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RESEARCH ARTICLE

Preliminary phytochemical and physicochemical Investigation and thin layer chromatography of ButeaMonosperma flower extract

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ABSTRACT

Almost all parts of the plant namely root, leaves, fruit, stem bark, flowers, gum, young branches are used as medicine. The fresh juice of Butea monosperma is applied to ulcers and for congested and septic sore throats (1). The gum is a powerful astringent given internally for diarrhea and dysentery. The bark is reported to possess astringent bitter, pungent, alliterative, aphrodisiac and anthelmintic properties also useful in tumors, bleeding piles and ulcers. The decoction is useful in cold, cough, fever and menstrual disorders. Roots are useful in elephantiasis and in curing night blindness and other eyesight defects. Also cause temporary sterility in women. They are also used to cure boils, pimples and tumors hemorrhoids and piles. Flowers are reported to possess astringent, diuretic, depurative, aphrodisiac and tonic properties. They are used to reduce swellings. Also effective in leprosy, leucorrhea and gout. In present study Preliminary phytochemical and physicochemical Investigation and thin layer chromatography of ButeaMonosperma flower extract. Various phytochemical studies are performed through which we get the knowledge about different phytoconstituent which are present in ethanolic extract of flower of Butea monosperma.

INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind (1). The use of herbs and medicinal plant as the first medicines is a universal phenomenon. Every culture on the earth, through written or oral tradition, has relied on the vast variety of natural chemistries found in plants for their therapeutic properties. All drugs from the plant are substances with a particular therapeutic action extracted from plants. Butea monosperma (Lam.) is a sacred tree, referred to as a treasurer of the gods, and used in sacrifice related rituals. From its wood, sacred utensils are made. The flowers are offered as in place of blood in sacrifice rituals to goddess Kali. The dry stem pieces are used to make sacred fire. It is an anthropogenic tree of several castes. 'Chakradatta' mentions the use of its gum in external astringent application. The leaves are believed to have astringent, depurate, diuretic and aphrodisiac properties. It promotes diuresis and menstrual flow. The seed is anthelmintic. The bark is also used in snakebite. When seeds are pounded with lemon juice and applied to the skin the act as a rubefacient. Arab horse dealers put one seed into each feed of corn to keep their horses in condition. The plant is used in ayurvedic, unani and siddha medicine for various ailments. Almost all parts of the plant namely root, leaves, fruit, stem bark, flowers, gum, young branches are used as medicine, food, fiber and for other miscellaneous purpose such as fish poison, dye etc (2, 3, 4). Almost 45 medicinal uses are associated with the plant and out of these claims almost half the number of claims have been scientifically studied and reported. The fresh juice of Butea monosperma is applied to ulcers and for congested and septic sore throats. The gum is a powerful astringent given internally for diarrhea and dysentery. The bark is reported to possess astringent bitter, pungent, alliterative, aphrodisiac and anthelmintic properties. Useful in tumors, bleeding piles and ulcers. The decoction is useful in cold, cough, fever and menstrual disorders. Roots are useful in elephantiasis and in curing night blindness and other eyesight defects. Also cause temporary sterility in women. Also applied in sprue, piles, ulcers, tumors. Leaves have astringent, tonic, diuretic and aphrodisiac properties (5). They are also used to cure boils, pimples and tumors hemorrhoids and piles. Flowers are reported to possess astringent, diuretic, depurative, aphrodisiac and tonic properties. They are used to reduce swellings. Also effective in leprosy, leucorrhea and gout. In 1938, TLC was

developed by Ismailoff and Schraiber. An adsorbent is coated on a glass plate which serves as a stationary support on which the mobile phase percolates and develops the chromatogram (6). This method is simple, rapid in separation and sensitive. The speed of separation is fast and it is easy to recover the separated compounds from plate (6).

MATRIALS AND METHOD:-

Materials

Fresh flowers about 150gm of *Butea monosperma* were collected in the flowering season is from March-April. Ethanol (95%) was purchased from CDH chemical laboratory, New Delhi, India, Silica Gel "G", Toluene, Ethyl Acetate and Vanillin-Sulphuric Acid Reagent were purchased from Spectrochem Mumbai, India. All other chemicals used were of analytical reagent grade and were utilized as they received.

Method

Preparation of Extract

The extraction process of flower of *Butea monosperma* was done by maceration process .In this process initially 150gm of dried flowers were weighed and put in 250ml conical flask then 200ml of extraction fluid (ethanol) was added on this and the fluid was poured on dried flowers of *Butea monosperma* previously weighed. The mixture was then kept for three days in a tightly sealed flask at room temperature protected from sunlight. The mixture was shaken several times daily. The mixture was filtered and filtrate was collected. The extract was concentrated under atmospheric pressure. The extract prepared was then transferred to suitable container and the further experiment work was started.

Standardization Parameters

All the parameters performed as per W.H.O. (WORLD HEALTH ORGANIZATION) Guidelines.

Determination of ash value

A 3gm of the grounded air dried material was taken in previously ignited and tarred crucible. The material was then spread in an even layer and ignited in by gradually increasing the heat to 500-600 until its white indicating an absence of carbon. Then it was cool in desiccators for 30min and then weighed. Then content of total ash was calculated in mg/gm of dried material (7, 8).

Acid Insoluble Ash

To the crucible containing the total ash 25ml of hydrochloric acid was added and cover with a watch glass and then boiled gently for

5min. Watch glass was then rinsed with hot water and this liquid was added to crucible. The insoluble material was collected on an ash less filter paper and then washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to original crucible dry on a hot plate and was ignited to a constant weight. The residue was allowed to cool in suitable desiccators for 30min and then weighed. The content of acid insoluble ash was collected in mg/gm of dried material (7, 8).

Water Soluble Ash

To the crucible containing the total ash 25ml of water added and then boiled for 5min. The insoluble matter was collected on a ash less filter paper. Then it was washed with hot water and ignited it in a crucible for 15min at a temperature not exceeding 450. The weight of this residue in mg is subtracted from the weight of total ash then the content of water soluble ash was calculated in mg/gm of air dried material (7, 8).

Determination of Extractable Matter (Extractive Value)

4gm of coarsely powdered air dried material accurately weighed in a glass stoppered conical flask. It was then macerated with 100ml of ethanol for 6 hours. Then it was allowed to stand for 18 hours. It was filtered and 25ml of the filtrate was transferred to a tared bottom dish and evaporated to dryness on a water bath. It was dried at 105 for 6 hours. It was cooled in desiccators for 30min and then it was weighed. Content of extractable matter was calculated in mg/gm of air dried material (7, 8).

Determination of Loss on Drying

Determination of loss on drying is also known as gravimetric determination. 3gm of material was accurate weighed place in a previously dried and tarred weighing bottle. The sample was dried by both the methods. In an oven at 100-105°C and in a desiccators over phosphorous pentoxide under atmospheric pressure or reduced pressure and at room temperature. It was dried till 2 consecutive readings don't differ by 5 mg. Then at last loss of weight in mg/gm of air dried material was calculated (7, 8).

Determination of Swelling Index

Simultaneously three determinations for the given material. The material was previously reduced to required fineness. 3gm of the material was accurately weighed. It was placed into a 25ml glass stoppered measuring cylinder. 25ml of water was added and the mixture was shaken thoroughly for

1hour. It was allowed to stand for 3 hours at room temperature. The volume occupied by the material was measured. The mean value of the individual determinations was carried out (7, 8).

Preliminary Phytochemical Screening

Test for Alkaloids (7, 8)

1) Dragondroff's test: - In 2-3ml of filtrate, few drops of previously prepared Dragondroff reagent was added.
2) Mayer's test: - In 2-3ml of filtrate, few drops of Mayer reagent was added.
3) Hager's test: - In 2-3ml of filtrate, few drops of Hager reagent was added.
4) Wagner's test: - In 2-3ml of filtrate, few drops of wagner reagent was added.

Test for Flavonoids (7, 8)

1) In 2-3ml of filtrate, Lead Acetate solution was added.
2) In Few drops of filtrate, Sodium Hydroxide solution was added
3) Shinoda Test: - In Few drops of filtrate, 5ml of Ethanol, Few drops of Conc. HCl and Magnesium Turnings were added.

Test for Tannins (7, 8)

1) In 2-3ml of alcoholic extract, Ferric Chloride solution was added.
2) In 2-3ml of alcoholic extract, Lead Acetate solution was added.
3) In 2-3ml of extract, Acetic Acid was added.
4) In 2-3ml of extract, dilute Nitric Acid was added.
5) In 2-3ml of ethanolic extract, Potassium Permagnate solution was added.

Test for Steroids (7, 8)

1) Salkowski Test: - In 2ml of extract, 2ml of Chloroform, 2ml of Conc. Sulphuric Acid were added and shaked well.
2) Lieberman Burchard Test: - In 2ml of extract, 2ml of Chloroform, 2ml of Conc. Sulphuric Acid and 2ml of Acetic Anhydride Solution were added.
3) Lieberman Test: - In3ml of extract, 3ml of Acetic Anhydride and few drops of Conc. Sulphuric Acid were added.

Test for Carbohydrate (7, 8)

1) Test for Reducing Sugar

   Fehling Test: - In Few drops of extract, 1ml of Fehling solution A and B was added
2) Test for Pentose

   Aniline Acetate Test: - The test solution (flower extract) was boiled and treated

with filter paper soaked in Aniline acetate solution solution was added

3) Test for Hexose:-

Cobalt Chloride Test: - In 3ml of test solution, 2ml of Cobalt Chloride solution was added and then boiled and cooled after that few drops of Sodium Hydroxide solution was added.

Test for Glycoside (7, 8)

1) Test for Saponin

Foam Test: - Drug extract was shaken vigorously with water.

2) Test for anthraquinone Glycoside: - In 3ml of extract, dilute sulphuric acid and equal volume of benzene was added and shaked.

Thin Layer Chromatography (7, 8)

Adsorbent - Silica Gel "G"

Mobile Phase - Toluene **:** Ethyl Acetate (9**:**1)

Detecting Agent - Vanillin-Sulphuric Acid Reagent

Sample For Detection - Ethanolic Extract of *Butea monosperma* Flower

TLC plates were prepared by mixing the adsorbent (silica gel"G") with a small amount of inert binder like calcium sulfate (gypsum) and water. This mixture was spread as thick slurry on glass slide. The TLC plates were dried and activated by heating in an oven for 30min at 110 °C. The thickness of the adsorbent layer is typically around 0.1–0.25mm for analytical purposes. A small spot of solution containing the sample (Extract) is applied to a TLC plate, about 1cm from the bottom edge. The solvent is allowed to completely evaporate off. A small amount (10ml) of Mobile Phase (Toluene**:**Ethyl Acetate) was poured in to a glass beaker and TLC plate was put into the chamber, so that its bottom touches the solvent, and the plate lies on the chamber wall and reaches almost to the top of the container. The container was closed with a cover glass and plate was left for a few minutes to move the mobile phase on TLC plate by capillary action. When the mobile phase was reached on the top of the TLC plate and separation was take place in the separating chamber, the plate was removed and dried. After dryness of the TLC plate the detecting agent (Vanillin-Sulphuric Acid Reagent) was sprayed and observed the plate under UV lamp. Then R_f Value was calculated.

RESULT AND DISCUSSION

The Standardization Parameters investigation of ethanolic extraxt of flower of *Butea monosperma* can be performed under the following methods like Total Ash Value, Extractive value, Swelling index and under

loss on drying categories. The data was obtained of following estimation was satisfactory and shows that the phyotochemical agents are present in the flower extract in effective quantity as well as in quality. The results of Standardization Parameters were shown in the table 1.

The phytochemical investigation ethanolic extraxt of flower of *Butea monosperma* can be performed under the following tests like Alkaloids, Flavonoids, Tannins, Steroid, Carbohydrates, and Glycoside categories. The result was obtained of following estimation was satisfactory and shows that the Alkaloids, Flavonoids, Carbohydrate, Tannins and Steroids like chemical constituets are present in the flower extract in effective quantity and quality. The results of phytochemical study were shown in the table 2.

Thin layer chromatography was carried out using Toluene: Ethyl Acetate (9:1). After running the solvent, R_f Values were calculated for the obtained two spot. On the basis of R_f Values (0.31 and 0.65), the compounds were identified as Butrin and Isobutrin.

Calculation of R_f Value

Rf Value = 1.8 / 5.8 = 0.31

Rf Value = 3.8 / 5.8 = 0.65.

CONCLUSION

The flower of *Butea monosperma* has many uses which include Anthelmintic, dysentery, cold, cough, fever, menstrual disorders, tumors, bleeding piles and ulcers, night blindness and other eyesight defects. In present study Preliminary phytochemical and physicochemical Investigation and thin layer chromatography of *ButeaMonosperma* flower extract. Various phytochemical studies are performed through which we get the knowledge about different phytoconstituent which are present in ethanolic extract of flower of *Butea monosperma*.

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Table 1. Standardization Parameters of ethanolic extraxt of flower of *Butea monosperma*

Standard parameter	Standard	Result
Ash value		
1) Total ash	Not more than 7%	6.8%
2) Acid insoluble ash	Not more than 1%	0.5%
3) Water soluble ash	Not more than 3%	2.3%
Extractive value		
1) Water soluble extractive	Not more than 20%	17.1%
2) Alcohol soluble extractive	Not more than 7%	6.23%
Loss on drying	-	10%
Swelling index	-	12%

Table 2. Preliminary Phytochemical Investigation ethanolic extraxt of flower of *Butea monosperma*

Experiment	Observation	Inference
Alkoloids		
Dragondroff's test	Orange brown ppt was formed	Present
Mayer's test	Ppt formed	Present
Hager's test	Yellow ppt was formed	Present
Wagner's test	Red ppt	Present
Flavonoids		
2-3ml of filtrate + lead acetate	Yellow color ppt was formed	Present
Few drops of filtrate + sodium hydroxide	Yellow color was formed which decolorizes after addition of acid	Present
Shinoda Test	Pink color was observed	Present
Tannins		
2-3ml of alcoholic extract + ferric chloride solution	Deep blue black color was formed	Present
2-3ml of extract + lead acetate	White ppt was formed	Present
2-3ml of extract + acetic acid	Red color solution was formed	Present
2-3ml of extract + dil. Nitric acid	Reddish to Yellow color was formed	Present
2-3ml of extract + potassium permagnate solution	Red ppt was formed	Present
Steroid		

Salkowski test	Chloroform appears Red and acid layer shows Greenish color	Present
Lieberman Burchard test	First Red color then Blue and finally Green color appears	Present
Lieberman test	Blue color was appeared	Present
Carbohydrate		
Fehling Test	No color change was observed	Absent
Aniline Acetate Test	No color change was observed	Absent
Cobalt Chloride Test	Solution appears Greenish Blue	Present
Glycosides		
Test for saponin	No change was observed	Absent
Test for anthraquinone Glycoside	No change was observed	Absent