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Pharmacognostic and pharmacological studies of *Bombax ceiba* thorn extract

[Estudios farmacognóstico y farmacológico del extracto de espina de *Bombax ceiba*]

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Abstract

Context: *Bombax ceiba* is a large deciduous tree found in tropical and sub-tropical regions of Asia, Africa, and Australia. Traditional systems of medicine such as Ayurveda, Siddha and Unani have been highlighted the use of *B. ceiba* parts (bark, leaves, and flower) for the treatment of numerous ailments like algesia, hepatotoxicity, hypertension, HIV infections, fever, dysentery, inflammation, catarrhal affection, ulcer, acne, gynecological disorders, piles and urinary infections. However, no scientific pharmacognostic, phytochemical and pharmacological study has been reported for *B. ceiba* thorn.

Aims: To study the pharmacognostic and pharmacological potentials of *B. ceiba* thorn extract.

Methods: The physicochemical properties were determined using pharmacopoeial tests. The phytochemical screening was carried out using standard protocols. The *in vitro* anti-inflammatory activity was performed by human red blood cells stabilization method, *in vitro* anthelmintic activity by *Pheretima posthuma* method, *in vitro* antioxidant activity by DPPH scavenging method, and anti-microbial studies by agar streak dilution method against bacteria *E. coli, B. subtilis*, *K. pneumonia*, and fungi *C. albicans* and *A. niger*.

Results: The hydroalcoholic thorn extract of *Bombax ceiba* (TEBC) showed significant anti-inflammatory (46.9% stabilization), anthelmintic (death at 59.22 min), *in vitro* anti-oxidant (41.62% inhibition), and anti-microbial activities (*B. subtilis* > *E. coli* > *K. pneumonia*; *A. niger* > *C. albicans*).

Conclusions: The study revealed the physicochemical, photochemical and pharmacognostic features of thorn of the *B. ceiba*. The study also revealed the possession of different pharmacological potentials of extract.

Keywords: anthelmintic; anti-inflammatory; antimicrobial; *Bombax ceiba*; pharmacognosy; thorn extract.

Resumen

Contexto: *Bombax ceiba* es un árbol de hoja caduca que se encuentra en las regiones tropicales y subtropicales de Asia, África, y Australia. Los sistemas tradicionales de la medicina como Ayurveda, Siddha y Unani han puesto de relieve el uso de las partes de *B. ceiba* (corteza, hojas y flores) para el tratamiento de numerosas enfermedades como la sensibilidad dolorosa, hepatotoxicidad, hipertensión, infecciones por VIH, fiebre, disentería, inflamación, catarros, úlceras, acné, trastornos ginecológicos, hemorroides y las infecciones urinarias. Sin embargo, no hay estudios científicos farmacognósticos, fitoquímicos y farmacológicos sobre la espina de *B. ceiba*.

Objetivos: Estudiar las potencialidades farmacognósticas y farmacológicas de extracto de espina de *B. ceiba*.

Métodos: Las propiedades fisicoquímicas se determinaron mediante pruebas reportadas en la farmacopea. El tamizaje fitoquímico se llevó a cabo utilizando protocolos estándar. La actividad anti- inflamatoria *in vitro* se realizó mediante el método de estabilización de glóbulos rojos humanos, la actividad antihelmíntica *in vitro* por el método de *Pheretima posthuma*, la actividad anti-oxidante *in vitro* por el método de secuestro de DPPH y los estudios anti-microbianos por el método de dilución en agar contra las bacterias *E. coli*, *B. subtilis*, *K. neumonía* y hongos *C. albicans* y *A. niger*.

Resultados: El extracto hidroalcohólico de espina de *Bombax ceiba* (TEBC) mostró significativa actividad anti- inflamatoria (46.9% de estabilización), vermífuga (muerte en 59.22 min), anti-oxidante (41.62% de inhibición) y anti-microbiana (*B. subtilis* > *E. coli* > *K. pneumonia*; *A. niger* > *C. albicans*).

Conclusiones: El studio reveló las características fisicoquímicas, fitoquímicas y farmacognósticas de la espina de *B. ceiba*. También reveló las potencialidades farmacológicas del extracto.

Palabras Clave: anti-inflamatorio; antimicrobiano; *Bombax ceiba*; extracto de la espina; farmacognosia; vermífugo.

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INTRODUCTION

Bombax ceiba (Bombacaceae), known as the silk cotton tree is a large deciduous tree found throughout India, tropical and sub-tropical regions of Asia, Africa and Australia (Chakraborty et al., 2010). It is an important medicinal plant of tropical and subtropical India and is well known for its traditional importance in society for centuries. Its medicinal usage has been reported in the traditional systems of medicine such as Ayurveda, Siddha and Unani where its potential have been highlighted for the treatment of numerous ailments like inflammation, microbial infections, algesia, hepatotoxicity, hypertension, angiogenesis, HIV, fever, dysentery, catarrhal affection, ulceration of the bladder, acne, gynecological disorders, piles and urinary infections (Jain et al., 2009; Chaudhary and Khadabadi, 2012). Its roots and flowers are regarded as having diuretic, laxative, tonic and restorative properties, while the leaves are reported to have application in the treatment of skin eruptions. The tender bark is used as famine food, demulcent, emetic and tonic, and its aqueous extract mixed with curd to check blood-dysentery (Jain and Verma, 2012). A variety of chemical components has been reported in *B. ceiba*. Ceibanaphthaquinone, isohemigossylic acid lactone-2-methyl ether, anthocyanin, shamimicin, lupeol, quercetin, hentriacontane, 7-hydroxycadalene, quercetin, rutin, quercetin-3-O-β-D-glucopyranoside, vicenin, fraxetin, quercetin-3-O-β-D-glucurono-pyranoside, sexangularetin-3-O-sophoroside, vitexin, isovitexin, kaempferol-3-O-rutinoside, kaemp-ferol-3-O-β-D-glucuronopyranoside, scopolin, iso-mangiferin, mangiferin, 7-O-methyl mangiferin, esculetin, scopoletin, blumenol C glucopyranoside, benzyl-β-D-glucopyranoside, phenylethyl rutinoside, protocatechuic acid, chlorogenic acid, methyl chlorogenate and vanillic acid have been the primary components present in plant (particularly; root, bark, stem, and flower) (Said et al., 2011; Chaudhary and Khadabadi, 2012; Joshi et al., 2013; Refaat et al., 2013).

Thorns or spines are small, sharp structural outgrowth of the epidermis or bark in plants, which either have physiological or anatomical advantages. In xerophytic plants, they are simply the modified leaves to prevent water loss and conserve water for an extended time in dry and harsh conditions. Apart from these advantages, protection of plant from grazing animals is a crucial role played by these thorny structures. Literature has highlighted these structures as 'horny' with no major studies conducted for exploring its significance. A thorough literature survey performed using several databases, like Traditional Knowledge Digital Library (India), Traditional Chinese Medicine Database (Taiwan), Natural Products Alert (NAPRALERT), Dictionary of Natural Products (CRCnetBase) and many other scientific databases on pharmacognostic and pharmacological importance of thorn of *B. ceiba*, has concluded that no significant scientific study has been reported yet. Dangi et al. (2014) and Shantha et al. (2009) have only mentioned some preliminary and very basic works on these thorns, which are moreover quite primitive. Therefore, the previously reported data encouraged to further investigate comprehensively and emphasize further on the pharmacognostic features and pharmacological potentials of these thorns.

This research explores (a) the pharmacognostic features (transverse section microscopy, powder characteristics, phytochemical screening) of *B. ceiba* thorns; (b) pharmacological activities like *in vitro* anti-inflammatory, *in vitro* anthelmintic, *in vitro* antioxidant, and anti-microbial studies of hydro-alcoholic thorn extract; and (c) to justify the traditional importance of *B. ceiba* in society using scientific approach.

MATERIAL AND METHODS

Instrumentation

Spectroscopic analysis was carried out using double-beam Shimadzu® Ultraviolet-Visible Spectrophotometer (Model UV-1800, Kyoto, Japan) connected to a computer having a spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm path length matched quartz cells was used. All weighing were performed using Shimadzu® electronic balance (Model AUW220D, Kyoto, Japan). Sonication was performed using Transonic Digital S (Sonicator), USA. Microscopy was performed using trinocular microscope CosLab®HL-24(B) equipped with Scope Image 9.0 software for recording.

Chemicals

All reagents, consumables, and chemicals for evaluation were purchased from Sigma-Aldrich (Germany) and HiMedia (India) through a local vendor at Nagpur. Double distilled water apparatus (Borosil®, India) was used for the experiment.

Collection of plant material

The thorns of *Bombax ceiba* were collected from the tree present at the medicinal plant garden of Kamla Nehru College of Pharmacy situated in Butibori area of Nagpur city in the Maharashtra state of India. The plant was authenticated (No. 9754) by Dr. Dongarwar, Department of Botany, Nagpur University, Nagpur, Maharashtra.

Preparation of extract

The thorns were collected from the tree, dried in the shade for a specified period, and powdered suitably. The dried powder (100 g, divided into multiple smaller amounts) was subjected to continuous hot Soxhlet extraction with 50 mL distilled water and 50 mL alcohol (ethanol 90%) in equal ratio at a temperature of 55-65°C during 32 cycles. The solvent was removed under reduced pressure and controlled temperature using a rotary vacuum evaporator. The hydroalcoholic thorn extract of *Bombax ceiba* (TEBC) yield was found to be 11.8% w/w.

Pharmacognostic evaluations

The powder form of thorns of *Bombax ceiba* was evaluated regarding organoleptic, physicochemical, histological and phytochemical aspects. In organoleptic part, the color, shape, size, texture and fracture were studied suitably. The physiochemical parameters like a loss on drying (LOD) at 105°C, total ash content, acid insoluble ash, water soluble ash and alcohol soluble extractive value were determined as per methods given in Indian Pharmacopoeia (2010). The LOD determination is highly significant since any excess of water in plant materials will promote bacterial growth, the presence of molds and cause deterioration by hydrolytic activity. The total ash value serves as an indicator for determination of chalk powder, earthy silica materials lime or other earthy matter. Acid insoluble ash is used to detect excessive earthy materials, which has a varying amount of calcium oxalate crystals in the cells while water-soluble ash is used to detect the presence of material exhausted by water. Alcohol soluble extractive values are indicative of the presence of the adulterants, defective processing and poor quality of the drug. Powder characteristic studies like bulk density and tapped density were performed according to the procedures specified in USP Pharmacopoeia (2010). The transverse section (TS) of thorn was subjected to histological identifications under a trinocular microscope at 30x resolution. The section was stained with concentrated sulfuric acid and phloroglucinol. The powder microscopy was performed by duly staining with and observed using trinocular microscope at 10x resolution. All-important features were detected and recorded suitably.

Phytochemical evaluation

Phytochemical screening of the TEBC was executed for the presence of sugars, alkaloids, glycosides, tannins, flavonoids, steroids, proteins and terpenes as per the given standard test procedures (Trease and Evans, 2008).

Alkaloids

Hager's test: Saturated solution of picric acid was added to TEBC (10 mg/mL), the formation of yellow precipitate indicates the presence of alkaloids.

Flavonoids

Shinoda's test: Few magnesium turnings and concentrated HCl was added dropwise to TEBC (10 mg/mL), appearance of a pink scarlet or crimson red color after few minutes confirmed the presence of flavonoids.

Carbohydrate

Fehling's test: Two mL of TEBC was mixed with equal volumes of Fehling A and Fehling B in different tubes and boiled for few minutes. Both the contents were mixed as they attain nearly the boiling point. The appearance of brownish-red precipitate formation indicated the presence of carbohydrates.

Cardiac glycoside

Legal's test: To TEBC (10 mg/mL), pyridine and alkaline sodium nitroprusside solution were added. An appearance of blood red color signified the presence of cardiac glycoside, but no blood red color appeared reflecting complete absence of cardiac glycoside.

Anthraquinone glycoside

Borntrager's test: The TEBC (10 mg/mL) was boiled with 1 mL of sulphuric acid in a test tube for 5 minutes and filtered while hot. The filtrate was cooled and shaken with an equal volume of dichloromethane. The lower layer of dichloromethane was separated and shaken with the half of its volume of dilute ammonia. A rose pink to red color was produced in the ammonia layer and indicated the presence of anthraquinone glycoside.

Tannin

Gelatin test: To the TEBC (10 mg/mL), 1% gelatin solution containing 10% NaCl was added, formation of buff-colored precipitate resulted due to the presence of tannins.

Saponin

Froth formation test: Two mL of TEBC was taken in a test tube and shaken until a stable froth or foam was formed for 5 minutes (in presence of saponin), however, no foam was formed for 5 minutes indicating the absence of saponin in TEBC.

Steroid

Libermann-Burchard's test: The TEBC (10 mg/mL) was treated with 7-8 drops of acetic anhydride solution, boiled, and cooled further. Concentrated sulphuric acid (5-6 drops) was further added from the side of the test tube, where a brown ring was formed at the junction of both layers; and upper layer changed to green, which demonstrated the presence of steroids.

Protein

Xanthoproteic test: To the extract (10 mg/mL), 1 mL of concentrated nitric acid was added and boiled to get a yellow precipitate, which after cooling, were added 2 mL of 40% sodium hydroxide solution, orange color appears (if protein is present). No orange color was formed with TEBC indicating absence of protein.

Phenol

Ferric trichloride: The extract (10 mg/mL) was dissolved in water, and 8-10 drops of dilute ferric trichloride were added, the formation of bluish-black color indicated the presence of phenol.

Diterpene

Copper acetate test: The TEBC (10 mg/mL) was treated with 3-4 drops of copper acetate solution, emerald green color appeared (in presence of diterpene). In TEBC, no emerald green color appeared, which confirmed the absence of diterpenes.

Triterpene

Salkowski's test: The TEBC (10 mg/mL) was treated with 5-6 drops of concentrated sulphuric acid, yellow color formation occurs in the lower layer (if triterpene is present), however no yellow color was formed describing absence of triterpene.

In vitro anti-inflammatory activity

Anti-inflammatory activity of TEBC was studied using *in vitro* method as described by Kar et al. (2012). This method is based on the fact that lysosomal enzymes released during inflammation, causes some disarray. Out of them, acute inflammation is the primary disorders results due to their extracellular function. By inhibiting these chemical mediators or by stabilizing the lysosomal membrane, the potential of experimental compounds is determined. Since human red blood cell membranes are similar to those of lysosomal membrane component, the prevention of hypotonicity-induced human red blood cells (HRBC) membrane lysis was taken as a measure in estimating anti-inflammatory property.

During this experiment, blood was suitably collected from a healthy individual who had not taken any anti-inflammatory drug for last 15 days. An equal volume of Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride) was mixed, and the content was

centrifuged at 3000 rpm. The plasma was separated carefully and preserved. The packed blood corpuscles were washed with saline solution (0.9%) and 10% suspension was prepared. Aliquots of plant extract were prepared using distilled water for concentrations 250, 500 and 1000 µg/mL. To each concentration, 1 mL phosphate buffer, 2 mL 0.18% saline (hyposaline) and 0.5 mL HRBC suspension were added. The above contents were incubated at 37°C for 30 min and were further centrifuged at 3000 rpm for 20 min. The hemoglobin content present in the supernatant solution was estimated spectrophotometrically at 560 nm using diclofenac sodium as the reference standard. A control was also prepared exempting the extract. The percentage hemolysis was calculated on the assumption that the control group would exhibit 100% hemolysis. The percentage of HRBC membrane stabilization by plant extract was calculated by using the formula:

$$\%\,Protection = 100 - \frac{\text{OD of Drug treated sample}}{\text{OD of Control}} \text{ x } 100$$

In vitro anthelmintic activity

The *in vitro* anthelmintic activity of TEBC was carried out according to the method by Manke et al. (2015) in Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline. Easy availability, anatomical and physiological resemblance with the intestinal roundworm parasite *Ascaris lumbricoides* of human beings made them to be used widely for the initial evaluation of the anthelmintic activity. Five groups of approximately equal size worms consisting of six earthworms individuals in each group were released in 10 mL of the desired concentration of the drug. The anthelmintic assay was carried out as per the method with minor modification. The experiment was designed into five groups, each containing six earthworms. Different concentration of extracts and the standard drug solution were poured into various Petri dishes. Observations were made for the time taken for paralysis, which may be defined as the condition when worm did not revive in normal saline. The time for the death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). For evaluation of anthelmintic activity of *Bombax ceiba* thorn extract, a group I was the control; group II received the standard drug (albendazole, 20 mg/mL); group III, IV and V received thorn extracts of *Bombax ceiba* at doses of 10, 15 and 20 mg/mL, respectively. All the results were expressed as mean ± SD of six worms in each group.

In vitro antioxidant capacity

1,1-diphenyl-2-picrylhydrazyl radical scavenging capacity

The potential of extract to scavenging the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was investigated according to Conforti et al. (2008). A stock solution of whole plant extract was prepared to the concentration of 1 mg/mL. The TEBC (100 µg/mL) was added at an equal quantity to a methanolic solution of DPPH (0.1 mM). The aliquot was incubated for 30 minutes at room temperature. The absorbance was recorded at 517 nm keeping ascorbic acid as a standard control.

In vitro *reducing capacity*

The reducing power of TEBC was determined according to the procedure described by Doughari et al. (2012). The experiment was carried out in triplicate. The plant extract (100 µg/mL) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 7.4) and 2.5 mL of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 minutes. After incubation, 2.5 mL of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. The increased absorbance of the reaction mixture indicated increased reducing power.

In vitro antimicrobial activity

The *in vitro* antibacterial activity of TEBC was evaluated against various pathogenic bacterial strains viz. *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumoniae*. Similarly, *in vitro* antifungal activity of TEBC was also evaluated against fungal strains viz. *Candida albicans* and *Aspergillus niger*.

The minimum inhibitory concentration (MIC) values were determined by comparison of different compounds with ciprofloxacin (anti-bacterial) and fluconazole (anti-fungal) as reference drugs.

Antibacterial activity

The *in vitro* antibacterial activity of TEBC was tested by disc diffusion method (Bharti et al., 2010b) under standard conditions using the Muller Hinton Agar medium. The test organisms were first cultured in nutrient broth, incubated for 24 h at 37°C, and then freshly prepared bacterial cells were spread onto the Muller Hinton agar plates in a laminar flow cabinet. The extract was dissolved in dimethylsulfoxide (DMSO) and soaked onto sterile discs of Whatman filter paper No. 1 (6 mm diameter). The discs were then placed onto the surface of the previously prepared bacterial plates and incubated. After 24 h of incubation at 37°C, the diameter of the zone of inhibition was measured for extract in mm. The activity was compared with standard antibiotic ciprofloxacin (positive control) and a disc impregnated with DMSO was used as a negative control. The tests were conducted for three times.

Antifungal activity

The *in vitro* antifungal activity of TEBC was tested by disc diffusion method (Bharti et al., 2010b) under standard conditions using Potato dextrose agar medium. Sterile discs of Whatman filter paper No. 1 (6 mm diameter) containing specific amounts of an antifungal agent fluconazole (50 µg/mL) and TEBC (100 µg/mL) were placed on the surface of an agar plate inoculated with a standardized suspension of the microorganisms tested. The plates were incubated at 28 ± 2°C for 72 h for evaluating antifungal activity. A paper disc impregnated with DMSO was utilized as a negative control.

Determination of the minimal inhibitory concentration (MIC)

The MIC of the TEBC was determined by agar streak dilution method (Bharti et al., 2010a). A stock solution of TEBC (100 µg/mL) in DMSO was prepared, and graded quantities of tested components were incorporated in a specified amount of sterile molten agar (Muller Hinton agar). A specified quantity of the medium (40-50°C) containing the extract was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. The microbial suspension was prepared (10^5 CFU/mL) and applied to plates with extract in DMSO to be tested and incubated at 37°C for 24 h. At the end of the incubation period, the MIC values were determined. All determinations were done in triplicates, and the average was taken as the final reading. The standard antibiotic, ciprofloxacin (100 µg/mL) used as positive control and 100 mL of DMSO used as a negative control. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

Statistical analysis

All experiments were carried out in a triplicate manner. The obtained data were expressed as mean ± standard deviation (SD). For statistical calculations, Minitab® ver. 17 was employed. For pharmacological activities, the unpaired Student t-test (two-tailed) was used to determine the difference between control and tested groups.

RESULTS

Physicochemical properties

The thorns were found to be pale ash to grey-brown color, 18 to 26 mm in dimension, conical shaped with a rough texture. The physicochemical studies showed that the loss on drying was found to be within pharmacopoeial specifications, i.e. 0.43%, which signifies that it has low water content and is merely free from any spoilage, browning, mold growth, which the water causes specifically. The ash values (total, acid insoluble and water soluble) were found to be 15.13, 1.98 and 6.56% w/w, respectively, which indicated that the impurities were in pharmacopoeial range. The alcohol soluble extractive value was found to be 9.82, which implied that the bulk and tapped densities were found to be 0.156 and 0.273, respectively, which represents that the powder has a very fine capillary network with a large number of inter- and intra-particulate spaces

and with high % compressibility index of 42.85. The physicochemical characteristics are given in Table 1.

Pharmacognostic analysis

The transverse section of thorns displayed cork cambium, cortex, stone cells, starch grains and parenchymatous cells. Brick or polygonal cork cells, impregnated with layer and suberin were observed. The phloem cells were found to be mostly parenchymatous in nature. Prominent polygonal stone cells were found in thorns, which are sclerenchymatous cells that are modified to provide mechanical strength. The cortex was found to be composed of thick-walled rigid and strongly lignified cells, which were isodiametric or polyhedral in appearance. The presence of brown-reddish brown appearance is due to the presence of high tannin content in thorn. Starch grains of variable sizes were detected. The thick walled parenchymatous cells were frequently observed in xylem portion of transverse section (Fig. 1).

The powder microscopy displayed a few very significant characteristics. Uni- and multi-seriate (1-11 seriate), heterogeneous rays composed of procumbent cells, sheath cells were observed. Abundant tiny starch grains of various sizes and non-separated fibers were found. Fibers were arranged alternately with narrowly banded or diffuse into aggregates located in parenchyma strands. Axial parenchyma, mostly apotracheal were abundant, but indistinct to naked eyes were arranged alternately with fibers. Single row of upright cells has often been present (Fig. 2).

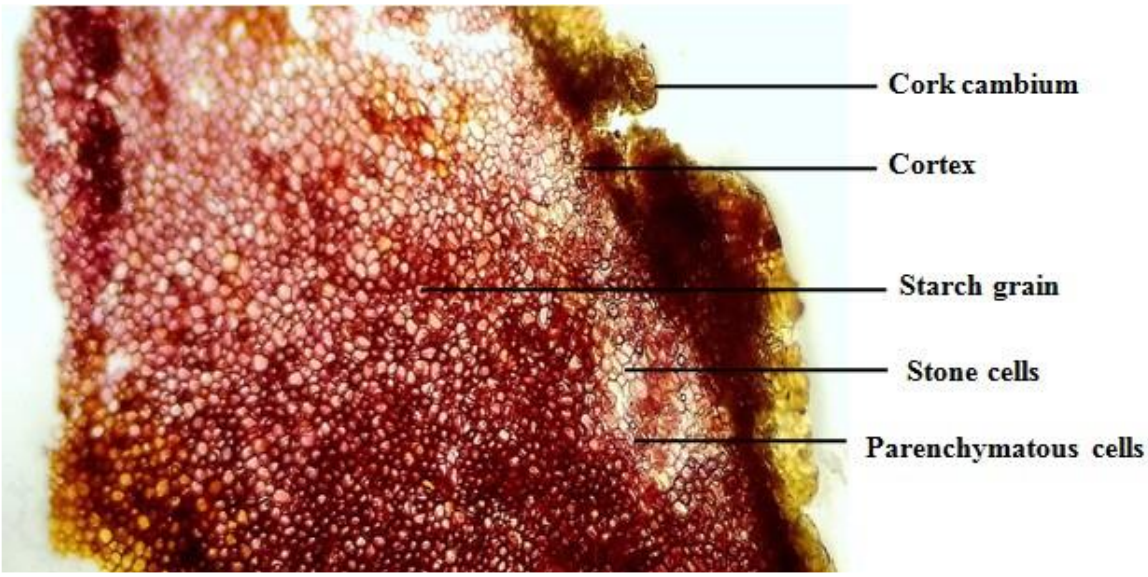

Figure 1. Transverse section of *B. ceiba* thorn.

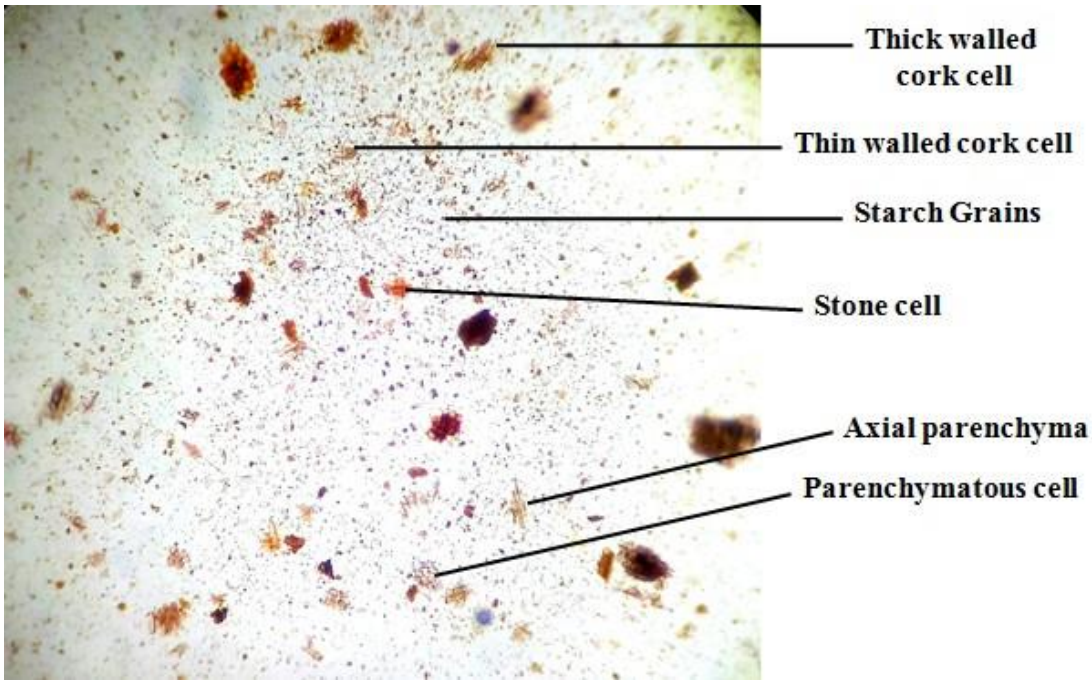

Figure 2. Powder microscopy of *B. ceiba* thorn.

Table 1. Physicochemical properties of *B. ceiba* thorn.

Parameters	Description
Color	Pale ash to grey-brown
Shape	Conical
Size	18 – 26 mm
Texture	Rough
Loss on drying (105°C) (%)	0.43
Total ash content (% w/w)	15.13
Acid insoluble ash (% w/w)	1.98
Water soluble ash (% w/w)	6.56
Alcohol soluble extractive value	9.82
Bulk density	0.156
Tapped density	0.273
% compressibility index	42.85

Phytochemical analysis

Phytochemical screening of the extract revealed the presence of carbohydrates, tannins, flavonoids, alkaloids, sterols, phenol, and glycosides (Table 2).

Pharmacology

In vitro anti-inflammatory activity showed that TEBC exhibited significant activity when compared to the standard diclofenac sodium. The percentage of HRBC membrane stabilization/protection offered was found to be 44.7% and 46.9% at concentrations of 500 and 1000 µg/mL of extract. The activity of the extract was observed to be concentration dependent where with increasing concentrations, the activity increased linearly. The inhibition of hypotonicity and heat induced red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity. These results may be due to the presence of phenolic and flavonoid content, which exhibited good antioxidant properties. Table 3 describes the *in vitro* anti-inflammatory potential of the extract.

The TEBC produced a dose-dependent paralysis, causing loss of motility at initial stage followed by loss of response to stimuli, and ultimately leading to the death of earthworms. The anthelmintic activity has been illustrated in Table 4. The TEBC at 10, 15 and 20 mg/mL concentrations demonstrated paralysis time of 46.16, 39.74, 33.91 min and death time of 87.54, 72.46, 59.22 min, respectively. The albendazole treated group at 10 mg/mL exhibited the paralysis time of 18.33 min and death time of 21.67 min. In the control group (normal saline), worms were observed for 24 h and no paralysis or death was found.

An anti-oxidant activity was shown by the TEBC by its potential in scavenging DPPH radical (41.62%). The reducing power of extract is based on the potential to reduce iron from ferric (Fe^{3+}) to ferrous form (Fe^{2+}), which was demonstrated by the intensity of absorbance. The thorn extract displayed an absorbance of 0.493, which was less than that of absorbance shown by ascorbic acid ($p<0.001$).The antioxidant potentials of the extract are depicted in Table 5.

The results of antibacterial and antifungal screening of TEBC are shown in Table 6. TEBC showed moderate anti-microbial activity against *E. coli*, *B. subtilis*, *K. pneumonia*, *C. albicans* and *A. niger*. TEBC displayed highest anti-bacterial activity against *B. subtilis* with a zone of inhibition (ZOI) 16.6 mm with MIC of 12.5 µg/mL, respectively. However, it was lower as compared to standard ciprofloxacin, which exhibited 26.6 mm at MIC value of 6.25 µg/mL. In contrast, TEBC exhibited poor anti-microbial activity against *K. pneumonia* with ZOI of 11.3 mm at MIC of 50 µg/mL. The extract also expressed a moderate activity against *E. coli* with ZOI of 13.6 mm at MIC of 12.5 µg/mL. In the case of evaluation of anti-fungal potentials, significant growth inhibition was presented by the thorn extract against *C. albicans* and *A. niger*. The ZOIs were found to be 18.3 mm in the case of *C. albicans* at MIC value of 25 µg/mL and 17.3 mm for *A. niger* at MIC 12.5 µg/mL. The extract presented higher activity against *A. niger* than *C. albicans*, although the activity is quite low as compared to fluconazole (standard), which showed 31.6 mm zone of inhibition.

DISCUSSION

In previous literature, the pharmacognostic studies on the transverse section of thorn have stated the presence of cork cells, phelloderm, periderm, sclerides, medullary rays and phloem fibers (Shantha et al., 2009; Dangi et al., 2014). The current study highlighted the presence of thick-walled cortex having strongly lignified isodiametric cells; thick-walled parenchymatous cells were observed in phloem and xylem portions with high tannin content are prominent features detected in microscopy. Additionally, polygonal cork cells impregnated within a layer of suberin, sclerenchymatous stone cells and various size starch grains (majority round in shape) are few other feature detected. Similarly, the previously reported powder microscopy has described the occurrence of starch cells and tannin containing parenchymatous cells (Shantha et al., 2009). In this study, the powder microscopy have detected the occurrence of axial parenchyma and sheath cells, uni- and multiseriate heterogeneous rays of procumbent cells, a single row of upright cells, and narrowly banded to non-separated fibers were found arranged alternately with the parenchyma strands.

Table 2. Phytochemical profile of hydroalcoholic extracts of *B. ceiba* thorns.

Chemical constituent	Test performed	Observations	Inference
Alkaloid	Hager's test	Yellow precipitate	Present
Flavonoid	Shinoda's test	Pinkish-red appeared	Present
Tannin	Gelatin test	Green color appeared	Present
Anthraquinone glycoside	Borntrager's test	Faint pink color observed	Present
Cardiac glycoside	Legal's test	No red color observed	Absent
Saponin	Froth formation test	A small height froth formed for 5 min	Absent
Carbohydrate	Fehling's test	Red precipitate	Present
Phenol	$FeCl_3$ test	Bluish-black color observed	Present
Protein	Xanthoproteic test	No yellow color observed	Absent
Sterol	Libermann-Burchard's test	Brown-ring formation	Present
Diterpene	Copper acetate test	No emerald green color observed	Absent
Triterpene	Salkowski's test	No yellow color observed	Absent

Table 3. *In vitro* anti-inflammatory activity of hydroalcoholic thorn extract of *Bombax ceiba* (TEBC) on the stabilization of human red blood cells membrane.

Treatment	Concentration (µg/mL)	Absorbance (560 nm)	% protection[#]
Control	-	0.482 ± 0.001	-
TEBC	250	0.311 ± 0.002***[a]	36.8
	500	0.267 ± 0.001***[a]	44.7
	1000	0.251 ± 0.001***[a]	46.9
Diclofenac sodium	100	0.154 ± 0.001***[a]	69.75

All values represent mean ± SD of n = 3;***$p<0.001$ with respect to control group. [a]Determined as compared with the control group (solution of 0.9% sodium chloride) using formula 100 - (OD of drug treated sample / OD of control) × 100. [#]% protection offered by the extract or standard refers to prevention of hypotonicity-induced HRBC membrane lysis.

Table 4. *In vitro* anthelmintic activity of hydroalcoholic thorn extract of *Bombax ceiba* (TEBC) on *Pheretima posthumad*.

Treatment	Concentration (mg/mL)	Paralysis Time# (min)	Death Time& (min)
Control	Saline	-	-
TEBC	10	46.16 ± 0.31***	87.54 ± 0.82**
	15	39.74 ± 0.47**	72.46 ± 0.86***
	20	33.91 ± 0.28***	59.22 ± 0.79**
Albendazole^	10	18.33 ± 0.27***	21.67 ± 0.18***

All values represent mean ± SD of n = 6;**p<0.01; ***p<0.001 with respect to control group. #Paralysis was the condition when worm did not revive in normal saline. &Death time was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water.^The standard reference for anthelmintic activity.

Table 5. *In vitro* antioxidant and reducing activities of hydroalcoholic thorn extract of *Bombax ceiba* (TEBC).

Treatment	Concentration (µg/mL)	% inhibition (n = 3)	Absorbance (n = 3) (700 nm)
TEBC	100	41.62 ± 0.44**	0.493 ± 0.003***
Ascorbic acid	100	83.27 ± 0.37	1.296 ± 0.001

Antioxidant capacity was carried out as per DPPH assay method. All values represent mean ± SD of n = 3;**p<0.01; ***p<0.001 with respect to standard group. Ascorbic acid was used as a standard reference for anti-oxidant activity.

Table 6. Anti-microbial activity of hydroalcoholic thorn extract of *B. ceiba* (TEBC).

Treatment	Concentration (µg/mL)	*E. coli*	*B. subtilis*	*K. pneumonia*	*C. albicans*	*A. niger*
TEBC	100	13.6 ± 0.57*** (12.5)	16.6 ± 0.57*** (12.5)	11.3 ± 1.15*** (50)	18.3 ± 0.66*** (25)	17.3 ± 0.57*** (12.5)
Ciprofloxacin	50	29.6 ± 0.57 (6.25)	26.6 ± 1.15 (6.25)	27.3 ± 0.57 (6.25)	-	-
Fluconazole	50	-	-	-	32.3 ± 1.15 (6.25)	31.6 ± 0.66 (6.25)

All values represent mean ± SD of n = 3;***p<0.001 with respect to the standard group. Zone of inhibition of test compounds against microbes were measured in mm. Values inside the bracket represent the minimum inhibitory concentration (MIC). Ciprofloxacin and fluconazole were used as standard references for antibacterial and antifungal activities, respectively.

The range of traditional applications of the *B. ceiba* in domestic medicine seems to be endless; they have been used as a medication against fever, inflammation, algesia, dysentery, microbial infections, hypertension, catarrhal affection, ulceration of the bladder, acne, gynecological disorders, piles and urinary infections. In this research, the thorn of traditional plant *B. ceiba* was studied with a rational and its pharmacological potentials were evaluated using suitable and simple models. The data presented in this study have exhaustively explored the potentials of thorn extract. The presence of flavonoids, glycosides, and polyphenols are key candidates for exerting *in vitro* anti-inflammatory and antioxidant activity. The anthelmintic activity may be due to flavonoid or chalcone content, which may have played an imperative role in killing earthworms by inhibiting any metabolic enzymes (Mahapatra et al., 2015). Thorns are hard, rigid structures well known for protection of plants from grazing animals and prevent the process of transpiration, where moisture is carried from roots (the main structure for absorbing ground water) to pores on the leaves (active area for evaporation), changes to vapor form and gets released to the atmosphere. With evolution, many inhabitants of dry or desert regions, modified their structure with due course of time to prevent transpiration and withstand harsh climatic conditions. The xerophytes are primarily characterized by these modified structures. In contrast, garden plants and herbs such as holly, rose, silverthorn, hawthorn, firethorn, pyracantha, bougainvillea, and blackberry have thorns, which may or may not have any physiological functions.

During inflammation, lysis of lysosomal component results in the discharge of enzymes that aggravate a large number of disorders (Kumar et al., 2012). Anti-inflammatory agents exert their beneficial effects by either inhibiting lysosomal enzyme release or by stabilizing the lysosomal membranes. Since human red blood cell membranes are very much similar to that of lysosomal membrane, the potential of thorn extract in preventing hypotonicity-induced HRBC membrane lysis was taken as a parameter for estimating anti-inflammatory property (Nagaharika and Rasheed, 2013). The extract containing rich phenolic and flavonoid contents exhibited dose-dependent stabilization of HRBC membrane. The phytoconstituents like flavonoid glycosides are believed to play a key role in combating inflammation. Vitexin, a significant flavone C-glycoside present in whole plant extract of *B. ceiba* may be present in the thorn. It has been reported to exhibit effective anti-inflammatory activity by targeting TRPV1, TNF-α, IL-1β, IL-6, IL-33, oxidative stress, modulating cytokine production, reducing neutrophil migration via inhibition of p38, ERK1/2 and JNK pathway (Borghi et al., 2013; Rosa et al., 2016). Vicenin, a flavonoid glycoside, displayed potential anti-inflammatory effect by suppressing TNF-α production (Marrassini et al., 2011). Isovitexin, a glycosyl flavonoid also demonstrated anti-inflammatory effect by inhibiting MAPK and NF-κB (Lv et al., 2016). Scopoletin, a coumarin compound has anti-inflammatory potential by inhibiting the release of PGE, TNF-2α, IL-1β and IL-6 and suppressing the expression of COX-2 (Kim et al., 2004). Flavonoids are well known to inhibit prostaglandin synthetase and produce an anti-inflammatory effect (Baumann et al., 1980).

Significant and dose-dependent anthelmintic activity of TEBC against *Pheretima posthuma* was detected. TEBC and albendazole, the reference drug, both caused paralysis of parasites that can be simulated with the expulsion of the worm from GIT by peristalsis. Albendazole causes flaccid paralysis of worms by inhibiting microtubule polymerization by binding to β-tubulin and is relatively non-toxic/less toxic to human due to its higher affinity for parasital β-tubulin than mammalian counterpart (Brunton et al., 2008). Anthelmintics having similarity with natural phenolic ring structures (niclosamide) have potent activity against a broad spectrum of worms by uncoupling oxidative phosphorylation (Katzung et al., 2012). Secondary metabolites like polyphenols and polyols have been reported to possess anthelmintic activity (Ndhlala et al., 2015). Therefore, it might be having a probability that the constituents present in thorn extract are phenol derivatives and may produce similar effects like niclosamide. However, further studies about phytoconstituents, their molecular target and mechanism of action(s) are needed.

Free radicals are very unstable molecules with an unpaired electron that react quickly with other compounds to capture surrounding electron to gain

stability and thus initiates a chain reaction, which cascades and lastly results in loss of cellular function (Halliwell and Gutteridge, 2007; Pala and Tabakçioglu, 2007; Pisoschi and Pop, 2015). Averagely, 10000–20000 free radicals attack body cell each day, of them oxygen free radicals, intermediates of dioxygen reduction resulting in damage deoxyribosyl backbone of DNA, accelerate oxidation of polydesaturated fatty acids, amino acids, co-factors (Stohs, 1995; Valko et al., 2006). Free radicals are main culprits for precipitation of diseases like cancer Alzheimer's disease, cardiac abnormalities, nephrotic disease, neurological complications, miscellaneous metabolic syndromes, aging, wrinkle formation and many other associated complications (Pala and Gürkan, 2008; Pham-Huy et al., 2008). Antioxidants and agents with potential to reduce free radicals scavenge these radicals and cease the chain reaction, thereby, preventing further damage (Fehér et al., 2012). The DPPH radical scavenging activity and reducing the power of any extracts reflects the potential as antioxidants and can be employed in different pathological conditions. At present, nearly 60% of the approved anticancer drugs globally or chemo-protectant agents are derived from nature. Recently, a step towards the exploration of natural antioxidants has revealed the perspective of TEBC in DPPH radical scavenging (41.62%). These results further suggested that the thorn extract has antioxidant properties along with low cytotoxicity and could exert protective effects against oxidative and free radical injuries occurring in oxidative stress-related diseases like cancer.

The result of this study showed that TEBC demonstrated varied anti-microbial activities against the tested organisms. On evaluation, it was observed that the thorn extract has shown highest ZOI against *B. subtilis*, in contrast, the extract exhibited moderate ZOIs against *E. coli* and *K. pnemoniae*. The activity against fungi *C. albicans* and *A. niger* were found to be lesser as compared to antibacterial with moderate ZOIs at both 50 and 100 µg/mL. When compared to standard drug samples, the extract did not exhibit powerful anti-microbial activity, and none of the ZOIs were found to be acceptable. However, extract showed satisfactory results against *B. subtilis*. *E. coli* is known to be resistant to drugs, have also demonstrated resistant to the tested thorn extract. The thorn extract exhibited higher activity in gram-positive bacteria as compared to gram-negative bacteria. The growth of *B. subtitlis*, a gram-positive mesophile, was inhibited better than gram-negative facultative anaerobics like *E. coli* and *K. pneumonia*. The probable reason for reduced activity may be due to the structural constitution of the cell wall of bacteria, which offers a solid barrier, causing restriction of certain antimicrobial phytochemicals to enter the cytoplasm. In gram-negative bacteria, a uniform 2–3 nm wide inner layer of peptidoglycan followed by a thicker 8–10 nm wide outer layer, composed of lipoproteins and lipopolysaccharides restricts the influx of components (Pelczar et al., 1993). The extract has a rich depot of antibacterial and antifungal components; namely, flavonoids, alkaloids, steroidal components, saponins, and terpenoids, which may be believed to play a crucial anti-microbial role.

CONCLUSIONS

The study revealed the physicochemical and pharmacognostic features of thorn of *B. ceiba* and highlighted key features. The presence of a wide range of phytochemical was preliminarily determined. The study also revealed the possession of various potentials like anti-inflammatory, antioxidant, anthelmintic, and antimicrobial activity against nosocomial infections opens new future perspectives of these thorns, which in early literature were considered hard structures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Concepts or Ideas	X	X		
Design		X		
Definition of intellectual content		X		
Literature search	X	X		
Experimental studies	X			
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Data analysis		X		
Statistical analysis			X	
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