Original Article

Antibacterial activity of methanolic extract of leaves *Indigofera Suffruticosa* naturally grown in Iraq

Omar Hussein Ahmed[1*], Alaa A. Bdulqader[2], Mohammed Ali Ezghayer[1]

[1]Department of Pharmaceutics, College of Pharmacy, Tikrit University, Tikrit, Iraq. [2]Department of Pharmacognosy, College of Pharmacy, Tikrit University, Tikrit, Iraq.

Correspondence: Omar Hussein Ahmed, Department of Pharmaceutics, College of Pharmacy, Tikrit University, Tikrit, Iraq. pharmacognosy88@gmail.com

ABSTRACT

To examine the leaves of *Indigofera suffruticosa's* methanolic extracts' antibacterial properties and phytochemical screening. By using the agar well diffusion technique, Many different concentrations of an extract from the Indigofera suffruticosa plant were pumped into bore wells. The average diameter of the zones of inhibition was observed after incubating the culture plates containing the extract and the test organisms at 37°C for 24 hours the antibacterial activity of extracts from Leaves of *Indigofera Suffruticosa* was assessed against two specific bacterial species. In the Leaves *Indigofera Suffruticosa* extract, the presence of flavonoids, alkaloids, coumarins, triterpenoids, and carbohydrates was confirmed. According to our findings, both Gram-positive and Gram-negative bacteria are significantly resistant to the antibacterial effects of the methanolic extract of plants produced by maceration. Escherichia coli and Staphylococcus aureus are both susceptible to the antibiotic effects of methanolic preparations of *Indigofera suffruticosa* leaves. The findings of this research indicate that extract from Leaves of *Indigofera Suffruticosa* may be utilized to treat illnesses brought on by the examined organisms.

Keywords: Leaves, *Indigofera Suffruticosa,* Methanolic extract, Organisms antibacterial activity

Introduction

The blue pigment is produced by *Indigofera suffruticosa* Mill as shown in **Figure 1**. (Fabaceae) is used for both yarn dyeing and traditional medical purposes, as a fever reducer, purgative, antispasmodic, abortive, diuretic, analgesic, or calming agent for gastrointestinal and urinary complaints, jaundice, ulcers, and other stomach and genitourinary disorders. The plant goes by many names. *I. suffruticosa* also has practical use beyond the kitchen; the plant may be fed to livestock [1]. The Fabaceae family is extremely abundant in Brazil; it is estimated that there are approximately 19,500 different species within this family, which is further subdivided into the Mimosoideae, Caesalpinioideae, and Papilionoideae subfamilies [2]. Pods are a characteristic shared by nearly all members of this family. Among the Fabaceae family of plants, the genus Indigofera stands out due to its several practical applications [3] and ground cover [4, 5]. *Indigofera truxillensis, Indigofera hirsuta*, and *Indigofera suffruticosa* are the three species in Brazil that have the common name "anileira" [6]. More than 700 species belong to this genus, which is found in tropical and subtropical regions worldwide. The biological properties of *I.suffruticosa*, such as its anticancer properties, were the primary focus of scientific investigation throughout the last several decades [7, 8], anti-inflammatory [9, 10], and antibacterial [9, 11] capabilities. Today, researchers are understanding this plant more broadly and deeply. Chemical compounds from extractions were separated using different solvents, and the biological potential of diverse plant components was assessed. The purpose of this review was to compile, from the available literature, crucial information on botany, geography, ethnopharmacology, phytochemistry, pharmacology, and toxicity of *I.suffruticos* [12]. Traditional Ethnopharmacology and Use *Indigofera suffruticosa* Purgative, sedative, and insecticidal characteristics, as well as antispasmodic, diuretic, abortive, and analgesic capabilities [13], have been ascribed to this plant for treating stomach and urinary issues, jaundice, ulcers, and fever.

Access this article online	
Website: www.japer.in	**E-ISSN:** 2249-3379

How to cite this article: Ahmed OH, Bdulqader AA, Ezghayer MA. Antibacterial activity of methanolic extract of leaves *Indigofera Suffruticosa* naturally grown in Iraq. J Adv Pharm Educ Res. 2023;13(4):99-103. https://doi.org/10.51847/fRbzdpKkBV

Flavonoids, triterpenoids, coumarins, alkaloids, and carbohydrates have all been found and isolated from *I. suffruticosa* by several studies [14]. From this plant, scientists have also extracted D-(+)-pinitol, -sitosterol, and louisfieserone [15-17]. Polyphenols (such as coumarin and chlorogenic acid), flavonoids (such as rutin, quercetin, and gallic acid), sugars, alkaloids, and triterpenoids are abundant in *I. suffruticosa* (from research on the leaves, seeds, and stems) [17]. Leaf oil from I. suffruticosa was also discovered to include chemicals such (z) -3-hexenyl benzoate, phytol, methyl linoleate, methyl hexadecanoic acid, linoleic acid, n-docosane, and n-tricosane [18-20]. The goal of this study was to learn more about the flavonoids, alkaloids, coumarins, triterpenoids, and carbohydrates that make *I. suffruticosa* by phytochemical screening and testing the Antibacterial Properties of Iraqi *I. suffruticosa* in Petri Dishes.

Figure 1. *Indigofera suffruticosa L* plant cultivated in Iraq.

Materials and Methods

Plant material

Indigofera suffruticosa leaves were collected from Baghdad in November 2021. University of Baghdad's Professor Dr. Sukaena Abass of the Department of Biology, College of Sciences, confirmed the plant's identity. After being cleaned and dried in the shade, leaves were crushed into a powder in a mechanical grinder.

Experimental work

Extraction method (cold method)

At room temperature, one hundred grams of the plant powder was soaked in one thousand five hundred milliliters of methanol, with intermittent shaking. After three days, anything that could be dissolved in methanol was filtered out. A rotary evaporator was used to remove all of the moisture from the filtrate while operating at very low pressure. Once the residue was processed, it turned out to be a dark green color. Dry the leftover residue off and check its antibacterial properties in the lab.

Preliminary phytochemical examination of crude extracts

Standard protocols for phytochemical analysis were applied to crude extracts, fractions, and powder specimens from the medicinal plants under investigation with the purpose of screening and identifying bioactive chemical ingredients.

Test of alkaloid: We warmed up 8 ml of 1% HCl, added 0.5-0.6 g of each plant extract and fraction, and filtered the resulting solution. Two milliliters of the filtrate were tested for the presence of alkaloids by treating it with Mayer's and Dragendorff's reagents one at a time and observing the resulting turbidity and precipitate development.

Coumarins test: Each plant extract or fraction was measured out to be 0.5 g in a test tube. Filter paper that had been soaked in a 1 N NaOH solution was used to seal the tube's opening. After placing the test tube in boiling water for a few minutes, the filter paper was removed and analyzed for coumarins using ultraviolet light, which revealed a yellow fluorescence.

Terpenoids test: Ascertained by (Salkowski) Two milliliters of chloroform were added to five milliliters of plant extract and fractions, and then three milliliters of concentrated (H_2SO_4). The presence of terpenoids was confirmed by the formation of a layer with the characteristic reddish-brown coloring at the contact.

Flavonoids test: The fatty materials were extracted from 0.5 g of each plant extract and fraction by shaking them with petroleum ether (lipid layer). The remaining fat was washed off and dissolved in twenty milliliters of 80% ethanol before being filtered. The following analyses were performed using the filtrate:

- Three milliliters of the filtrate were added to four milliliters of aluminum chloride in methanol (1%). Indicating the existence of flavonoids, the yellowing process revealed their presence.
- An observation of the color was made by combining three milliliters of the filtrate with four milliliters of 1% potassium hydroxide in a test tube. Flavonoids were present if the color was a deep yellow.

Antibacterial activity

The extracted Indigofera suffruticosa was evaluated for its antibacterial activity against Gram-negative and Gram-positive bacterial strains using an agar well diffusion experiment [21, 22]. Muller-Hinton (MH) agar was poured onto sterile Petri dishes to a volume of about 20 ml. We were able to separate

the individual bacterial species from their respective stock cultures by using a sterile wire loop [23]. After the cultures were established, wells were drilled into the agar plates at a depth of 6 mm using a sterile tip. Many different concentrations of an extract from the Indigofera suffruticosa plant were pumped into bore wells. The average diameter of the zones of inhibition was observed after incubating the culture plates containing the extract and the test organisms at 37°C for 24 hours [24, 25].

Statistical analysis

Statistical analysis was performed using GraphPad Prism [26]. The data are shown as the mean standard deviation of three separate tests, with Statistical significance at the p 0.05 level [27, 28].

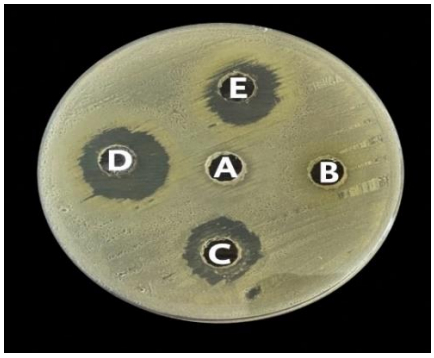
Results and Discussion

Extraction methods

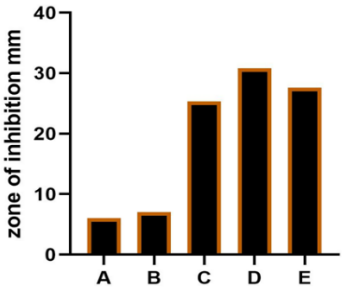
The first stage in creating the finished natural product from its basic elements is extraction. Which specific extraction method used to remove a chemical is determined by the nature of the substance. After employing two extraction techniques, one at a lower temperature and the other at a higher one, for the extraction of a single plant component, the optimal solvent and extraction technique were determined by comparing the percentage yield obtained from each technique and analyzing the crude extract for constituents using thin-layer chromatography. Selectivity, solubility, cost, and safety should all be taken into account when choosing a solvent for solvent extraction according to the law of similarity and impermissibility (like dissolves like) It is generally true that solvents having a polarity value close to that of the solute will work better, and vice versa. Unfortunately, the lengthy extraction time and poor extraction efficiency of this approach are the prices you pay for its simplicity. Thermolabile components may be extracted using this method which requires two days of leaf maceration This strategy was opted for in the event of pharmaceutical interest.

Antibacterial activity

Extracts and physiologically active chemicals derived from common plant species have garnered a lot of interest as of late. Medicinal plants have a significant role in helping people in developing countries satisfy their most basic healthcare needs, and they may be a unique source of chemicals with strong activity against infectious germs. We found that the macerated methanolic extract of *I. suffruticosa* had strong antibacterial activity against both Gram-positive and Gram-negative bacteria as shown in **Figures 2 and 3**. Methanol extracts of *I. suffruticosa* are effective in preventing the spread of Staphylococcus aureus and Escherichia coli, two types of Gram-negative bacteria. This research lends credence to the traditional usage of this herb to cure infectious disorders, particularly those caused by bacteria. The extracts are now being purified to isolate the bioactive component(s), and future research may shed light on their potential antibacterial and antifungal properties.

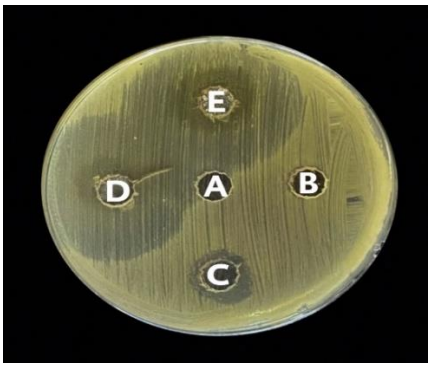


a)

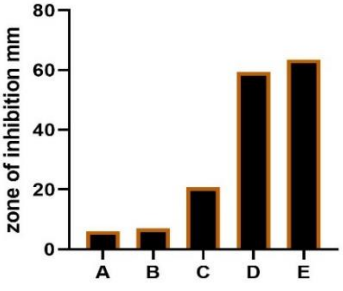


b)

Figure 2. Antibacterial activity of *I.suffruticosa* against *E. coli.* A, control. B, 62.5 microgram/ml. C, 125 microgram/ml. D, 250 microgram/ml. E, 500 microgram/ml.



a)



b)

Figure 3. Antibacterial activity of *I.suffruticosa* against *S. aureus.* A, control. B, 62.5 microgram/ml. C, 125 microgram/ml. D, 250 microgram/ml. E, 500 microgram/ml.

Conclusion

The findings of this research indicate that extract from Leaves of *Indigofera Suffruticosa* may have great potential as antimicrobial compounds against microorganisms and may be utilized to treat illnesses brought on by the examined organisms.

Acknowledgments: This study was supported by the College of Pharmacy, Department of Pharmacognosy, University of Tikrit, Tikrit, Iraq.

Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. Oliveira DM, Paiva EA. Anatomy and ontogeny of Pterodon emarginatus (Fabaceae: Faboideae) seed. Braz J Biol. 2005;65(3):483-94.
2. Dutra VF, Messias MC, Garcia FC. Papilionoideae (Leguminosae) of" campos ferruginosos" of Itacolomi State Park, Minas Gerais, Brazil. Braz Jo Bot. 2005;28:493-504.
3. Campos JKL, Araújo TFDS, Brito TGDS, da Silva APS, da Cunha RX, Martins MB, et al. *Indigofera suffruticosa* Mill. (Anil): Plant profile, phytochemistry, and pharmacology review. Adv Pharmacol Sci. 2018;2018:8168526.
4. Pesavento F. O azul fluminense: Um estudo sobre o comércio do anil no Rio de Janeiro colonial, 1749-1818. Rev Econ. 2005;7(2):207-31.
5. Vieira JR, De Souza IA, Do Nascimento SC, Leite SP. Indigofera suffruticosa: An alternative anticancer therapy. Evid-based Complementary Altern Med. 2007;4(3):355-9.
6. Leite SP, Silva LL, Catanho MT, Lima EO, Lima VL. Atividade antiinflamatória do extrato de indigofera suffruticosa. Rev Bras Ciênc Saúde. 2003;7(1):47-52.
7. Leite SP, de Medeiros PL, da Silva EC, de Souza Maia MB, de Menezes Lima VL, Saul DE. Embryotoxicity in vitro with extract of Indigofera suffruticosa leaves. Reprod Toxicol. 2004;18(5):701-5.
8. Leite SP, Vieira JR, de Medeiros PL, Leite RM, de Menezes Lima VL, Xavier HS, et al. Antimicrobial activity of Indigofera suffruticosa. Evid-Based Complementary Altern Med. 2006;3(2):261-5.
9. Roig T, Mesa JT. Plantas Medicinales Arómaticas y Venenosas de Cuba, Editorial Ciencia y Técnica Instituto del Libro, La Habana Cuba. 1974.
10. Braga R. Plantas do Nordeste Especialmente do Ceara, Escola Superior de Agricultura, Mossoró, Rio Grande do Norte, Brazil, 3rd edition. 1976.
11. Mioto ST, Iganci JR. Iganci, Indigofera in: Lista de Espécies da Flora do Brasil, Jardim Botânico do Rio de Janeiro, Rio de Janeiro, Brazil. 2015.
12. Riet-Correa F. Comunicação Pessoal, Lab Diagnóstico, UFPel, Pelotas, Brazil. 2000.
13. Barbosa Neto JD, Oliveira CM, Peixoto PV, Barbosa IB, Ávila SC, Tokarnia CH. Anemia hemolítica causada por Indigofera suffruticosa (Leg. Papilionoideae) em bovinos. Pesq Vet Bras. 2001;21(1):18-22.
14. NAPRALERT, Natural Products Alert, Illinois University, Chicago, IL, USA, 2015. Available from: http://www.uic.edu/phamacy/depts/pcrps/napralert.html.
15. Silva CB, Moraes MM, da Camara CA, Ribeiro ND, de Melo JP, de Lima VL, et al. Chemical composition and acaricidal activities of Indigofera suffruticosa essential oil against two-spotted spider mite. Quím Nova. 2019;42(3):313-8.
16. Ribeiro NC, da Camara CAG, Ramos CSJ. Chemical composition and acaricidal activities of indigofera suffruticosa essential oilagainst two-spotted spider mite. Agr Res. 2016;76:71.
17. Calvo TR. Uso sustentável de biodiversidade brasileira-prospecção químico-farmacológica em plantas superiores: Alchornea glandulosa, Alchorneatriplinervia (Euphorbiaceae), Indigofera truxillensis e Indigofera suffruticosa (Fabaceae). Doctoral thesis, Universidade Estadual Paulista, São Paulo, Brazil. 2007.
18. Vijisaral ED, Arumugam S. GC-MS analysis of bioactive constituents of Indigofera suffruticosa leaves. J Chem Pharm Res. 2014;6(8):294-300.
19. Chen TY, Sun HL, Yao HT, Lii CK, Chen HW, Chen PY, et al. Suppressive effects of Indigofera suffruticosa Mill extracts on lipopolysaccharide-induced inflammatory responses in murine RAW 264.7 macrophages. Food Chem Toxicol. 2013;55:257-64.
20. Arriaga AM, Lemos TL, Santiago GM, Andrade-Neto M, Braga MA, de Almeida MC, et al. Chemical composition and antioxidant activity of Indigofera suffruticosa. Chem Nat Compd. 2013;49(1):150-1.
21. Yazbek PB, Tezoto J, Cassas F, Rodrigues E. Plants used during maternity, menstrual cycle and other women's health conditions among Brazilian cultures. J Ethnopharmacol. 2016;179:310-31.
22. Bahjat HH, Ismail RA, Sulaiman GM, Jabir MS. Magnetic field-assisted laser ablation of titanium dioxide nanoparticles in water for anti-bacterial applications. J Inorg Organomet Polym Mater. 2021;31:3649-56.
23. Khashan KS, Abdulameer FA, Jabir MS, Hadi AA, Sulaiman GM. Anticancer activity and toxicity of carbon nanoparticles produced by pulsed laser ablation of graphite in water. Adv Nat Sci: Nanosci Nanotechnol. 2020;11(3):035010.

24. Khashan KS, Badr BA, Sulaiman GM, Jabir MS, Hussain SA. Antibacterial activity of Zinc Oxide nanostructured materials synthesis by laser ablation method. InJournal of Physics: Conference Series 2021 Mar 1 (Vol. 1795, No. 1, p. 012040). IOP Publishing.
25. Jihad MA, Noori FT, Jabir MS, Albukhaty S, AlMalki FA, Alyamani AA. Polyethylene glycol functionalized graphene oxide nanoparticles loaded with nigella sativa extract: A smart antibacterial therapeutic drug delivery system. Molecules. 2021;26(11):3067.
26. Mohammed MK, Mohammad MR, Jabir MS, Ahmed DS. Functionalization, characterization, and antibacterial activity of single wall and multi wall carbon nanotubes. InIOP Conference Series: Materials Science and Engineering 2020 Mar 1 (Vol. 757, No. 1, p. 012028). IOP Publishing.
27. Ali IH, Jabir MS, Al-Shmgani HS, Sulaiman GM, Sadoon AH. Pathological and immunological study on infection with Escherichia coli in ale balb/c mice. InJournal of Physics: Conference Series 2018 May 1 (Vol. 1003, p. 012009). IOP Publishing.
28. Younus A, Al-Ahmer S, Jabir M. Evaluation of some immunological markers in children with bacterial meningitis caused by Streptococcus pneumoniae. Res J Biotechnol. 2019;14:131-3.