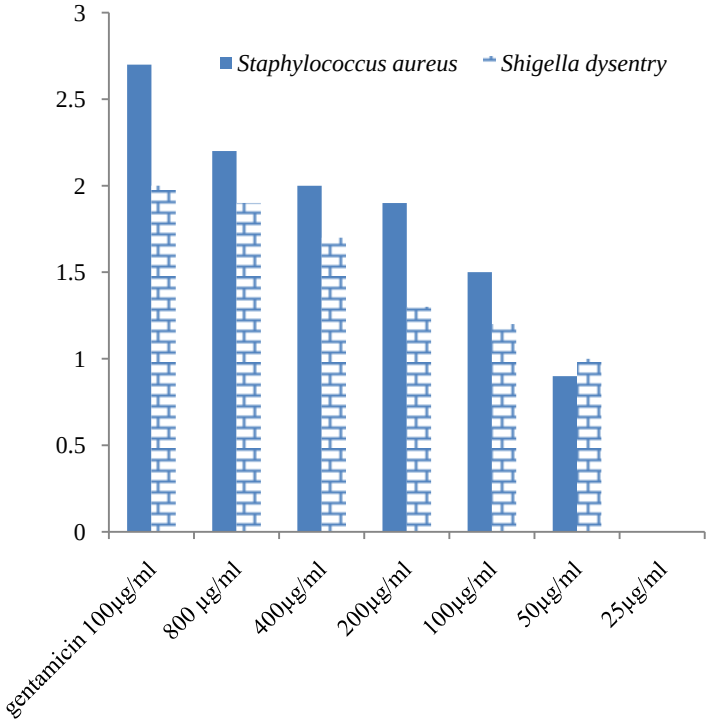

Figure 3: zone of inhibition (mm) of ethanolic Leaves extract in different concentrations Vs standard

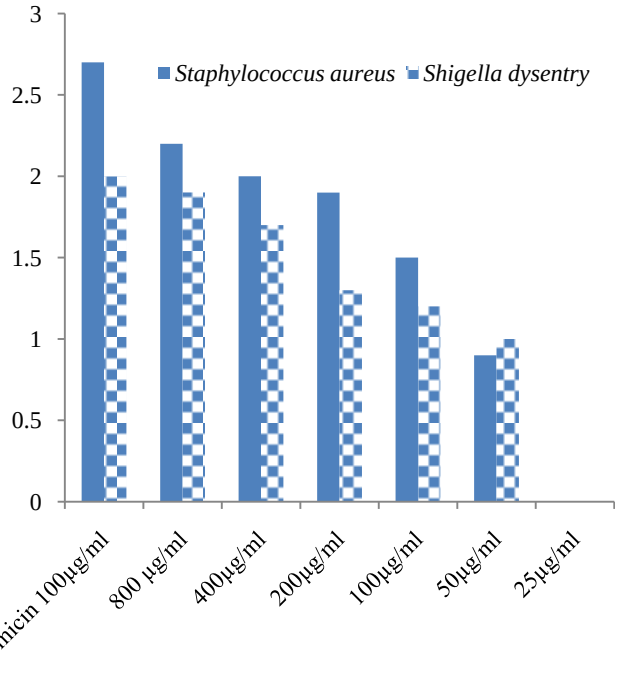

Figure 4: zone of inhibition (mm) of ethanolic Barks extract in different concentrations Vs standard

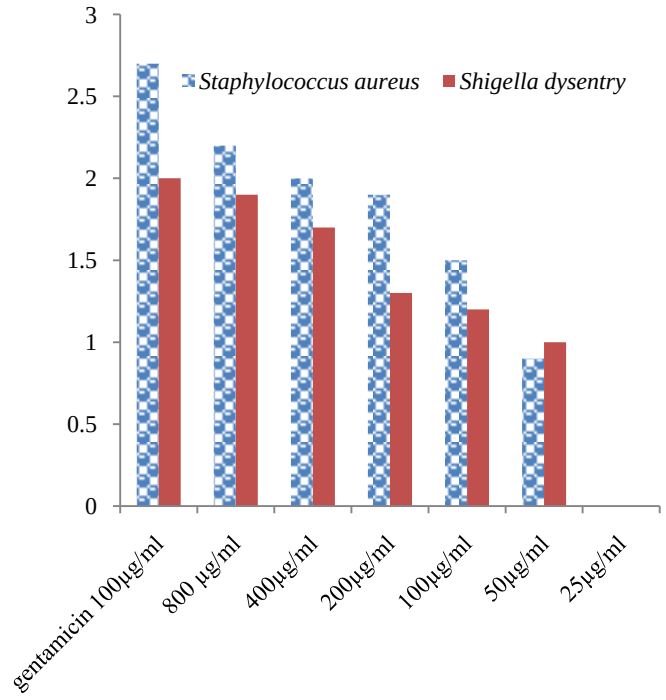

Figure 5: zone of inhibition(mm) of D/W: ethanolic Leaves extract in different concentrations Vs Standard

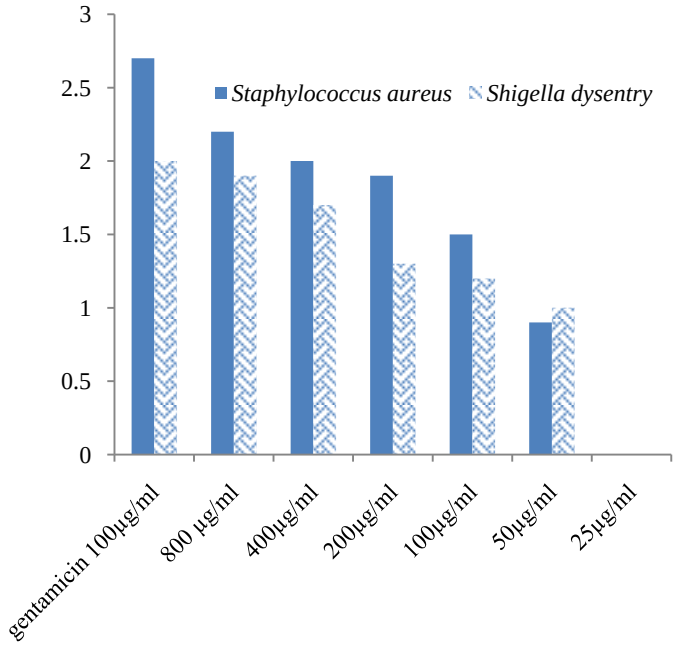

Figure 6: zone of inhibition (mm)D/W: ethanolic Barks extract in different concentrations Vs standard

The antihelmenthic activity was found to be moderate in ethanolic leaves extract. Polyphenolic compounds possess anthelmintic activity[9]. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol interfere with energy generation in helminth parasites. Antihelmenthic activities of other solvent extracts like distilled water and mixture distilled water and ethanol were carried out earlier by other researcher, so here only ethanolic extracts was subjected to study.

Table 6: Antihelmintic activity of ethanolic leaves extracts

Plant extracts	Antihelmintic activity		
	Concentration (mg/ml)	Paralyzing time(min)	Death time(min)
Ethanolic Leaves	15mg/ml	27	85
	25mg/ml	25	80
	50mg/ml	18	70
Mebendazole (Standard)	10mg/ml	5	20
Blank (6% DMSO)	-	-	-

CONCLUSION

This study developed certain standard methodologies for proper identification of isolated plant parts and adulteration. Using different solvents different yield values were obtained, alcoholic extract of both leaves and barks produced highest yield value followed by distilled water: ethanol extract and lastly by distilled water extract. Glycosides, Carbohydrates, proteins, saponin and phytosterol, polyphenol were found to be active phytoconstituents in all different extract of leaves and barks of the same plant, all extract were devoid of alkaloids.

The antibacterial activity of the distilled water: ethanol extract showed higher activity against *Staphylococcus aureus* as compared with other followed by aqueous bark extract against *Shigella dysentery*. The activity was increased with increased concentration of the extracts but less than the standard. The study hopefully provides some important informations for considering the plants for further works.

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Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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