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ORIGINAL ARTICLE **OPEN ACCESS**

Extraction and Phytochemical analysis of *Butea superba* Roxb using Successive Solvent Extraction and Direct Ethanolic Extraction methods

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ABSTRACT
Herbal medicine is a popular therapeutic approach, with medicinal plants playing a significant role in traditional medicine. Butea superba L., also known as Ghandhali, Bakuchi, Khip, and Prasaran, is a leafy vegetable native to India. Traditional uses of the plant include enhancing strength, power, and male sexual performance. The study of bioactivity for each constituent is crucial as it plays a significant role in regulating bodily functions and addressing various diseases, including diabetes, hypertension, asthma, hepatomas, psoriasis, and potentially cancer. The present study focused on the extraction and phytochemical analysis of Butea superba Roxb, employing both Successive Solvent Extraction (SSE) and Direct Ethanolic Extraction (DEE) methods. The SSE method involved a systematic extraction process using solvents of increasing polarity, while DEE utilized ethanol directly. The extracted components were subjected to phytochemical analysis to identify and quantify the bioactive compounds present in Butea superba. Various analytical techniques, including chromatography, were employed to explore the chemical composition. This comprehensive investigation aims to provide insights into the efficiency of SSE and DEE methods for extracting phytochemicals from Butea superba and to elucidate the diversity of bioactive compounds present in this plant.
Keywords: *Butea superba, Bioactives, Chromatography, Successive Solvent Extraction, Ethanolic Extraction,*

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INTRODUCTION

Herbal medicine is a triumph of popular therapeutic diversity. Almost in all the traditional medicine, the medicinal plants play a major role and constitute the backbone for the same. In order to make sure the safe use of these medicines, a necessary first step is the establishment of standards of quality, safety and efficacy. *Butea superba* L. (b. superba) is locally known in India as a Ghandhali, Bakuchi, Khip, Prasaran. This aromatic climbing plant is a leafy vegetable that can be eaten raw or steamed [1].

The examination of Indigenous practices and a comprehensive literature review on the plant *Butea superba* opens up avenues for further exploration of its phytochemical and pharmacological properties. Traditional applications involve the use of the plant's tuber and stem in medicinal practices, where it is believed to confer strength, enhance power, and improve male sexual performance. This has earned *Butea superba* Roxb. the reputation of a miraculous herb [2].

Given its potential to contribute to human health, there is considerable interest in scrutinizing the chemical constituents of *Butea superba* and understanding their biological activities. The study of bioactivity for each constituent is particularly crucial, as it plays a significant role in regulating bodily functions and addressing a spectrum of diseases, including diabetes, hypertension, asthma, hepatomas, psoriasis, and potentially cancer. Compounds that inhibit phosphodiesterase have demonstrated effects on platelet-aggregation inhibition, stimulation of the central nervous system (CNS), and activation of cellular functions [3].

Research findings by scholars and academics confirm the non-mutagenic nature of the chemicals derived from Butea, highlighting its safety profile. Notably, it induces a potent genital vasodilating effect akin to Viagra but with a smooth action that avoids nervous, muscular, or cardiac overstimulation, thereby safely enhancing erectile performance through nitric oxide production [4].

The bioactivity testing of flavonoids and flavonoid glycosides has revealed higher inhibitory effects on cAMP phosphodiesterase compared to caffeine and theophylline. In light of the serious adverse effects associated with commercial erectile dysfunction drugs, the herbal alternative in Butea superba, with its

gentle yet safe action, emerges as a promising option. Further investigations into the bioactive extracts/fractions of *Butea superba* substantiate its potential benefits. Hence, in consideration of these findings and for various other reasons, it is strongly recommended to conduct additional research to unveil the diverse uses and benefits of this herb [5].

MATERIAL AND METHODS

Ethyl acetate and Petroleum ether were acquired from SD Fine Chemicals in Mumbai. Chloroform and ethanol were sourced from Merck in Mumbai. The leaves of the *Butea superba* plant were collected from the University campus in Sagar, Madhya Pradesh, India. All other chemicals utilized in the study were of analytical grade. Double distilled water (DDW) was consistently employed whenever necessary throughout the research.

Collection and Identification of Drug

The leaves of the *Butea superba* plant were gathered from the University campus in Sagar, Madhya Pradesh, India, during the months of January to March. The authentication process was conducted by Dr. P. Tiwari, with the assigned Herbarium number Bot/1019, at the Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.). The authenticated leaves were then meticulously preserved in the herbarium of the institute for reference and documentation.

Organoleptic Characters of Powdered Drug

A small amount of *Butea superba* each powdered drug was spread on a white tile and physically examined for general appearance i.e. nature, colour, odour, taste, texture etc.

Extraction Procedure

Following procedure was applied for the preparation of extracts from the shade dried and powdered leaves of Butea superba.

Successive Solvent Extraction

Defatting of Plant Material

Leaves of *Butea superba* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in a soxhlet apparatus. The extraction was continued till the defatting of the material had taken place [6].

Extraction with Chloroform

The defatted marc of the drugs was subjected to extraction with chloroform in a soxhlet apparatus. The extraction was continued for a period of 6-7 days. The extract was then concentrated and finally dried to a constant weight [7].

Extraction with Ethyl Acetate

The marc obtained after chloroform extraction was subjected to extraction with ethyl acetate. The extraction was continued for a period of 6-7 days for complete extraction. The extract was concentrated and finally dried.

Extraction with Ethanol

The marc after ethyl acetate extraction was subjected to ethanol extraction in soxhlet apparatus. The extraction was performed for a period of 6-7 days to achieve complete extraction. Further extract was concentrated and dried to a constant weight.

Extraction with water

Lastly, the marc was subjected to hot water maceration. The maceration was continued for a period of 24 hrs. The aqueous extract was filtered and concentrated [8].

Direct Ethanolic Extraction

Defatting of Plant Material

Leaves of *Butea superba* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether (60-80°C) in soxhlet apparatus. The extraction was continued till the defatting of the material had taken place. Complete defatting was ensured, by collecting few drops from the thimble on the filter paper showed no oily spot.

Extraction with Ethanol

The marc obtained after petroleum ether was subjected to ethanol extraction in soxhlet apparatus. The extraction was continued for a period of 6-7 days to achieve complete extraction. The extract was concentrated under reduced pressure up to dryness. Thus obtained semisolid dark green extract was designated as ethanolic extracts [9].

Fractionation of Ethanolic Extract

The dried ethanolic extract was suspended in a small quantity of water and fractionated with butanol by using separating funnel. The resultant butanolic fraction of ethanolic extract was used for further study [10].

Physical Examination of the Butanolic Fraction of Ethanolic Extract

The dried butanolic fraction of the drugs was evaluated for physical parameters such as consistency, Colour, odour and taste.

Phytochemical Analysis of the Prepared Extracts [11-13]

The extracts obtained by solvent extraction were subjected to various qualitative test to detect the presence of plant constituents.

Test for Carbohydrates

Fehling's Test (for reducing sugars)

Test solutions were treated with few drops of Fehling's reagent [Dissolve 34.66g of Copper sulphate in distilled water and make upto 500 ml (solution A). Dissolve 173 g of potassium sodium tartarate and 50 g of sodium hydroxide in distilled water and make volume upto 500 ml (solution B). Mix two solutions in equal volume prior to use for detection of reducing sugars. It gives brick red Colour on warming with the test sample

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 ammonical silver nitrate solution (Tollen' 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 bullk 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 were 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 dthe paper was then sprayed with Ninhydrin reagent. The paper was then allowed to

dry and then heated in an oven at 115ºC for 5 minutes. Appearance of violet coloured spots indicated the precence 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

A useful procedure for initial trials is to run two separates' plates on using very polar solvents, e.g., ethanol, and the other employing a non-polar liquid such as hexane. After observing which type of mobile phase moves the solutes from the origin and determining their Rf values, the solvents may be modified to increase selectivity and resolution in number of ways. The polarity may be altered by adding other solvents keeping in view their dielectric constants. Substance with functional groups similar to those of the solutes such as ether, alcohols or carboxyl's, may be added to increase the Rf value by promoting solubility in the mobile phase [14]. Acids or bases (acetic acid or ammonia) may be added to affect the charges on the solutes to prevent tailing (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

About 50 mg of Petroleum ether extract was dissolved in 2 mL of petroleum ether and was used for TLC studies. Similarly samples of Ethanolic extract, Butanolic fraction of Ethanolic extract were prepared for TLC.

Detecting Reagent

Anisaldehyde – Sulphuric acid reagent: 0.5 ml Anisaldehyde was mixed with 10 ml of Glacial Acetic acid followed by 85 ml methanol and 5 ml conc. Sulphuric acid drop wise. 5% ferric chloride solution in methanol. 10% sulphuric acid in methanol [15].

RESULTS AND DISCUSSION

All the extracts of *Butea superba* were evaluated for physical parameters such as consistency, color, odor and taste. The extracts obtained were greenis yellow in case of petroleum ether extraction, dark green in case of direct ethanolic extraction but in case of butanolic fraction of ethanolic extract was show brown in colour. Both the ethanolic extracts and butanolic fraction of ethanolic extract had a fetid odor and a bitter taste.

Qualitative chemical tests were carried out for all the extracts of *Butea superba* The results of the tests showed the presence of alkaloids, fixed oils, triterpenoids and phytosterols in the petroleum ether extract, chloroform extract showed the presence of alkaloids, phenols, tannins, flavonoids, triterpenoids and phytosterols. Ethyl acetate extract showed the presence of phenols, tannins and flavonoids. The ethanolic extract showed that it contains alkaloids, glycosides, carbohydrates, gums and mucilage, amino acid, saponins, phenols, tannins, flavonoids, triterpenoids and phytosterols. Finally aqueous extract of the drug was found to be glycosides, carbohydrates, gums and mucilage, amino acid, saponin and flavonoids and butanolic fraction of ethanolic extract contain alkaloids, phenols, tannins, phytosterols, saponins, and triterpenoids .

On the basis of all the qualitative tests performed in each extract ethanolic extract and butanolic fraction of ethanolic extract was subjected for the further phytochemical and pharmacological studies because only the ethanolic extract and butanolic fraction of ethanolic extract showed the presence of desired phytochemicals i.e. flavonoids, phenols, tannins, saponins, triterpenoids and phytosterols.

Table 1: Qualitative chemical tests performed on different extracts

Chemical Tests	Pet. ether	Chloroform	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 2: 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	+	-

+ Present; - Absent

TLC was performed to find out the number of constituents in the respective extracts. Different solvent systems of different polarity were tried and the best solvent system, which gives best resolution, was selected. Thin layer chromatography of the extraction performed with different solvents using Silica gel G as adsorbent suggesting the separation pattern of the constituents. Different solvents systems of different

polarity were tried and the best solvent system, which gives best resolution, was selected. For petroleum ether extract solvent system Toluene: Ethyl acetate (95:5) give best separation give 8 spots and for the ethanolic extract and butanolic fraction of ethanolic extract best resolution was obtained in the mobile phase, Chloroform: Methanol (22.5:7.5) give 8 and 4 spots respectively. The TLC plates were derivatized with anisaldehyde-sulphuric acid reagent. After using these spraying reagents showed good resolution of the spots.

CONCLUSION

Herbal medicine is a popular therapeutic approach, with medicinal plants playing a significant role in traditional medicine. Butea superba, a leafy vegetable native to India, was used in a study to evaluate the extracts of Butea superba. The extracts were evaluated for physical parameters, color, odor, and taste. The results showed the presence 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 ethanolic extract were subjected to further phytochemical and pharmacological studies. Thin layer chromatography (TLC) was performed to determine the number of constituents in the extracts, with the best resolution obtained in the mobile phase. The TLC plates were derivatized with anisaldehyde-sulphuric acid reagent, resulting in good resolution of spots.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of the paper.

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