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Preliminary investigation of phytochemical analysis and antibacterial activity of Methanolic extract of *Indigofera linnaei* Ali roots (Fabaceae)

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Abstract

Natural products have played a key role in drug discovery, especially for cancer and infectious diseases. The aim of study was to investigate the preliminary phytochemical analysis and antibacterial potential of methanolic extract of *Indigofera linnaei* roots using disk diffusion method against human bacterial pathogens. The report of the present work observed that methanolic extract of roots revealed that active compounds of flavonoids, tannin and saponin were detected in coloring test. The methanolic extract of *Indigofera linnaei* roots observed that maximum activity of both bacterial strains of *S. aureus* and *P. aeruginosa* were observed by the present study.

Keywords: *Indigofera linnaei;* Fabaceae; roots; extracts; active compounds; antibacterial activity

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1. INTRODUCTION

Genus of Indigofera is belongs to the family Fabaceae, which is comprised more than 750 species and it is mainly herbs, shrubs or small trees, distributed in forests, savannas and disturbed areas (Kaushik et al., 2016; Marquiafável et al., 2009). They are widely distributed throughout the tropical and subtropical regions of the world (Gao and Schrire,2017). About 75% of these species are restricted to Africa and Madagascar, and many species are also encountered in the Sino-Himalayan region, Australia, and Central and South America (Schrire, 2013). A few species can be found in temperate areas of East Asia (Ponmari et al., 2014). Several investigations have appraised the phytochemistry and pharmacology of *Indigofera* species. Approximately 200 compounds have been identified, including phenolic compounds, terpenoids, alkaloids and glucose esters of nitropropanoic acid. Some of them have been described as possessing biological activities, and this is notably the case with indirubin (Han, 1988; Meijer et al., 2007) and indigocarpan (Mahajan et al., 2016). Pharmacological studies have revealed that crude extracts and purified fractions of Indigofera species have various properties, including anti-arthritic (Rajkapoor et al., 2009), anthelmintic (Meenakshisundaram et al., 2016), anticancer (Agarwal and Sharma, 2017).

Indigofera linnaei Ali (Synonyme : I. enneaphylla) *Indigofera linnaei* Ali is belongs to the family Fabaceae and is a reputed indigenous medicine. *Indigofera linnaei* is a spreading, usually prostrate woody herb, 14–51 cm high with a long taproot, which forms a flat mat up to 1.6 m across, and up to 42 cm high. It is distributed in the throughout India. The medicinal value of this plants is extracted juice is used as antiscorbutic and diuretic, for burns and epilepsy. It has long been used by the ethnic people and native medical practitioners to treat rheumatism, arthritis, inflammation, tumor and liver diseases (Wealth, 1992). Literature review previous studies revealed that three active compounds of nitropropanoyl esters of glucose namely 1,2,6-tri-*O*-(3-nitropropanoyl)-β-D-glucopyranose, 2,3,4,6-tetra-O-(3-nitropropanoyl)-α-D-glucopyranose and 3,4,6-tri-*O*-(3-nitropropanoyl)-α-D-glucopyranose were isolated from the aerial parts of *Indigofera linnaei* (Majak *et al.,* 1992). A new isoflavone namely 7,8-methylenedioxy-4'-methoxyisoflavone has been isolated from the entire plant of *Indigofera linnaei* (Rajendraprasad Chakradhar, 2004**).** In our earlier studies, the plant extracts exhibited a potent free radical scavenging and antioxidant activity **(**Kumar *et al.,* 2011**)**, anticancer (Kumar *et al.,* 2011b) and wound healing activity **(**Hemalatha *et al.,* 2001**)**. In the present study was aimed to evaluate the antibacterial effect of 80% of methanol extract of *Indigofera linnaei* roots on human bacterial pathogens.

2. MATERIAL AND METHODS

2.1.1 Plant Collection and Extraction

The plant materials of *Indigofera linnaei* roots were collected from the local area of Palayamkottai region of Tamilnadu. The collected plant materials were identified in the regional flora and air dried for 15 days and powdered. The powdered materials (250g) of *Indigofera linnaei* roots were extracted with 80% methanol for 5hr in soxhlet apparatus. Collected crude extract was concentrated in the evaporation of solvent. The collected crude extract was stored in the refrigerator and further studies.

2.1.2 Identification of active compounds

The preliminary investigation of active compounds of the roots of *Indigofera linnaei* were identified by the according to standard method (Horborne,1984).

2.3 Investigation of Antibacterial activity

2.3.1 Bacterial pathogen selected for the study

The experimental studies of preliminary investigation of antibacterial activities were tested for the extract of *Indigofera linnaei* roots against human bacterial pathogen strain used in this study. The selected bacterial strains were *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhi.*

2.3.2 Experimental design and Disk diffusion method

The nutrient agar medium was used for the disk diffusion method. All the tested bacteria strains were cultured for overnight at 37°C in nutrient

broth. Suspension of bacteria (1 ml) with an optical density of McFarland 0.5 was prepared in physiological saline solution (0.9%) and it was adjusted to 90% of transmittance (530 nm) in a spectrophotometer. All the bacterial suspensions were evenly spread on the nutrient agar plates. The tested extract was prepared for different concentrations of 10, 20 and 40 mg/ ml dissolved in 1 ml of methanol. All the disk was drop wise injected in the extract and dried. Control disk as a methanol was used. All these experiments were performed in triplicate. The bacterial plates were incubated for 37°C at 12h and 12 h after naked observation of the results of the inhibition zones produced by the root extracts and measured in the zone of inhibition in mm.

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Analysis

In the present study observed that results of the methanolic extracts of *I. Indigofera linnaei* roots shown in the table-1. The methanolic extract of *I. Indigofera linnaei* observed the result of the active compounds present in the alkaloids, flavonoids, triterpenes, saponins and tannins were indicated in the color test. Previous studies, detected in methanolic extract of *Indigofera trita* L.F. SPP. *subulata* (vahl) did not show the presence of iridoids and saponins and tannin was present in leaf, stem and root extracts of *Indigofera trita* L.F. SPP. *subulata* (vahl ex poir) on water, petroleum ether and chloroform extracts (Vinoth et al.,2011). Water and chloroform fractions of stem and root indicated the presence of saponins (Vinoth et al.,2011). Fixed oil was not detected in the methanolic extract of both parts of leaf and root o *I. tinctoria* L. (Swaminathan,2018).

3.2 Antibacterial activity

In the present study, antibacterial activity of *Indigofera linnaei* was maximum activity recorded against *S. aureus* and *P. aeruginosa and* moderately activity was observed against *Salmonella typhi* and *Klebsiella pneumoniae* (Table-2). Previous results observed similar results of ethanolic extracts of the same species have been mentioned in the literature for their antibacterial activity on *S. aureus* and *P. aeruginosa* (Ali et al.,1993). Similar results of previous studies, aqueous extract of *I. dendroides* has significant antimicrobial activity against *S. aureus, Bacillus subtilis, K. Pneumoniae and E. coli* (Esimone et al.,1999). Renukadevi and Sultana (2011) observed the results of methanol extract of *I. tinctoria* L. showed antibacterial activity against S. aureus and *Bacillus subtillis*. The conclusion of the observed results obtained from the present study suggest that *Indigofera linnaei* is a potential source of antibacterial activity. Further studies will be isolation of active compounds from the roots of *Indigofera linnaei*.

Table 1: Results of the preliminary investigation of the phytochemicals present in the methanolic extract of *Indigofera linnaei* roots.

Sl.No.	Active compounds	Present/Absent
1	Alkaloids	*** (Hight)
2	triterpenes /essential oils	*(Low)
3	Flavonoids	** (Medium)
4	Tannins	* (Low)
5	Saponnins	*(Low)

Table-2: Results of Antibacterial potential of roots of *Indigofera linnaei*

Sl.No.	Pathogens	Zone of Inhibition (mm)
1	Bacillus subtilis	13
2	*Escherichia coli*	12
3	*Klebsiella pneumoniae*	15
4	*Salmonella typhi*	15
5	*Staphylococcus aureus*	**17**
6	*Pseudomonas aeruginosa*	**17**

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