

**Phytochemical Study of *Butea superba* Roxb. and its Extraction by
Successive Solvent and Direct Ethanolic Extraction**

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ABSTRACT

Herbal therapy is a widely accepted therapeutic method, where the use of medicinal herbs has great importance in traditional medicine. *Butea superba* L., often referred to as Ghandhali, Bakuchi, Khup, and Prasaran, is an indigenous green vegetable originating from India. The herb has been traditionally used to augment physical strength, potency, and male sexual prowess. An essential aspect of research is examining the bioactivity of each component, since it has a substantial impact on the regulation of biological processes and the treatment of numerous conditions such as diabetes, hypertension, asthma, hepatomas, psoriasis, and perhaps cancer. The current investigation centered on the extraction and phytochemical analysis of *Butea superba* Roxb, using both Successive Solvent Extraction (SSE) and Direct Ethanolic Extraction (DEE) techniques. The SSE technique included a methodical extraction procedure using solvents with progressively higher polarity, while DEE immediately employed ethanol. Phytochemical analysis was performed on the extracted components of *Butea superba* to determine the presence and amount of bioactive substances. Diverse analytical techniques, such as chromatography, were used to investigate the chemical makeup. This extensive study seeks to provide valuable insights into the efficacy of SSE (Solvent Extraction) and DEE (Deep Eutectic Solvent Extraction) techniques for extracting phytochemicals from *Butea superba*, as well as to clarify the range of bioactive substances found in this plant.

Keywords: *Butea superba*, Bioactives, Chromatography, Successive Extraction, Ethanolic Extraction

INTRODUCTION

Herbal therapy is a remarkable achievement in the realm of diverse and widely accepted medicinal practices. Medicinal plants are integral to traditional medicine, serving as a fundamental component. To ensure the safe use of these medications, it is essential to first set guidelines pertaining to their quality, safety, and effectiveness. *Butea superba* L. (b. superba) is often referred to in India by the native names Ghandhali, Bakuchi, Khup, and Prasaran. This fragrant vine is a verdant vegetable that may be consumed in its raw state or cooked by steaming.^[1]

Examining Indigenous customs and doing a thorough literature research on the plant *Butea superba* provides opportunities for future

investigation into its phytochemical and pharmacological qualities. Conventional uses include using the tuber and stem of the plant in medical traditions, since it is considered to provide vigor, augment potency, and increase male sexual prowess. *Butea superba* Roxb. has gained a reputation as a miracle herb.^[2]

Due to its possible impact on human health, there is significant interest in examining the chemical components of *Butea superba* and comprehending their biological properties. Examining the bioactivity of each component is of utmost importance, as it has a substantial impact on controlling biological processes and treating a range of conditions such as diabetes, hypertension, asthma, hepatomas, psoriasis, and perhaps cancer. Phosphodiesterase inhibitors have been shown to

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have impacts on inhibiting platelet aggregation, stimulating the central nervous system (CNS), and activating cellular activities.^[3]

Research conducted by researchers and academics has shown that the chemicals obtained from *Butea* are non-mutagenic, indicating that they do not cause genetic mutations. This underscores the safety of these compounds. Significantly, it produces a strong dilation of the vaginal blood vessels similar to Viagra, but with a gentle impact that prevents excessive stimulation of the nerves, muscles, or heart, thereby safely improving erectile function by increasing the generation of nitric oxide.^[4]

Flavonoids and flavonoid glycosides have been shown to have stronger inhibitory effects on cAMP phosphodiesterase in comparison to caffeine and theophylline, as seen in bioactivity testing. Considering the significant negative consequences linked to commercial medications for erectile dysfunction, the herbal substitute found in *Butea superba* presents itself as a hopeful choice due to its mild but secure effects. Additional research on the bioactive extracts/fractions of *Butea superba* confirms its potential advantages. Therefore, based on these results and other compelling factors, it is very advisable to carry out more investigation in order to uncover the different applications and advantages of this plant.^[5]

MATERIALS AND METHODS

The procurement of ethyl acetate and petroleum ether was made from SD Fine Chemicals in Mumbai. The chloroform and ethanol were obtained from Merck, a supplier located in Mumbai. The foliage of the *Butea superba* plant was gathered from the University campus located in Sagar, Madhya Pradesh, India. All the other compounds used in the investigation were of analytical grade. Double distilled water (DDW) was routinely used as needed throughout the study.

Collection and Identification of Drug

The foliage of the *Butea superba* plant was collected at the University campus in Sagar, Madhya Pradesh, India, during the months of January and March. Dr. P. Tiwari, from the Department of Botany at Dr. H. S. Gour Vishwavidyalaya in Sagar (M.P.), undertook the authentication procedure. The allotted Herbarium number is Bot/1019. The verified foliage was then carefully conserved at the institute's herbarium for the purpose of reference and documentation.

Organoleptic Characters of Powdered Drug

A minute quantity of powdered *Butea superba* medication was applied onto a white tile and subjected to a physical examination to assess its overall characteristics, such as its nature, color, odor, taste, and texture.

Extraction Procedure

The following protocol was used to produce extracts from the leaves of *Butea superba*, which were dried in the shade and then ground into a powder.

Successive Solvent Extraction

Defatting of Plant Material

The leaves of *Butea superba* were air-dried in a shaded area at the ambient temperature. The plant material, which had been dried in the shade, was finely ground and then extracted using petroleum ether in a soxhlet system. The extraction process was maintained until the removal of fat from the material had occurred.^[6]

Extraction with Chloroform

The residual solids of the medicines were extracted using chloroform in a soxhlet device. The extraction process was prolonged for a duration of 6-7 days. The extract was further condensed and ultimately desiccated until a consistent weight was achieved.^[7]

Extraction with Ethyl Acetate

The residue obtained following chloroform extraction was further extracted using ethyl acetate. The extraction process was extended for a duration

of 6-7 days to ensure thorough extraction. The extract was condensed and then dehydrated.

Extraction with Ethanol

The residue obtained after extracting with ethyl acetate was further extracted with ethanol using a soxhlet device. The extraction process was conducted over a duration of 6-7 days in order to ensure thorough extraction. The additional extract was condensed and desiccated until reaching a consistent weight.^[7]

Extraction with water

Finally, the marc underwent hot water maceration. The maceration process was prolonged for a duration of 24 hours. The solution was passed through a filter and then reduced in volume.^[8]

Direct Ethanolic Extraction

Defatting of Plant Material

The foliage of *Butea superba* was desiccated in a shaded environment at the ambient temperature. The plant material that had been dried in the shade was finely ground and then extracted using petroleum ether (60-80°C) in a soxhlet device. The extraction process was maintained until the removal of fat from the material had occurred. Thorough defatting was achieved by collecting a little amount of liquid from the thimble onto the filter paper, which did not exhibit any oily residue.

Extraction with Ethanol

The residue produced after using petroleum ether was further extracted with ethanol using a soxhlet device. The extraction process was prolonged for a duration of 6-7 days in order to get full extraction. The extract was evaporated under vacuum until it was completely dry. The resulting substance, which had a thick consistency and a dark green color, was identified as ethanolic extracts.^[9]

Fractionation of Ethanolic Extract

The dehydrated ethanolic extract was dissolved in a tiny amount of water and separated into fractions using a separating funnel with butanol. The butanolic fraction obtained from the ethanolic extract was used for further analysis.^[10]

Physical Examination of the Butanolic Fraction of Ethanolic Extract

The physical properties of the dried butanolic portion of the medicines were assessed, including consistency, color, odor, and taste.

Phytochemical Analysis of the Prepared Extracts^[11-13]

The solvent-extracted extracts underwent many qualitative tests to identify the presence of plant components.

Test for Carbohydrates

Fehling's Test (for reducing sugars)

The test solutions were treated with a little amount of Fehling's reagent. Prepare solution A by dissolving 34.66g of Copper sulfate in distilled water and adjusting the volume to 500 ml. Combine 173 g of potassium sodium tartarate and 50 g of sodium hydroxide in distilled water and adjust the volume to 500 ml to create solution B. Prior to detection of reducing sugars, combine two solutions in equal volumes. Upon heating the test sample, it exhibits a brick red color.

Molisch's Test

Test solution treated with few drops of Molisch's reagent (10 g α -naphthol in 100 ml of 95% ethanol). Then 2 ml of conc. sulphuric acid is added slowly from sides of the test tube. Shows purple ring at the junction of two layers.

Tollen's test

A little portion from each extract was taken separately with a small amount of the distilled water and filtered. A few drops of the ammoniacal silver nitrate solution (Tollen's reagent) was added to each filtrate and kept in a boiling water bath for 5 minutes. Appearance of a silver mirror along the sides of the test tubes indicated the presence of reducing sugars.

Barfoed's reagent test

A little portion from each extract was taken separately with 2 ml of distilled water and filtered. Then a small volume of Barfoed's reagent was added to each test tube and kept in a boiling water

bath for 2 minutes. Appearance of a red precipitate indicated the presence of monosaccharides

Hasch's Test

In this test extract is dissolved in water and then conc. H₂SO₄ was added from the sidewalls. Formation of a brown ring suggests presence of carbohydrates.

Test for Proteins

On concentrating the aqueous extract to a small bulk a translucent mass was separated, which did not redissolve. This mass gave positive test for proteins.

Millons Test

Test solutions treated with Millon's reagent [Dissolve 1 g of mercury in 9 ml of fuming nitric acid, after cooling; add equal volume of distilled water]. Protein is stained red on warming.

Xanthoproteic Test

Test solutions treated with conc. nitric acid and boiled gives yellow precipitate.

Biuret Test

Test solutions treated with 10% sodium chloride and 1% copper sulphate (1 drop). Solution gives violet / purple Colour. On addition of alkali, it becomes dark violet.

Test for Alkaloids

A small portion from the respective extract was shaken with about 3 ml of 1.5 % v/v hydrochloric acid and filtered. The filtrate was tested with the alkaloidal reagents.

Dragendorff's Test

The acidic solution treated with Dragendorff's reagent (potassium bismuth iodide) gives orange precipitate.

Mayer's Test

Test solution with Mayer's reagent (potassium mercuric iodide) gives cream Coloured precipitate.

Hager's Test

The acidic solution treated with Hager's reagent (saturated picric acid solution) gives yellow precipitate.

Wagner's Test

Test solution treated with Wagner's reagent (iodine - potassium iodide solution) gives reddish brown precipitate.

Test for Phytosterols

The extracts were refluxed separately with alcoholic solution of potassium hydroxide till complete saponification. The saponified extract was diluted with water and unsaponifiable matter was extracted with diethyl ether. The ethereal extract was evaporated and the residue (unsaponifiable matter) was subjected to the following test by dissolving the residue in the chloroform.

Libermann Burchard Test

To the test solution few drops of acetic anhydride were added and conc. sulphuric acid added from sides of test tube, shaken and allowed to stand. Lower layer turns bluish green indicating the presence of sterols.

Salkowski Test

Test extract solution was treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Lower layer turns red indicating the presence of sterols.

Test for Glycosides

Keller Killiani Test (for digitoxose)

Test solution is treated with few drops of Ferric chloride solution and mixed, and then sulphuric acid containing ferric chloride solution is added, it forms two layers. Lower layer shows reddish brown Colour while upper layer turns bluish green.

Baljet Test

Test solution treated with sodium picrate gives yellow to orange colour.

Legal Test

Test solution treated with a drop of 2% sodium nitroprusside and a drop of sodium hydroxide is then added. Production of a deep red Colour constitutes a positive test.

Test for Saponins

The presence of saponins is usually indicated in the alcoholic and water extracts of the drug. 1 ml

of both the extracts were diluted to 20 ml by the respective liquids and shaken well. The presence of saponins was indicative by the formation of dense foam. However, other extracts were also tested for the presence of saponins.

Foam Test

Test solution on shaking shows foam formation, which is stable for at least 15-20 minutes.

Hemolysis Test

The test extract solutions were subjected to hemolytic test. 1ml of blood was diluted with 10 ml of sodium citrate (36.5 g/l) and separately 10 mg of extract was dissolved in phosphate buffer pH 7.4 and made up to 100 ml. Drop of blood was taken over slide and observed under microscope (40x) for the presence of intact RBC, then 1-2 drops of saponin solution was put over the blood and observed under microscope for hemolysis.

Test for Tannins

A small fraction of the residue from each extract was dissolved in about 2 ml of distilled water separately and filtered. The filtrate was tested with following reagents.

Gelatin- Lead Acetate Test

Test solution treated with lead acetate solution. This solution gives a white precipitate, when a 1% solution of gelatin containing 10% sodium chloride is added.

Test for Phenolics

A small fraction of the residue from each extract was dissolved in about 2 ml of distilled water separately and filtered. The filtrate was tested with following reagents. The solution treated with few drops of ferric chloride solution gives dark Colour.

Test for Flavonoids

Magnesium Ribbon test

Test solution taken in a test tube. Few magnesium ribbons are dipped and conc. HCl is added over them. Magenta (brick red) Colour develops indicating presence of flavonoids.

Test for Terpenoids

The test solution was prepared by dissolving extracts in chloroform.

Libermann Burchard Test

To the test solution few drops of acetic anhydride were added and mixed well. Then few drops of conc. sulphuric acid added from sides of test tube. Red Colour is produced in the lower layer indicates the presence of triterpenes.

Salkowski Test

Test extract solution was treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Lower layer turns golden yellow indicating the presence of triterpenoids.

Test for free Amino acids

A little fraction from each extract was taken in water and filtered. The filtrate was used as such and also after removing tannins by lead acetate method. Then applied the spots on chromatographic paper. The spots were dried and the paper was then sprayed with Ninhydrin reagent. The paper was then allowed to dry and then heated in an oven at 115°C for 5 minutes. Appearance of violet coloured spots indicated the presence of free amino acids.

Test for Fixed oils, Fats and Waxes

A drop of the ethanolic solution of each extract was placed separately on filter paper strips. The solvent was allowed to evaporate. Appearance of oily spots indicated the presence of fixed oils, fats or waxes.

Thin Layer Chromatography

Preparation and activation of plates

Silica gel G with distilled water (1:3) was triturated in a glass pestle mortar and spread over the glass plates (10 cm × 20 cm) by pouring till a uniform layer was obtained and allowed to air-dry. The plates were activated for one hour at 110°-120° C before application of sample.

Selection of solvents

An effective method for preliminary experiments is to conduct two distinct trials utilizing highly polar liquids, such as ethanol, for one trial, and a

nonpolar liquid, such as hexane, for the second trial. By analyzing the mobile phase that effectively transports the solutes from the starting point and calculating their R_f values, the solvents may be altered in various ways to enhance selectivity and resolution. The polarity may be modified by adding additional solvents, taking into consideration their respective dielectric constants. A substance containing functional groups that like those of the solutes, such as ethers, alcohols, or carboxyl groups, may be introduced to enhance the R_f value by facilitating solubility in the mobile phase.^[14] To avoid tailing, the charges on the solutes may be modified by adding acids or bases such as acetic acid or ammonia (Table 1-4).

Table 1: Different Solvents Systems for TLC of Petroleum Ether Extract of *Butea superba* Leaves

S. No.	Solvent System	No. of Spots	Conclusion
1.	Toluene : Ethyl acetate (90:10)	7	Good
2.	Toluene : Ethyl acetate (95:5)	8	Best
3.	Toluene: Ethyl acetate: Acetic acid (82: 15: 3)	5	Fair
4.	Hexane : Ethyl acetate (90:10)	7	Good
5.	Hexane: Ethyl acetate: Acetic acid (95:4.9:0.1)	4	Not Significant
6.	Benzene: Ethyl acetate (85:15)	4	Not Significant

Table 2: Different Solvents Systems for TLC of Ethanolic Extract of *Butea superba* Leaves

S. No.	Solvent System	No. of Spots	Conclusion
1	Chloroform:Methanol:water (65:35:10)	7	Good
2	Chloroform: Methanol (22.5:7.5)	8	Best

3	Chloroform: Methanol (40:10)	5	Fair
4	Butanol: Glacial acetic acid: Water (5:1:4)	3	Average
5	Toluene : Ethyl acetate (90:10)	2	Average
6	Chloroform: Methanol (74:26)	7	Good

Table 3: Different Solvents Systems for TLC of Butanolic Fraction of Ethanolic Extract of *Butea superba* Leaves

S. No.	Solvent System	No. of Spots	Conclusion
1.	Chloroform : Methanol : water (65:35:10)	3	Good
2.	Chloroform: Methanol (22.5:7.5)	4	Best
3.	Butanol : Glacial acetic acid : Water (5:1:4)	2	Average
4.	Toluene : Ethyl acetate (90:10)	2	Average
5.	Chloroform : Methanol (74:26)	3	Good

Table 4: Different Solvents Systems for TLC of Aqueous Fraction of Ethanolic Extract *Butea superba* Leaves

S. No.	Solvent System	No. of Spots	Conclusion
1.	Chloroform : Methanol : water (65:35:10)	2	Good
2.	Chloroform: Methanol (22.5:7.5)	4	Best
3.	Butanol : Glacial acetic acid : Water (5:1:4)	3	Average
4.	Toluene : Ethyl acetate (90:10)	2	Not Significant
5.	Chloroform : Methanol (74:26)	3	Good

Preparation of sample solution

A solution containing about 50 milligrams of Petroleum ether extract was prepared by dissolving it in 2 milliliters of petroleum ether. This solution was then used for conducting Thin Layer Chromatography (TLC) experiments. Additionally,

samples of the Ethanolic extract and the Butanolic fraction of the Ethanolic extract were produced for Thin Layer Chromatography (TLC).

Detecting Reagent

The reagent used is a combination of anisaldehyde and sulphuric acid. A solution containing 0.5 ml of Anisaldehyde was prepared by mixing it with 10 ml of Glacial Acetic acid, followed by the addition of 85 ml of methanol. Finally, 5 ml of concentrated Sulphuric acid was added drop by drop. A solution containing 5% ferric chloride in methanol. A solution containing 10% sulfuric acid dissolved in methanol.^[15]

RESULTS AND DISCUSSION

The physical characteristics of various extracts of *Butea superba* were assessed, including consistency, color, odor, and taste. The obtained extracts exhibited a greenish-yellow hue for the petroleum ether extraction, a dark green hue for the direct ethanolic extraction, and a brown hue for the butanolic fraction of the ethanolic extract. Both the ethanolic extracts and butanolic fraction of the ethanolic extract exhibited a malodorous scent and a bitter flavor.

A series of qualitative chemical tests were conducted on each of the extracts derived from *Butea superba*. The test results indicated the detection of alkaloids, fixed oils, triterpenoids, and phytosterols in the extract obtained from petroleum ether. Similarly, the extract obtained from chloroform revealed the presence of alkaloids, phenols, tannins, flavonoids, triterpenoids, and phytosterols. The ethyl acetate extract exhibited the presence of phenolic compounds, tannins, and flavonoids. The ethanolic extract exhibited the presence of alkaloids, glycosides, carbohydrates, gums and mucilage, amino acid, saponins, phenols, tannins, flavonoids, triterpenoids, and phytosterols. The analysis revealed that the aqueous extract of the drug consists of glycosides, carbohydrates,

gums, mucilage, amino acids, saponins, and flavonoids. On the other hand, the butanolic fraction of the ethanolic extract contains alkaloids, phenols, tannins, phytosterols, saponins, and triterpenoids.

Based on the results of the qualitative tests conducted on the various extracts, it was determined that the ethanolic extract and butanolic fraction of the ethanolic extract contained the desired phytochemicals, including flavonoids, phenols, tannins, saponins, triterpenoids, and phytosterols. Therefore, these specific extracts were chosen for further phytochemical and pharmacological studies.

Table 5: Qualitative chemical tests performed on different extracts

Chemical Tests	Pet. ether	Chloro form	Ethyl acetate	Ethanol	Aqueous
Alkaloids					
Dragendorff's reagent	+	+	-	+	-
Mayer's reagent	+	+	-	+	-
Hager's reagent	+	+	-	+	-
Wagner's reagent	+	+	-	+	-
Glycosides					
Brontanger's	-	-	-	+	+
Legal's	-	-	-	+	+
Phenols/Tannins					
Ferric chloride	-	+	+	+	-
Gelatin Solution	-	+	+	+	-
Flavonoids					
Magnesium Ribbon Test	-	+	+	-	+
Saponins					
Foam	-	-	-	+	+
Hemolysis	-	-	-	+	+
Fixed oil/Fats					
Spot	+	-	-	-	-
Gums & Mucilage					

Water	-	-	-	+	+
Carbohydrates					
Molisch	-	-	-	+	+
Fehling's	-	-	-	+	+
Tollen's	-	-	-	+	+
Amino acids					
Ninhydrin	-	-	-	+	+
Millon's	-	-	-	+	+
Biuret	-	-	-	+	+
Triterpenoids					
Lieberman Burchard	+	+	-	+	-
Salkowski	+	+	-	+	-
Molescott's	-	+	-	+	-
Phytosterols					
Lieberman	+	+	-	+	-

Table 6: Chemical Constituents present in the different fractions of extract of *Butea superba*

Chemical Constituents	Butanolic Fraction	Aqueous Fraction
Alkaloids	+	-
Glycosides	-	+
Phenols/Tannins	+	-
Flavonoids	-	+
Saponins	+	+
Fixed oil/Fats	-	-
Gums & Mucilage	-	+
Carbohydrates	-	+
Amino acids/Protein	-	+
Triterpenoids	+	-
Phytosterol	+	-

Thin-layer chromatography (TLC) was conducted to determine the quantity of components present in the individual extracts. Various solvent systems with varying polarities were tested, and the most optimal solvent system, which yielded the highest resolution, was chosen. The extraction was subjected to thin layer chromatography using Silica gel G as the adsorbent to determine the separation pattern of the constituents when different solvents were used. Various solvent systems with varying polarities were tested, and

the most optimal solvent system, which provided the highest resolution, was chosen. The solvent system used for extracting petroleum ether is a mixture of toluene and ethyl acetate in a ratio of 95:5. This solvent system provides the most effective separation, resulting in the detection of 8 distinct spots. On the other hand, when extracting the ethanolic extract and the butanolic fraction of the ethanolic extract, the best resolution is achieved using a mobile phase consisting of chloroform and methanol in a ratio of 22.5:7.5. This mobile phase results in the detection of 8 spots for the ethanolic extract and 4 spots for the butanolic fraction. The thin-layer chromatography (TLC) plates underwent derivatization using the anisaldehyde-sulphuric acid reagent. Upon application of the aforementioned spraying reagents, a notable enhancement in the clarity and distinctiveness of the spots was observed.

CONCLUSION

Herbal medicine is a widely utilized therapeutic modality, wherein the utilization of medicinal plants holds substantial prominence within the realm of traditional medicine. *Butea superba*, a botanical species indigenous to India, was employed in a scientific investigation to assess the efficacy of *Butea superba* extracts. The extracts underwent assessment for physical parameters, including color, odor, and taste. The analysis revealed the existence of alkaloids, fixed oils, triterpenoids, and phytosterols in the petroleum ether extract, chloroform extract, ethyl acetate extract, ethanolic extract, and butanolic fraction of ethanolic extract. The ethanolic extract and butanolic fraction of the ethanolic extract were further analyzed through phytochemical and pharmacological studies. A thin layer chromatography (TLC) analysis was conducted to ascertain the quantity of constituents present in the extracts. The mobile phase yielded the most optimal resolution during the experiment. The

thin-layer chromatography (TLC) plates underwent derivatization using the anisaldehyde-sulphuric acid reagent, which led to the successful separation of spots with high resolution.

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REFERENCES

1. Sirichaiwetchakoon K, Suksuphew S, Srisawat R, Eumkeb G. *Butea superba* Roxb. Extract ameliorates scopolamine-induced cognitive and memory impairment in aged male rats. *Evid Based Complement Alternat Med* [Internet]. 2021; 2021:1–15. Available from: <http://dx.doi.org/10.1155/2021/2703138>
2. Binalee F, Udomuksorn W, Kumarnsit E, Vongvatcharanon U, Vongvatcharanon S. Anabolic effect of *Butea superba* Roxb. On improving skeletal muscle atrophy and strength via androgen receptors and increasing parvalbumin levels in orchidectomized rats. *Sci Asia* [Internet]. 2022;48(6):718. Available from: <http://dx.doi.org/10.2306/scienceasia1513-1874.2022.102>
3. B S, K K. Qualitative analysis of inorganic acid, basic radicals, and estimation of biomolecules in the flower extract of *Butea superba* roxb. *Asian J Pharm Clin Res* [Internet]. 2018;11(10):161. Available from: <http://dx.doi.org/10.22159/ajpcr.2018.v11i10.27087>
4. Eumkeb G, Tanphonkrang S, Sirichaiwetchakoon K, Hengpratom T, Naknarong W. The synergy effect of daidzein and genistein isolated from *Butea superba* Roxb. on the reproductive system of male mice. *Nat Prod Res* [Internet]. 2017;31(6):672–5. Available from: <http://dx.doi.org/10.1080/14786419.2016.1180603>
5. Smitasiri Y, Pinmongkholgul S. Acute effect of *Butea superba* roxb. Extract on intracavernous pressure and blood pressure in adult male rats. *Acta Horti* [Internet]. 2014;(1023):149–53. Available from: <http://dx.doi.org/10.17660/actahortic.2014.1023.21>
6. .Bagchi B, Department of Botany, Bangabasi College, Kolkata-700009, West Bengal, India. A relative study of diversity of endophytic fungi in a Lianas *Butea superba* from Belpahari and their seasonal variation. *International Journal of Experimental Research and Review* [Internet]. 2020; 23:27–34. Available from: <http://dx.doi.org/10.52756/ijerr.2020.v23.003>
7. .Malaivijitnond S, Ketsuwan A, Watanabe G, Taya K, Cherdshewasart W. Luteinizing hormone reduction by the male potency herb, *Butea superba* Roxb. *Braz J Med Biol Res* [Internet]. 2010;43(9):843–52. Available from: <http://dx.doi.org/10.1590/s0100-879x2010007500076>
8. Boonsombat J, Prachyawarakorn V, Pansanit A, Mahidol C, Ruchirawat S, Thongnest S. Superbanone, A New 2-Aryl-3-benzofuranone and Other Bioactive Constituents from the Tube Roots of *Butea superba*. *Chem Biodivers* [Internet]. 2017;14(7):e1700044. Available from: <http://dx.doi.org/10.1002/cbdv.201700044>
9. Leelahawong C, Srisomsap C, Cherdshewasart W, Chokchaichamnankit D, Vinayavekhin N, Sangvanich P. Proteomic analysis of isozymes in *Butea superba* leaves. *Russ J Plant Physiol* [Internet]. 2017;64(1):100–8. Available from: <http://dx.doi.org/10.1134/s1021443717010101>
10. Mizuki D, Qi Z, Tanaka K, Fujiwara H, Ishikawa T, Higuchi Y, et al. *Butea superba*-induced amelioration of cognitive and emotional deficits in olfactory bulbectomized mice and putative mechanisms underlying its actions. *J Pharmacol Sci* [Internet]. 2014;124(4):457–67. Available from: <http://dx.doi.org/10.1254/jphs.13252fp>
11. Mizuki D, Matsumoto K, Tanaka K, Thi Le X, Fujiwara H, Ishikawa T, et al. Antidepressant-like effect of *Butea superba* in mice exposed to chronic mild stress and its possible mechanism of action. *J*

- Ethnopharmacol [Internet]. 2014;156:16–25. Available from: <http://dx.doi.org/10.1016/j.jep.2014.08.014>
12. Arai MA, Koryudzu K, Koyano T, Kowithayakorn T, Ishibashi M. Naturally occurring Ngn2 promoter activators from *Butea superba*. Mol Biosyst [Internet]. 2013;9(10):2489–97. Available from: <http://dx.doi.org/10.1039/c3mb70083f>
 13. Kumar Goswami S, Jamwal R, Balachandran J, Kumar Pandre M, Dethe S, Agarwal A, et al. Multiple enzyme inhibition potential of *Butea superba* for management of erectile dysfunction. Planta Med [Internet]. 2012;78(11). Available from: <http://dx.doi.org/10.1055/s-0032-1320492>
 14. Tocharus C, Sooksaen P, Shimbhu D, Tocharus J. *Butea superba* (Roxb.) improves penile erection in diabetic rats: *B. superba* improves penile erection. Andrologia [Internet]. 2012;44 Suppl 1:728–33. Available from: <http://dx.doi.org/10.1111/j.1439-0272.2011.01257.x>
 15. Cherdshewasart W, Sutjit W, Pulcharoen K, Chulasiri M. Mutagenic and antimutagenic effects of the traditional herb used for treating erectile dysfunction, *Butea superba* Roxb. Biosci Biotechnol Biochem [Internet]. 2010;74(5):923–7. Available from: <http://dx.doi.org/10.1271/bbb.90727>