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INVESTIGATION OF PHYTOCHEMICALS AND ANTIMICROBIAL PROPERTIES OF *Indigofera tinctoria* LEAVES

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ABSTRACT

In the present study to investigate the phytochemical and antimicrobial activity of *Indigofera tinctoria* leaves extract. *Indigofera tinctoria* leaves showed that the presence of tannin, saponin, flavonoids, terpenoids, triterpenoids, anthraquinone, steroids, polyphenol, glycosides and coumarins in in both extract. Alkaloids presence only methanol extract. Quantitative analysis showed that significant amount of phytochemicals such as flavonoids, phenol and terpenoids were present in *Indigofera tinctoria* leaves. Histochemical studies further proved the presence of phytochemicals in *Indigofera tinctoria* leaves. *Indigofera tinctoria* leaves was potential antibacterial activity confirmed against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* species of bacteria strains. *Indigofera tinctoria* leaves was promising antifungal activity evidenced against *Candida albicans* and *Aspergillus flavus* species of fungi strains. The *Indigofera tinctoria* leaves has rich source of phytochemicals and possess potential antimicrobial activity. The results of the study concluded that *Indigofera tinctoria* leaves may be used for the treatment of microbial infections.

Keywords: *Indigofera tinctoria* leaves, Phytochemical, Antimicrobial activity

INTRODUCTION

Microorganisms are very diverse and include all bacteria, archaea and most protozoa. This group also contains some fungi, algae, and some micro-animals such as rotifers. Many macroscopic animals and plants have microscopic juvenile stages. Some microbiologists classify viruses and viroids as microorganisms, but others consider these as nonliving (Madigan and Martinko, 2006).

The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. However, the situation is alarming in developing as well as developed countries due to indiscriminate use of

antibiotics. In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from natural sources including plants. Keeping in view, the present study to investigate the phytochemical and antimicrobial activity of *Indigofera tinctoria* leaves.

MATERIALS AND METHODS

Collection of plant materials

The leaves powder of *Indigofera tinctoria* were purchased in January 2021 from siddha medicinal shop, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation of plant extract

1 gram of the powder of *Indigofera tinctoria* leaves were transferred in to different

conical flask (250ml). The conical flask containing 50ml of different solution (methanol and water). The conical flask containing *Indigofera tinctoria* leaves were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984). Total phenols estimated by the method of Edeoga *et al.*, (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956). Histochemical tests (John Peter Paul, 2014; Gersbach *et al.*, 2001). The antibacterial activity was performed by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007).

RESULTS AND DISCUSSION

Table.1: Qualitative phytochemical analysis of *Indigofera tinctoria* leaves extract

S. No	Phytochemicals	Methanol extract	Aqueous extract
1	Tannin	+	+
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	++	++
5	Terpenoids	++	++
6	Triterpenoids	++	++
7	Alkaloids	+	-
8	Antroquinone	++	+
9	Polyphenol	++	++
10	Glycosides	+	+
11	Coumarins	++	++

(+) Presence, (++) High concentrations and (-) Absences

Quantitative analysis

Quantitative analysis revealed that the *Indigofera tinctoria* leaves has flavonoids, terpenoids and phenol. Significant amount of flavonoids (60.00mg/gm), terpenoids

Qualitative and quantitative analysis

A number of phytochemicals isolated from plant material are used in the pharmaceutical drug industry today. The phytochemicals under investigation include secondary metabolites, many which are synthesized for plant defense and adaption to environmental stress. The phytochemicals can range from medicinally useful agents to treat varieties of diseases such as diabetes, malaria, anaemia (Fola., 1993).

In the present study was carried out on the *Indigofera tinctoria* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Indigofera tinctoria* leaves investigated and summarized in Table-1 and figure 2 and 3. The phytochemical screening *Indigofera tinctoria* leaves showed that the presence of tannin, saponin, flavonoids, terpenoids, triterpenoids, antroquinone, steroids, polyphenol, glycosides and coumarins in in both extract. Alkaloids presence only methanol extract.

(30.00mg/gm), and phenol (215.00mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.

Table.2: Quantitative analysis of phytochemicals in *Indigofera tinctoria* leaves powder

Phytochemicals	Result (mg/gm)
Phenol	215.00 ± 4.97
Flavonoids	60.00 ± 2.75
Terpenoid	30.00 ± 1.69

Value were expressed as Mean ± SD for triplicate

Phenol and flavonoids have become an intense focus of research interest because of their perceived beneficial effects for health, including antidiabetic, anticarcinogenic, antiatherogenic, antiulcer, anti-thrombotic, anti-inflammatory, immunomodulating, antimicrobial, varodialatory, and analgesic effects (Dewick, 2001). In the present study, *Leucaena leucocphala* leaves contain phenol and flavonoids which may possess potential antidiabetic activity.

Similarly Kumar *et al.*, (2013) investigated the preliminary phytochemical screening of the leaves of the plant *Lasia spinosa* (Lour) Thwaites. The phytochemical screening showed that the methanol extract contained flavonoids, phenol, terpenoids, tannin, saponin, glycosides and alkaloid which are responsible for the biological activities.

Histochemical analysis

Histochemistry is the branch of histology dealing with the identification of chemical components of cells and tissues; it is

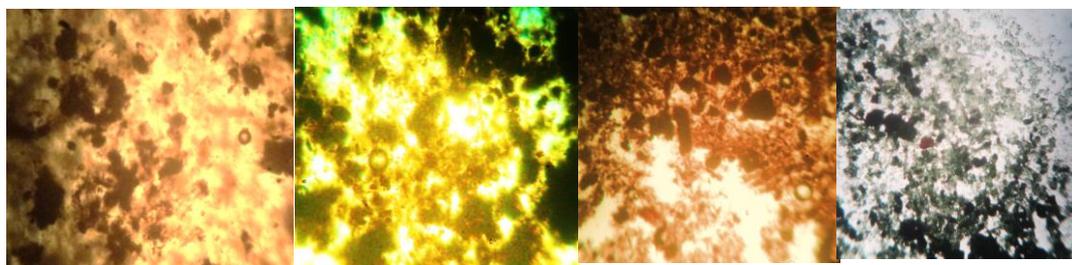
a powerful tool for localization of trace quantities of substances present in biological tissues. Histochemical techniques have been employed to characterize structure and development, and to study time course of deposition and distribution of major phytochemicals (Krishnan *et al.*, 2001).

In the present study, *Indigofera tinctoria* leaves were treated with specific chemicals and reagents. The *Indigofera tinctoria* leaves powder treated with diluted ammonia and H₂SO₄ gave yellow colour indicates flavonoids. The *Indigofera tinctoria* leaves powder treated with few drops of H₂SO₄ gave yellow color indicates the presence of saponins. Plant powder treated with Toluidine blue gave Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenol hydrazine (few drops) gave Orange colour indicates the presence of terpenoids. (Table 3 and Figure 5). These results further confirmed the presence of phytochemicals.

Table 3: Histochemical analysis of *Indigofera tinctoria* leaves powder

Phytochemical	Result
Saponins	++
Flavonoids	++
Terpenoids	+
Polyphenol	++

Single plus (+) represents presence and double plus (++) represents high concentrations



Saponins

Flavonoids

Terpenoids

Polyphenol

Figure 5: Histochemical analysis of *Indigofera tinctoria* leaves powder

Antimicrobial activity of *Indigofera tinctoria* leaves

Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions,

and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman *et al.*, 2003). This situation forced scientists to search for new antimicrobial substances with plant origin.

Plants produce a diverse array of secondary metabolites. Some of these metabolites have antifungal activity and are considered as a potential source of active principles in the formulation of new pesticides (Vyvyan 2002). Plant extracts with antifungal properties were used for controlling phytopathogens at laboratory, greenhouse and

field level (Bergeron et al. 1995). More than 2000 plant species have been reported to have antimicrobial and antifungal properties (Grainge and Ahmed 1988). Among 29 plant species, 15 plant extracts showed antifungal activity, with *Acer nikoense* (Nikko maple), *Glycyrrhiza glabra* and *Thea sinensis* being

the most effective plants (Sato et al. 2000). There are 4 major types of Microbes: bacteria, fungi, protists and viruses. Recently, many investigators (Punnagai et al. 2016) have identified the antifungal properties of plant extracts.

Table. 4: Antimicrobial activity of *Indigofera tinctoria* leaves

Microbial Strains	Concentration (µl)			Std. (30µl)
	50µl	100µl	150µl	
Bacteria				
<i>Escherichia coli</i> (mm)	2.25±0.15	5.50±0.38	7.00±0.79	10.00±0.70
<i>Pseudomonas aeruginosa</i> (mm)	1.50±1.05	3.25±0.22	6.00±0.42	9.00±0.63
<i>Staphylococcus aureus</i> (mm)	2.00±0.14	4.75±0.33	6.50±0.45	9.50±0.66
Fungal				
<i>Candida albicans</i> (mm)	0.75±0.04	3.00±0.21	5.75±0.40	8.25±0.57
<i>Aspergillus flavus</i> (mm)	0.50±0.03	2.50±0.17	5.00±0.35	8.00±0.56

Values expressed as Mean ± SD for triplicates

Bacterial standard: Chloramphenicol

Fungal standard: Fluconazole

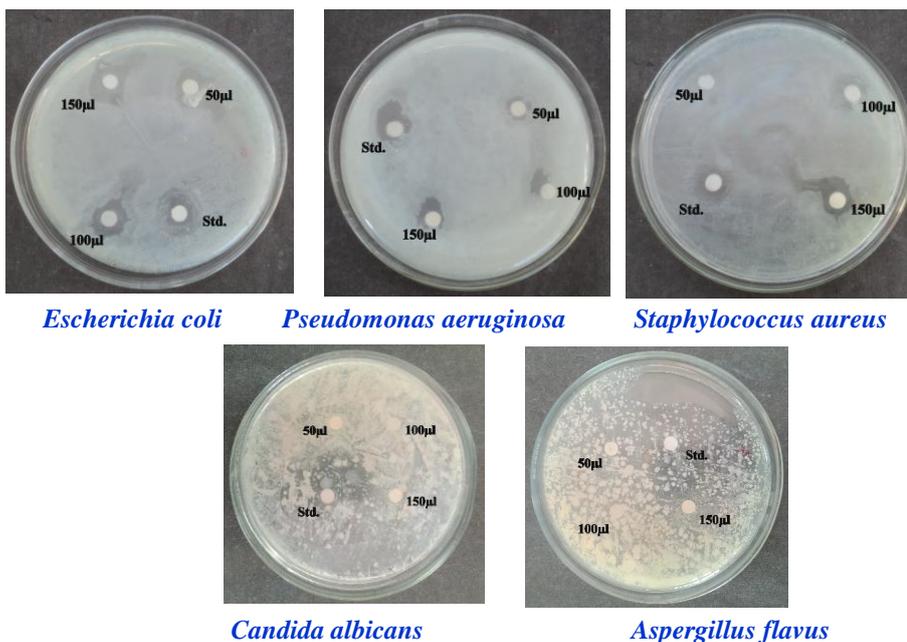


Fig. 6: Antimicrobial activity of *Indigofera tinctoria* leaves

Conclusion

The *Indigofera tinctoria* leaves has rich source of phytochemicals and possess

potential antimicrobial activity. The results of the study concluded that *Indigofera tinctoria* leaves may be used for the treatment of microbial infections.

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