MAT JOURNALS

**Journal of Pharma and Drug Regulatory Affairs**
Volume 2, Issue 1

# Evaluation of Antimicrobial Potential of *Anisochilus carnosus* L. (Wall)

**Reshi N A*, Raytekar N A**
*Assistant Professor, Department of Life Science, School of Science, Sandip University, Nashik, Maharashtra, India*

***INFO***
***Corresponding Author:***
***E-mail Id:***
**nissarreshi@gmail.com*

***DOI:*** *10.5281/zenodo.3405732*

## *ABSTRACT*

*An investigation was carried out to assess the antimicrobial potential of Anisochilus carnosus against Proteus vulgaris, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candia albicans, Microsporum canis, Microsporum gypseum and Alternaria alternata. Aqueous and different organic solvent extracts were screened against test microorganisms by Disc diffusion method followed by determination of Minimum inhibitory concentration (MIC) of the active extracts by two fold serial dilution method. Significant antibacterial activity was observed in methanol extract against P. aeruginosa with 36±0.42 mm zone of inhibition and MIC value of 0.32 mg/ml. Ethyl acetate exhibited the maximum antifungal property against A. alternata with the maximum of 32±0.52 mm zone of inhibition and MIC value of 0.45 mg/ml. The study confirms the presence of potent antimicrobial agents in A. carnosus.*

***Keywords:*** *Antimicrobial property, A. alternata, A. carnosus, minimum inhibitory concentration, P. aeruginosa*

## INTRODUCTION

There has been a continuous battle between humans and the diseases ever since the existence of civilization. Pathogenic diseases are considered as most threatening and of grave concern especially from the last three decades [1]. After the discovery of penicillin, people were of the hope that it is the end of the infectious disease era but their joy was short lived due to the development of resistance by the pathogens [2]. With the development of resistance and the intrinsic mutations, most of the available antibiotics have become obsolete. Development of resistance by microorganism against available antibiotics is seen as most dreadful which demands for the immediate alternatives, else the human life may be at risk in coming decades [3].

Natural resources are considered to be the best available alternatives to combat drug resistance menace. Plants are considered as the factories of complex chemicals with potential biological activities. From last few decades, the use of plants as medicine has increased to manifolds owing to their effective and diverse medicinal properties. Plants are believed to be the source of


Page 1-5 © MAT Journals. All Rights Reserved

potent antimicrobial agents and a wide range of plants have been reported to treat several infectious diseases which is attributed to the presence of biologically potent secondary metabolites in them. Today, bioactive molecules are screened, characterized and traded in a market as a raw material for pharmaceutical industries [4].

Tribal people rely only on plants for their medicinal needs. Scientific evaluation of traditional medicinal plant will not only authenticate their medicinal use but may also lead to the discovery of novel drugs. It is being estimated that out of 422127 species of plants known worldwide, 35000 to 70000 plants are used for the treatment of various ailments but only small number of medicinal plants have been scientifically evaluated [5]. In India, 50% of the higher flowering plants species are being used as medicine and till date only 20% have been scientifically evaluated [6]. The development of microbial resistance is alarmingly growing and the outlook for the use of antimicrobials in future is still floating. Therefore, a proper approach needs to be adopted to stop the deadly antimicrobial resistance by banning the over the counter availability of antibiotics and at the same time a search for antimicrobials from natural resources needs to be intensified which may lead to the discovery of novel and potential drugs. In the present investigation we have carried out the assessment of antibacterial potential of Anisochilus carnosus.

*A. carnosus* is a multipurpose annual medicinal herb which grows in the regions of Hong Kong, Macao, Shangai, Tianjin, India, Myanmar and Sri Lanka. The plant is traditionally used for the treatment of various ailments like anticancer [7], anti-inflammatory [8], antifungal [9]. The leaf paste of *Anisochilus carnosus* in combination with the *Ocimum sanctum*, *Mentha piperita* and *Alpinia galangal* is used for the treatment of influenza, dermatitis and illness derived from the bites of bugs [10].

## MATERIALS AND METHODS

Anisochilus carnosus plants were collected from Northern Western Ghats of Maharashtra. Aqueous extraction was carried out by mixing the deionized water with dry coarse leaf powder in the ratio of 1: 5 (w/v). The flask containing the aqueous extract was allowed to settle down and kept at 50°C in an oven with intermittent shaking [11]. The filtrate was lyophilized and stored for further use. Hot extraction of leaf was carried out by Soxhlet apparatus sequentially with petroleum ether, chloroform, ethyl acetate, ethanol and methanol [12]. The solvent extracts were concentrated under reduced pressure and were stored at 5°C in vials for further use. The test bacteria; *Proteus vulgaris, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and fungi Candia albicans, Microsporum canis, Microsporum gypseum and Alternaria alternata* were procured from MTCC Chandigarh, India. The bacteria and fungi were maintained on nutrient agar and potato dextrose agar respectively. Antimicrobial potency assessment was carried out by disc diffusion method as described by Anonymous, (1996) [13]; Hoffman *et al.*, (2004) [14]. Streptomycin and nystatin were used as positive controls for antibacterial and antifungal assessment respectively. Negative controls were maintained by using the respective solvents. Discs were impregnated with 10 mg extracts and loaded on nutrient agar and PDA plates seeded with test bacteria and fungi respectively. The plates were wrapped with cellophane tape and kept in refrigerator at 5°C for 20 minutes to allow the diffusion of extract into the medium. Thereafter, plates were incubated at 37°C for 24 hrs in incubator. Antimicrobial activity was evaluated by measuring the zone of inhibition after 24 hrs. The


Page 1-5 © MAT Journals. All Rights Reserved

experiment was repeated thrice and statistical analysis was carried out by using SPSS, DMRT with P≤0.5. The Minimal Inhibitory concentration was evaluated by 96 well plate two fold serial dilution method as described by Klancnik *et al.*, 2010 [15]; Johnson *et al.*, 2011[16]; Raveesha, 2015 [17]. The microbial growth in each well was determined by reading the respective absorbance at 600 nm by using Universal Micro Plate Reader.

## RESULTS AND DISCUSSION

### Antibacterial Activity

The results of antibacterial activity assay are presented in the Table 1. Non polar solvents like chloroform, petroleum ether and ethyl acetate have shown less antibacterial activity against the test organisms. Methanol extract exhibited the maximum antibacterial potential against *P.aeruginosa* with 36±0.42 mm zone of inhibition and MIC value of 0.32 mg/ml. Ethyl acetate and ethanol extract also showed significant antibacterial efficacy with maximum of 28±45 and 26±34 mm zone of inhibition against *Proteus vulgaris* and *Klebsiella pneumoniae* respectively.

### Antifungal Activity

The results of antifungal assay are presented in Table 2. Ethyl acetate showed the maximum antifungal activity against *A. alternata* with the maximum of 32±0.52 mm zone of inhibition and MIC value of 0.45 mg/ml. Non polar solvents like Chloroform and Petroleum ether did not show any significant antifungal activity; however methanol and ethanol extracts inhibited the growth of *Candida albicans* significantly.

*Moreno et al., 2006* reported that non polar solvents like chloroform and petroleum ether being hydrophobic diffuse slowly in to the medium due to which they exhibit less antibacterial property. The same has been reported in the present study wherein the least activity is exhibited by the non-polar solvents.

***Table 1.*** *Antibacterial Activity (zone of inhibition in mm) of Leaf Extracts of A. carnosus*

| | **Test Bacteria** | | | |
|---|---|---|---|---|
| **Extract** | ***P. vulgaris*** | ***E. coli*** | ***K. pneumoniae*** | ***P. aeruginosa*** |
| Aqueous | 15±0.43 | 08±0.30 | 12±0.44 | 14±0.78 |
| Chloroform | 11±0.28 | 13±0.28 | 12±0.40 | 18±0.44 |
| Petroleum Ether | 14±0.65 | 10±0.65 | 16±0.55 | 16±0.70 |
| Ethyl Acetate | 28±0.45 | 23±0.55 | 20±0.58 | 20±0.60 |
| Methanol | 26±0.76 | 27±0.48 | 28±0.65 | 36±0.42 |
| Ethanol | 25±0.70 | 23±0.60 | 26±0.34 | 25±0.68 |
| Streptomycin | 42±0.80 | 38±0.42 | 38±0.46 | 40±0.80 |

**Each test was repeated thrice. Each value represents Mean±S.D, statistical analysis done by SPSS, DMRT (P≤0.5)*

***Table 2.*** *Antifungal Activity (zone of inhibition in mm) of Leaf Extracts of A. carnosus*

| | **Test Fungi** | | | |
|---|---|---|---|---|
| **Extract** | ***Candia albicans*** | ***Microsporum canis*** | ***Microsporum gypseum*** | ***Alternaria alternata*** |
| Aqueous | 06±0.44 | 08±0.50 | 10±0.50 | 11±0.25 |
| Chloroform | 08±0.20 | 11±0.20 | 13±0.40 | 15±0.66 |
| Petroleum Ether | 13±0.55 | 14±0.65 | 16±0.45 | 21±0.58 |
| Ethyl Acetate | 22±0.50 | 20±0.35 | 25±0.58 | 32±0.52 |
| Methanol | 24±0.66 | 27±0.30 | 24±0.78 | 28±0.52 |
| Ethanol | 20±0.77 | 25±0.60 | 22±0.88 | 24±0.88 |
| Nystatin | 21.5±0.28 | 16.5±0.28 | 22.5±0.28 | 22.5±0.28 |

**Each test was repeated thrice. Each value represents Mean±S.D, statistical analysis done by SPSS, DMRT (P≤0.5)*


Page 1-5 © MAT Journals. All Rights Reserved

Plants are considered to be the rich source of broad spectrum antibiotics owing to the presence of potent bioactive compounds. In the present study we have reported that *A. carnosus* is highly potent antimicrobial source. Methanol and ethanol extracts exhibited the maximum antibacterial activity which is attributed to the presence of complex bioactive molecules. In our study, more extracts exhibited antibacterial activity than antifungal activity which is supported by earlier studies that fungi are more resistant to plant extracts than bacteria [18-20]. It is worth to be noted that aqueous extract has sown less activity compared to the organic solvents. Extraction of secondary metabolites largely depends upon the type of solvent used and it has been reported earlier that aqueous extracts are less effective than other organic solvents [21]. Traditional healers use drugs mostly prepared with water as they do not have access to other lipophilic solvents. This is of serious concern as it is impossible for healers to extract all the active compounds which might be present in the plant [16]. Dosage is important with regard to the type solvent used, if water is used, the dosage has to be higher, whereas the same dosage using a lipophilic solvent may be dangerous [22]. Screening of traditional medicinal plants has revealed that they are rich sources of bioactive compounds. Determination of antimicrobial potential of plants used by tribal people would be helpful for the rural communities. Fractionation, purification and characterisation of bioactive molecules may lead to the development of novel and natural antibiotics.

## CONCLUSION

The study confirms the antimicrobial potential of leaf extracts and thereby confirms the traditional medicinal use of *A. carnosus*. Characterization and identification of biologically active molecules would pave a way for the development of novel antibiotics.

## ACKNOWLEDGEMENT

Authors are grateful to Sandip University, Nashik for providing all the necessary facilities to carry out this research.

## REFERENCES

1. Almagboul AZ, Bashir AK, Farouk A, Salih AKM (1985), "Antimicrobial activity of certain Sudanese plants used in folkloric medicine. Screening for antibacterial activity", *Fitoterapia,* Volume 56, pp. 331−337.
2. Mahesh B, Satish S (2008), "Antimicrobial activity of some important medicinal plant extract against plant and human pathogens", *World J. of Agri.Sci.,* Volume 4, pp. 839−843.
3. Gopalkrishnan S, George S, Benny PJ (2010), "Antimicrobial effect of *Punica grantum* on pyogenic bacteria", *J. of Pharma and Biomed Sci.,* Volume 3, Issue 6, pp. 81−86.
4. Jain SC, Jian R, Vlietinck JA (2004), "In vitro and in vivo antimicrobial efficacy of Mimosa hamate", *Indian J Biotechnology*, Volume 3, pp. 271−273.
5. Buwa LV, Staden JV (2006), "Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. *J.of Ethnopharmacol,* Volume 103, pp. 139−142.
6. Usman AK, Hazir R, Zeeshan N, Muhammad Q, Jafar K, Bushra R (2013), "Antibacterial activity of some medicinal plants against selected human pathogenic bacteria", *Eur J MicrobiolImmunol*., Volume 3, pp. 272−274.
7. Muthuraman MS, Santharam L, Ariraman S, Pemaiah B (2012), "Studies on anticancer and antimicrobial efficacy of Anisochilus carnosus extracts", *International Journal of Pharmacy and Pharmaceutical Science,* Volume 4, Issue 2, pp. 132−135.
8. Kumar AS, Samanta KC, Chipa RC


Page 1-5 © MAT Journals. All Rights Reserved

(2010), "Hepatoprotective activity of alcoholic and aqueous extracts of leaves of Anisochilus carnosus (Linn.) Wall", *International Journal of Pharmacy and Life Sciences,* Volume 1, Issue 2, pp. 99−104.
9. Kulandhaivel M, Sooraj SN, Nathiya K, Palaniswamy M (2011), "Antifungal activity and phytochemical screening of Anisochilus carnosus and Melaleuca alternifolia against opportunistic pathogen Candida albicans", *International Journal of Pharmaceutical and Biological Archives,* Volume 2, Issue 6, pp. 1668−1670.
10. Subramanian SS, Nair AGR (1972), "Flavonoids of the leaves of *Mentha spicata* and *Anisochilus carnosus", Phytochemistry,* Volume 11, Issue 1, pp. 452−453.
11. Harborne JB (1998), "Phytochemical Methods-A guide to modern techniques of plant analysis", *Chapman and Hall,* pp. 182−190. London
12. Suffredini JB, Sader HS, Goncalves AG, Reis AO, Gales AC, Varella AD and Younes RN (2004), "Screening of antimicrobial extracts from plants native to Brazilian Amazon rainforest and Atlantic forest Brazil", *Journal of Medical and Biological Research,* Volume 37, pp. 379−384.
13. Anonymous (1996), "Pharmacopeia of India (The Indian Pharmacopeia), 3rd Edn., Govt. of India, New Delhi, Ministry of Family and Health Welfare.
14. Hoffman BR, Delas AH, Blanco K, Wiederhold N, Lewis RE and Williams L (2004), "Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana", *Pharmaceutical Biology,* Volume 42, Issue 1, pp. 13−17
15. Klancnik A, Piskernik S, Jeršek B, Mozina SS (2010), "Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts", *Journal of Microbiological Methods,* Volume 81, Issue 2, pp. 121−126.
16. Johnson M, Wesely EG, Kavitha MS, Uma V (2011), "Antibacterial activity of leaves and inter- nodal callus extracts of *Mentha arvensis* L", *Asian Pacific journal of tropical Medicine,* Volume 4, Issue 3, pp. 196−200.
17. Raveesha KA (2015), "Evaluation of antifungal potential of selected medicinal plants against human pathogenic fungi", *International Journal of Green Pharmacy,* Volume 9, Issue 2, pp. 110−117.
18. Leven M, Vanden Berghe DA, Mertens F, Vlietinck A, Lammens E (1979), "Screening of higher plants for biological activities-Antimicrobial activity", *Planta Medica,* Volume 36, pp. 311−321.
19. Naqvi SAH, Khan MSY, Vohora SB (1991), "Antibacterial, anti-fungal and anthelmintic investigations on Indian medicinal plants", *Fitoterapia,* Volume 62, pp. 221–228.
20. Heisey RM, Gorham BK (1992), "Antimicrobial elects of plant extracts on *Streptococcus mutans, Candida albicans, Trichophyton rubrum* and other microorganisms", *Letters in Applied Microbiology,* Volume 14, pp. 136–139.
21. Ficker CE, Arnason JT, Vindas PS, Alvarez LP, Akpagana K, Gbeassor M, Smith, ML (2003), "Inhibition of human pathogenic fungi by ethno botanically selected plant extracts", *Mycoses,* Volume 46, Issue 1−2, pp. 29−37.
22. Murugan S, Uma Devi P, Parameswari NK, Mani KR (2011), "Antimicrobial activity of *S. jambos* against selected human pathogens", *Int J Pharm PharmSci,* Volume 3, pp. 44−47.


Page 1-5 © MAT Journals. All Rights Reserved