IJPSR (2022), Volume 13, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 June 2021; received in revised form, 12 July 2021; accepted, 24 July 2021; published 01 March 2022

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF BARK OF *HARDWICKIA BINATA* ROXB (FABACEAE / CAESALPINIACEAE)

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Keywords:

Antimicrobial activity, Antifungal activity, Bark, *Hardwickia binata* Roxb, Microorganisms.

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ABSTRACT: Background and Objectives: This study was carried out with the objective to investigate the antibacterial and antifungal activity of the bark of *Hardwickia binata* Roxb. **Materials and Methods:** Methanolic extract of bark of *Hardwickia binata* Roxb. exhibited an inhibitory effect towards the pathogenic organisms. Antimicrobial activity the zone of inhibition values of methanolic extract of bark of *Hardwickia binata* Roxb. in against two Gram-positive microorganisms *viz. Staphytococcus aureus* and *Bacillus subtilis* and two Gram negative microorganisms *viz. Escherichia coli* and *Pseudomonas aeruginosa* showed significant activity. Antifungal activity of methanolic extract of bark of *Hardwickia binata* Roxb. in against two microorganism, Candida albicans, and *Aspergillus niger*, showed significant activity. **Results:** The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The microbial activity of the *Hardwickia binata* was due to the presence of various secondary metabolites. **Conclusion:** Hence, these plants can be used to discover bioactive natural products that may serve AS leads in the development of new pharmaceuticals research activities.

INTRODUCTION: Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat AS many commonly used antibiotics have become less effective against certain illnesses not only because many of them produce toxic reactions but also due to the emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(3).1189-93

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(3).1189-93

In many developing countries, traditional medicine is one of the primary healthcare systems [1, 4]. Herbs are widely exploited in traditional medicine, and their curative potentials are well documented [5, 6]. About 61% of new drugs developed between 1981 and 2002 were based on natural products, and they have been very successful, especially in the areas

of infectious disease and cancer [7, 8]. Recent trends, however, show that the discovery rate of active novel chemical entities is declining [9]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action [10, 11]. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [12]. Much work has been done on ethnomedicinal plants in India [13]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, *etc.*, which have been found *in-vitro* to have antimicrobial properties [14, 15].

The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [16]. The harmful microorganisms can be controlled with drugs, which results in the emergence of multiple drug-resistant bacteria. It has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs, which are utilized as therapeutic agents [17]. Therefore, there is need to search for new infection-fighting strategies to control microbial infections. *Hardwickia binata* is a deciduous, moderate to large-sized tree. The bark of saplings is almost silvery-white and smooth. Leaves are alternate, bifoliolate resembling Bauhinias. Leaflets are almost kidney-shaped and grayish-green in color, sessile, entire, obliquely ovate, and coriaceous testa [18, 19].

Previous researchers worked out tannins from the bark to produce medicines for the treatment of diarrhea, worms, indigestion, and leprosy and produce an appetizer [20]. The leaves, pods, and bark contain tannins [21]. Bark used for gums and resins [22]. The leaves are used for fodder and manure [23]. The leaves extract showed activity against both gram-positive and gram-negative bacteria and fungi. Bioactive substances showed antimicrobial agents for treating various bacterial and fungal infections, including gonorrhea, pneumonia, eye infections, mycotic infections, and tannins traditionally used to protect catarrh, wounds, and hemorrhoids, diarrhea, and antidote in heavy metal poisoning. Flavonoids possess anti-inflammatory, anti-allergic, antithrombotic, antimicrobial and vaso-protective effects. The leaves are used for headache and treatment of constipation [24]. Therefore, the present study aims to evaluate the antimicrobial activity and antifungal activity of Hardwickia binata Roxb and the literature survey reveals that no reports were found on the antimicrobial and antifungal activity of the bark extracts of *Hardwickia binata* Roxb.

MATERIAL AND METHOD:

Plant Material: The plant *Hardwickia binata* Roxb. is widely found throughout India. For my work the plant was collected from in the deep forest of Satpuda hills with the help of forest officers of Chopda Tahsil, Dist. Jalgaon, Maharashtra (India) and authenticated by Prof. (Dr.) Priyanka A. Ingle, scientist, BSI (Botanical Survey of India), Pune (M.S.). The stems of the plant were dried under shade and then coarsely powdered with the help of a mechanical grinder. The powder was passed through sieve no. 40 and stored in an airtight container for further studies. Extraction was carried out by a continuous soxhlet extraction process for 72 h.

Tests Microorganisms for Antimicrobial Activity:

Gram-Positive: Clostridium tetani**,** Bacillus subtilis.

Gram-Negative: Salmonella typhi**,** Pseudomonas aeruginosa.

Test Microorganism for Antifungal Activity: Candida albicans**,** Aspergillus niger.

Anti Microbial and Antifungal Activity:

Preparation and Standardization of Stock Culture: From the cultures maintained on nutrient agar slants, one loop full of the respective organisms was taken and aseptically transferred to 100 ml of sterile nutrient broth in a flask that was shaken thoroughly and incubated at 370 °C for 24 h. One ml of this seeded broth was then diluted with 9 ml of sterile water in a culture tube. This was shaken thoroughly, and about 1ml of this suspension was transferred to a second culture tube, which in addition to 9 ml of sterile water. This was

shaken thoroughly and further diluted 10 times with sterile water till 1010 dilution was obtained (up to 10 culture tube). Incubating 0.2 ml of each dilution on solidified nutrient agar medium by spread plate method did standardization of the seeded broth. After incubation at 370 °C for 48 h, the number of wells formed colonies on the plate was counted. The seeded broth was then suitably diluted to contain between 107 to 108 microorganisms c.f.u./ml (colony forming unit per ml). This was designated as the working stock that was used for antimicrobial studies.

Preparation of Test Solution: The test solution of methanol extract of bark of *Hardwickia binata* Roxb. was prepared by dissolving 1 gm of dried methanol extract of bark of *Hardwickia binata* Roxb. in 1 ml of solvent and the filter paper soaked in respective solvent was used as control.

Procedure: Both antibacterial and antifungal activity of methanol extract was screened by filter paper disc method. A previously liquefied Muller Hinton Agar media was inoculated with the requisite quantity of the microorganism's suspension. The suspension was added to the medium at a temperature between 400-500C and the inoculated medium was poured immediately into dried petri dishes to occupy a depth of 3 to 4 mm. The paper disc (No.2 Whatman) was cut down into a small disc (6mm in diameter) and sterilized at 1800C/30' m in a hot air oven and then impregnated with the test solution, standard solution, and paper disc soaked with solvent as control. The dried discs were placed on the surface of the medium.

The dishes were left standing for 1-4 h at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions. Subsequently incubated for about 18 h at about 370 °C, and the diameter of the circular inhibition zones was measured [25].

RESULTS AND DISCUSSION: Methanol extract of the bark of Hardwickia binata Roxb exhibited an inhibitory effect on pathogenic organisms. The antimicrobial activity of *Hardwickia binata* extract was evaluated against Gram-negative *Escherichia coli* & *Pseudomonas aeruginosa* and Gram-positive bacteria namely, *Staphylococcus aureus* & *Bacillus subtilis* by disc method. The zone of inhibition of synthesized HB extracts good antimicrobial activity against gram negative bacteria such as *Escherichia coli* & *Pseudomonas aeruginos*a with zone of inhibition 8.3 mm & 7.9 mm and 9.1 mm & 8.8 mm respectively. *Hardwickia binata* Extract is most effective against both the fungi, i.e., Candida albicans and Aspergillus niger. The results are depicted in **Table 1, 2,** and **Fig. 1, 2.** Recently, much attention has been directed towards extract and biologically active compounds 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 may offer a new source of antibacterial agents with significant activity against infective microorganisms. Our results concerning the methanol extract of bark of *Hardwickia binata* Roxb. showed a significant antibacterial and antifungal activity against both Gram-positive and Gram-negative bacteria.

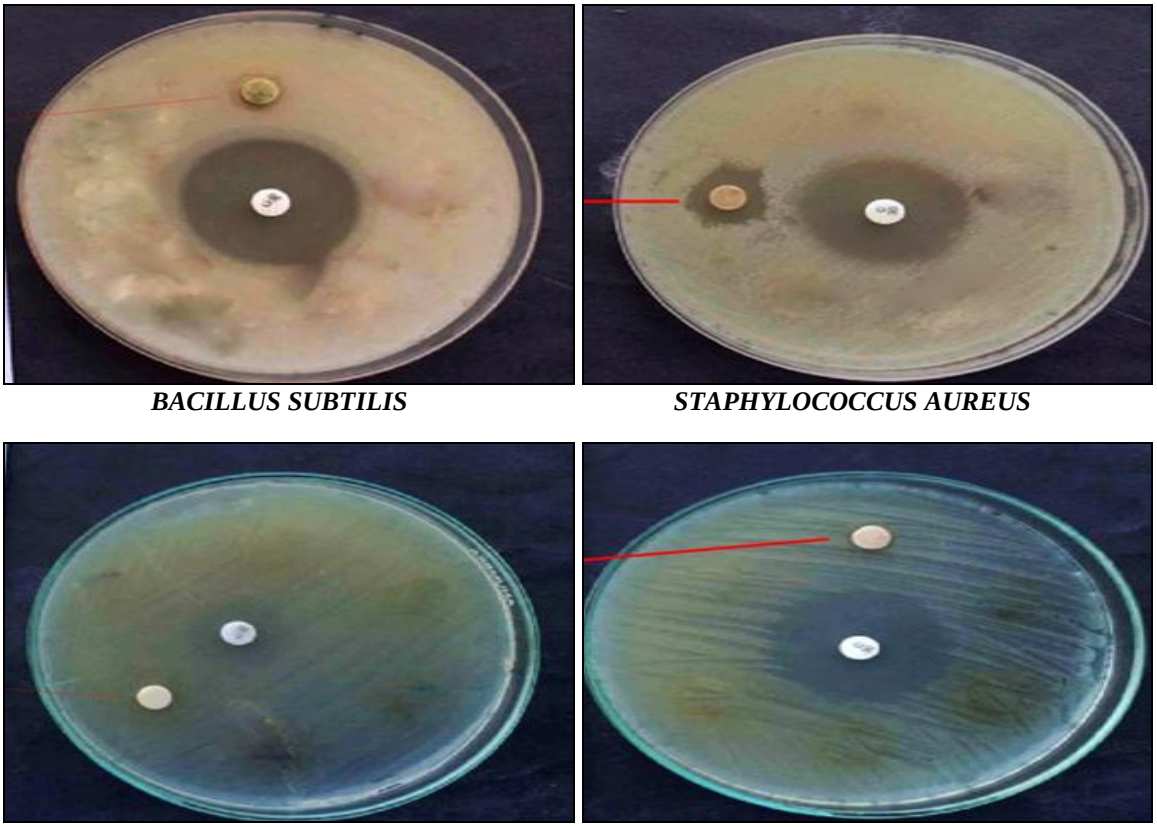
TABLE 1: MICROBIAL CULTURE AND COLLECTION CENTRE

S. no	Name of culture	Culture collection centre
	Bacteria	
1	*Pseudomonas aeruginosa* (NCIM 2036)	NCIM, Pune
2	*Escherichia coli* (NCIM 2109)	NCIM, Pune
3	*Staphylococcus aureus*	NCIM, Pune
4	*Bacillus subtilis*	NCIM, Pune
	Fungi	
1	*Candida albicans*	NCIM, Pune
2	*Aspergillusniger*	NCIM, Pune

TABLE 2: ANTIMICROBIAL ACTIVITY AND ANTI-FUNGAL ACTIVITY

Microorganisms	Diameter of zone of inhibition(mm)	
	Extract	Standard
Gram-Negative bacteria		
Pseudomonas aeruginosa	8.3	12.46
Escherichia coli	7.9	29.12
Gram-Positive bacteria		
Staphylococcus aureus	9.1	15.32
Bacillus subtilis	8.8	18.02
Fungi		
Candida albicans	4.6	11.59
Aspergillus niger	5.8	12.10

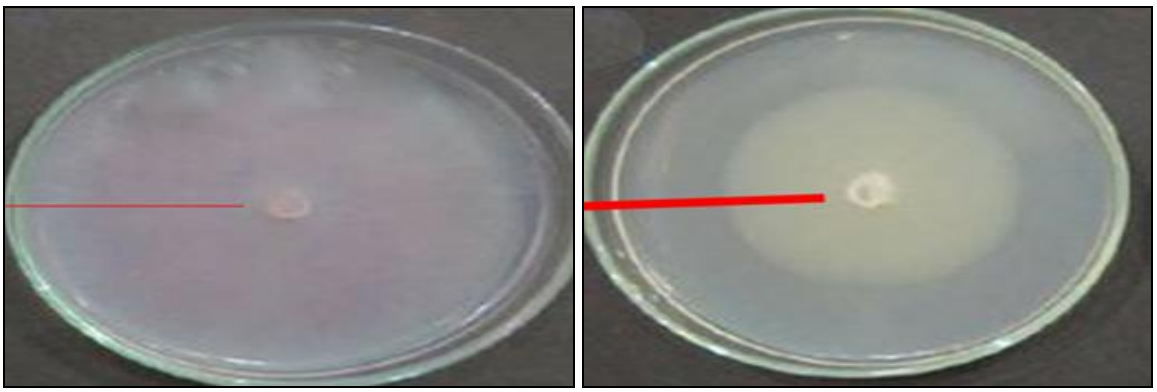
Diameter in mm calculated by Vernier Caliper; '-' means no zone of inhibition; Well diameter - 6 mm; NCIM-National Collection of Industrial Micro-organisms; Standard-Chloramphenicol



BACILLUS SUBTILIS ***STAPHYLOCOCCUS AUREUS***

PSEUDOMONAS AERUGINOSA ***ESCHERICHIA COLI***

FIG. 1: ANTIMICROBIAL ACTIVITY OF METHANOL EXTRACT OF BARK OF *HARDWICKIA BINATA* ROXB



CANDIDA ALBICANS ***ASPERGILLUS NIGER***

FIG. 2: ANTIFUNGAL ACTIVITY OF METHANOL EXTRACT OF BARK OF *HARDWICKIA BINATA* ROXB

CONCLUSION: The present study justified the claimed uses of barks in the traditional system of medicine to treat various infectious diseases caused by microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as antimicrobial agents.

The present results will form the basis for the selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

ACKNOWLEDGMENT: The authors are grateful thanks to the Management and Principal of Smt. Sharad chandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, for providing timely support for the research work.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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How to cite this article:
Rageeb M Usman M and Patil: R Antimicrobial and antifungal activity of bark of hardwickia binata roxb (fabaceae / caesalpiniaceae). Int J Pharm Sci & Res 2022; 13(3): 1189-93. doi: 10.13040/IJPSR.0975-8232.13(3).1189-93.