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Preliminary phytochemical analysis and biological screening of *Indigofera hochestetteri*

Prachi P Parvatikar and SB Madagi

Abstract
The objective of the present work was to evaluate *in vitro* antimicrobial and antioxidant activity of *Indigofera hochestetteri*. The selected plant was collected in the vicinity of the University campus. Herbal extraction was done with leaf and rhizome of *Indigofera hochestetteri* by Soxhlet extraction method with use of ethanol as solvent. Phytochemical analysis was done using different biochemical tests. Antimicrobial activity was performed by using standard agar well diffusion method whereas antioxidant potential of plant extract was analyzed by following 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The preliminary phytochemical analysis of ethanolic leaf extract and legume extract of *Indigofera hochestetteri* contained broad spectrum of secondary metabolites such as flavonoids, saponin, steroids, amino acid and carbohydrate. The extracts from being screened against six microorganism including fugal strain. The antimicrobial screening showed good activity in both extracts against *S. aureus*, *B. Subtillis*, *E. coli* and antifungal activity against *A. niger*. The both extracts exhibited dose dependant antioxidant activity. Further study requires isolation, Characterization and structural elucidation of specific bioactive compounds in both extracts that may help in the development of new drugs.

Keywords: *I. hochestetteri*, Phytochemical, flavonoids, antimicrobial, antioxidant

Introduction
Plants are such wonderful gift of nature to mankind. Plants provide all essential things to human such as food, shelter, medicine etc. The edible plants provide nutrients essential for energy, body building, maintenance and regulation of body processes. From last few decades it has been shown that wild or semi-wild plants are also important because they are also rich in vitamins, minerals, essential fatty acids and fibre contents. Some of the plants also enhance taste and colour in diets. From ancient time most plants are used in the treatment of many diseases. Today about 25% of all prescribed medicines substances derived from plants (Kokate *et al* 1994) [3]. In recent years, there has been rejuvenation interest in "rediscovering natural products" (Cragg *et al 2005*) [4]. The reason may be hasty 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) [7].

The developing antimicrobial drug of plant origin from higher plant has good scope in developing phytomedicines to act against microbes (Kumaraswamy *et al 2008*) [8]. Several active principles of many medicinal plants have been isolated and introduced as valuable drugs in modern system of medicine. Subsequently, researchers are involved in development of new bioactive molecule from natural products (Saravanan, R., *et al*. 2011) [9].

The genus *Indigofera* L. is the largest genus of the tribe Indigofereae other than tribe Galegeae of family Leguminosae (Fabaceae). The genus contains around 700 species found in the tropics and subtropics of the Old and New World. The genus is of considerable economic importance. Blue indigo dye is obtained from the stem and foliage of the plant. The dye contains Indigotin and Indican pigments which imparts blue colour. Important dye yielding species are *I. articulata, I. suffruticosa, I. tinctoria* and *I. caerulea*. Other species are used as medicine, fodder, cover crops, green manure, human food, erosion control and ornamentals. Some species of the economically important Indigofera species are *I. arrecta, I. suffruticosa* and *I. tinctoria*, especially the last one is widely used in the traditional manufacture of the dye indigo. The other species like *I. caerulea* used as anthelmintic, *I. cordifolia* and *I. glandulosa* considered as good fodder for domestic animals. The decoction leaves of *I. hirsuta* used in diarrhoeal conditions. Beside this, *I. oblongifolia* used to increase appetite by the peoples of

Doni area, Kappat hills, Gadag. The seeds of *I. trita* used as nutritive tonic (Kotresha and Kambhar, 2016) [11].

The aim of present study is to highlight the phytochemical and pharmacological investigation of *I. hochestetteri* was selected to investigate the phytochemical and biological activities against different microorganism of ethanolic extracts of the *I. hochestetteri* (fig 1). This could be conducted to investigate the unexploited potential.

Result

Preliminary Phytochemical analysis

The extractive yield of leaf and legume from ethanolic extracts were 2.8% and 2.5% respectively (Table 1). Both extracts were solid and sticky in nature. The preliminary phytochemical analysis showed 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	Color	Nature	% dry weight w/w
Pod	Brown	Solid, sticky	2.5
Leaf	Dark green	Solid, slightly sticky	2.8

Table 2: Result of phytochemical screening of plant extracts (+ present; - absent)

Phytoconstituents	Pod	Leaf
Alkaloids	-	-
Cardiac glycosides	-	-
Anthraquinone glycosides	-	-
Saponins	-	-
Cynogenetic glyoidcosides	-	-
Steroids	+	+
Triterpenoids	+	+
Tanins	+	+
Flavonoids	+	+
Carbohydrates	+	+
Fats & Oils	+	-
Amino Acids or Protein	+	+

Antibacterial and antifungal activity

The antimicrobial activity of both ethanolic extracts of *Ihochestetteri* were determined against 8 microorganisms including two fungi. It shows maximum zone of inhibition on leaf extract in activity *S. aureus, B. subtillus, E. coli* (22, 25, 18 mm respectively) and in pod extract *B. subtillus, S. aureus E. coli* (19,16,12mm respectively*).* While *A. niger* (25,22mm) shows good activity in both extract as compare to *F. graminaum* (25,20 mm). In overall assessment all the extracts were found to leafpossess antimicrobial potentiality and it should be further established with isolated compounds (Tab 3).

Table 3: Antimicrobial of activity against selected microorganisms

S. No.	Name of organism	Std antibiotic	Zone of inhibition (mm)	Zone of inhibition(mm)							
				100 µg/ml		50 µg/ml		25 µg/ml		10 µg/ml	
				Leaf	Pod	Leaf	Pod	Leaf	Pod	Leaf	Pod
1	*S. aureus*	Amoxycillin.	28	20.1 ± 1.15	19.1 ± 1.01	14.2 ± 1.10	16.3 ± 1.30	8.3 ± 1.25	4.95± 0.91	3.1 ± 1.08	2.0 ± 1.00
2	*B. subtillus*	Tetracyclin	23	25.3 ± 0.75	20.4± 1.41	19.2 ± 1.15	19.5 ± 1.84	-	-	-	-
3	*E. coli*	Tetracyclin	23	18.8 ± 0.50	16.7 ± 0.32	19.2 ± 1.25	12.8± 1.11	6.5 ± 1.45	4.2 ± .067	-	-
4	*P. gingivalis*	Chloromphenicol	25	14.6 ± 1.37	16.8 ± 1.08	-	-	-	-	-	-
5	*P. arigenosa*	Tetracyclin	23	11.2 ± 1.47	10.8± 1.15	7.3 ± 0.75	5.1 ± 0.85	3.97 ± 0.10	-	-	-
6	*K. pneumonia*	Amoxycillin	28	11.8 ± 1.10	14.2 ± 1.15	4.9 ± 1.15	3.5± 0.64	-	-	-	-
7.	*A. nigar*	Penicillin	23	25.2 ± 0.90	22.8± 1.78	23.7 ± 0.90	20.6± 0.90	18.0 ± 1.50	15.4 ± 1.90	-	-
8.	*F. graminearum*	Tetracycline	23	20.7 ± 1.78	17.6 ± 1.59	12.0 ± 1.00	10.98± 1.35	6.05 ± 1.69	2.9± 0.84	-	-

DPPH Free Radical activity

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

Discussion and Conclusion

Nearly 80% of the world population relay on traditional medicine for primary health care, most of which involves the use of natural products [13]. In developing countries plant origin drug plays important role in fulfilment of basic health care. The plant extract especially secondary metabolite are good source of therapeutic agents, they showed good inhibitory activity against many pathogenic organism [14]. The first step is phytochemical analysis and the *in vitro* screening of antimicrobial activity [15]. Some of new findings have contributing in identification of the new active molecule which is associated with antimicrobial activities and also in developing therapeutic drug against many diseases [16]. Now a day's bacterial strains have become resistant to many broad spectrum antibiotics [17]. This is the main reason which makes chemotherapy appear to be complicated and costly effective to human kind [7]. Many substances may be antimicrobial, but only few of them will be potential therapeutic agents for the simple reasons that mammalian cells are more sensitive to

chemical inhibitions than microbial cells [18]. The crude products obtained from cheaper sources are often associated with a large number of compounds that have discomforting abilities due to toxicity of drugs [19]. Hence the herbal drugs have to be subjected to extensive toxicological and clinical tests to confirm the prescribed status.

The plant extract containing antioxidants interacts with 2, 2-diphenyl-1-picrylhydrazyl (DPPH) which transfers hydrogen atoms or electrons or DPPH radical, thus scavenging its free radical nature converting it to 1-1diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the extract [20]. The decrease in absorbance of DPPH radical in spectrophotometric measurements caused by antioxidants is due to the progress of reaction between radicals and antioxidant molecules which results in the scavenging of the DPPH radical by hydrogen donation. DPPH reaction mixture change in colour from purple to yellow is evidently visual. Hence, DPPH is usually used as a substance to generate free radicals to evaluate antioxidant activity [21].

In this present study antimicrobial properties evaluated with eight microorganisms and considerable results showed moderate inhibitory activity against almost all microorganisms. It is probably due to presence of phytochemicals in the extracts. While result of DPPH activity clearly indicates the ability of *I. hochesterri* 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. So results indicate that noticeable hydroxyl and super oxide radical scavenging effect was observed in ethanolic extracts.

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Conflicts of interests

The author has no conflicts of interest.

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