

**ANTIDERMATOPHYTIC ACTIVITY OF YOUNG LEAVES METHANOLIC EXTRACT
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ABSTRACT

The main aim and object to calculate in-vitro antidermatophytic activity of methanolic young leaves extracts of *Bixa orellana*, this is the first report on antidermatophytic assessment. The antidermatophytic activity of high-polar methanolic young leaves extracts of *Bixa orellana* was evaluated by agar well diffusion scheme, the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), minimum bactericidal concentration (MBC) were determined using broth dilution method. The preliminary phytochemical tests for secondary metabolites were carried out in both the extracts. The antidermatophytic examination through the five fungal and five bacterial strains were experimented to determine the effective concentration from methanolic young leaves extracts of *Bixa orellana*. The high-polar methanolic extract was exhibited an efficient antidermatophytic activity. The maximum antidermatophytic activity was observed against *C. albicans* (20.00±0.00mm) followed by *T. rubrum* (12.66±1.15mm), *M. gypseum* (10.33±1.15mm), *T. tonsurans* (09.33±0.57mm) and *T. Mentagrophytes* (09.00±0.00mm). Whereas, the maximum antibacterial activity of 20.00±0.00 mm inhibition was observed in *E. coli* followed by *B. subtilis* (18.66±0.57mm), *P. aeruginosa* (17.33±0.57mm) and *S. aureus* (16.00±00mm). The zone of inhibition was found to be concentration dependent relative. The preliminary phytochemical tests for the detection of secondary metabolites were performed. The present first report provides a foundation for the isolation and purification of anti-dermatophytic molecule(s) from the young leaves methanolic extracts of *Bixa orellana*.

KEYWORDS: antidermatophytic activity, *Bixa orellana*, methanolic extract.**INTRODUCTION**

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind.^[1] *Bixa orellana* belongs to family Bixaceae; it is frequently refined in India.^[2] *Bixa orellana* was extensively utilize for obtaining colouring agent that had purpose in foods, body paints, arts, crafts and murals. *Bixa orellana* congaing "annatto" dye and is been largely used as predictable rinse since lengthened Instance. Dye have been using textile industries^[3] as therapeutics agents in pharmaceuticals.^[4,5] So numerous investigations have been report on neurological, anticonvulsant, analgesic, antimicrobial activity^[4] and diuretic activity.^[5] Various studies focus on administration and anticipation of diabetes mellitus,^[6] antiperiodic and antipyretic^[7] activates.

The traditional healers claim that some medicinal plants such as *Bixa sps* are more efficient to treat infectious

diseases than synthetic antibiotics. It is necessary to evaluate in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants represent an alternative treatment for non severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. In Indian traditional systems like Ayurveda this plant is being used popularly as an astringent and mild purgative and is measured by them as a superior remedy for treating dysentery and kidney diseases. The root bark is used for ant periodic and antipyretic.^[8,9]

Diverse plant parts were being used in treating various diseases like wise, the seeds and root were used in treating gonorrhoea.^[10] The roots and young leaves used in epilepsy, dysentery, fever, jaundice and snake bite.^[11-13] *B. orellana* was shown activity against protozoan, helminths and had platelet antiaggregant activity.^[14-15]

The present report mainly aimed at the valuation of the antidermatophytic potentiality of *Bixa orellana* using high polar methanolic successive solvent extract of young leaves, with the trust that such an effort will produce an attention among the people to investigate for novel active phyto-drug molecule.

MATERIALS AND METHODS

Collection of plant material

Plant material was collected from Botanical garden, Gulbarga University, Gulbarga during the month of May and June 2014. The collected material was authenticated with the help of Flora of Gulbarga, The voucher specimen (HGUG-863) deposited in herbarium centre, department of Botany, Gulbarga University, Karnataka, India. The Collected materials were brought to the laboratory, washed with tap water followed by distilled water and surface sterilized with 1% HgCl₂ shade dried and used for extraction. The dried leaves were homogenized to a fine powder and stored in a air tight bottles.

Extraction of extract through plant material by soxhlet apparatus: The young leaves materials after drying were ground in a grinding machine in the laboratory. 25g of shade dried powder was weighed and extracted successively with ethanol in soxhlet extractor for 48h. The extracts were concentrated under reduced pressure and preserved in refrigerator in airtight bottles for further use.

Test microorganisms: Five fungal cultures strains, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Candida albicans* and five bacterial strains, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* obtained from M.R. medical college, Gulbarga, Karnataka, India were used in the present study. Bacterial cultures were grown in nutrient broth (Himedia, M002) at 37°C and maintained on nutrient agar slants at 4°C, fungal cultures were grown in potato dextrose broth at 28 °C and maintained on potato dextrose agar slants at 4°C.

Statistical Analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using STATISTICA 5.5 (Stat Soft Inc, Tulsa, Oklahoma, USA) software. A probability value of difference $p \sim 0.05$ was considered to denote a statistically significance All data were presented as mean values \pm standard deviation (SD).

Agar-well diffusion method: The assay was conducted by agar well diffusion method. About 15 to 20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using 5 days old culture strains. The fungal strains were suspended in a saline solution (0.85% NaCl)

and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations of serially diluted extract (0.62, 1.25, 2.5, 5, 10, 20 and 40mg/ml) was added to the 20 μ l to each wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37°C. After incubation for 48h, the plates were observed for zone of inhibition. Diameter zone of inhibition was measured and expressed in millimetres. Dimethyl formamide (DMF) was used as a negative control. The experiments were conducted in triplicates. The same method was followed for testing antidermatophytic activity using nutrient agar medium incubated at 37°C for 18h.^[14]

Minimum Inhibitory Concentration:^[16] One ml of sterile liquid Sabouraud medium was added to 11 sterile capped tubes, 1 ml of each solvent extracts suspension was added to tube 1. The contents were mixed and 1 ml was transferred to tube 2. This serial dilution was repeated through to tube nine and 1 ml was discarded from tube 9. Fifty μ l of inoculum was added to tubes 1-10 and the contents were mixed. Medium control (no inoculum and no drug) and inoculum control (no drug) tubes were prepared. The final concentrations of each plant solvent extracts ranged from 05mg/ml to 0.02 mg/ml. The tubes were incubated at 30° C for 96 h. The fungal growth in each tube was evaluated visually depending up on the turbidity in the tubes. MIC was defined as the drug concentration at which the turbidity of the medium was the same as the medium control.

Ten μ l aliquot of cell suspension from the tube without observed growth of dermatophytes was inoculated on to Sabouraud dextrose agar. Minimum fungicidal concentration (MFC), Minimum bactericidal concentration (MBC) of test compound were determined as the lowest concentration of the agent at which no colonies were seen after 4 days at 30°C. Triplicate sets were maintained for each experiment.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the methanolic young leaves extracts of *Bixa orellana* by adopting standard methods^[17] (Harborne, 1998). The results were represented in table-2 which reveals the presence of various phytochemical such as alkaloids, flavonoids, phenols, triterpenoids, steroids and saponins. The high polar extract shows strapping optimistic reaction to flavonoids, phenols and saponins. Whereas the rest of the tests shown negative response to alkaloids, steroid and tannin.

The antidermatophytic examination through the five fungal and five bacterial strains were experimented to determine the effective concentration from methanolic young leaves extracts of *Bixa orellana* (Table 1). The

high-polar methanolic extract was exhibited an efficient antidermatophytic activity. The maximum antidermatophytic activity was observed against *C. albicans* (20.00±0.00mm) followed by *T. rubrum* (12.66±1.15mm), *M. gypseum* (10.33±1.15mm), *T. tonsurans* (09.33±0.57mm) and *T. Mentagrophytes* (09.00±0.00mm). Whereas, the maximum antibacterial activity of 20.00±0.00 mm inhibition was observed in *E. coli* followed by *B. subtilis* (18.66±0.57mm), *P. aeruginosa* (17.33±0.57mm) and *S. aureus* (16.00±00mm). The zone of inhibition was found to be concentration dependent relative.

The negative control (DMF) was not shown inhibition against all the tested fungal and bacterial strains. Ketoconazole used as a positive control at conc. 2 mg/ml showed 24.00±0.00 to 26.33±1.15 mm in antifungal activity, whereas the streptomycin sulphate standard antibacterial drug showed 26.00±0.00 to 29.33±0.57 mm inhibition zone.

The MIC value 0.15 mg⁻¹ recorded against *E.coli* followed by 0.15 mg⁻¹ was recorded against *Ca* 0.62 mg⁻¹, *Sa* 0.15 mg⁻¹, *Bs* 0.31 mg⁻¹ showed, While against *Tr* 1.25 mg⁻¹, *Tt* and *Tm* 2.5 mg⁻¹, and against *Mg* 0.62 mg⁻¹ concentrations detected. Similarly, the MFC value against *Tt*, *Ca* was 0.6 mg⁻¹. While 0.3 mg⁻¹ MBC value recorded against *Sa*, *Ec*. (Figure 1, 2).

The analogous antimicrobial results were obtained by T.C Fleischer *et al.*,^[18] using different bacterial strains. Veronique Seidel *et al.*,^[19] reported preliminary pharmacological screening of *B. orellana*. young leaves. The little bit of similar antimicrobial results reported from previous records,^[20-24] but yet there is no previous reports on in-vitro antidermatophytic activity, this is the first and novel report using a successive high polar methanolic young leaves extract used for this outcome.

Table 1: Antidermatophytic activity of young leaves methanolic extract of *Bixa orellana*.

Concentrations mg/ml	Test organisms & inhibition of zones in mm								
	Fungal strains					Bacterial strains			
	<i>T. rubrum</i>	<i>T.tonsurans</i>	<i>T.mentagrophytes</i>	<i>M .gypseum</i>	<i>C .albicans</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>E. coli</i>
40	12.66±1.15	09.33±0.57	09.00±0.00	10.33±1.15	20.00±0.00	18.66±0.57	17.33±0.57	16.00±0.00	20.00±0.00
20	11.00±0.00	07.66±0.57	08.66±1.15	09.00±0.00	18.33±1.15	16.66±1.52	14.00±0.00	15.33±0.57	18.66±0.57
10	08.00±0.00	07.33±1.15	06.66±0.57	07.66±1.15	15.33±0.57	12.00±0.00	13.66±1.15	14.66±1.52	15.33±1.15
5	06.66±1.52	05.33±0.57	05.00±0.00	06.33±0.57	11.66±1.52	10.66±0.57	11.00±0.00	13.00±0.00	14.66±0.57
2.5	06.66±0.57	NA	NA	06.00±0.57	10.33±1.15	07.66±1.15	10.66±1.15	10.33±1.15	12.66±1.15
1.25	NA	NA	NA	05.00±0.00	09.00±0.00	06.33±0.57	08.66±0.57	09.66±1.52	10.00±0.00
0.62	NA	NA	NA	NA	05.33±0.57	05.00±0.00	06.66±1.15	07.33±0.57	08.00±0.00
K 02	24.00±0.57	26.33±1.15	26.00±1.00	24.66±1.52	24.00±0.00	-	-	-	-
S 02	-	-	-	-	-	29.33±0.57	28.33±1.15	26.00±0.00	26.33±1.15

A- Petroleum ether extract, B- 98% methanolic extract, *Tr* - *Trichophyton rubrum*, *Tt* - *Trichophyton tonsurans*, *Tm*- *Trichophyton mentagrophytes*, *Mg*- *Microsporium gypseum*, *Ca* - *Candida albicans*, *Sa*- *Staphylococcus aureus*, *P a*- *Pseudomonas aeruginosa*, *Bs*- *Bacillus subtilis*, *Ec*- *Escherichia coli*, *NA*-Not Active, *K*-Ketoconazole, *S*-*Streptomycin Sulphate*.

Table 2: Preliminary tests for the occurrence of secondary metabolites of methanolic leaf extract of *Bixa orellana*.

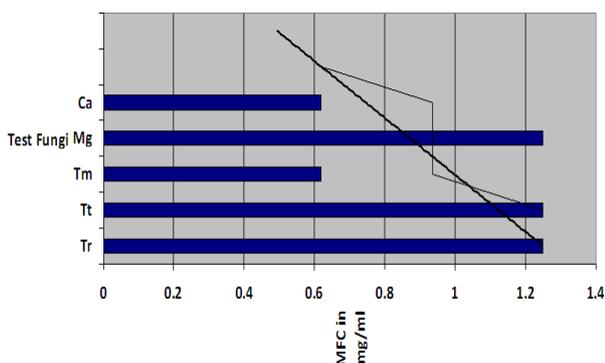
Secondary metabolites	Name of the test	Methanolic extract
Alkaloids	Dragendoff's test	--
	Wagner's test	--
Phenol	Hot water test	++
	Ellagic acid test	++
Flavonoids	Shinoda test	+
	NaoH test	-
Tannins	Salkowski's test	-
Steroids	Salkowski's test	-
	Liebermann-Burchard test	-
Saponins	Foam test	++

+ Present, - Absent.

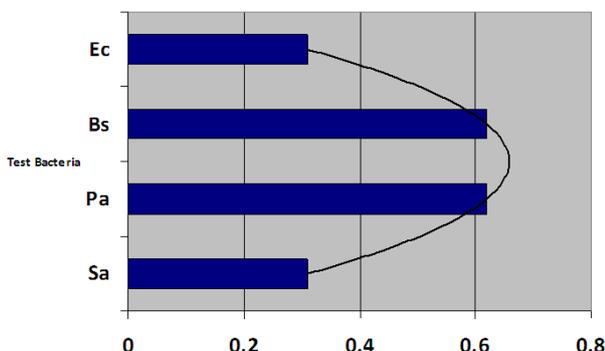
Table 3: Minimum Inhibitory Concentrations against test strains of young leaves methanolic extract of *Bixa orellana*.

Botanical name of the medicinal plant crude extracts	Test organisms & Minimum Inhibitory Concentrations in mg/ml								
	Fungal strains					Bacterial strains			
	<i>Tr</i>	<i>Tt</i>	<i>Tm</i>	<i>Mg</i>	<i>Ca</i>	<i>Sa</i>	<i>Pa</i>	<i>Bs</i>	<i>Ec</i>
<i>B. orellana</i>	1.25≤	2.5≤	2.5≤	0.62≤	0.62≤	0.15≤	0.31≤	0.62≤	0.15≤

Tr - *Trichophyton rubrum*, *Tt* - *Trichophyton tonsurans*, *Tm*- *Trichophyton mentagrophytes*, *Mg*- *Microsporium gypseum*, *Ca* - *Candida albicans*, *Sa*- *Staphylococcus aureus*, *Pa* - *Pseudomonas aeruginosa*, *Bs*- *Bacillus subtilis*, *Ec*- *Escherichia coli*

**Figure 1: Minimum Fungicidal Concentration (mg/ml) of young leaves methanolic extract of *Bixa orellana*.**

Sf- *Sterculia foetida* *Tr* - *Trichophyton rubrum*, *Mg*- *Microsporium gypseum*, *Ca* - *Candida albicans*, *Tt* - *Trichophyton tonsurans*, *Tm* - *Trichophyton mentagrophytes*.

**Figure 2: Minimum Bactericidal Concentration (mg/ml) of young leaves methanolic extract of *Bixa orellana*.**

Sf- *Sterculia foetida*. *Sa*- *Staphylococcus aureus*, *Bs*- *Bacillus subtilis*, *Ec*- *Escherichia coli*, *Pa*- *Pseudomonas aeruginosa*.

CONCLUSION

This present report established that in the field of phyto-pharmacognocny. The present plant *Bixa orellana* methanolic leaf extract can be most effective against human pathogenic microorganisms. The phytochemical compound may have even more potency with respect to inhibition of dermatophytes of crude. This work to be spreads a fundamental approach to discover novel drug to the antidermatophytes using young leaves extract of

Bixa orellana. The expectations studies on the isolation of pure compound(s) from this plant young leaves will be performed. This could be of considerable interest to the development of novel active drugs molecule.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

The present work carried out in the department of Botany, Gulbarga University, Kalabuge, Karnataka. In the year of 2014, Prof. GM Vidyasagar supervised to the present work and first author carried out the results.

SIGNIFICANCE STATEMENT

The present report concentrated to evaluate the antidermatophytic potentiality of *Bixa orellana* using high polar methanolic successive solvent extract of young leaves, with the trust that such a effort was produced. This present report established that in the field of phyto-pharmacognocny. The present plant *Bixa orellana* methanolic leaf extract can be most effective against human pathogenic microorganisms. The phytochemical compound may have even more potency with respect to inhibition of dermatophytes of crude. This work to be spreads a fundamental approach to discover novel drug to the antidermatophytes using young leaves extract of *Bixa orellana*. The expectations studies on the isolation of pure compound(s) from this plant young leaves will be performed.

REFERENCES

1. Thomson, W.A.R. Medicines from the Earth. Maidenhead, United Kingdom. McGraw-Hill Book, 1978; 158: 353-357. Co.
2. Patnaik, B.R. Annatto can fetch foreign exchange. Indian Farming, 1971; 20(1): 28-30.
3. Morton. J. Atlas of Medicinal Plants of Middle America. Springfield, Illinois, 1982; 572-573.
4. Shilpi, J.A. Taufiq-Ur-Rahman, M.D. Uddin, S.J. Alam, M.S. Sadhu, S.K. and Seidel, V. Preliminary pharmacological screening of *Bixa orellana* L young leaves. Journal of Ethnopharmacology, 2006; 108(2): 264-271.
5. Radhika, B. Nasreen B. Srisailam, K. and V. M. Reddy, Diuretic activity of *Bixa orellana* Linn Leaf extract. Indian journal of Natural product and

- Research, 2010; 1(3): 353-355.
6. Morrison, E.Y. Thompson, H. Pascoe, K. West, M. and Fletcher, C, Extraction of an hyperglycaemic principle from the Annatto (*Bixa orellana*), a medicinal plant in the West Indies. *Tropical and Geographical Medicine*, 1991; 43(1-2): 184-188.
 7. Russel, K.R. Omoruyi, F.O. Pascoe, K.O. and Morrison, E.Y. Hypoglycaemic activity of *Bixa orellana* extract in the dog. *Methods and Findings in Experimental and Clinical Pharmacology*, 2008; 30(4): 301-305.
 8. Metta, Ongsakul. Arunsri, Jindarat. and Chanapong, Rojanaworarit., Antibacterial effect of crude alcoholic and aqueousextracts of six medicinal plants against *Staphylococcus aureus* and *Escherichiacoli*. *J. Health Res*, 2009; 23(3): 153-156,
 9. Fabricant, D.S. and Farnsworth, N.R., The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspective Supplements*, 109: 69-75.
 10. Yusuf, M. Chowdhury, J.U. Yahab, M.A. and Begum. J. *Medicinal Plants of Bangladesh*. BCSIR Laboratories Bangladesh, 1994; 38.
 11. Joshi, S.G. *Medicinal Plants*. Oxford and IBH Publishing, Co Pvt Ltd. India, 99.
 12. Ghani, A. *Medicinal Plants of Bangladesh*. 2ed. Asiatic Society of Bangladesh, 2003; 127.
 13. Nunez, V. Otero, R. Barona, J. Saldarriaga, M. Osorio, R.G. Fonnegra, R. Jimenez. S.L. Diaz, A. Neutralization of the edema forming, defibrinating and coagulant effects of *Bothrops asper* venom by extracts of plants used by healers in Colombia. *Brazilian Journal of Medical and Biological Research*, 2004; 37(7): 969-977.
 14. Villar, R. Calleja, J.M. Morales, C. and Caceres, A. Screening of 17 Guatemalan medicinal plants for platelet antiaggregant activity. *Phytotherapy Research*, 1997; 11(6): 441-445.
 15. Barrio, A.G. Grueiro, M.M.M. Montero. D. Nogal. J.J. Escario, J.A. Muelas, S. *In vitro* antiparasitic activity of plant extracts from Panama. *Pharmaceutical Biology*, 2004; 42(4-5): 332-337.
 16. Shivakumar Singh, P. and Vidyasagar, G.M. In-vitro antidermatophytic activity of low polar petroleum ether and inter polar methanolic seed extracts of *Sterculia foetida*. *Int J Pharm Bio Sci*, 2006; 5(2): (B) 872-879.
 17. Horborne, J.B. *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis* 3rd Eds. Chapman and Hall. London, 1998.
 18. Fleischer, T.C. antidermatophytic activity of the young leaves and seeds of *Bixa orellana*, *Fitoterapia*, 2003; 74(1-2): 136-138.
 19. Véronique, Seidel. Preliminary pharmacological screening of *Bixa orellana* L. young leaves, *Journal of Ethnopharmacology*, 2006; 108 (2): 264-271.
 20. Castello, M. Phatak, A. Chandra, N. Sharon, M. Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa orellana* L.. *Ind J Exp Bio*, 2002; 40: 1378-81.
 21. Stohs, S.J. Safety and efficacy of *Bixa orellana* (achiote, annatto) leaf extracts. *Phytother Res*, 2014; 28(7): 956-60.
 22. Galindo-Cuspinera, V. Lubran, M.B. Rankin, S.A. Comparison of the volatile compounds in water- and oil-soluble annatto (*Bixa orellana* L.) extracts. *J Agric Food Chem*, 2002; 50: 2010-2015.
 23. Fleischer, T.C. Ameade, E.P.K. Mensah, M. LK. Sawyer, I.K. Antimicrobial activity of the leaves and seeds of *Bixa orellana*. *Fitoterapia*, 2003; 74: 136-8.
 24. Shahid-ul-Islam, Luqman, J. Rather, Faqeer Mohammad, *Phytochemistry biological activities and potential of annatto in natural colorant production for industrial applications – A review*. *Journal of Advanced Research*, 2016; 7: 499-514.