ISSN 2348-0319 International Journal of Innovative and Applied Research [2024]
(Volume 12, Issue 03)
23-29

Journal home page: http://www.journalijiar.com

INTERNATIONAL JOURNAL
OF INNOVATIVE AND APPLIED RESEARCH

RESEARCH ARTICLE

Article DOI: 10.58538/IJIAR/2086
DOI URL: *http://dx.doi.org/10.58538/IJIAR/2086*

SCREENING OF PHYTOCHEMICALS AND ANTIMICROBIAL POTENTIAL *OF Indigofera linnaei* ALI., LEAVES

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Manuscript Info

Manuscript History
Received: 21 February 2023
Final Accepted: 23 March 2024
Published: March 2024

Keywords:
Indigofera Linnaei, Phytochemicals, Anti-Microbial Potential

Abstract

Indigofera linnaei, Ali, belongs to the family Fabaceae, is a medicinal plant growing wild in tropical countries.In the present investigation, the aerial leaf sections of *Indigofera linnaei* were examined for Triterpenoids, steroids, saponins, tannins, phenols, flavonoids, alkaloids, glycosides and coumarins were found in the ethanol and aqueous extracts of the leaves of *Indigofera linnaei*. The quantitative estimation of phytoconstituents in the powdered samples of *Indigofera linnaei* revealed that flavonoids were found in significant quantities when compared to alkaloids, tannins, phenols, and saponins. To evaluate the antimicrobial activity of ethanol extract using the standard disc diffusion method against three organisms. The antibacterial activities of the ethanolic extracts were compared favorably with that standard bacterial antibiotic (Chloramphenicol) and fungal antibiotic (Fluconazole). The Ethanolic extract of leaf showed a maximum zone of inhibition in *Escherichia coli*(7.65±0.53) against*Staphylococcus aureus*(4.71±0.32)*andCandida albicans* (5.12±0.35).

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Introduction:-

Plants also hold different inorganic nutrients which are essential for growth development and proper functioning of human body ingestions of this inorganic compound in excess or limited amount can cause various health issues Vasanth *et al.*, 2023.

Plants have great potential uses, especially as traditional medicine and pharmacopoeia drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine. (Hill,1952),. Medicinal plants have provided the modern medicine with numerous plant-derived therapeutic agents

(Okwu1999), many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases (Tagboto *et.al*., 2001).,

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 (Bandaranayake 2006, Govindarajan et al., 2008).

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 (Duke 1981). 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. The plant also possesses antibacterial, antidepressant, and nootropic activity (Renukadevi and Sultana 2011).

Hence the objectives of this presence study was to perform a phytochemical study and to evaluate the antimicrobial activity of*Indigofera linnaei* Ali (family-Fabaceae), commonly known as Birdsville indigo is a Trailing, branched, slender annual or perennial herbs with woody rootstock, 15–50 cm high with a long taproot. Leaves impair pinnate .*Indigofera* is a large genus of over 750 species of flowering plants belonging to the family Fabaceae.

Material And Methods:-

Plant Material

A collected plant species *Indigofera linnaei* Ali., leaves were collected December 2023 from Thanjavur district, Tamil Nadu, India. The plant was identified based on the Morphological characters by using standard manual of Flora of Presidency of Madras by J.S Gamble

Preparation of plant powder [Krishnamurthy 1993**]**

Indigofera linnaei (leaves) was first cleaned using tap water in order to remove any dirt or debris and later using sterile distilled water. They were dried in laminar flow biological safety cabinet. They were crushed in a sterile mortar pestle until affine paste was obtained. Similarly, the plant all the species cleaned and a fine powder was made. Required concentration of the plant extracts was made using ethanol.

Qualitative Phytochemical Analysis

The qualitative phytochemical tests for Alkaloids, flavonoids, terpenoids, steroids, phenols compounds, tannins, saponins and coumarins were carried out on the concentrated extracts using the standard procedures to identify the constituents as described by the methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Quantitative Phytochemical Analysis

Quantitative estimation of phytoconstituents Quantitative estimation of phytoconstituents like alkaloids [Harborne 1973], flavonoids [Bohm and Kocipai-Abyazan 1994], tannins [Van-Burden and Robinson 1981] and phenols [Edeoga *et al*., 2006], saponin [Obadoni and Ochuko 2001] of *Indigofera linnaei*

Antimicrobial activity

Antimicrobial activity of the extracts and fractions were tested using the agar diffusion method described by Collin *et al.,* (1970). Varying concentrations of the extracts and fractions were prepared and tested against test pathogen. The plates were incubated at 37°C for 24 hours and the zone of inhibition measured.

Test Microorganisms:

Three microbial species were used as test organisms. These include *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*, all of which were isolated from people with skin infections.

Determination of antimicrobial activity

The antimicrobial activity was performed by disc diffusion method.

Escherichia coli

E. coli is gram negative, Facultative anaerobic and Non-sporulating organism. The cells are about 2µ long and 0.5µ in diameter with a cell volume of 0.6 to 0.7µm^3. The optimal growth of *E coli* occurs at 37˚Cbut some laboratory strains can multiply at temperature of up to 49˚C (Fotadar *et al.*, 2005).

Some virulent strains of *E. coli* causes Gastroenteritis, Urinary tract infections, and neonatal meningitis, In rare cases, virulent strains are also responsible for Hemolytic-uremic syndrome (HUS), Peritonitis, Mastitis, Septicemia and Gram negative Pneumonia.

Staphylococcus aureus

Staphylococcus aureus may occur as a commensal on human skin sometimes it infect other tissue when normal barrier have been breached, this leads to furuncles (boils) and carbuncles (a collection of furuncles). In infant *Staphylococcus* infection can cause a severe disease *Staphylococcus* scalded skin syndrome (Curran and Al-Salihi, 1980).

Candida albicans

Candida albicans is a dimorphic fungus that exists as a commensal of warm-blooded animals including humans. It colonizes mucosal surfaces of the oral and vaginal cavities and the digestive tract and is also able to cause a variety of infections, depending on the nature of the underlying host defect. Therefore, *C. albicans* infections (candidiasis) are very infrequent in healthy individuals. (Corner and Magee, 1997).

Preparation of Media

Nutrient Agar (NA-Himedia) Media for Bacteria

Composition of Media

Animal's tissue	:	5.00 g
Sodium chloride	:	5.00g
Beef extract	:	1.50g
Yeast extract	:	1.50g
Agar	:	15.0g

Preparation of medium:

Suspend 28.0 grams in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mix well and pour into sterile Petriplates.

Potato Dextrose Agar (PDA-Himedia) Media for Fungi

Composition of Media.

Potatoes infusion from	:	200.00g
Dextrose	:	20.00g
Agar	:	15.00g

Preparation of medium:

Suspend 39.0 grams in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚c) for 15 minutes. Mix well before dispensing in specific work, when pH 3.5 is required; acidify the medium with sterile 10 % tartaric acid. The amount of acid required for 100 ml of sterile cooled medium is approximately 1 ml. do not heat the medium after addition of acid.

Results and Discussion:-

Leaf extracts of the species analyzed in fig: 1,2 were found to include alkaloids, flavonoids, terpenoids, steroids, phenols, tannins, saponins, glycosides and coumarins. Among alkaloids, flavonoids, phenols, tannins and saponins were found in high concentrations in the ethanol solvent and also flavonoids, Phenols, saponins were present high concentration in aqueous solvent. Terpenoids, steroids, glycosides and coumarins were moderately present in ethanol solvent, whereas the aqueous solvent alkaloids, terpenoids, steroids, tannins, glycosides, coumarins were moderately present. Out of these three solvents, ethanol is the most efficient. Consequently, we decided to undertake additional research with ethanol as a solvent. The presence of these potent phytochemicals made it abundantly evident that *Indigofera linnaei*leaf extract possesses significant anti- microbial, anti-inflammatory, anti-diabetic,

antioxidant analysis etc.,.Awoyinka *et al*. (2007) extracted eight bioactive compounds from dry leaf of *Indigofera linnaei* using water and ethanol.

Table 1:- Phytochemical screening of *Indigofera linnaei*leaf.

S.no	Phytochemical compounds	Aqueous	Ethanol
1	Alkaloids	+	++
2	Flavonoids	++	++
3	Terpenoids	+	+
4	Steroids	+	+
5	Phenols	++	++
6	Tannins	+	++
7	Saponins	++	++
8	Glycosides	+	+
9	Coumarins	+	+

(++)Strongly present,(+) moderate present,(-)absent

Fig 1:- Phytochemical screening of *Indigofera linnaei*in ethanol solvent.

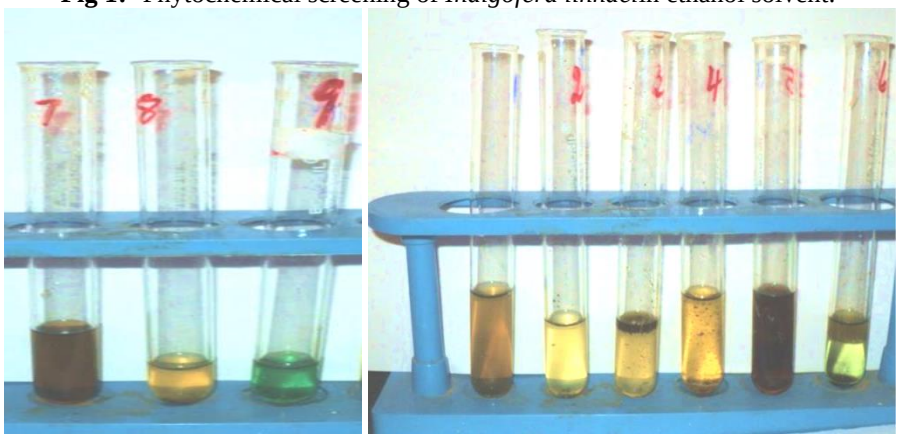
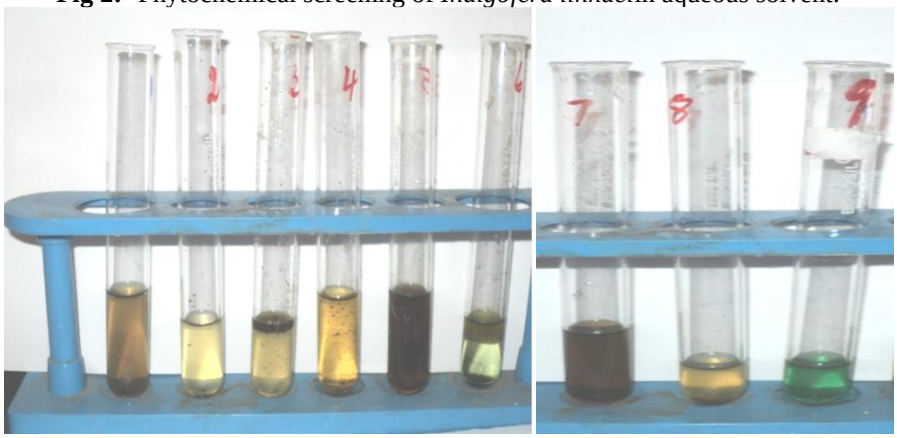


Fig 2:- Phytochemical screening of *Indigofera linnaei*in aqueous solvent.



1. Tannin, 2.Glycoside, 3.Saponin, 4.Flavonoids, 5. Steroids, 6.Terepenoids, 7.phenols, 8. Alkaloids, 9.coumarins**.**

Quantitative analysis

Quantitative analysis exposed that the *Indigofera linnaei* leaf contains significant amount of phenols, flavonoids, alkaloids, saponin and tannin. Significant amount of total phenol (70.78mg/gm), tannin (25.12mg/gm), alkaloids (103.45mg/gm), saponin (80.32mg/gm) and flavonoids (120 .25mg/gm) were presented (Table 2).

Table 2:- Quantitative analysis of *Indigofera linnaei* leaf extract.

S.No	Name of the Test	Result (mg/gm)
1.	Phenol	70.78
2.	Flavonoids	120 .25
3.	Alkaloids	103.45
4.	Saponin	80.32
5.	Tannin	25.12

Antimicrobial activity

The antimicrobial activity of plant extracts were detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Indigofera linnaei* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 3. The inhibitory activities in culture media of the *Indigofera linnaei* reported in Table 3 were comparable with standard antimicrobiotic viz. chloromphenical and fluconazole.

The *in vitro* antimicrobial activity of the *Indigofera linnaei* leaves extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 3. The inhibitory activities in culture media of the *Candida albicans* reported in Table 3 were comparable with standard antimicrobiotic viz. chloromphenical and fluconazole.

The leaves of *Indigofera linnaei* showed antimicrobial potential aganist the bacteria such as *Escherichia coli*, *Staphylococcus aureus* and thefungi *Candida albicans.* Among the 3 organisms *Escherichia coli* (7.65±0.53) showed maximum zone of inhibition followed by *Candida albicans* (5.12±0.35)and *Staphylococcus aureus* (4.71±0.32) respectively.

According to Sakthivel *et al*., 2015aqueous extracts of *Iindigofera sp.*,plant were tested for the antimicrobial activity against pathogenic isolates isolated from various clinical sources. The extract was tested at different concentration like 64, 128, 256, and 512 mg/ml by BDM. MIC of crude extracts of *Iindigofera* leaves was found to be satisfactory. MIC obtained against test isolates indirectly depicts the amount of antimicrobials present in the aqueous extract.

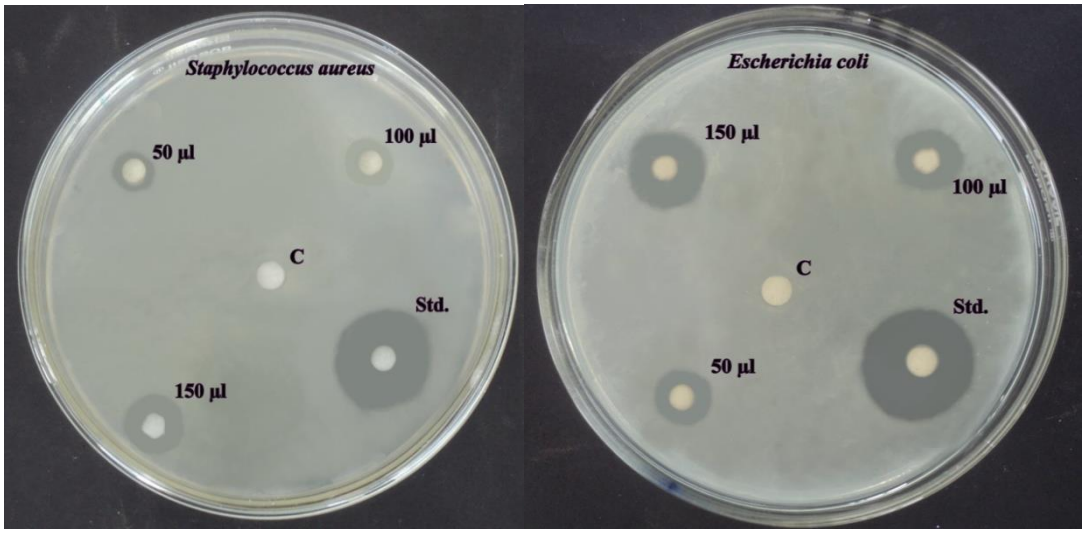
Table 3:- Antimicrobial activity of *Indigofera linnaei* leaf Ethanolic extract.

Microbial Organism	50µl	100 µl	150 µl	Standard	Control
Escherichia coli (mm)	2.20±0.15	3.84±0.26	7.65±0.53	11.42±0.79	0
Staphylococcus aureus (mm)	2.09±0.14	2.63±0.18	4.71±0.32	11.26±0.78	0
Candida albicans (mm)	1.37±0.09	2.41±0.16	5.12±0.35	10.28±0.71	0

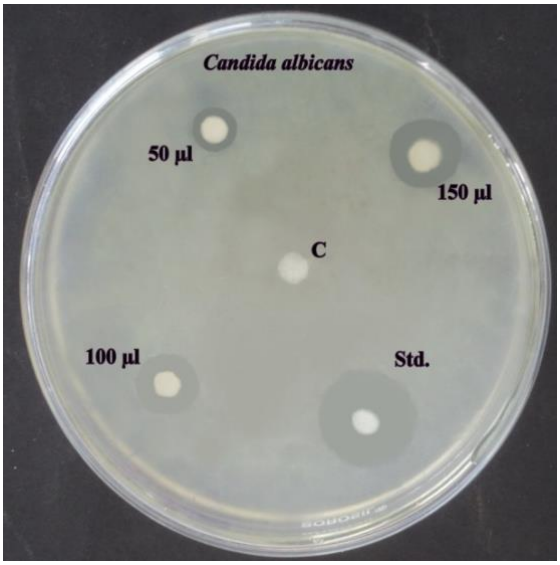
Values were expressed as Mean ± SD.
Bacterial standard - Chloromphenical
Fungal standard - Fluconazole

Fig 3:- Antimicrobial activity of*Indigofera linnaei*leaf.

Staphylococcus aureus ***Escherichia coli***



Candida albicans



Conclusion:-

Recently much attention has been directed toward extract and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants covering the basic health needs in developing countries, and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.

Reports showed that the ethanolic extract of *Indigofera linnaei* Ali., has significant antimicrobial activity against the Gram-positive bacterial (*Staphylococcus. aureus)*, Gram-negative bacterial (*E. coli*) and fungal species (*C. albicans*).From this study we can conclude that the traditional use of this plant for the treatment of infectious diseases is promising, mainly against bacteria, fungi and further investigations may improve our understanding of possible anti- microbial and antifungal activities.

Acknowledgements:-

The Author greatly thankful to PG and Research Department of Botany, K.N.G.A.College, Thanjavur. The authors deeply express their gratitude to Bharathidasan University, Trichy, India.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

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