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# Phytochemical analysis and antimicrobial behavior of Indigo plant (*Indigofera hirshuta* L.)

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**Abstract**

This study was carried to estimate the antimicrobial and antioxidant activity of *Indigofera hirshuta* under laboratory conditions. The sampled plants from the culture were transferred to laboratory for leaf and rhizome extraction by a Soxhlet method with the use of ethanol as solvent. A phytochemical analysis was performed and standard agar well diffusion was used to evaluate antimicrobial activity of the plant. The antioxidant potential of plant extract was analyzed by following 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The phytochemical estimation of ethanolic leaf and legume extracts revealed several secondary metabolites, and, antimicrobial screening showed required activity in both extracts against *S. aureus*, *B. Subtillis*, *E. coli* and antifungal activity against *A. niger*. There is scope for isolation, characterization and structural study of specific bioactive compounds in extracts that may help in the development of new drugs.

**Keywords :** *Indigofera hirshuta*, Phytochemical, flavonoids, antimicrobial, antioxidant, *S. aureus*, *B. Subtillis*, *E. coli* and antifungal activity.

## Introduction

Plants provide all essential products to human like as food, shelter, medicine, etc. The edible plants provide nutrients for energy, protein, maintenance and regulation of homeostasis in our body. In this context, recent researches have shown that wild or semi-wild plants are also important as enriched with vitamins, minerals, essential fatty acids and fibre contents. The plants are also used as medicine from ancient periods and presently about 25% of all prescribed drug materials are derived from plants (Kokate *et al.*, 1994). There is increasing interest in the investigations of a drug potential in natural products (Cragg and Newman, 2007). The reason behind this interest may be a vast increase in the rate of infections, antibiotic resistance in microorganisms and side effects of synthetic antibiotics. In contrast to synthetic drug, herbal drug has no side effect and have vast potential to cure many infectious diseases. Furthermore the costs of chemotherapeutics are too expensive to the public especially in developing countries (Sarala *et al.*, 2010). The antimicrobial drug of plant origin has good scope in the design of plant extracted medicines to act against microbes (Kumaraswamy *et al.*, 2008). Several active products of several medicinal plants have been isolated and introduced as valuable drugs in modern system of medicine. Researchers are increasingly involved in the development of new bioactive molecule from natural products (Saravanan *et al.*, 2011).

The genus *Indigofera* L. is the largest genus of the tribe Indigofereae other than tribe Galegeae of the family Leguminosae (Fabaceae). The genus contains around 700 species found in the tropics and subtropics of the world. The genus is of considerable economic importance. Blue indigo dye is obtained from the stem and foliage of the plant, while some species are also used in medicinal value in practices.

The present study was aimed to focus on the phytochemical activities using ethanolic extracts of *I. hirshuta* against different microorganisms.

**Methods and Materials**

The plant pods and leaves were collected from local experimental fields during the study period. Organic extracts were prepared by successively extracting dried pods sand, leaves (100 g) with 200 ml of diethyl ether, chloroform or acetone, common solvents arranged in order of increasing polarity. Briefly, the leaf powder was homogenized firstly with 200 ml of diethyl ether for 2 hrs in a mechanical stirrer, kept refrigerated overnight (4°C) and filtered with Whatman no.1 paper. The solvent was then removed under reduced pressure in a rotary evaporator at 45°C to produce diethyl-ether extract. The plant material which was not extracted by diethyl-ether was then homogenized with 200 ml chloroform and all extraction process was repeated generating the chloroform extract. Finally, the remaining powder was submitted to acetone extract ion to produce acetone extract. All dried organic extracts of were stored at −20°C until use and dissolved in dimethyl sulfoxide (DMSO,1%) before each test.

An approximate amount of diethyl-ether, chloroform and acetone extracts from plant pods and leaves were subjected to phytochemical analysis to ascertain the presence of secondary metabolites in according to Wagner and Bladt (2009).

The anti-microbial activity was tested against the microorganisms as originally obtained from vaginal secretion; urine sample and blood sample, respectively. The anti-bacterial activity of the organic extracts of the pod and leaves were determined by the disc diffusion method (De Oliveira *et al.*, 2012).

The minimal inhibitory concentration (MIC) was determined by a micro-dilution broth susceptibility assay (Clinical and Laboratory Standards Institute, 2011). Two-fold serial dilutions of the organic extracts of the plant containing 50–0.20 mg/ml in DMSO were prepared in Mueller-Hinton Broth in a 96-well micro-titer plate. Bacterial suspensions were prepared from each strains freshly grown in Mueller-Hinton broth (approximately 1.5 × 108 CFU/ml) and 10µL of this suspension was added to each well. After incubation at 37°C for24h, bacterial growth was recorded using a Resazur in solution (0.01%). MIC was the lowest concentration at which no color change (from purple to pink) was observed. Afterwards, cultures were seeded in MHA medium and incubated for24h at 37°C to determine the minimum bactericidal concentration (MBC), which corresponds to the lowest amount of extract that kills bacteria. All experiments were performed in triplicate.

The results are expressed as the mean ± standard deviation (SD). Statistical analyses were performed by ANOVA and unpaired Student's t-test through Minitab software.

**Results**

The ethanolic extracts percentage in the leaf and legume were 2.8% and 2.5%, respectively (Table - 1) and solid and sticky in nature.

The phytochemical estimation exhibited the presence of flavonoids, steroid, tannin, triterpenoids, amino acid, carbohydrate and fat (Table - 2).

**Table 1. Nature and percentage yield of the plant extracts**

| Extract | Colour | Nature | Percent dry weight (w/w) |
|---|---|---|---|
| Pod | Brown | Solid and sticky | 2.6 |
| Leaf | Dark green | Solid and slight sticky | 2.9 |

**Antibacterial and antifungal activity**

Both ethanolic extracts of *I. hirshuta* were estimated against 8 microorganisms including two fungi. It exhibits highest zone of inhibition on leaf extract in activity in *S. aureus*, *B. subtillus* and *E. coli* (22, 25, 18 mm, respectively) and in the pod extract of *B. subtillus*, *S. aureus* and *E. coli* (19,16,12 mm respectively). While *A. niger* (25, 22mm) shows good activity in both extract when compared to *F. graminaum* (25, 20 mm). All the leaf extracts were found to contain antimicrobial activity and it was further confirmed with isolated compounds (Table - 3).

**Table – 2. Result of phytochemical screening of plant extracts (+ present; - absent)**

| Phytochemicals | Pod | Leaf |
|---|---|---|
| Alkaloids | _ | _ |
| Glycosides | _ | _ |
| Saponins | _ | _ |
| Steroids | + | + |
| Triterpenoids | + | + |
| Tannins | + | + |
| Flavonoids | + | + |
| Carbohydrates | + | + |
| Fats and Oils | + | _ |
| Amino acids or Protein | + | + |

**Table – 3. Antimicrobial of activity against selected microorganisms**

| S.No. | Organism | Inhibit. zone (mm) | Zone of Inhibition in different concentration | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 100 µg/ml | | 50 µg/ml | | 25 µg/ml | |
| | | | Leaf | Pod | Leaf | Pod | Leaf | Pod |
| 1 | *S.aureus* | 27 | 20.1±1.15 | 19.1±1.01 | 14.3±1.09 | 16.3±1.25 | 8.3±1.25 | 4.95±0.91 |
| 2 | *B. subtilis* | 22 | 25.01±0.70 | 20.5±1.35 | 19±1.13 | 19.3±1.15 | - | - |
| 3 | *E. coli* | 22 | 18.5±0.55 | 16.5±0.30 | 19±1.30 | 12.10±1.05 | 6.4±1.40 | 4.19±.062 |
| 4 | *P. arigenosa* | 22 | 11.1±1.45 | 10.6±1.12 | 7.2±0.72 | 5.2±0.80 | 3.95±.08 | - |
| 5 | *K. pneumonia* | 27 | 11.6±1.07 | 14.02±1.12 | 4.7±1.12 | 3.4±0.62 | - | - |
| 6 | *A. niger* | 22 | 25±0.85 | 22.6±1.75 | 23.4±0.92 | 20.4±0.91 | 17.96±1.52 | 15.3±1.88 |
| 7 | *F. graminearum* | 22 | 20.6±1.74 | 14.42±1.57 | 11.90±1.02 | 10.94±1.30 | 6.03±1.64 | 2.7±0.82 |

Radical losing activities are very important due to the deleterious role of free radical in food and biological systems. The chemical estimations are based on the loss of synthetic free radicals using a variety of radical generating system and method for detection of oxidation end point. DPPH with an odd electron shows strong absorption band at 515 nm in ethanol. The ethanolic extract of leaf shows good activity (1mg/ml) as compared to legume. The activity goes on increasing as concentration of extract increased.

**Discussion and Conclusion**

Nearly 80% of the population depends on traditional medicine for primary health care related to the natural products, especially in marginal economic countries where phytochemicals plays major a role in requirement of basic health care. The plant extract

particularly secondary metabolites are good sources of therapeutic agents and also exhibited good inhibitory property against several pathogens (Joshi Bishnu *et al.*, 2011).

The research on plant medicines requires primary stage of phytochemical estimation and laboratory screening of antimicrobial activity (Tona *et al.*, 1998). Recent findings have been contributing to the identification of new bioactive material which is associated with antimicrobial activities and also in designing therapeutic drug against several diseases (Mahesh and Satish, 2008). In recent years, bacterial strains have become resistant to many broad spectrum antibiotics which require chemotherapy as complicated and costly effective medicine to human kind (Davies, 1994). Also, there is limited chemicals may be prescribed as potential therapeutic antimicrobial agents for the simple reason about the sensitivity of animal cells against a pathogen (Govindarajan *et al.*, 2013). The crude products derived from chemicals are often associated with such compounds that are conjoined with drug toxicity (Singh *et al.*, 2007). Hence the plant derived drugs require extensive toxicological and clinical tests to confirm prior to public use in practice.

The plant extract containing antioxidants interacts with 2,2-diphenyl-1-picrylhydrazyl (DPPH) which transfers DPPH radical, thus loosed its free radical nature and changes as 1-1diphenyl-2-picryl hydrazine confirmed by the degree of discoloration for applied extract. DPPH reaction mixture change in colour from purple to yellow is evidently visual and so usually used as a substance to generate free radicals to estimate antioxidant activity (Al-Soqueer, 2011).

In this study, antimicrobial activities estimated with eight pathogens and the results showed moderate inhibitory activity against almost all microorganisms probably due to the presence of phytochemicals in the extracts. While the result of DPPH activity clearly indicates the ability of *I. hirshuta* leaf and legume extracts to inhibit hydroxyl radicals mediated by deoxy-ribose degradation which was increased in a concentration dependent manner. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive species.

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