ORIGINAL ARTICLE

Pharmacognostic evaluation and free radical scavenging activity of *Bombax ceiba* leaf extracts

Radhika Chikatipalli[1], K. Saravanakumar[2], Chandra Sekhar Kothapalli Bannoth[3]

[1]Research Scholar, Pharmaceutical Sciences, Jawaharlal Nehru Technological University Ananthapur, Ananthapuramu, Andhra Pradesh, India, [2]Department of Pharmaceutical Sciences, Sree Vidyanikethan College of Pharmacy, A. Rangampet, Tirupati, Andhra Pradesh, India, [3]Department of Chemistry, Krishna University, Machilipatnam, Krishna, Andhra Pradesh, India

Abstract

Objective: The present study aims pharmacognostic investigations and antioxidant activity of *Bombax ceiba* leaf extracts. **Materials and Methods:** The materials and methods for standardization parameters were performed by the Association of Official Analytical Chemist (AOAC) methods. The antioxidant activity was evaluated by taking ascorbic acid as standard, total phenolic content and total flavonoid content were determined by Folin–Ciocalteu method. **Results:** The results of qualitative phytochemical screening of *Bombax ceiba* aerial part extracts showed the presence of important phytochemical compounds, namely, flavonoids, saponins, tannins, and phenolic compounds. The results of total phenolic content and total flavonoid content of different *Bombax ceiba* leaf extracts showed the highest TPC and TFC for pet. ether extract and the free radical scavenging activity of extracts is in concentration gradient. **Conclusion:** It is concluded that *Bombax ceiba* leaf extracts have a rich profile of phytochemicals with significant proximate composition and good antioxidant activity. The findings indicate that *Bombax ceiba* leaf extract can be taken as an initiative step for finding out the promising agents responsible for its phytochemical and antioxidant activities and could be an important source of natural compounds for the development of new drug.

Key words: Antioxidant activity, preliminary phytochemical analysis, total flavonoid content, total phenolic content

INTRODUCTION

Bombax ceiba (syn. *Bombax malabaricum* DC. *Salmalia malabarica*) of the family Bombacaceae is an important medicinal plant of tropical and subtropical India commonly known as Silk Cotton Tree, Indian Red Kapok tree, Semal, Shimul, Shalmali, etc. Almost every part of this plant is used as medicine for curing maximum number of ailments.[1] The pharmacological studies in this plant provided the information that *B. ceiba* exhibits various biological activities such as astringent, cooling, stimulant, diuretic, aphrodisiac, demulcent, and tonic activities.[2] It was found that the young roots of *B. ceiba* have hypoglycemic, hypolipidemic, and hepatoprotective activities and confirm the traditional uses of this plant to manage diabetes and its associated liver toxicity.[3] In addition, the male rat treated with young root extracts of this plant has improved sexual performances and behaviors. The methanolic stem bark extract of this plant has excellent antiobesity activity in rats induced by a high-fat diet. Bark and seeds powder has hyperlipidemic activity with a reduction in serum and tissue lipid profiles.[4] The lupeol from stem bark exhibits the inhibitory effect on human umbilical venous endothelial cells (HUVECs) tube formation without affecting the growth of tumor cell lines.[5] It is reported that there are triterpenoid compounds in the stem bark extract, which could be responsible for lowering blood glucose levels. The leaf extracts displayed significant wound healing activity. They also showed a remarkable hypoglycemic and hypolipidemic activity which is related

Address for correspondence:
Radhika Chikatipalli, Pharmaceutical Sciences, Jawaharlal Nehru Technological University Ananthapur, Ananthapuramu, Andhra Pradesh, India.
E-mail: radhikapharma11@gmail.com

Received: 27-01-2021
Revised: 19-03-2021
Accepted: 28-03-2021

to Type 2 diabetes. Moreover, the antidiabetic activity of *B. ceiba* leaf extracts may due to the antioxidant activity and pancreatic cell histology improvement.[6] The mangiferin in leaf extracts improved the diabetic complications in the kidney and decreased free radicals in meningeal cells. In addition, the leaf extracts exhibit the antianxiety activity. The leaf extracts of this plant improved the stress behaviors in animal models. The leaf extracts also increased the antioxidant values in brain tissues of rats. It is reported that the flower extract has an excellent antioxidant activities.[7] The phytochemical screening revealed that flower extracts of this plant consist of three xanthones and nine flavonoids.[8] Another study found that the flowers comprise 10 flavonoids, quercetin, four coumarins, and seven other compounds. For ethnobotanical uses, it was found that androecium of this plant is used as a food ingredient for Indian people.[9] Some parts of this plant are edible that is famous in the Northern part Thailand. People use flowers to cook as curry soup, called "Nam Ngiao" served with rice noodles. However, there are a few scientific reports on the biological activities of *B. ceiba* leaves.[10]

Plants have been an important source of remedies from the beginning of culture.[11] Plants have been an important source of remedies from the beginning of culture. Many parts of the plant (root, stem bark, gum, leaf, prickles, flower, fruit, seed, and heartwood) are used by various tribal communities and forest dwellers for the treatment of a variety of ailments.[12] The plant literature survey shows the plant possesses astringent, cooling, stimulant, diuretic, aphrodisiac, demulcent, and tonic effects and also helps in dysentery. It also possesses important pharmacological activity such as aphrodisiac, anti-inflammatory, and hepatoprotective activity in addition to anticancer and anti-HIV activity, anti-*Helicobacter pylori*, antiangiogenic, analgesic, and antioxidant activity and hypotensive, hypoglycemic, and antimicrobial activity.[13] It is reported to contain important phytoconstituents such as naphthol, naphthoquinones, polysaccharides, anthocyanins, shamimin, and lupeol.[14] Mostly, the selection of a candidate species for investigations can be done on the basis of long-term use by humans (ethnomedicine). This approach is based on an assumption that the active compounds isolated from such plants are likely to be safer than those derived from plant species with no history of human use.[15]

MATERIALS AND METHODS

Collection and Preparation of Samples

Bombax ceiba plant was obtained from nearby areas of Tirumala hills. The collected plants were identified and authenticated by plant Taxonomist Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, with voucher Ref. No. 1656, dated September 11, 2017. All the chemicals used for the study are of Sigma grade.

Fresh leaves of the plant were collected, cleaned, and washed under running water to remove soil and dirt, then air-dried. The dried samples were milled with an electric blender before being ground into powder and stored in a desiccator until required for analysis. The 50% and 95% ethanol extracts were prepared by adding 100 g of dry powder of leaf and flower in 400 mL of 50% and 95% ethanol and macerated for 7 days. The crude residues were filtered using filter paper Whatman No. 1. The filtrates were obtained by removing the solvents in a rotary evaporator and then freeze-dried as a fine powder and kept at −20°C until used.[16]

Physicochemical Analysis

Proximate analysis

All the standard procedures of proximate analysis were carried out according to the procedure of the Association of Official Analytical Chemist. The proximate composition of *Bombax ceiba* was analyzed to find out the moisture content, ash content, protein composition, lipid content, crude fiber, and carbohydrate composition.[17]

Mineral Analysis

The mineral concentration of the test samples was determined using the dry ash extraction method.[18]

Fluorescence Analysis

The plant powder was treated with various chemicals such as concentrated sulfuric acid, aqueous ferric chloride solution, iodine solution, ammonia solution, and aqueous potassium hydroxide solution for fluorescence analysis.

Preliminary Phytochemical Investigation

Phytochemical tests were carried out first on the samples to establish the presence or absence of the chemical constituents using standard procedures by Harborne.[19]

Determination of Total Phenolic Content

The total phenolic content (TPC) was investigated by Folin–Ciocalteu method. The TPC value was expressed as mg gallic acid equivalents (GAE)/g sample.[20]

Determination of Total Flavonoid Content

The quantitative estimation of total flavonoids in the extracts of *B. ceiba* was done by spectroscopic method using rutin as reference standard.

Determination of *in vitro* Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out and the results were expressed as a percentage inhibition of DPPH calculated from the following formula.

% radical inhibition = ([A0− A1]/A0) ×100, whereas, A0= Absorbance of blank and A1= Absorbance of sample and half maximal inhibitory concentration (IC_{50}) was calculated from the calibration curve by linear regression.[21]

Table 1: *Bombax ceiba* leaves physicochemical parameters

Parameters	Results
Foreign matter	None
Total ash (mg/g)	5.1±0.06
Acid-insoluble ash (mg/g)	0.61±0.36
Water-soluble ash (mg/g)	5.15±0.07
Loss on drying (mg/g)	9.42±0.22
Bitterness	Mild

Table 2: % yield of different *Bombax ceiba* leaf extracts

Parameters	Results
Pet. ether	5.65±0.12
Ethyl acetate	7.12±0.28
Hydroalcohol	9.45±0.16
Aqueous	9.15±0.08

Table 3: Nutritional composition of *Bombax ceiba* leaves

Nutrients	Values (%)
Mineral	13.23
Carbohydrate	1.48
Protein	0.7
Fats	0.75
Fiber	2.85
Sodium	19.07 (mg/100 g)
K	153.66 (mg/100 g)
Ca	177 (g/100 g)

Hydroxyl Radical Scavenging Activity

Hydrogen peroxide scavenging activity is the ability of the plant extract to remove hydrogen peroxide which was determined according to the method of Ruch *et al*.[22,23]

Statistical Analysis

All data were expressed as mean ± standard error of mean with $n = 3$. The difference among means was tested using one-way analysis of variance followed by Duncan multiple range test. $P < 0.05$ was considered statistically significance.

RESULTS AND DISCUSSION

Physicochemical Analysis

The physicochemical parameters showed absence of foreign matter with mild bitterness % yield.

The % yield of different *Bombax ceiba* leaves reported in the Tables 1 and 2 showed maximum yield with hydroalcoholic extract.

Nutritional Composition

B. ceiba leaves contain carbohydrate, fiber, and rich source of minerals. The Nutritional composition of *B. ceiba* leaves is depicted in Table 3.

Table 4: Fluorescence studies of *Bombax ceiba* leaves powdered

Sample + reagents	UV light		Visible light
	Short 254 nm	Long 365 nm	
Drug powder	-	-	Pale brown
Powder + sodium hydroxide	-	Green fluorescence	Green fluorescence
Powder + 1 N HCl	-	Green fluorescence	Green fluorescence
Powder + 50% nitric acid	-	-	Brownish-orange
Powder + 50% H_2SO_4	-	-	Reddish-brown
Powder + 50% picric acid	-	-	Yellowish-orange
Powder + ammonia + nitric acid	-	-	Yellowish-orange
Powder + ferric chloride	-	-	Black

-: Indicates no fluorescence

Table 5: Phytochemical constituents of different *Bombax ceiba* extracts

Extracts	Pet. ether	Ethyl acetate	Hydroalcoholic	Aqueous
Flavonoids	–	+	+	+
Alkaloids	–	–	–	–
Phenols	–	+	+	+
Sterols	–	–	–	–
Carbohydrates	–	+	+	+
Fixed oils and fats	+	–	–	–
Mucilage	–	–	–	–
Tannins	+	+	+	+
Saponins	–	–	–	+

+: Present; –: Absent

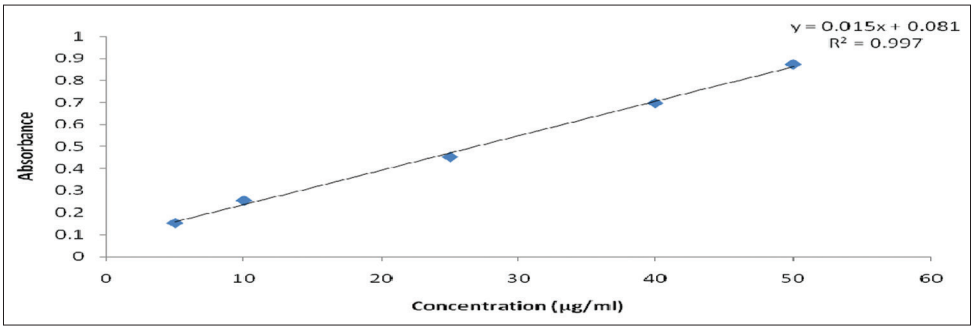

Figure 1: Calibration curve of standard gallic acid for the determination of total phenolic content

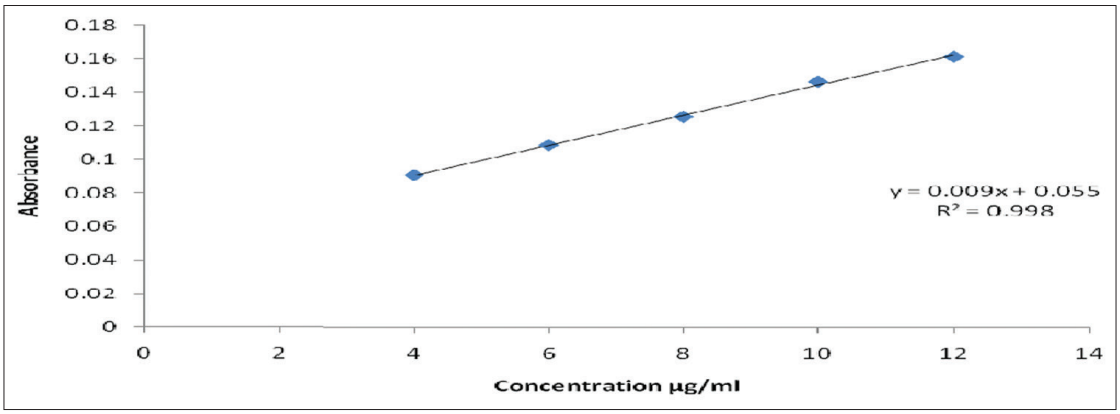

Figure 2: Total flavonoids content µg/mg equiv (to rutin)

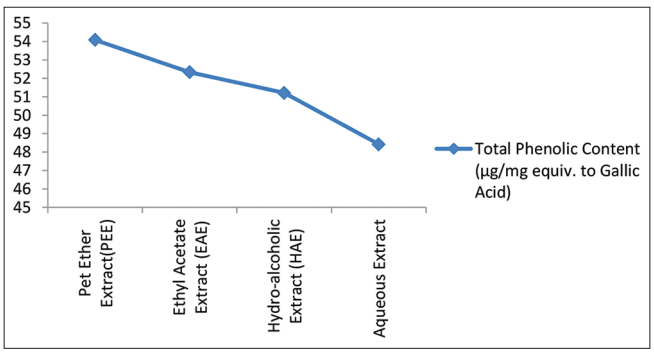

Figure 3: Total phenolic content of *Bombax ceiba* extracts in µg/mg equiv. to gallic acid

Fluorescence Analysis

Fluorescence studies of powder with various reagents revealed the presence of green fluorescence with concentrated HCl and NaOH, under UV and daylight [Table 4].

Phytochemical Analysis

Preliminary chemical analysis indicated that the presence of phenols, tannins, flavonoids, and carbohydrates in hydroalcoholic and aqueous extract [Table 5].

Estimation of Total Phenolic and Flavonoid Content

The calibration curves were plotted using various concentrations of gallic acid and rutin for determining the TPC and rutin for determining the TFC. The calibration curves of gallic acid and rutin are depicted in [Figures 1 and 2] respectively.

Estimation of Total Flavonoids

The results of total phenolic content and total flavonoid content of different *Bombax ceiba* leaf extracts showed the highest TPC and TFC for pet. ether extract while aqueous extract showed the lowest TPC and TFC compared with other extracts [Figures 3 and 4].

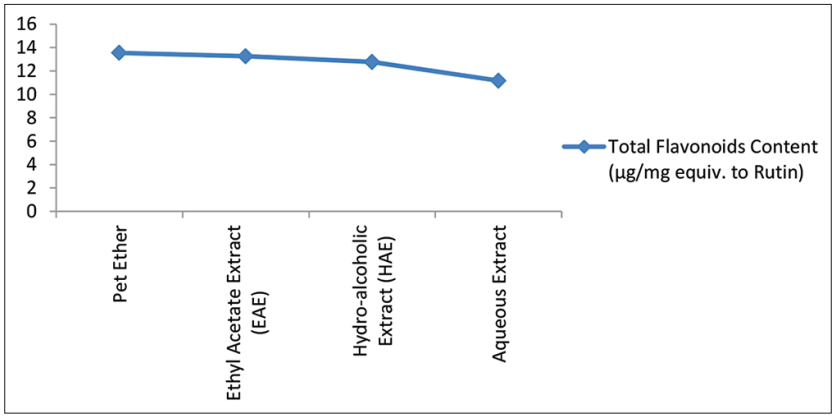

Figure 4: Total phenolic content of *Bombax ceiba* extracts in µg/mg equiv. to gallic acid

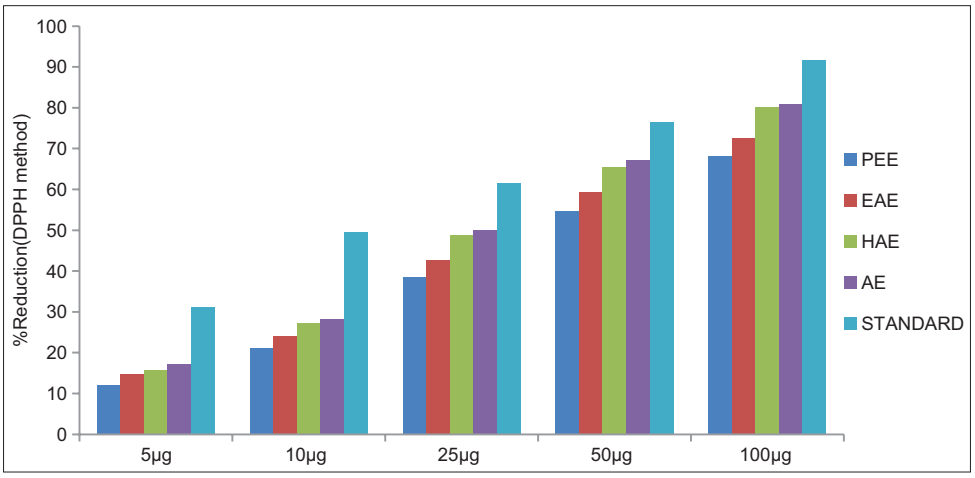

Figure 5: *Bombax ceiba* extracts DPPH scavenging activity

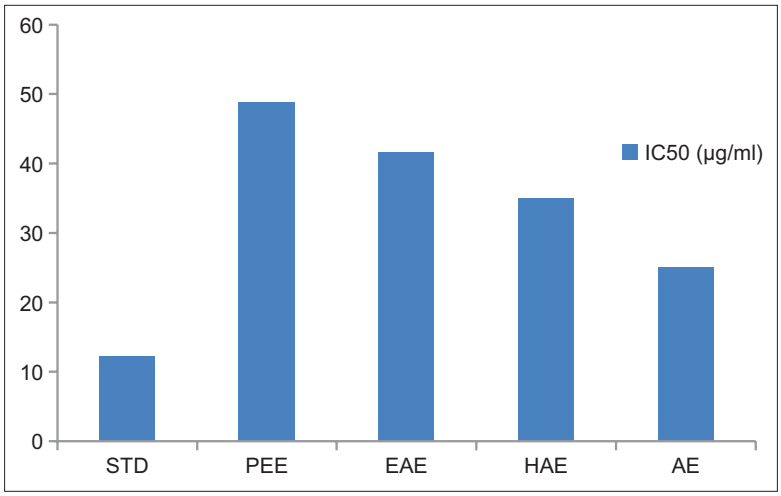

Figure 6: Different extracts of *Bombax ceiba* antioxidant activity (IC_{50}) of DPPH method

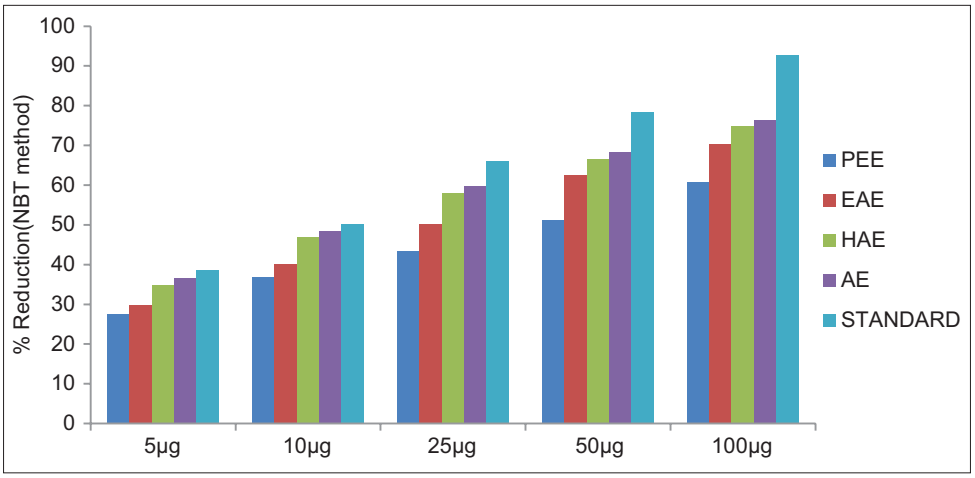

Figure 7: Superoxide scavenging activity of different *Bombax ceiba* extracts

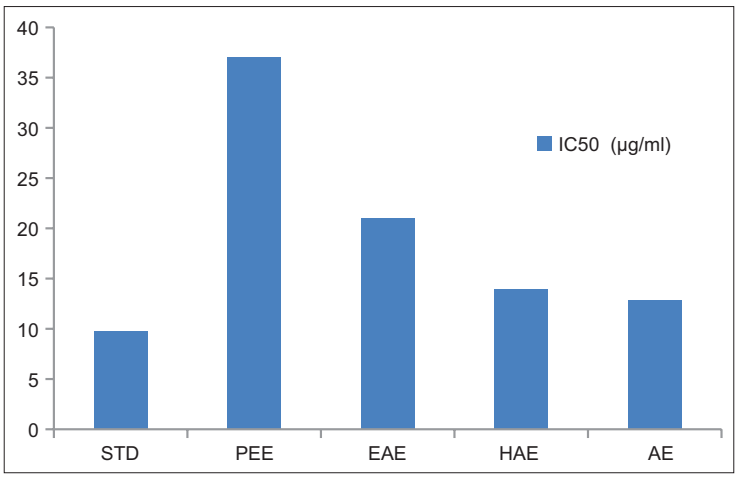

Figure 8: Antioxidant activity (IC_{50}) of different *Bombax ceiba* extracts (NBT method)

In vitro Antioxidant Activities

2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity

These reports demonstrated that radical recovery activities were in direct proportion to extract concentrations [Figures 5 and 6].

Superoxide Scavenging Activity (NBT Method)

A number of concentrations ranging from 5 to 100 µg/ml of familiar ether, ethyl acetate, hydroalcohols, and aqueous extracts were compared for their antioxidant activity in various *in vitro* models and the results are in the concentration gradient [Figures 7 and 8].

CONCLUSION

Nature was the source of rich phytochemical diversity that possesses important pharmacological and biological activities. In the present era of widespread of various diseases, there is a need to find out novel agents with therapeutic properties to rule out the adverse side effects of conventional medicine system. The current investigation results have shown that *Bombax ceiba* leaves have good proximate, preliminary, and phytochemical profile with antioxidant activity. With regard to the results, the different extracts of *Bombax ceiba* leaves could be an important source of natural compounds with antioxidant capacity. The phytochemical profiling and antioxidant activity provide a promising area of research in natural therapeutics and further studies are to be carried out to find the components responsible for its various pharmacological activities. The study will take a long way in analyzing potent clinical and phytochemical applications of *Bombax ceiba* extract.

ACKNOWLEDGMENTS

The authors are thankful to Principal and Management of Sree Vidyanikethan College of Pharmacy, Tirupati, for providing necessary infrastructure and facilities to conduct this research work.

CONFLICTS OF INTEREST

No conflicts of interest.

REFERENCES

1. Chaudhary PH, Khadabadi SS. *Bombax ceiba* Linn.: Pharmacognosy, ethnobotany and phyto-pharmacology. Pharmacogn Commun 2012;2:2-9.
2. Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, *et al*. Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. Int J Mol Sci 2018;19:1578.
3. Jain V, Verma SK. Assessment of credibility of some folk medicinal claims on *Bombax ceiba* L. In: Khandelwal KR, editors. Practical Pharmacognosy Technique and Experiments. 23rd ed. Maharashtra: Nirali Prakashan; 2005.
4. Arafa AF, Foda DS, Mahmoud AH, Metwally NS, Farrag AR. *Bombax ceiba* flowers extract ameliorates hepatosteatosis induced by ethanol and relatively moderate fat diet in rats. Toxicol Rep 2019;6:401-8.
5. Bora MM, Deka C, Tapadar SA, Jha DK, Kakati DK. Alkyds from red silk-cotton (*Bombax ceiba*) seed oil and investigation on their microbial degradation. Prog Org Coat 2018;124:71-9.
6. Xu GK, Qin XY, Wang GK, Xie GY, Li XS, Sun CY, *et al*. Antihyperglycemic, antihyperlipidemic and antioxidant effects of standard ethanol extract of *Bombax ceiba* leaves in high-fat-diet-and streptozotocin-induced Type 2 diabetic rats. Chin J Nat Med 2017;15:168-77.
7. Li G, Lou HX. Strategies to diversify natural products for drug discovery. Med Res Rev 2018;38:1255-94.
8. Guo-Kai W, Bin-Bin L, Xin-Xin L, Ya-Jun S, Min-Jian Q. Phonemic constituents from the leaves of *Bombax malabaricum*. Nat Prod Res Dev 2012;24:336-8.
9. Hossain E, Mandal SC, Gupta JK. Phytochemical screening and *in vivo* antipyretic activity of the methanol leaf-extract of *Bombax malabaricum* DC (*Bombacaceae*). Trop J Pharm Res 2011;10:55-60.
10. Anandarajagopal K, Sunilson JA, Ajaykumar TV, Ananth R, Kamal S. *In vitro* anti-inflammatory evaluation of crude *Bombax ceiba* extracts. Eur J Med Plants 2013;3:99-104.
11. Mishra A, Thakur M, Alok S. Evaluation of immunomodulatory activity of polysacchride fraction of *Inula racemosa*, *Bombax ceiba* and *Allium sativum*. Int J Pharm Sci Res 2016;7:3749.
12. Molinari G. Natural products in drug discovery: Present status and perspectives pharmaceutical biotechnology. Adv Exp Med Biol 2009;655:13-27.
13. Sithara NV, Komathi S, Rajalakshmi G, Queen J, Bharathi D. Phytochemical analysis of *Andrographis paniculata* using different solvents. Eur J Biotechnol Biosci 2016;4:28-3.
14. Nagarathna PK, Reena K, Reddy S, Wesley J. Evaluation of immunomodulatory activity of the flavanoid from *Kigelia africana*. Indian J Pharm Biol Res 2014;2:41-8.
15. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Nirali Prakashan; 1994.
16. Lv Y, Glahn RP, Hebb RL, Rizvi SS. Physico-chemical properties, phytochemicals and DPPH radical scavenging activity of supercritical fluid extruded lentils. LWT 2018;89:315-21.
17. Maurya SK, Seth A, Laloo D, Singh NK, Gautam DN, Singh AK. Śodhana: An ayurvedic process for detoxification and modification of therapeutic activities of poisonous medicinal plants. Anc Sci Life 2015;34:188-97.
18. Khandelwal KR. Practical Pharmacognosy: Techiques and Experiments. 13th ed. Pune, India: Nirali Prakashan, 2005. p. 149-56.
19. Harborne JB. Phytochemical Methods. 2nd ed. London, New York: Chapman and Hall; 1984. p. 288.
20. Meena V, Chaudhary AK. Shalmali (*Bombax ceiba*): Versatility in its therapeutics. Int J Green Pharm 2017;11:S401-6.
21. Fernando CD, Soysa P. Optimized enzymatic colorimetric assay for determination of hydrogen peroxide ($H2O_2$) scavenging activity of plant extracts. MethodsX 2015;2:283-91.
22. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: A comparative overview. Evid Based Complement Alternat Med 2005;2:465-73.
23. Zhang YB, Wu P, Zhang XL, Xia C, Li GQ, Ye WC, *et al*. Phenolic compounds from the flowers of *Bombax malabaricum* and their antioxidant and antiviral activities. Molecules 2015;20:19947-57.

Source of Support: Nil. **Conflicts of Interest:** None declared.