Antibacterial assay of leaf of Dalbrgia sisso roxb

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ABSTRACT

Different parts of Dalbrgia sisso roxb. has been traditionally used for medicinal purposes to cure various diseases. In the present study of antibacterial activity of leaves and their various extracts (methanolic, pet ether, citric acid and ethanolic) has been investigated against Escherichia coli (gram negative species) by using disc diffusion method. Antibacterial activity of citric acid extracts has been found more potent against E. coli. But the methanolic extract of leaves of Dalbrgia sisso roxb. showed antibacterial activity against the bacterial test species second more potent drug. Methanolic extract of leaves as well as panicilline are have less potent antibacterial activity against Escherichia colis.

Key Words: Antibacterial activity, Dalbrgia sisso roxb., Escherichia coli.

INTRODUCTION

Synonyms: English: Indian rosewood, Bombay black wood, **Hindi:** Shisham, sissoo, sissu, **Manipuri:** sissu, **Bengali:** Sitral, shishu, shisu, sisu, **Nepali:** sisham, sisso, **Sanskrit:** aguru, shinshapa, **Spanish:** sissu, **Tamil:** gette, nukku kattai, sissuti, yette, **Thai:** du-khaek, pradu-khaek, **Trade name:** sisso [1]

Fig. Plant of Dalbergia

Bilogiacal Source: It is obtained from **Stem bark** and **heartwood** of "Dalbergi sisso roxb" blonging to **family** Fabeaceae.

Propogation:-

It takes place most commonly by root suckers and also by seeds. The seeds remain viable for only a few months. Seeds should be soaked in water for 48 hours before sowing60 – 80% germination can be expected in 1 – 3 weeks. Seedlings require partial sun or fullsun[2-3].

Taxonomical Classification

Kingdom: Plantae, **Division**: Magnoliphyta, **Class**: Magnoliopsida, **Order**: Fabales **Family**: Fabiaceae, Leguminoceae**, Subfamily**: Faboideae, **Genus**: Dalbergia, **Species**: D.sisso, **Phylum**: Tracheophyta, **Scientific name:** Dalbergia sisso[2-3].

Plant Dscription:-

Shisham, Dalbergia sissoo Roxb. (Leguminosae, subfamily Papilionoideae) is a medium to large deciduous tree with a light crown which reproduces by seeds and suckers. It can grow up to a maximum of 25m in height and 2 to 3m in diameter, but is usually smaller. Trunks are often crooked when grown in the open. Leaves are leathery, alternate, pinnately compound and about 15cm long. Flowers are whitish to pink, fragrant, nearly sessile, up to 1.5cm long and in dense clusters 5 – 10cm in length. Pods are oblong, flat, thin, strap-like 4 – 8cm long, 1cm wide and light brown. They contain 1 – 5 flat bean-shaped seeds 8 – 10mm long. They have a long taproot and numerous surface roots which produce suckers. Young shoots are downy and drooping; established stems with light brown to dark gray bark to 2.5cm thick, shed in narrow strips; large upper branches support a spreading crown[2-3]**.**

MATERIALS AND METHODS

Selection of plant

Dalbergia sisso Roxb.belongs to family Fabeaceae is medicinally important plant, commonly grown in some parts of our country and used in the treatment of various disease and disorders of human ailments by tribal and rural people of our country. So, far no any systematic work was carried out to investigate the anti-bacterial activity of leaf of the selected plant therefore, the plant was selected for present investigation[4].

Collection of plant material

The leaves of the selected plant were collected in the months of August 2011 from the botanical gardens of Ujjain District of Madhya Pradesh.

Extraction of plant material:

Process: Drug + whole of menstruum, Shake occasionally during 7 days, Strain of liquid, and press the marc, Mix the liquid, clarify by↓↓, subsidence for filtratiy

Extraction: 25 gm. Of leaf powder drug was taken and dissolved in 200 ml of citric acid, Ethanol, Methanol, Pet ether Solvent in glass stopper flask, Than solution was stirred with magnetic stirrer for 3 hours and shake occasionally during 7 days, Than solution was filtered and the filtrate was evaporated on water bath to get the extract.

Preliminary Phytochemical screening

The aqueous extract obtained after decoction of leaves was subjected to various Phytochemical screening as per the standard procedure to reveals various active phytoconstituents.

1. Tests for fixed oils and fats, Spot test A small quantity of extract solution was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5 N alcoholic potassium hydroxide was added to a small quantity of sample along with a drop of phenolphthalein, the mixture was heated on a water bath for 1-2 hours, formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

2. Tests for steroids and triterpenoids, Libermann-burchard test The extract solution was treated with few drops of acetic anhydride, boiled and cooled. Then con. Sulphuric acid was added from the side of test tube, brown ring was formed at the junction two layers and upper layer turn green which showed presence of steroids and formation of deep red colour indicated presence of triterpenoid.

Salkowski test The extract solution was treated with few drop of conc. sulphuric acid, red colour at lower layer indicated presence of steroids and formation of yellow coloured lower layer indicated presence of triterpenoids.

3. Test for proteins and free amino acids A small quantity of the extract solution was dissolved in few ml of water and treated with following reagents.

- **Million's reagent:** Small quantity of extract solution was taken, added few drops of millions reagent red colour was obtained (mercury fuming nitric acid).
- **Ninhydrin reagent:** Small quantity of extract solution was taken, added few drops of ninhydrin reagent (0.1% solution in butanol).
- **Biuret's test:** Small quantity of extract solution was taken, added 5% of sodium hydroxide and 1% of copper sulphate solution pink or purple colour was obtained (sodium hydroxide and copper sulphate solution).

4. Test for tannins.

- **Ferric chloride solution:** Treated the extract solution with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.
- **Lead acetate solution:** Treated the extract solution with 10% lead acetate solution white precipitate was obtained.

5. Test for Flavonoides

- **Alkaline reagent test**To the extract solution added few drops of magnesium hydroxide solution, intense yellow colour was formed which turn to colourless on addition of few drops of dilute acid indicated presence of Flavonoides.
- **Shinoda test** To the extract solution added few magnesium turnings and concentrated hydrochloride drop wise pink, crimson red colour appeared after

6. Carbohydrates:-

- **Molisch's test:-**To the extract solution added few drops of α-naphthol, and then added few drops of sulphuric acid through the side of test tube. Purple to violet colour appears at the junction.

7. Glycosides

Borntrager's test:-Boiled the extract solution with 1ml of sulphuric acid in test tube for 5 min. Filtered while hot. Cooled the filtrate and shake with equal volume of dichloromethane or chloroform.Saperate the lower layer of dichloromethane or Chloroform & shake it with half of its volume of dilute ammonia shows the presence of anthraquinone glycosides. A rose pink to red colour is produced in the ammonical layer few minutes.

8. Alkaloids

Dragendorff's reagent:-To the extract solution added few drops of Dragendorff's reagent (potassium bismuth iodide solution). Reddish brown precipitate obtained.

- **Mayer's reagent: -** To the extract solution added few drops of Mayer's reagent (potassium mercuric iodide solution) Cream colour precipitate obtained.
- **Wagner's reagent: -** To the extract solution added few drops of Wagner's reagent (iodine-potassium iodide solution) Reddish brown precipitate obtained.
- **Hager's reagent: -** To the extract solution added few drops of Hager's reagent (saturated solution of picric acid) Yellow precipitate obtained.

9. Carbohydrates

- **Molisch's test:-**To the extract solution added few drops of α-naphthol, and then added few drops of sulphuric acid through the side of test tube. Purple to violet colour appears at the junction.

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Culture media plate was prepared:-

Table.2 Nutrient agar media for bacteria:-

S.No	Ingredients	Quantity Prescribed	Quantity Taken
1	Beef Extract	10 gm	2.5 gm
2	Peptone	10 gm	2.5 gm
3	Sodium chloride	5.0 gm	1.25 gm
4	Agar	20 gm	5 gm
5	Distilled Water	1000 ml	250 ml

- **Procedure for bacteria media:**

1) 5 gm of agar was dissolved in 250 ml of water.

2) Then beef extract, peptone and sodium chloride was added in above agar solution with continuous stirring.

3) The media was heated to dissolve the agar to form the clear liquid.

4) Then pH was maintained at 7.2-7.4 by pH paper.

5) Sterilized by autoclave at 115^{o}c at 15 lb pressure for 15 minutes.

Procedure for Antibacterial Activity:-

1) Collection of micro-Organism: - one bacterial Escherichia-coli (gram –ve) from Ujjain.

2) Drug Entrapped disc were Prepared: - Whatman filter paper was pieces intosmall disc one quarter inch diameter. All dilutions were applied to autoclaved filter paper disc using micro pipette with sterile pipette tip.

3) Culture for Micro-organism: - The identified organism was applied to media for sensitivity test by streaking of medium with help of swab.

4) Application of disc: - All the disc of sample, standard, control with different dilution was placed on the inoculation plate with the help of flame sterilized forceps. After application of disc lead of petri-plate was closed, petri-plate was inoculated at 37^{o}c for 24 hours for bacteria and 72 hours for fungi.

5) Procedure for dilution:-

1) 100 mg drug was dissolved in 100 ml of distilled water to prepared stock solution (1000 µg /ml).

2) From the above solution 10 ml was taken in volumetric flask and diluted upto 100 ml to prepared sub-stock solution (100 µg/ml).

3) From the above solution the adequate concentration2% 5% and10% was

Prepared and in the same way standard drug concentration 100 µg/ml was

Prepared.

6) Zone Of Inhibition: - After incubation the plate were inspected to identify zone of inhibition. The diameter of zone of inhibition of each compound and the diameter of disc was recorded and zone of inhibition was calculated by using formula below:

Zone of inhibition= Diameter of sample/ standard **-** diameter of disc

RESULTS AND DISCUSSION

This attempt was made to study the pharmacognostical, phytochemical and antimicrobial activities of plant. The study was divided into three major parts viz.

- Phytochemical screening
- Antibacterial activities

Table no. 3 Extract yield and characterization

S/No.	Solvent	Color of extract	Yield
1.	Citric acid	Light green	1g
2.	Ethanol	Dark brown	200mg
3.	Pet ether	Dark green	100mg
4.	Methanol	Dark Green	150mg

Phytochemical Screening

The various extract of the plant of Dalbergia sisso were subjected to phytochemical screening which reveal the presence of various pharmacological active components. Phytochemical Screening of sample Dalbergia sisso was done as follows:

Table no. 4 List of Phytochemical screening.

Constituents	**Test**	**EXTRACT**			
		Citric acid	**Ethanol**	**Pet. ether**	**Methanol**
Alkaloids	Mayer's test	+ve	+ve	+ve	+ve
	Dragondroff's test	+ve	+ve	+ve	+ve
Carbohydrates	Molisch's test	+ve	+ve	+ve	+ve
Glycosides	Borentragers test	+ve	+ve	+ve	+ve
Fixed oil and fats	Spot test	+ve	-ve	-ve	-ve
Tannins	Fecl3	-ve	+ve	-ve	-ve
Protein	Million's test	+ve	+ve	+ve	+ve
	Biuret test	+ve	+ve	+ve	+ve
Flavanoids	Shinoda test	+ve	+ve	+ve	+ve
Triterpenoids	Salkowski's test	-ve	+ve	-ve	-ve

The different concentrations (2µg/ml,5µg/ml,100µg,/ml) of extracts were tested for antibacterial activity. The most effective concentration was found to be (citric acid extract of Dalbergia sisso) because it gave the Zone of inhibition.

Table no.5 Zone of Inhibition

Strain	**Escherichia Coli**		
Concentration(µg / ml)	20	50	100
Sample Dalbergia sisso drug			
Citric acid	_	9	8
Ethanol	_	4	5
Mathanol	_	2	7
Pet-ether	_	3	6
Standard drug			
Penicillin (100 / ml)			24

Zone of Inhibition

Zone of Inhibition was less as Compared to Standard drugs and it appears that when Concentration of sample solution increases than zone of inhibition was increases.

Fig:9. Antibacterial culture media of zone of inhibition 1

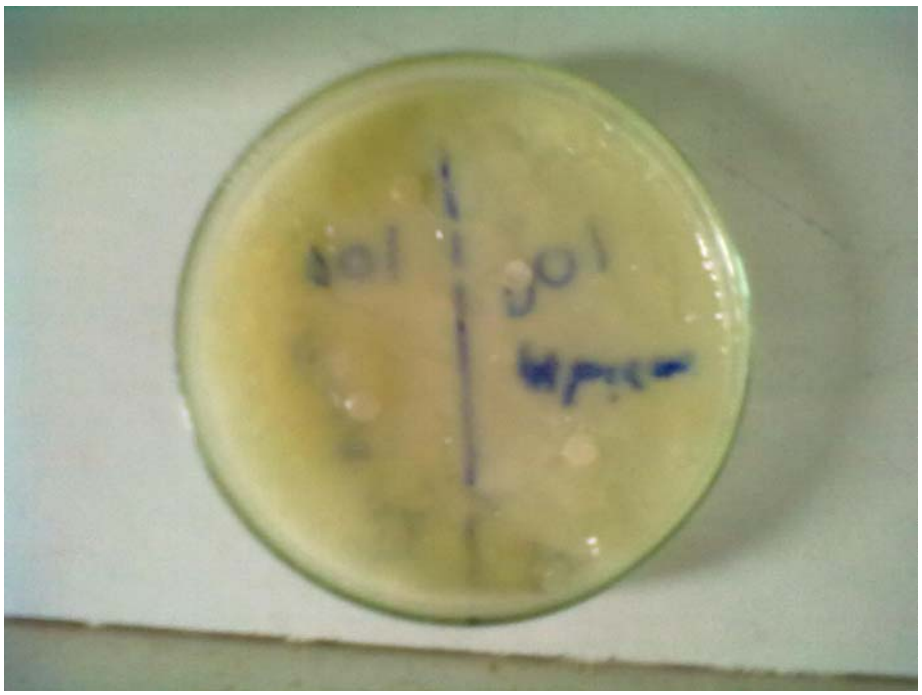

Fig :10. Antibacterial culture media of zone of inhibition 2

Conclusion

Then the preliminary phytochemical screening was done to determine the presence of its active constituents. Due to preliminary phytochemical screening it shows the presence of proteins, Flavonoides, alkaloids, carbohydrates and glycosides. Further, the antibacterial activity of plant was performed and reported. For the antibacterial activity ofDalbergia sisso, the antibacterial and was done. For the antibacterial activity the one bacterial strains were used i.e. Escherichia-coli. If the concentration was increases the zone of Inhibition was increases.

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