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PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF AERIAL PARTS OF *INDIGOFERA GERARDIANA* FOR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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Abstract

The crude methanolic extract of aerial Indigofera gerardiana (1mg/ml) was tested for antibacterial and antifungal activities. The plant's phytochemical screening found carbohydrates, starch, steroids, glycosides, triterpenoids, and flavonoids. The crude methanolic extract, n-hexane, butanol, and aqueous fractions of Indigofera gerardiana were effective against bacteria, fungus, and leishmaniasis. The zone of inhibition (ZOI) for Escherichia coli and Staphylococcus aureus was 11mm. The ZOI is compared to imipenum, administered a single time at 3 mg/ml. The anti-fungal activity against fungal strains, including Trichophyton longifusus, Candida albicans, Microspoumcanis, and Fusarium solani, is inhibited by 60%, 50%, 50%, and 40%. Amphotericin B, a single 400µg/ml dose, was administered, and it was the standard treatment for Aspergillus flavus and miconazole for other fungal species.

Keywords: *Indigofera gerardiana, antibacterial activity and antifungal activity.*

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1. INTRODUCTION:

Traditional medicine involves the use of plants for medicinal purposes. The term 'herb' includes leaves, stems, flowers, fruits, seeds, roots, rhizomes, and bark of any medicinal plant [(1)]. The World Health Organization (WHO) estimates that about 80% of the world population uses herbal medicine for some aspect of primary health care. Herbal medicine is a significant component in all Indigenous peoples' traditional medicine and a common element in homoeopathic, naturopathic and conventional oriental [(2)].

The plant *Indigofera gerardiana*, commonly known as Ghorega, belongs to Leguminoseae. It is widely distributed in northern parts of India and finds various medicinal uses in the indigenous system of medicine. In northern areas of India, this plant is traditionally used for relieving abdominal and spastic pains and infectious diseases, especially skin infections involving microorganisms [(3)].

The present study aimed to determine the potential antibacterial and antifungal activities of methanolic extract from aerial parts of *Indigofera gerardiana* against different bacterial and fungal strains.

2. MATERIALS AND METHODS:

2.1. Microbial strains

The bacterial test strains used in this antibacterial activity study were *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Shigella flexenari* (clinical isolate), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella typhi* (ATCC 19430). Fungal strains are *Candida albicans* (ATCC 2091), *Candida glabarata* (ATCC 90030, *Aspergillus flavus* (ATCC 32611), *Trichophyton longifusis* (clinical isolate), *Mycosporum canis* (ATCC 11622) and *Fusarium solani* (ATCC 11712) were obtained from Institute of Microbiology Technology, Chandigarh. The bacterial strains were maintained on an agar slant at 4ºC in the same laboratory where the antimicrobial tests were performed.

2.2. Collection and identification of plant material

The fresh specimens of plants *Indigofera gerardiana* Wall was collected from upper Dir and Chitral (Bonni), respectively. Each plant sample was washed, separated, and dried in air for about two weeks at room temperature. Then, it was used for the following tests and analyses. These plants were ground to 60 mesh size and were preserved in airtight bottles. Some fresh specimens were used to study morphological characters, and some were utilized for section cutting [(4)].

2.3. Extract preparation

The preparation method is adopted for preparing plant extracts with little modifications. The 20 g portions of the powdered plant material were soaked separately in 100 ml of water, hexane, and alcohol for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each extract was passed through Whatman filter paper no. 1. The filtrate obtained was concentrated in a vacuum using a rotary evaporator at 32°C [(5)].

2.4. Determination of antimicrobial activity

The agar well diffusion method was modified. Soybean casein digest agar was used for bacterial cultures. The culture medium was inoculated with the microorganisms suspended in soybean casein digest broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (distilled water, hexane and alcohol, as the case may be). A standard antibiotic (imipenum, dose 3 mg/ml) was used as a positive control simultaneously. The plates were then incubated at 37°C for 18 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter observed [(6)].

2.5. Minimum inhibitory concentration (MIC)

The extract showing antibacterial activity in the agar well assay will be subjected to a MIC assay. The antimicrobial MIC studies will be carried out using the broth dilution method. The MIC was taken as the lowest extract concentration in the well plate, and it showed no turbidity after 24 h of incubation at 37°C. The turbidity of the wells was interpreted as the visible growth of microorganisms [(7)].

2.6. Processing of plant material

The plant materials were dried in the shade for 5-6 days and then dried in a hot air oven at 40ºC. After drying, the plant materials were milled to powder and passed through the sieve (mesh size 40), which was used to identify plant metabolites [(8)].

2.7. Screening of phytochemicals

The phytochemical studies were performed to test the different chemical groups present in the drugs. Unless otherwise mentioned in the respective individual test, a 10% (w/v) extract solution was taken. The chemical group tests were performed, and the results are shown in tables. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them [(9)].

2.8. Antibacterial activity

It was performed using the agar well diffusion method. Methanolic extract and different portions dissolved in an organic solvent were used in 3 mg/mL of DMSO, and pure compounds were used in a dose of 1 mg/mL[10]. Antibacterial activity was carried out against various bacterial strains, including *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. In this activity, 3 types of media are required: solid medium, semi-solid medium and liquid medium [11].

2.8.1. Culture media

Solid medium (Nutrient agar)

Nutrient agar (28 gm) was dissolved in distilled water, and the volume was 1 litre. It was then placed in an autoclave at 121^{0}C for 15 min. Media was then chilled to 40^{0}C and poured into sterile Petri dishes, and media was then left to solidify at room temperature [12].

Semisolid medium (Soft agar)

Soft agar (0.8 gm) was dissolved in distilled water, and volume was 100 mL. It was then dispensed in 7mL quantity to screw-capped test tubes. Then, it was placed in an autoclave at 121^{0}C for 15 minutes and cooled [13].

Liquid medium (Nutrient broth)

Nutrient broth (0.8 grams) was dissolved in distilled water to 100 mL. Screw-capped test tubes were filled with 3 ml of prepared broth, autoclaved at 121^{0}C for 15 min, and chilled. Bacteria were grown on stock culture agar. Bacteria were implanted in nutrient bisque and incubated at 37^{0}C for 24 hours. Soft agar was melted and chilled the next day, and 100 µL of bacterial culture was added. Shake well and place onto a nutritional agar plate. Rotate the plate to distribute culture evenly and harden the grass. A sterile sharp tool made a 6 mm medium hole in each plate with centres at least 24 mm apart. Using sterile dropping pipettes, 3 mg/ml of DMSO was applied to each well. Other holes received DMSO and a conventional antibacterial medication as -ve and +ve controls. Plates were incubated at 37^{0}C for 24 hours. Activity was estimated by measuring resistance zone diameter (mm) [14].

2.9. Antifungal activity

It was carried out following agar tube dilution protocol. Methanolic extract and various fractions were used in a 24 mg/mL dose, and pure compounds 12 mg/mL of sterile DMSO were provided as stock solution. The agar dilution method is the most convenient method for routine testing of samples such as plant extracts. The process is suitable for testing nonsterile plant extracts because aerobic organisms do not develop well under solidified agar. This method has an advantage: unlike the diffusion method, no concentration gradient occurs during the testing procedure [15]. Antifungal activity was conducted against clinical specimens of human pathogens, namely *Candida albicans*, *Candida glabarata*, *Aspergillus flavus*, *Trichophyton longifusis*, *Mycosporum canis* and *Fusarium solani*.

3. RESULTS AND DISCUSSION:

3.1. Phyto-chemical screening of *Indigofera gerardiana*

Preliminary phytochemical screening was carried out by using standard procedure. The phytochemical screening of the plant revealed the presence of carbohydrates, starch, steroids, glycosides, triterpenoids, and flavonoids [16]. Results are summarized in **Table 1.**

Table 1: Phytochemical analysis of *Indigofera gerardiana*

Phytochemical tests	***Indigofera gerardiana* extract of aerial parts**			
Active constituents	**IGCR**	**IGPE**	**IGAC**	**IGME**
Alkalodids	-	-	-	-
Flavonoids	++	+++	++	++
Saponins	+	++	++	++
Tannins	+	+++	+++	++
Steroids	+	++	-	++
Cardiac Glycosides	+	++	++	++
Proteins	+	++	++	++
Resins	+	+++	++	++
Starch	+++	++	++	+
Triterpenoids	+	++	+++	++

(-) No presence, (+) Less presence, (++) Moderate Presence, (+++) High presence, IGCR: Crude powder, IGPE: Petroleum ether extract, IGAC: Acetone extract, IGME: Methanol Extract.

3.2. Antibacterial activity

The tests were executed on six bacteria-indicated strains: *Escherichia coli*, *Bacillus subtilis*, *Shigella flexeneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. They were maintained on an agar slant at 4ºC. Before the screening, the strains were activated for bacteria at 37oC for 24 h on nutrient agar (NA) or Sabouraud glucose agar (SGA), respectively. Crude methanolic extract and other portions exhibited ZOI in mm against different bacterial strains compared to standard drugs, i.e. Imipenum. The dose of 3mg/ml was given in a single concentration. Crude methanolic extract of *Indigofera gerardiana*, n-hexane fraction, butanol fraction and the aqueous fraction showed good to significant action against *Staphylococcus aureus*, *Shigella flexeneri*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* [17]. The results are presented in Table 2 and Fig 1.

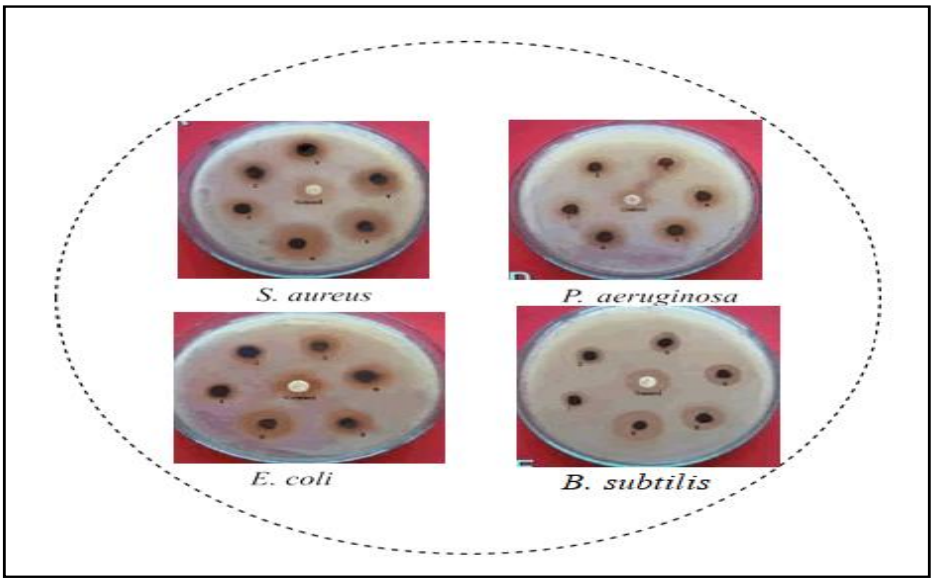

Fig1: Antibacterial activity of microorganism

The crude methanolic extract of *Indigofera gerardiana* exhibited anti-bacterial activity only against *Escherichia coli* & *Staphylococcus aureus*, and the zone of inhibition observed was 11 mm and 11mm respectively [18].

Table 2: Antibacterial activity on organisms

Sample	*S. Flexenari*		*B. Subtilus*		*E. Coli*		*S. Aureus*		*P. Aeruginosa*		*S. Typhi*	
	1	2	1	2	1	2	1	2	1	2	1	2
1	-	-	-	-	11	39	11	39	-	-	8	36
2	10	41	-	-	-	-	13	48	-	-	15	57
3	9	37	-	-	-	-	9	37	-	-	-	-
4	-	-	-	-	12	48	-	-	-	-	-	-
5	-	-	-	-	9	37	-	-	11	39	7	26
6	11	39	-	-	-	-	-	-	9	37	12	48
STD	24	-	23	-	28	-	27	-	20	-	26	-

3.3. Antifungal activity

It was tested on *Trichophyton longifusus*, *Candida albica*, Aspergillusflavus, Micro spoumcanis, Fusarium solani, and Candida glaberata. They were kept on 4^0C agar. The strains were activated at 37^0C for 24 hours on nutritional agar (NA) or Sabouraud glucose agar (SGA) for fungi before viewing. Amphotericin-B was the standard dose for *Aspergillus flavus* and miconazole for other fungal species [19]. A single 400µg/ml dosage was administered. The most effective inhibitors of *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microspoumcanis*, and *Fusariumsolani* were *Indigofera gerardiana* crude extract, n-hexane, chloroform, and aqueous. *Indigofera gerardiana* crude extract inhibited *Trichophyton longifusus*, *Candida albicans*, *Microspoumcanis*, and *Fusariumsolani* by 60%, 50%, 50%, and 40%, respectively. Similarly, the n-hexane fraction suppressed *Aspergillus flavus*, *Microspoumcanis*, and *Fusarium solani* growth by 20%, 90%, and 70%. The chloroform fraction inhibited *Candida albicans*, *Aspergillus flavus*, *Microspoumcanis*, and *Fusarium solani* by 10%, 60%, 50%, and 20%. The butanol fraction inhibited *Aspergillus flavus*, *Mycosporum canis*, and *Fusarium solani* by 20%, 90%, and 20%. The aqueous portion inhibited *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Mycosporumcanis*, and *Fusarium solani* by %. All fractions and crude methanolic extract were inactive against *Candida glaberata* [20]. The results are depicted in Table 3 and Fig 2.

Table 3: Anti-fungal assay of crude extract and fractions

Fungal Strain	% of inhibition						Standard Drug
	1	2	3	4	5	6	
T. longifusis	60	-	-	50	-	30	Miconazole
C. albicans	50	-	11	80	-	10	Miconazole
A. flavus	-	20	60	-	20	40	Amphotericin-B
M.canis	50	91	50	40	90	70	Miconazole
F. solani	40	72	21	40	21	42	Miconazole
C. glabarata	-	-	-	-	-	-	Miconazole

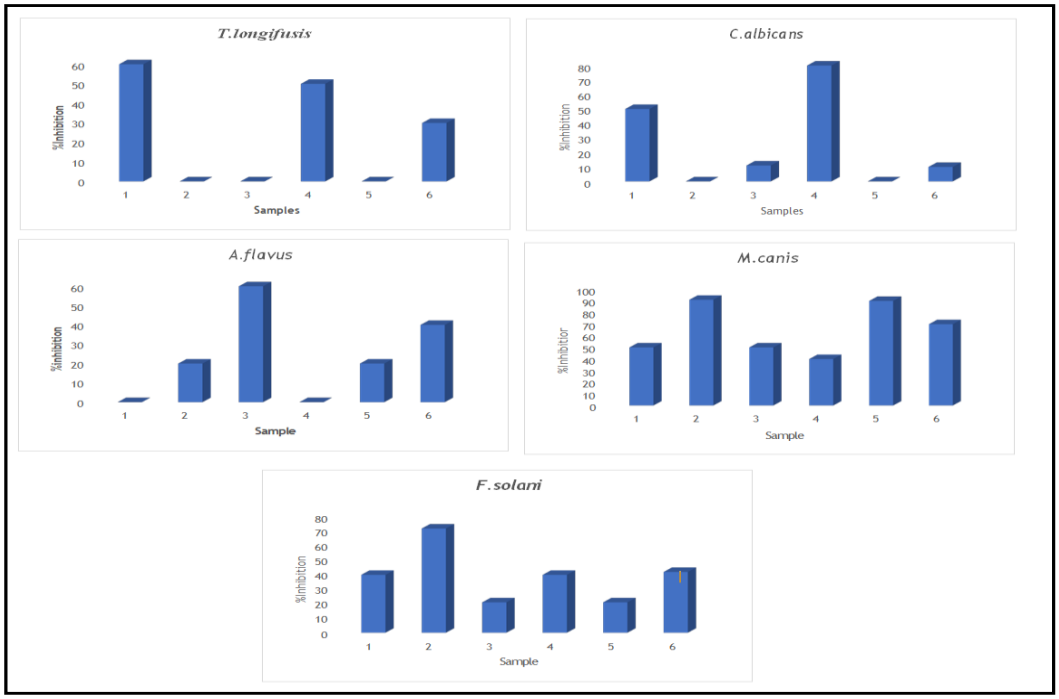

Fig 2: Percentage inhibition of fungal strains

4. CONCLUSION:

The current investigation strongly supported using *Indigofera Gerardiana* for various activities. Interestingly, antibacterial and antifungal activity was found. These activities may be attributed to carbohydrates, starch, steroids, glycosides, triterpenoids, and flavonoids found in the crude extract of aerial parts of *Indigofera Gerardiana*. These phytochemical groups of natural products are known to display various activities. This study has shown that extracts of aerial parts of *Indigofera Gerardiana* exhibited antibacterial and anti-fungal activities. Methanolic extract of aerial parts is the most effective against bacterial strains, *P. aeruginosa*, *B. subtilis*, *S. aureus*, and *E. coli* and fungal strains *Trichophyton longifusus Microspoumcanis*, *Fusarium solani* and *Candida albicans.* The extracts of this plant were effective against *S. aureus*, known for its increased resistance to antibiotics. Therefore, this plant can treat various diseases caused by antibiotic-resistant bacteria. However, further research is required to understand the mechanisms involved in antimicrobial activity.

REFERENCES:

1. Akerele. Medicinal plants and primary health care: An agenda for action. Fitoterapia, 59: 355-363 (1988).
2. Ahmed, A. S. Phytochemical screening of some Malaysian plants. Journal of Tropical Forests Products, 1(1): 38 (1995).
3. Chakrabarti, R., Damarla, R. K. B., Mullangi, R., Sharma, V. M., Vikramadithyan, R. K and Rajagopalan, R. Insulin sensitizing property of Indigofera mysorensis extract. J. Ethnopharmacol. 105, 102-106 (2006).
4. Amala .B. E., Ganga. N, Arivudainambi R, Anti-inflammatory activity of Indigofera aspalathoides Vahl. Indian JMedRes. 76.115 (1982).
5. Shanson, DC Classification and pathogenicity of microbes. In: Shanson, D.C., (ed.) Microbiology in clinical Practice, Wright, Great Britain (1989).
6. Dahot, M. U. Antibacterial and antifungal activity of small protein of Indigofera oblongifolia leaves. J. of Ethnopharmacol, 64, 277-282 (1999).
7. Esimon, .C.O.A., Dikwu., MU and Muko., K. N. Antibacterial properties of Indigofera dendroides leaves. Fitroterapia. 70 (5): 517-520 (1999).
8. Kivack B., Mert T., Tansel H. Antibacterial and cytotoxic activities of Ceratonia siliqua L extracts. Turk. J. Biol, 26:197-200 (2001).
9. Nyarko. K. Alexander., Archibald A. Sittie J. Phytotherapia Research Indigofera arrecta: safety evaluation of an antidiabetic plant extract in non-diabetic human volunteers. 12(1), 52-54 (1998).
10. Solomon Habtemariam. In vitro antileishmanial effects of antibacterial diterpenes from two Ethiopian Premna species: P. Schimperi and P. Oligotricha" BMC Pharmacology, 3:6 (2003).
11. Nisar M, Tariq SA, Marwat IK, Shah MR, Khan IA. Antibacterial, antifungal, insecticidal, cytotoxicity and phytotoxicity studies on Indigofera gerardiana. J. Enzyme Inhib. Med. Chem., 24: 224-229 (2009).
12. Mathabe, M. C., Nikolova, R. V., Lall, N and Nyazema, N. Z. Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. J. Ethnopharmacol, 105, 286-293. (2006).
13. Gilani . A.H., Molla. V. Atta-ur-Rehman and shah. BH. Role of natural products in medicine, J. Pharmaceutic. Kecl; 2; 111-118 (1992),
14. Naseem, R., Mahmud., K and Arshad., M. Chemical composition and antibacterial activity of Crotalaria burhia from Cholistan Desert, Pakistan. Hamdard Medicus. 49 (4): 49-52 (2006).
15. Shahjahan, M., Vani, G and Devi, C. S. Protective Effect of Indigofera oblongifolia in CCl4-Induced Hepatotoxicity; J Med Food., 8: 261-265 (2005).
16. Kivack B., Mert T., Tansel H. Antibacterial and cytotoxic activities of Ceratonia siliqua L extracts. Turk. J. Biol, 26:197-200 (2001).
17. Paxton, J.D. Assay for antifungal activity, In: methods in Plant Biochemistry, Vol.6 (Hostemann, K., Harborne, J.b., dey, P.M., eds), Academic Press, London, UK (1991).
18. Umar, M. Antibacterial and antifungal activity of small protein of Indigofera oblongifolia leaves Journal of Ethanopharmacology 64: 277-282.(1999).
19. Prakash, D., Suri, S., Upadhyay, G and Singh, B. N. Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. International Journal of Food Science and Nutrition. 58, 18 - 28 (2007).
20. Puri,A. Khaliq,T. Ragebdran . S .M .Bhatia.G, Chandra.R. Narender. T. Antispasmodic activity of Indigofera gerardiana . J.Herb Pharacother 59- 64 (2007).