

Evaluate the Efficacy of *Indigofera aspalathoides* against Bacteria

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ABSTRACT

Plants contain chemical compounds that may be in one way or another responsible for healing properties and other functions. Phytochemistry deals with the analysis of plant chemicals called natural products, and with changes occurring in such chemicals due to alterations in environmental conditions. Many plants have been used because of their antimicrobial traits, which are chiefly due to the synthesis of secondary metabolites. The present study is aimed to investigate the phytochemical and antibacterial activity of the medicinal plant *Indigofera aspalathoides* against *Escherichia coli*, *salmonella typhi*, *shigella*, and *staphylococcus aureus*. The initial screening of antibacterial activity for the plant extracts was studied using the ethanolic, hexane and diethyl ether extracts. The ethanolic extracts of *Indigofera aspalathoides* leaves showed prominent antibacterial activity against *E.coli* and *salmonella typhi*. The total soluble proteins analysed in control and extract treated *E.coli* exhibited protein synthetic machinery. The ethanolic, hexane and diethyl ether extracts produce very little changes in inhibiting *shigella* and not that much in *staphylococcus aureus* cultures. Our results indicate that the ethanolic extract of *Indigofera aspalathoides* leaves have potent antibacterial components against *E. coli* and *salmonella typhi*.

Keywords: *Indigofera aspalathoides*, Antibacterial activity, Efficacy, Secondary metabolites.

Abbreviations: MTCC – Microbial Type Culture Collection, LBA – Luria Bertani Agar, MSA – Mannitol Salt Agar, SSA – Salmonella Shigella Agar

1. INTRODUCTION

Indigofera is a large genus of about 700 species of flowering plants belonging to the family Fabaceae. The species are mostly shrubs, though some are herbaceous, and a few can become small trees up to 5–6 m tall. Most are dry-season or winter deciduous. The leaves are pinnate with 5–31 leaflets and the terminal leaflet present. Leaf sizes vary from 3–25 cm. The flowers are small, produced on racemes 2–15 cm long. The plant *Indigofera aspalathoides* (Leguminosae) is commonly known as 'Shivanarbembu' in Tamil. In the traditional medicinal system, the leaves, flowers and tender shoot are said to be cooling and demulcent; they are used in the form of decoction for leprosy and cancerous affections. The leaves are also applied to abscesses. The whole plant is used in oedematous tumors and the ashes are used in preparations for dandruff's. The methanol extract of *Indigofera aspalathoides* also possess hepatoprotective activity (Robards et al., 1999). The chemical components includes: Steroids, triterpenes, alkaloids, phenolic groups, flavones, saponin, tannin, sugar and aminoacids are present (Katalinic et al., 2006). It also shows the presence of almost all minerals such as sulphur, phosphorous, irons and calcium except chlorine. Total ash content is 18%, the acid insoluble, sulphated ash; water soluble ash content is 1.13%, 17.89% and 1.24% respectively. A decoction of the leaves and flowers is given for leprosy and cancerous affections. The root is an instant remedy for toothache. A decoction of the whole plant is given as an alternative in secondary syphilis, psoriasis, etc. It is also used for abscesses and for skin diseases (Matkowski et al., 2008). The main objective of this work is to screen and evaluate the antibacterial activity of *Indigofera aspalathoides* against *Escherichia coli*, *Salmonella typhi*, *Shigella*, and *Staphylococcus aureus*. The screening of antibacterial activity for the plant extracts was studied using the ethanolic, hexane and diethyl ether extracts.

2. MATERIALS AND METHODS

2.1. Collection of the Sample

The plant (*Indigofera aspalathoids*) was collected from Trichy, District of Tamil Nadu. The bacterial strains used for this study were collected from MTCC (Chandigarh).

2.2. Preparation of Plant Extracts

Mature and healthy plants were collected and washed thoroughly. It was then air dried for about two weeks. The dried plants were ground to powder. From that 3grams of the powder were taken in each conical flask and solvents such as diethyl ether, hexane and ethanol were added and then kept in a shaking incubator for 24 hours. It was then filtered with whatman No. 1 filter paper to obtain the extract. The extract was collected in a screw cap glass bottle (Fig.1).

2.3. Preparation of Microbial Culture

The collected bacteria's were sub cultured in the respective agar medium (Fig.2). The bacterial strains used in this study are: *Escherichia coli* MTCC 249 (LB Agar), *Salmonella typhi* MTCC 3216(LB Agar), *Staphylococcus aureus* MTCC 3160 (MSA), *Shigella* MTCC 1457 (SS Agar).

2.4. Antimicrobial Activity Test

Bacterial strains were tested for antibacterial activity in test tubes containing ethanol, hexane, and diethyl ether extract. Incubate the tubes at 37 °C for 24 hours. After the incubation the readings were taken in spectrophotometry at 750nm.

2.5. Extraction of carotenoids from *Indigofera aspalathoides*

Extraction of carotenoids from *Indigofera aspalathoides* involves 4 process including purification step. 1) Dehydration 2) Saponification 3) Salting out and 4) Purification.

Secondary metabolites:

It is an organic compound that is not directly involved in the normal growth, development, or reproduction of an organism.

ANTIMICROBIAL ACTIVITY

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic).



Figure 1
Plant Extract (1) Diethyl ether (2) Hexane and (3) Ethanol

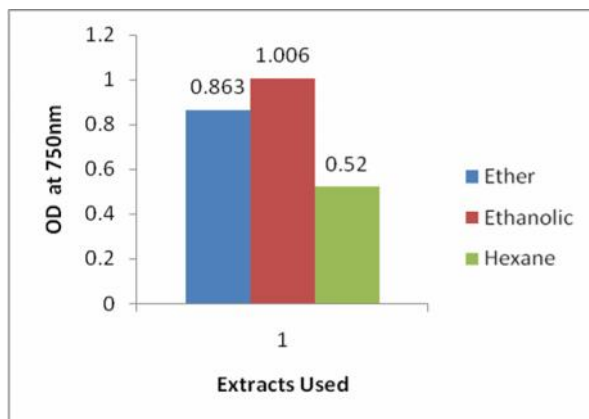


Figure 3
Spectroscopic analysis of ethanol, ether and hexane extract against the microbes



Figure 2
Microbial cultures in the respective agar medium

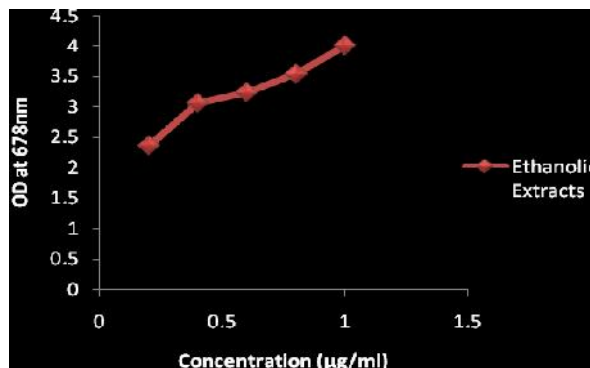


Figure 4
Antioxidant Assay

Carotenoids: Carotenoids are tetraterpenoid organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some bacteria, and some types of fungus.

Antioxidant: It is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent.

2.5.1. Dehydration

It's the process of removal of water from the plant and the process is done by air drying.

2.5.2. Saponification

It involves hydrolysis of esters under basic conditions. During saponification, the esters are hydrolyzed and free pigments released.

2.5.3. Salting out

It's the process in which the impurities of the plant extract will settle down at the bottom of the tube.

2.5.4. Purification

It is done by column chromatography.

2.6. Extraction of Carotenoids

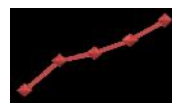
To the dehydrated plant material add 3 vol (v/w) of extractant and mix for 15 mins to make a paste. The type of extractant should be chosen based on the carotenoids of interest, No heating is necessary. Filter the extract using Whatman no. 42 filter paper. Concentrate the extracts to ~40 ml in a rotary evaporator attached to a vacuum pump at

≤55°C. To the concentrate add 3ml of saponifying solution and stir 45 min at 56°C. Transfer the saponified extract to a 125-ml separatory funnel and add 1 vol salting-out solution. Remove the bottom layer and wash upper layer three times with 10 ml water. Add 3 g anhydrous Na₂SO₄ and filter using Whatman no. 42 filter paper. Save filtrate and separate carotenoids by column chromatography.

2.7. Separation of carotenoids by column chromatography

Plug the bottom of a 600 × 40-mm chromatography column with glass wool. Mount the column on a vacuum filtration device, using a 1-liter filtration flask as a receiving vessel. Add adsorbent to obtain a 20-cm layer while applying vacuum. Level the surface of the adsorbent and place a firm 2-cm layer of anhydrous Na₂SO₄ on top. Pour carotene eluant into column until eluant wets all of the adsorbent. Replace receiving vessel with a clean flask and pour filtrate into column. Allow the entire sample to penetrate into the adsorbent and then add carotene eluant until the first carotenoid band that separates is completely collected in the flask. The carotenes and esters present in the sample are contained in this fraction. Other carotenoids remain on the column. The chromatography process is monitored visually. The exact volume of eluant added to the column will vary depending on sample concentration and composition. Elute the monohydroxy pigments with MHP eluant and dihydroxy pigments and more polar pigments with DHP eluant, using a clean receiving flask for each. If necessary, Store pigments ≤ 24 hr at 0° to 5°C and protect from light.

2.8. Total Antioxidant Assay



To find out the antioxidant activity in the plant, the ethanolic extract of *Indigofera aspalathoides* is taken the method is as follows: **TAC reagent:** 7.45ml of sulphuric acid, 0.9942gm of sodium sulphate, 1.234gm of Ammonium molybdate and made up to 250ml with distilled water. 3 gm of the plant was weighed and grinded with 30ml of ethanol to get the ethanolic extract. The grinded plant was filtered with whatman paper. The ethanolic extract was taken at the concentration of 0.2, 0.4, 0.6, 0.8 and 1ml respectively in each test tube. The final volume is made up to 1ml by adding distilled water. 1ml of distilled water is considered as blank. Add 1ml of PBS and 1ml of TAC reagent to each tubes including blank. Incubate all the tubes for 60mins at 50°C in a water bath. After cooling OD is taken at 678nm and the reading was observed.

3. RESULTS AND DISCUSSION

The medicinal plant *Indigofera aspalathoides* is having various properties such as anticancer, antioxidant, antihepatotoxicity etc. The works on antimicrobial activities of *Indigofera aspalathoides* are very few. Therefore, in the present study we made an attempt to find out the antibacterial efficacy of *Indigofera aspalathoides* on *E.coli*, *Salmonella typhi*, *Shigella* and *Staphylococcus aureus*. We followed the natural method of dehydration (shadow drying) to prepare the extracts. Previously Chah et al., (2006) and Li et al., (2008) followed solar drying and boiling (100°C for 2

hours) respectively. The high temperature may affect the bioactive compounds of the plant, so the method used is shadow drying in which all the compounds will not be affected. Ethanolic extract of *Indigofera aspalathoides* showed the highest inhibitory action meanwhile the ether and hexane extracts doesn't showed much effect against the microbes shown in Fig.3. The pigment extracted from the leaf ethanolic extract of the plant *Indigofera aspalathoides* shows the presence of the pigment carotenoid. This pigment can also be used as drug. The ethanolic extract of the *Indigofera aspalathoides* shows the antioxidant property to scavenge the free radicals (Fig.4).

4. CONCLUSION

The present study forms a good platform for the investigation of antimicrobial agents from *Indigofera aspalathoides* and its efficacy on different group of bacteria. The analysis of carotenoids and anti-oxidant assay explores the antibacterial properties of *Indigofera aspalathoides* and can be used as pharmacological compounds against infectious diseases caused by pathogens.

SUMMARY OF RESEARCH

1. The plant (*Indigofera aspalathoids*) was collected from Trichy and the bacterial strains were collected from MTCC (Chandigarh).
2. The extracts of the plant were collected using various solvents such as ethanol, ether and hexane.
3. The efficacy of the extract was checked against the bacteria such as *E.coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Shigella*.
4. Antioxidant assay and the pigment analysis were performed to analyse the activity of the plant.

FUTURE ISSUES

The compound present in the leaf of the plant *Indigofera aspalathoids* can be analyze by GS-MS and the function of each compound can be study.

DISCLOSURE STATEMENT

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RELATED RESOURCE

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