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Research Article

PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF *INDIGOFERA TINCTORIA* L.

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ABSTRACT

Objective: The present study was designed to evaluate the phytochemical composition and antibacterial and antioxidant potential of methanolic leaf and root extracts of *Indigofera tinctoria* L.

Methods: Phytochemical analysis was done using standard methods. The methanolic leaf and root extracts of the plant were tested against *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Enterobacter aerogenes,* and *Salmonella paratyphi* B by cup-plate agar diffusion method. The free radical scavenging activities of the methanol extracts of leaves and roots were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Results: Phytochemical screening revealed the presence of carbohydrates, reducing sugars, alkaloids, saponins, phenolic compounds, and flavonoids in methanolic leaf and root extracts. Methanolic leaf extracts of the plant exhibited antibacterial activity against a wide range of bacteria, but the root extracts failed to inhibit the tested bacterial pathogens. The antioxidant activity determination revealed that at 100 µg/ml, methanolic root extracts had the highest antioxidant activity (89.10%) on DPPH free radicals followed by methanolic leaf extracts (46.74%).

Conclusion: The results of the present study conclude that the studied plant possesses broad-spectrum antibacterial and antioxidant properties and may act as a potent antioxidant for biological systems susceptible to free radical-mediated reactions.

Keywords: *Indigofera tinctoria* L., Methanol extract, Phytochemical analysis, Antibacterial activity, 2,2-diphenyl-1-picrylhydrazyl assay.

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INTRODUCTION

The search for novel antimicrobial and antioxidants from natural sources has increased in response to the emergence of drug-resistant microorganisms and negative health effects of synthetic antioxidants [1,2]. Herbal drugs have gained a reputation in recent years because of their safety, efficacy, and cost-effectiveness. In the present day, nearly four billion people living in the developing world depend on plant-derived medicines as their first line of action for combating diseases and maintaining health [3-5].

Indigofera tinctoria Linn. (*Fabaceae*) is a leguminous plant which is widespread across tropical regions around the globe, as it had been cultivated and highly valued for centuries as the main source of indigo dye, leading to its common names "true indigo" and "common indigo." In traditional medicine, the plant is useful in the treatment of cancer, hydrophobia, gout, rheumatoid arthritis, cephalalgia, lumbago, epilepsy, insanity, blennorrhagia, urinary complaints, cough, bronchitis, rhinitis, asthma, palpitation, hepatitis, splenomegaly, hemorrhoids, sores, old ulcers, constipation, leucoderma, grey hairs, snake bite, scorpion bite, and insect bite [6-8]. In Africa, the indigo powder used in calendering also served as a disinfectant and cicatrizing drug to aid in the formation of scar tissue as part of the practice of tattooing. In Cameroon, the twigs are still commonly used as a toothbrush, and the roots have been widely applied as a treatment for a toothache. A root preparation is applied in Tanzania as a remedy against syphilis, gonorrhea, and kidney stone [9]. The plant also possesses antibacterial [10], anti-inflammatory [11], antidiabetic [12], anti-HIV [13], immunomodulatory [14], antidepressant, and nootropic activity [15]. However, few reports are currently available in the literature regarding the antimicrobial and antioxidant activities of leaves and roots of the plant. In the continuation of the strategy of new drug discovery, the present study investigates the phytochemical constituents, antibacterial and antioxidant properties of the methanolic leaf and root extracts of *I. tinctoria* L.

METHODS

Collection and processing of plant materials

The plant *I. tinctoria* L. free from disease was collected from Kolli hills, Tamil Nadu, India. The identification of the plant was carried out by Dr. A. Balasubramanian, ABS Botanical Conservation, Research, and Training Centre, Salem. The leaves and roots were washed under running tap water, air dried, and homogenized to powder form and stored in the airtight bottle.

Preparation of plant extracts

Leaf powder (20 g) and root powder (20 g) were soaked separately in 200 ml of methanol in a conical flask and kept for 2 days at room temperature in laboratory shaker with a shaking speed of 120 rpm. The extracts so obtained were filtered through Whatman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator under reduced pressure and then stored at 4°C for further analyses [2].

Phytochemical screening

The crude methanolic leaf and root extracts of *I. tinctoria* L. were tested for the presence of phytochemicals using standard phytochemical methods [16-18].

Test bacterial strains

Clinical isolates of *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Enterobacter aerogenes,* and *Salmonella paratyphi* B were obtained from Sri Gokulam Hospital, Salem, India. Pure bacterial cultures were maintained on nutrient agar slants at 4°C.

Determination of antibacterial activity

To determine the antibacterial activity of methanolic leaf and root extracts, cup-plate agar diffusion method was adopted [19]. The bacterial cultures were grown in brain–heart infusion (BHI) broth and incubated at 37°C for 24 h. The cultures were later diluted with sterile medium, adjusted to 0.5 McFarland turbidity standard and inoculated on BHI agar plates by streaking the organisms over the surface of the medium using a sterile cotton swab and allowed to dry for about 10 min. Four wells of 6 mm in diameter and 4 mm in depth were cut using a sterile cork borer, maintaining a distance of 3 cm between them. The wells were filled with 100 µl of methanolic leaf and root extracts (100 mg/ml) dissolved in dimethyl sulfoxide (DMSO). Wells filled with 100 µl of nalidixic acid (500 µg/ml) and 100 µl of DMSO served as positive and negative controls, respectively. The plates were then kept at room temperature in an upright position for 2 h for diffusion of extracts and then incubated under the same growth conditions as mentioned above. Antibacterial activity was determined by measuring the inhibition zones formed around each well, averaged and the mean values were noted.

Antioxidant activity determination by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Free radical scavenging ability of methanolic leaf and root extracts and standard synthetic antioxidant used in the study prepared in methanol at concentrations of 20–100 µg/ml was determined in accordance with the Shimada *et al.*'s method [20] which is based on the principle of scavenging the DPPH. DPPH was added to the solutions prepared with plant extracts and standard antioxidant substance ascorbic acid and stirred. The samples were kept in the dark for 30 min and the absorbance was measured at 517 nm against a blank. Radical scavenging activity was calculated by the following formula.

Percentage Inhibition = (control OD-sample OD/(control OD)×100

Table 1: Phytochemical screening of methanolic leaf and root extracts of *I. tinctoria* L.

Phytochemical tests	Methanolic leaf extract	Methanolic root extract
Carbohydrates		
Molisch's test	+	+
Reducing sugars		
Fehling's test	+	+
Benedict's test	+	+
Alkaloids		
Mayer's test	+	+
Dragendorff's test	+	+
Hager's test	+	+
Wagner's test	+	+
Phytosterol		
Salkowski test	+	–
Fixed oil		
Spot test	–	–
Saponins		
Froth test	+	+
Proteins and free amino acids		
Xanthoprotein test	+	–
Phenolic compounds		
Ferric chloride test	+	+
Flavonoids		
Alkaline reagent test	+	+

+Present, –Absent. *I. tinctoria: Indigofera tinctoria*

RESULTS

The results of the phytochemical screening of the investigated methanolic leaf and root extracts of *I. tinctoria* L. showed the presence of carbohydrates, reducing sugars, alkaloids, saponins, phenolic compounds, and flavonoids in methanolic leaf and root extracts, whereas, phytosterol, proteins, and free amino acids were detected only in methanolic leaf extracts. Fixed oil was not detected in both methanolic leaf and root extracts (Table 1).

The antibacterial activity of methanolic leaf and root extracts of *I. tinctoria* L. was tested against five bacterial pathogens with nalidixic acid as a positive control and DMSO as a negative control (Table 2). The methanolic leaf extracts of *I. tinctoria* L. showed promising antibacterial activity against a wide range of bacteria, while methanolic root extracts inhibited none of the tested bacterial pathogens. Maximum *in vitro* inhibition was scored by *E. faecalis*, followed by *S. aureus*, *S. paratyphi* B, *E. aerogenes,* and *K. pneumoniae*, which presented inhibition zone diameter of 26.66±0.57 mm, 24.33±0.57 mm, 22.33±0.57 mm, 20.66±0.57 mm, and 18.33±0.57 mm, respectively.

The antioxidant activity of methanolic leaf and root extracts of *I. tinctoria* L. as measured by the ability to scavenge DPPH free radicals was compared with the standard ascorbic acid. The results of DPPH assay revealed that methanolic leaf and root extracts exhibited significant antioxidant activity (Fig. 1). The DPPH scavenging effect was found to increase with increasing concentrations. At 100 µg/ml, the highest percentage inhibition (89.10%) was shown by methanolic root extract compared to the reference antioxidant ascorbic acid (77.71% inhibition) followed by 46.74% inhibition by methanolic leaf extracts.

DISCUSSION

Plants are the important source of bioactive compounds for the development of potential chemotherapeutic agents [21]. Phytochemical investigation of methanolic leaf and root extracts of *I. tinctoria* L. revealed the presence of various phytochemicals. Flavonoid is known to have a wide range of therapeutic properties as antimicrobial, antioxidative, antirheumatism, and chemopreventive agents. Phenolic compounds are identified as antioxidative and scavenging agents against oxidative damage associated with free radicals [8,22]. As phytochemicals often play a significant role in plant defense against plant pathogens, stress as well as interspecies protections, these phytochemicals have been used as drugs for millennia [23].

By cup-plate agar diffusion method, the methanolic leaf extracts showed good antibacterial activity, evidencing that methanol is an efficient organic solvent to be used for the extraction of bioactive plant materials. Renukadevi and Sultana [10] reported that methanol extract of *I. tinctoria* L. showed antibacterial activity against *S. aureus*, *Streptococcus pyogenes*, and *Bacillus pumilus*. Vijayan *et al.* [24]

Table 2: Antibacterial activity of methanolic leaf and root extracts of *I. tinctoria* L.

Bacterial pathogens	Diameter of zone of inhibition (mm)			
	Nalidixic acid	DMSO	Methanolic leaf extract	Methanolic root extract
S. aureus	29.33±1.15	Nil	24.33±0.57	Nil
E. faecalis	22.66±1.52	Nil	26.66±0.57	Nil
K. pneumoniae	26.33±0.57	Nil	18.33±0.57	Nil
E. aerogenes	20.66±0.57	Nil	20.66±0.57	Nil
S. paratyphi B	27.33±1.15	Nil	22.33±0.57	Nil

Values are means of triplicate determinations±standard deviation. DMSO: Dimethyl sulfoxide, *I. tinctoria: Indigofera tinctoria, S. aureus: Staphylococcus aureus, E. faecalis: Enterococcus faecalis, K. pneumoniae: Klebsiella pneumoniae, E. aerogenes: Enterobacter aerogenes, S.paratyphi B: Salmonella paratyphi B*

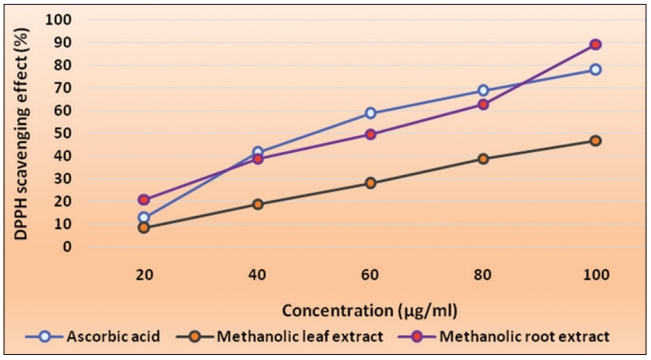

Fig. 1: 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of methanolic leaf and root extracts of *Indigofera tinctoria* L. Values are means of triplicate determinations ± standard deviation. DPPH: 2,2-diphenyl-1-picrylhydrazyl

have reported that methanol extract of leaves of the plant showed antibacterial activity against methicillin-resistant *S. aureus*, *E. faecalis*, *Moraxella catarrhalis*, *Haemophilus influenzae,* and anaerobes. In the present study, the differences in sensitivity of tested bacterial pathogens to methanolic leaf and root extracts of *I. tinctoria* L. may be explained by the differences in cell wall composition and/or in genetic content of plasmids that can be easily transferred among microbial strains [25]. It may also be described by differences in the mechanism by which the active principles of the plant extracts exert their action and the concentration of the extract employed [26,27].

The DPPH scavenging activity is due to the neutralization of free radical by the methanolic leaf and root extracts of *I. tinctoria* L. either by transfer of hydrogen or of an electron [28]. Free radical scavenging is the recognized mechanism for antioxidant-inhibiting lipid oxidation [29]. The highest free radical scavenging activity of methanolic leaf and root extracts of the plant corroborates with the results of Srinivasan *et al.* [8] and Bakasso *et al.* [30].

CONCLUSION

The results obtained from the present study suggest that *I. tinctoria* L. is a potential source of antibacterial and antioxidant molecules. Bioassay-guided fractionation procedure should be performed to characterize and isolate the phytochemical responsible for the antibacterial and antioxidant activity. Furthermore, they should be subjected to pharmacological evaluation with the objective of assessing theirs *in vivo* efficacy, toxicity, potential adverse effects, interactions, and contraindications.

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