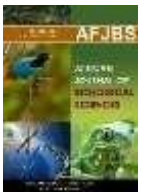
https://doi.org/10.48047/AFJBS.6.4.2024.761-767

African Journal of Biological Sciences



Study of the Antimicrobial potential of some Ethno-medicinal plants from High altitude areas of North West Himalayan Garhwal region, Uttarakhand State, India

Meenakshi Sharma[1*], Abhishek Mathur[2], Sunita Bishnoi[1]

[1*,3]Department Of Chemistry, Fbas, Vivekananda Global University, Jaipur- 302012, Rajasthan, India;
Meenakshi0228@Gmail.Com[1], Bishnoi.Sunita@Vgu.Ac.In[3]
[2]dept. Of Research And Development, Prathista Industries Limited, Telangana State, India
Drabhishekmathur123@Gmail.Com;
*Corresponding Authors: Meenakshi Sharma
*Meenakshi0228@Gmail.Com

Article History
Volume 6, Issue 3, May 2024
Received: 12 Apr 2024
Accepted:19 May 2024
Doi:10.48047/AFJBS.6.3.2024.761-767

Abstract
Objectives: Plants are meant to have treasures of miraculous molecules of pharmacological importance. The present study was confined to understand the antimicrobial potential of medicinal plants of high altitude region of North West Himalayan Garhwal region.
Methods: The antimicrobial activity was determined via well diffusion method of plant extracts of plants viz. Andrographis paniculata, Artemis annua, Xanthium strumarium, Rheum australes and Valeriana jatamansi prepared in different solvents as per increasing polarity viz hexane, methanol and water. The Minimum inhibitory concentration was also determined of the potent antimicrobial extracts against E. coli, S. aureus, B. subtilis, A. niger, S. cerevisiae and C. albicans.
Findings: The results of the study suggests the significant antimicrobial potential of plant extracts of the plants a doses of 85.0 to 180.0 micro gram/ml. The study provides a way to identify the active principles/ molecules such as alkaloids, flavonoid, phenol in the plant extracts and to isolate and characterize the active molecules responsible for antimicrobial spectrum. The present study is one of its kind to screen the medicinal plants of high altitude region and to determine their microbial potential against variety of pathogens.
Novelty: The results of the study is novel and unique of its kind and is not published anywhere.
Keywords: Antimicrobial activity, Medicinal plants, polar and non polar solvent extracts, High altitude, North West Himalayan Garhwal Region, minimum inhibitory concentration

1. Introduction

Plants are known to have medicinal properties since ancient times in India and thus, the country is meant to have repositories of significant diversity of medicinal plants. As per mythology, four Vedas include the oldest known references in Indian literature to the medicinal qualities of plants. [1] Sacred texts of Aryan knowledge from 600-800 B.C., the Atharvaveda (about 1200 B.C.) and the Rig-Veda (written between 3500 and 1600 B.C.) are the two Vedas with the greatest references to healing. Some plants and their effects on humans were detailed in the Rig-Veda, one of the oldest collections of human knowledge. [2-5] The Atharvaveda describes in quite considerable detail the

usage of 129 plant medicines, while the majority of the medicines mentioned in Charaka, Sushrut, and Vagbhata are derived from plants.[6-8] Numerous medical conditions are treated for in humans by employing thousands of medicinal plants, really, the drug's medicinal value is dependent upon the compositional ingredients being used in the formulation. The traditional local healers also have a vast knowledge and experience of using a variety of medicinal herbs for treating the illness and infections. With the information of local healers, the present study was focused on the collection of some medicinal plants and their parts from high altitude areas of North west Himalayan Garhwal region for antimicrobial properties against different pathogens causing severe diseases and infections in humans. [9-10]

2. Materials and Methods

2.1 Collection and identification of plant material

The selected plants were collected from High altitude region of Garhwal ranges of Uttarakhand, India. The plants will be further identified from Botanists/Taxonomists.

2.2 Preparation of different solvent extracts

Plant parts were separated, washed with distilled water, dried under shade and pulverized. The method[11] was adopted for preparation of plant extracts with little modifications. Briefly 20 g portions of the powdered plant material were soaked separately in different solvents on the basis of increasing polarity (petroleum ether, hexane, chloroform, mehanol, hydro-alcoholic and water) for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatman filter paper No. 1 (Whatman, England) The filtrates obtained were concentrated in vacuo using water bath at 30 ^{0}C.

2.3 Determination of antimicrobial activity of the solvent extracts

2.3.1 Culture Media

For antibacterial test, Soyabean Casein Digest agar/broth and Sabouraud's dextrose agar/broth of Hi Media Pvt. Bombay, India were used for antifungal test.

2.3.2 Inoculum

The bacteria was inoculated into Soyabean Casein Digest broth and incubated at 37 ^{0}C for 18 h and suspension was checked to provide approximately, 10^5 CFU/ml. The same procedure was done for fungal strains and the strains were inoculated into Sabouraud's dextrose broth but the fungal broth cultures were incubated at 48-72 h.

2.3.3 Determination of diameter of zone of inhibition by well diffusion method

The agar well diffusion method was modified.[12] Soyabean Casein Digest agar medium (SCDM) was used for bacterial cultures. The culture medium was inoculated with the bacteria separately suspended in nutrient broth. Sabouraud's dextrose agar/broth was used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks. Standard antibiotic (Azithromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were incubated at 37 ^{0}C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. For assaying, antifungal activity of plant extracts, Sabouraud's dextrose agar/ broth medium plates was used. The same procedure as that for determination of antibacterial property was adopted and then after the

diameter of zone of inhibition was observed after 48-72 h. Flucanazole (1mg/ml) was used as standard for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

2.3.4 Determination of Minimum Inhibitory Concentration (MIC)

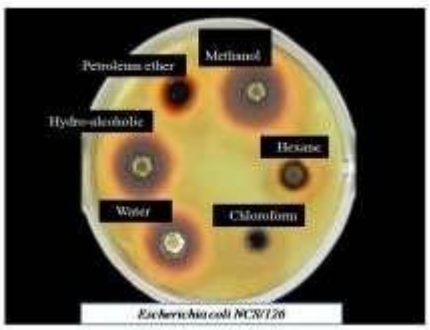
MIC value of metabolites was determined by the method adopted with some modifications. [13] Metabolites extracted prepared in highest concentration (200 μg/ml) in sterile distilled water and was serially diluted with N-saline (0.85 % NaCl) and similar quantity of bacterial suspension was added to different test tubes and incubated for 48 h. The inhibition of turbidity appeared in the minimum dose at which total growth of bacteria gets killed was considered as minimum lethal concentration (MLC) while little turbidity appeared in the minimum amount of dose of plant extract which inhibits the growth of bacteria was known as Minimum Inhibitory Concentration (MIC).

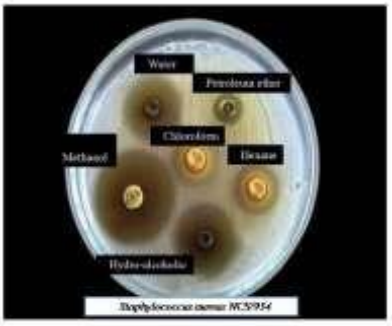
3. Results and Discussion

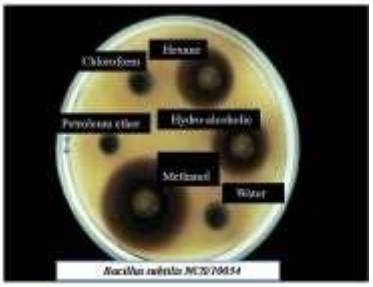
The present study illustrates the antibacterial and anti fungal activity of the solvent extracts against the pathogens. Different solvent extracts of these plants were studied for antimicrobial activity. Although the results of methanolic, aqueous and petroleum ether of Andrographis paniculata, Artemis annua, Xanthium strumarium, Rheum australes and Valeriana jatamansi were only reported as these possessed significant antimicrobial potential at 200 micro gram per ml against E. coli, S. aureus, B. subtilis, A. niger, S. cerevisiae and C. albicans. It was also found that some extracts did not possessed any antimicrobial characteristics. The study demonstrated that, the methanolic extracts possessed effective antimicrobial potential in comparison to other extracts. The antimicrobial spectrum follows the order viz. Methanolic extracts>queous extracts>Petroleum ether extracts. The results of antimicrobial activity are shown in Table 1, 2, 3 and Figure 1. The potent extracts showed the MIC values from 85.0 to 180.0 micro gram/ml. Further the potent extracts were screened for MIC values, the results of the same are reported in Table 4. The results of the study are in correlation with the previous findings. [14-18]

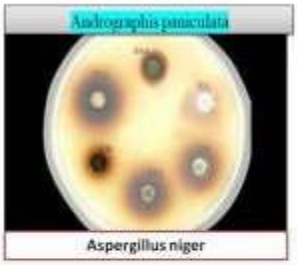
4. Conclusion

The results of the study suggest the importance of plant extracts for significant antimicrobial activity. Further studies will lead to the isolation of active principle (s) from plant extracts to formulate antimicrobial formulation against the studied pathogens.



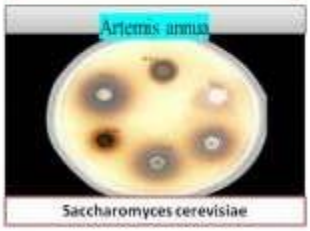

Water
Petroleum ether
Chloroform
Methanol
Benzene
Hydro-alcoholic


Chloroform
Benzene
Petroleum ether
Hydro-alcoholic
Methanol
Water


Andrographis paniculata
Aspergillus niger


Andrographis paniculata
Saccharomyces cerevisiao


Artemis annua
Aspergillus niger


Artemis annua
Saccharomyces cerevisiae


Andrographis paniculata
Methanol
Aqueous
Petroleum ether
Candida albicans


Xanthium strumarium
Aqueous
Methanol
Candida albicans


Artemis annua
Aqueous
Methanol
Candida albicans


Rheum australes
Methanol
Petroleum ether
Aqueous
Candida albicans

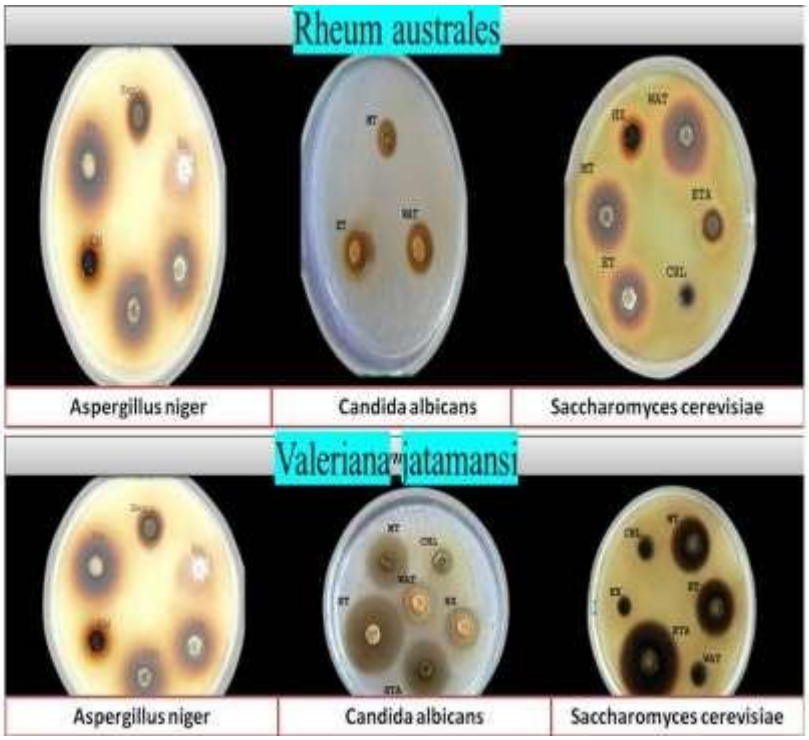


Figure 1: Screening of antimicrobial activity of solvent extracts of plants

Table 1: Antimicrobial activity of methanolic solvent extract of plants

Plants/Methanolic Solvent extracts	Diameter of zone of inhibition (mm) Microbial strains					
	E. coli	S. aureus	B. subtilis	A. niger	S.cerevisiae	C. albicans
Andrographis paniculata	20.0	28.0	30.0	15.0	18.0	22.0
Artemis annua	25.0	27.0	30.0	22.0	18.0	18.0
Xanthium strumarium	20.0	28.0	35.0	28.0	18.0	20.0
Rheum australes	20.0	28.0	30.0	15.0	18.0	22.0
Valeriana jatamansi	20.0	35.0	40.0	22.0	25.0	27.0
Azithromycin (1 mg/ml)	25.0	28.0	35.0	NT	NT	NT
Flucanazole (1 mg/ml)	NT	NT	NT	25.0	22.0	30.0
Methanol solvent	0.0	0.0	0.0	0.0	0.0	0.0

*NT, Not tested

Table 2: Antimicrobial activity of aqueous extract of plants

Plants/Aqueous extracts	Diameter of zone of inhibition (mm) Microbial strains					
	E. coli	S.aureus	B.subtilis	A.niger	S.cerevisiae	C. albicans
Andrographis paniculata	15.0	20.0	20.0	10.0	15.0	15.0
Artemis annua	18.0	15.0	18.0	15.0	12.0	12.0
Xanthium strumarium	15.0	22.0	14.0	20.0	14.0	12.0
Rheum australes	18.0	18.0	20.0	12.0	12.0	15.0
Valeriana jatamansi	18.0	22.0	25.0	15.0	15.0	20.0
Azithromycin (1 mg/ml)	25.0	28.0	35.0	NT	NT	NT
Flucanazole (1 mg/ml)	NT	NT	NT	25.0	22.0	25.0
Distilled water	0.0	0.0	0.0	0.0	0.0	0.0

*NT, Not tested

Table 3: Antimicrobial activity of petroleum ether extract of plants

Plants/Petroleum ether extracts	Diameter of zone of inhibition (mm) Microbial strains					
	E. coli	S.aureus	B.subtilis	A.niger	S.cerevisiae	C. albicans
Andrographis paniculata	8.0	16.0	15.0	0.0	12.0	10.0
Artemis annua	10.0	10.0	12.0	12.0	15.0	12.0
Xanthium strumarium	12.0	15.0	15.0	15.0	12.0	10.0
Rheum australes	0.0	13.0	13.0	0.0	0.0	15.0
Valeriana jatamansi	15.0	12.0	18.0	15.0	10.0	15.0
Azithromycin (1 mg/ml)	25.0	28.0	35.0	NT	NT	NT
Flucanazole (1 mg/ml)	NT	NT	NT	25.0	22.0	25.0
Petroleum ether solvent	0.0	0.0	0.0	0.0	0.0	0.0

*NT, Not tested

Table 4: Minimum Inhibitory Concentration of Plant extracts

Plants/Methanolic extracts	Minimum Inhibitory Concentration (micro gram/ ml)					
	E. coli	S.aureus	B.subtilis	A.niger	S.cerevisiae	C. albicans
Andrographis paniculata	150.0	110.0	90.0	180.0	180.0	130.0
Artemis annua	120.0	120.0	90.0	150.0	180.0	180.0
Xanthium strumarium	150.0	110.0	85.0	130.0	180.0	150.0
Rheum australes	150.0	110.0	90.0	180.0	180.0	150.0
Valeriana jatamansi	150.0	100.0	80.0	120.0	100.0	120.0
Azithromycin (1 mg/ml)	120.0	110.0	90.0	NT	NT	NT
Flucanazole (1 mg/ml)	NT	NT	NT	100.0	22.0	25.0

*NT, Not tested

References

1. Uniyal B, Shiva V, Traditional knowledge on medicinal plants among rural women of the Garhwal Himalaya, Uttaranchal, Ind. J. Trad. Knowl. 2005; 4: 259-266.
2. Khan H, Saeed M, Khan MA, Khan I, Ahmad M, Antimalarial and free radical scavenging activities of rhizomes of Polygonatum verticillatum supported by isolated metabolites, Medicinal Chemistry Research. 2012a; 21: 1278- 1282.
3. Khan H, Saeed M, Muhammad N, Pharmacological and phytochemical updates of genus Polygonatum, Phytopharmacology. 2012b; 3: 286-308.
4. Mushtaq S, Abbasi B H, Uzair B, Abbasi R, Natural products as reservoirs of novel therapeutic agents, Excli J. 2018; 17: 420-451.
5. Kochhar SL, Tropical crops: A textbook of economy botany. Macmillan Pub Ltd. London. 1981: 268-71.
6. Saklani S, Kawra M and Parcha, Herbal-derived anticancer phytoconstituents: tradition to molecular mechanisms, International Journal of Pharmaceutical Science Research. 2019; 10: 1632-1639.
7. Saklani S, Rawat Y, Plygun S, Shariati MA, Nigam M, Maurya VK, Yadav A and Mishra, AP, Biological activity and preliminary phytochemical screening of terminalia alata heyne ex roth., The Journal of Microbiology, Biotechnology and Food Sciences. 2019; 8: 1010.
8. Orech FO, Hansen AJ, and Friis, Ethnoecology of traditional leafy vegetables of the Luo people of Bondo district, Western Kenya, Int. J. Food Sci. Nutr. 2007; 58: 522-530.

9. Kamble V, Gaikwad N, Fourier transform infrared spectroscopy spectroscopic studies in embelia ribes burm. F.: a vulnerable medicinal plant, Asian Journal of Pharmaceutical and Clinical Research. 2016; 9: 41-47.
10. Droge W. Free radicals in the physiological control of cell function, Physiological Reviews. 2002; 82:47–95.
11. Mathur A, Purohit R, Mathur D, Prasad GBKS, Dua VK, Pharmacological investigation of methanol extract of Mentha piperita L. roots on the basis of antimicrobial, antioxidant and anti-inflammatory properties. Der Pharmacia Sinica. 2011; 2: 208-216.
12. Mathur, A, Bhat R, Prasad G.B.K.S, Dua VK, Prasad GBKS, Verma S.K, Agarwal PK, Antimicrobial activity of plants traditionally used as medicines against some pathogens, Rasayan Journal of Chemistry. 2010; 3: 615-620.
13. Bashir ZA. In-Vitro Antimicrobial Activity of Membrane-Acting Antibiotics Action against Streptococci, Journal of Applied Pharmaceutical Sciences. 2012; 2: 042- 047.
14. Negi YS, Rawat MSM, Pant-Joshi G and Badoni S, Biochemical Investigation of Fruits of Some Common Ficus Species, J. Food Science and Technology. 1992; 25: 582-584
15. Mishra A, Saklani S, Bahare S, Parcha V, Mehdi Sharifi-Rad, Satyrium nepalense, a high altitude medicinal orchid of Indian Himalayan region: chemical profile and biological activities of tuber extracts, J. Chil. Chem. Soc. 2016;
16. Mishra AP and Saklani S. Satyrium nepalense: a rare medicinal orchid of western Himalaya (India); phytochemical screening, antimicrobial evaluation and conservation studies, Indonesian Journal of Pharmacy. 2012; 23: 162-170.
17. Saklani S and Chandra S. In vitro antimicrobial activity, nutritional profile and phytochemical screening of wild edible fruit of Garhwal Himalaya (Ficus auriculata). International Journal of Pharmaceutical Sciences Review and Research. 2012; 12: 61-64.
18. Bhatnagar M, Sarkar N, Nigam G, Apang O, Singh S, Ghosal S, Evaluation of antimycobacterial, leishmanicidal and antibacterial activity of three medicinal orchids of Arunachal Pradesh, India, BMC Complimentary and Alternative Medicine. 2017; 17 : 1-10.