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Original Article

Evaluation of the antifungal, antibacterial, and anti-inflammatory activity of a halophyte plant *Arthrocnemum macrostachyum* Koch extracts.

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Abstract

Background: Indiscriminate use of conventional antibiotics has become a major factor responsible for the emergence and dissemination of multidrug-resistant strains of microorganisms. In this regard, we analyzed the plant extracts as an alternative solution for the inhibition of the resistant bugs.

Methodology: This study was planned to investigate the antifungal, antibacterial and anti-inflammatory activities of hexane, ethyl acetate, chloroform, and methanol extracts of *Arthrocnemum macrostachyum*. Soxhlet apparatus was used to prepare different extracts of the whole plant using hexane, ethyl acetate, chloroform, and methanol. The antifungal and antibacterial activity was analyzed with the help of a good diffusion technique using different concentrations of plant extracts. The anti-inflammatory activity was also checked by observing the inhibition of albumin denaturation.

Results: Maximum antifungal activity was observed by hexane, methanol, and chloroform extracts, and maximum antibacterial activity showed by the methanol extract. The most sensitive fungi found were *Mucor*, *Aspergillus flavus, Saccharomyces,* and *Candida. Trichophyton mentagrophytes* were found resistant to all the extracts. The most sensitive bacterial isolates found were *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa,* and *Citrobacter freundii*. The most resistant organisms were *Streptococcus fecalis* and *Shigella dysenteriae*. Methanol and chloroform extracts produced observable anti-inflammatory potential by inhibiting albumin denaturation. Statistical analysis was performed on SPSS version 23.0.

Conclusion: The plant extracts showed significant antifungal, antibacterial and anti-inflammatory activity. These findings may support using the *Arthrocnemum macrostachyum* whole plant as an anti-inflammatory, antibacterial, and traditional antifungal plant. Our results demonstrate that this plant has considerable therapeutic effects on potential infectious agents and can be used to alternative conventional medicines.

Keywords

Arthrocnemum macrostachyum, Extracts, Anti-Inflammatory, Antifungal, Antibacterial, Well-Diffusion Assay

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Introduction

In the modern world, antibiotics are used to treat infections, but now antibiotic resistance has become the biggest challenge for researchers and clinicians[1,2]. Thousands of people died due to the improper treatment of bacterial and fungal infections. The emergence of resistant species due to the spread of antibiotic resistance is a major human threat and requires special attention[3,4]. This threat leads researchers to analyze new alternative treatments[5].

Pathogenic fungi are the causative agents of various infections in humans, and these infections are difficult to treat[6]. *Aspergillus flavus*, *Aspergillus niger*, *Candida* species, *Mucor* species, *Penicillium* species, *Saccharomyces cerevisiae*, *Trichophyton mentagrophytes*, and M*icrosporum gypseum* are among the most important human pathogens causing serious infections. *Trichophyton mentagrophytes* and *Microsporum gypseum* are well-known dermatophytes and cause the infection of nails, hairs, and skin in humans[7]. Herbal extracts are widely used for the treatment of fungal infections[8].

In the present era, the globe is facing increasing bacterial infections caused by different species of pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus (MRSA*), and many more[9]. These organisms become more hazardous when they acquire resistance to the limited treatment options[10]. For instance, *carbapenem-resistant Klebsiella pneumoniae* (CRKP) are the strains that produce carbapenemase and thus hydrolyze carbapenem antibiotics[11]. *Pseudomonas aeruginosa* is an opportunistic pathogen and mostly causes infections in immunocompromised individuals. This bacterium has now become resistant to several antibiotics, especially due to the production of β-lactamase enzymes[12]. The mortality rate is also much higher due to the antibiotic-resistant strains as compared to the sensitive strains. Inflammation is the process linked with tissue damage and in which various chemical mediators are produced[13]. These mediators are strongly associated with several diseases, including autoimmune and heart diseases, cancer, arthritis, diabetes, etc[14,15]. Thus, by reducing the inflammation process, these diseases may be treated. Many medicines are available for this, but still, there is a need to design more drugs[16-18]. In underdeveloped and developed countries, the desire for plant-based therapies is growing because of the increasing awareness that they are natural products, non-narcotic, simply biodegradable, have no injurious side effects, and easily accessible at affordable price[19]. The parts of the plant utilized in herbal therapy include seeds, berries, roots, leaves, fruits, bark, flower, and the whole plant is also used[20]. Complementary alternative medicine (CAM) seems useful and good for cost-saving, replacing high technology, providing inexpensive remedies, and boosting up the power of the body's natural ability to heal.

Arthrocnemum macrostachyum or glaucous glasswort is an edible halophyte and grows on beaches and in extreme locations like high salinity, drought, cold, and heat[21]. *Arthrocnemum macrostachyum* is usually found in coastal and saline areas of Sindh and Baluchistan, Pakistan. In Karachi coastal area, this plant is abundantly growing. It is a succulent shrub with a jointed leafless stem. It is distributed widely in saline marshes and sea coasts of the Mediterrane and the Irano-Turanian region. Five species of *Arthrocnemum* are present throughout the world, and in Pakistan, it is represented by two species[22]. It is disseminated on the coast from South West Iberia through the Mediterranean region to the Middle East and Asia. It is also present in marshes of the southwest of Spain, in the middle to high tidal elevations range, where it is intermittently subjected to the tidal influx and seasonal hyper-salinity. It can hyper-tolerate cadmium and due to which it is used in soil remediation technique[23]. It contains about 25% oil, mainly α-linolenic and linoleic acids[24]. Moreover, it bears anticholinesterase activity; hence it can be used to treat Alzheimer's disease[25].

In our study, *Arthrocnemum macrostachyum* plant extracts were used, and they showed antibacterial

activity against both gram-positive and gram-negative bacteria and showed antifungal effects against different fungi. Moreover, the used extracts also possess anti-inflammatory action.

Methodology

Collection of plant

Arthrocnemum macrostachyum (whole plant) was collected from the coastal area of Hawkes Bay, Karachi, and the voucher specimen was deposited in Karachi University Herbarium, Centre for Plant Conservation, University of Karachi. The voucher number was issued as G.H.No. 86615.

Preparation of plant extracts

After collection, the plant was dried, crushed, and ground to convert into powdered form. The Soxhlet apparatus and BUCHI Rota-vapour R-200 were used to make the extracts. The concentrated extract was partitioned in the extract tube and left open to remove any residual solvent. The dried form of all the extracts was kept at 4°C for experimental procedures.

Effect of plant extracts on bacterial & fungal isolates

To prepare plant extracts, different solvents were used, including methanol, ethyl acetate, hexane, and chloroform. DMSO (Dimethyl sulfoxide) was used to make different concentrations of four types of extracts, i.e., 250 µg, 500 µg, 750 µg, 1000 µg, 5000 µg for antibacterial activity and, 250 µg, 750 µg, 1500 µg, 3000 µg, 4000 µg and 5000 µg for antifungal activity. DMSO was used as a negative control. Extract concentrations were selected after performing MIC (Minimum Inhibitory Concentration) assay.

Collection and identification of fungal & bacterial isolates

The fungal and bacterial isolates were collected from the microbial culture bank (University of Karachi) and clinical diagnostic lab. The isolates were identified with the help of morphological, colonial, and biochemical characteristics. Different staining techniques and media were used to observe the morphological and colonial characteristics, respectively. The fungal isolates identified and used were *Penicillium, Mucor, Aspergillusniger, Aspergillus flavus, Microsporum gypseum, Trichophyton mentagrophytes, Candida, and Saccharomyces* species. The bacterial isolates identified and used were Methicillin-resistant *Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterobacter aerogenes, Streptococcus faecalis, Shigella dysenteriae, Salmonella typhi, Salmonella paratyphi A, Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, and Citrobacter freundii.*

Inoculums preparation

A pure fungal and bacterial suspension was prepared in 0.85% saline to ensure a uniform count throughout the experiment by comparing the turbidity of each fungal and bacterial suspension with 0.5 McFarland's index[26].

Preparation of lawns and well-diffusion assay

Mueller Hinton and Sabouraud's dextrose agar were used to observe the antibacterial and antifungal activity, respectively, by performing a well-diffusion technique. 0.5 McFarland's index was prepared to get the inoculum size of 1.5 x 10^8CFU/ml (colony-forming unit)[26]. 0.1ml was added, and the lawn was prepared with the help of a spreader. After a certain period of time, wells were made on each plate with the help of a borer, and 50 µl of different concentrations of extracts were transferred to the wells. 50 µl of DMSO was added in one well as a negative control[27, 28]. 50µl of nystatin was added in another well as a positive control for fungi. In bacteria, vancomycin, ampicillin, streptomycin, methicillin, erythromycin, rifampin, novobiocin, and gentamycin were used as the positive control. The plates were incubated at room temperature for 48 hours to one week for fungi and at 37°C for 24 hours for bacteria.

Measurements of the zone of inhibition

After incubation, the plates were observed for the zone of inhibition around the wells, and the diameter was measured around each well in millimeters (mm).

In vitro anti-inflammatory activity (Inhibition of albumin denaturation)

The reaction mixture was comprised of test extracts and 1% aqueous solution of bovine albumin fraction. Extracts were used in 200ug concentration as aspirin was used. All the samples were incubated at 37°C for 20 minutes and then heated at 51°C for 20 minutes. After cooling, the turbidity of the samples was measured using spectrophotometer at 660 nm. Percent inhibition of protein denaturation was calculated by applying the formula, i.e., % inhibition= [{Abs control- Abs sample}/Abs control] x 100, (Abs control = the absorbance without sample, Abs sample = the absorbance of sample extract/standard)[18].

Statistical analysis

Statistical analysis was performed by using the SPSS version 23.0. One way analysis of variance (ANOVA) followed by Bonferroni post hoc test and student's t-test were performed to compare the groups with level of confidence P <0.05; (where * = P < 0.05, ** = P < 0.01, *** = P < 0.001). Data were presented as mean ± SEM.

Results

Anti-inflammatory activity (Inhibition of albumin denaturation)

A leading cause of inflammation is the denaturation of proteins. We analyzed the anti-inflammatory effect of different extracts of *Arthrocnemum macrostachyum*on protein denaturation. The results of our preliminary assay showed that few of our extracts (200 ug/mL) were effective in inhibiting heat-induced albumin denaturation. Maximum inhibition was observed by methanol and chloroform extracts, i.e., 52.13 ± 0.05 and 48.02 ± 0.07, respectively. However, other extracts did not show observable inhibition as hexane showed 28.14 ± 0.09 and ethyl acetate as 1.33 ± 0.08 inhibition. Aspirin was used as a standard anti-inflammatory drug[11] and showed the maximum inhibition, i.e., 76.69 ± 0.09 at of 200 µg concentration.

Antifungal activity of *Arthrocnemum macrostachyum*

In order to study the effect of *Arthrocnemum macrostachyum,* four solvents were used, and six concentrations of each extract were prepared in DMSO, i.e., 250µg, 750µg, 1500µg, 3000µg, 4000µg, and 5000µg. DMSO was used as a negative control and nystatin as a positive control. The effect was investigated against *Penicillium, Aspergillus niger, Aspergillus flavus, Mucor, Candida, Saccharomyces, Microsporum gypseum, Trichophyton mentagrophytes*. The results revealed that the plant extracts could suppress fungal growth with variable potency shown in Table 1.

Table 1: Highest zone of inhibition (mm) of *Arthrocnemum macrostachyum* extracts against different fungal isolates.

Fungal isolates	Hexane	Chloroform	Ethyl acetate	Methanol	Nystatin
Penicillium	1.5	1.3	0.0	1.6	4.5
Mucor	1.8	1.2	1.0	1.8	3.5
A. niger	4.5	1.0	0.0	2.8	5.0
A. flavus	1.0	1.5	1.0	1.0	4.0
Saccharomyces	2.1	1.0	1.3	1.5	4.0
Candida	1.0	1.2	1.0	1.3	4.5
M. gypseum	0.0	0.0	0.8	0.0	6.0
T. mentagrophytes	0.0	0.0	0.0	0.0	3.0

All the fungal cultures were treated with hexane extract, and the result showed that in the case of *Penicillium, Aspergillus niger, Aspergillus flavus, Mucor,* and *Saccharomyces, a* significant difference was observed at all the concentrations of the extract ($p<0.001$***) as compared to nystatin. While in the case of *Candida,* no zone was observed at 250 µg ($p<0.05$*) concentration, while at all other concentrations less significant zone was observed ($p<0.05$*). The extract showed no activity against *Microsporum gypseum* and *Trichophyton mentagrophytes.* Among all the extracts, hexane extract showed significantly larger antifungal zones (Figure 1).

In the same manner, we treated the fungal cultures with different concentrations of chloroform extract and showed a significant reduction in the growth of *Penicillium, Mucor, Aspergillus flavus,* and *Candida* ($p<0.001$***). *Aspergillus niger* and *Saccharomyces* showed a significant difference when compared with nystatin ($p<0.05$*). In case of *Microsporum gypseum* and *Trichophyton mentagrophytes*, no zone of inhibition was observed.

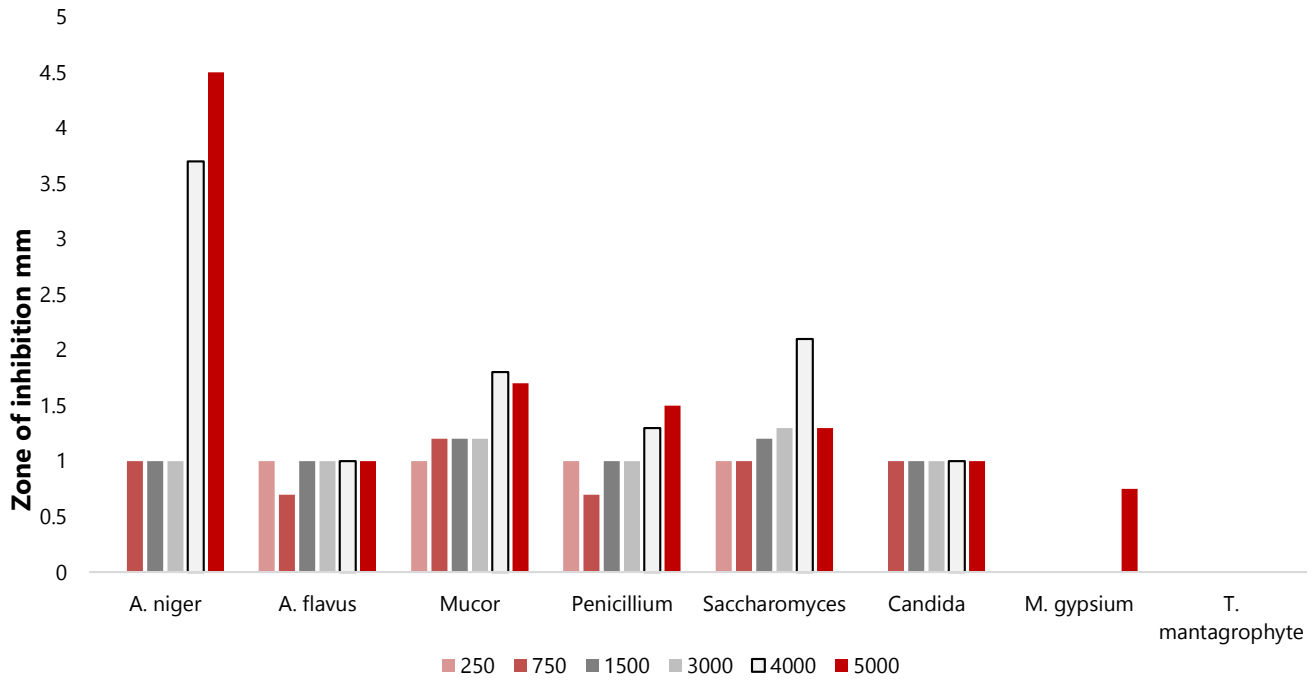

Figure 1: Antifungal effect of different concentrations of hexane extract

To evaluate the effect of ethyl acetate extract of *Arthrocnemum macrostachyum,* the same method was followed, and it was observed that *Penicillium, Mucor, Aspergillus flavus, Saccharomyces, Candida,* and *Microsporuum* were significantly sensitive to all the concentrations of extract ($p<0.001$***). No zone of inhibition was observed in case of *Aspergillus niger* ($p<0.05$*) and *Trichophyton mentagrophytes.*

The result of methanol extract of *Arthrocnemum macrostachyum* showed that this extract affected the growth of *Penicillium, Mucor, Aspergillus niger, Aspergillus flavus, Saccharomyces,* and *Candida* as a significant zone was observed ($p<0.001$***). *Microsporum gypseum* and *Trichophyton mentagrophytes* remained insensitive to all the concentrations of the extract.

Antibacterial activity of *Arthrocnemum macrostachyum*

The antibacterial effect was observed against Methicillin-resistant *Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Staphylococcus epidermidis, Staphylococcus saprophyticus, Enterobacter aerogenes, Streptococcus faecalis, Shigella dysenteriae, Salmonella typhi, Salmonella para typhi A, Escherichia coli*, *Klebsiella pneumonia, Proteus mirabilis, and Citrobacter freundii.* Six different concentrations of each extract were prepared in DMSO, i.e., 250 µg, 500 µg, 750 µg, 1000 µg, and 5000 µg. DMSO was used as a negative control, and different antibiotics i.e., vancomycin, ampicillin, streptomycin, methicillin, erythromycin, rifampin, novobiocin, and gentamycin, were used as a positive control. The maximum activity of various extracts against bacterial growth is shown in table 2.

The methanol extract was significantly effective against *Staphylococcus saprophyticus,* and *Staphylococcus epidermidis* (p <0.001***). In the case of *Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Citrobacter freundii, Klebsiella pneumoniae, Escherichia coli,* Methicillin-resistant *Staphylococcus aureus (MRSA*), the inhibitory effect of the extract was observed but statistically non-significant. In the case of *Streptococcus faecalis, Shigella dysenteriae, Enterobacter aerogenes*, and *Salmonella paratyphi A,* no inhibitory effect was observed at any concentration of the extract (Figure 2).

Table 2: Highest zone of inhibition (mm) of *Arthrocnemum macrostachyum* extracts against different bacterial isolates

Bacterial Isolates	Chloroform	Ethyl Acetate	Hexane	Methanol	Antibiotics
Salmonella typhi	20	8.1	4.2	24	van = 6
Proteus mirabilis	13	15	5.0	15	van = 8
Pseudomonas aeruginosa	19	13	18.1	19.2	van = 5
Citrobacter freundii	13.4	14.2	8.0	23	van = 8
Enterobacter aerogenes	8.1	0	0	0	van = 7
Klebsiella pneumonia	0	12.2	0	11	amp = 12
Salmonella typhi para A	0	4.1	10	0	strept = 13
Staphylococcus saprophyticus	15.1	0	0	34	eryth = 14
MRSA	0	4	0	4	met = 6
Staphylococcus epidermidis	0	0	9	14.0	rif = 10
Escherichia coli	0	13.4	5	8	van = 8
Streptococcus fecalis	0	0	0	0	nov = 17
Shigella dysenteriae	0	0	0	0	gen = 19

Van: Vancomycin, Amp: Ampicillin, Gen: Gentamycin, Strep: Streptomycin, Met: Methicillin, Nov: Novobiocin, Rif: Rifampin, Eryth: Erythromycin

Ethyl acetate extract was significantly effective against *Proteus mirabilis* (p <0.001***) and, in the case of *Citrobacter freundii* and *Escherichia coli,* the extract was less significant (p <0.01**). In the case of *Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae,* Methicillin-resistant *Staphylococcus aureus (MRSA*), *Salmonella paratyphi A,* the inhibitory effect of the extract was not significant. In the case of *Staphylococcus epidermidis, Streptococcus faecalis, Shigella dysenteriae, Enterobacter aerogenes, and Staphylococcus saprophyticus,* no inhibitory effect was observed at any concentration of the extract (Figure 3).

The clinical bacterial isolates were treated with hexane extracts, and *Pseudomonas aeruginosa* showed a significant zone of inhibition (p<0.01**). In the case of *Proteus mirabilis, Citrobacter freundii, Escherichia coli,*

Salmonella typhi, Salmonella paratyphi A, and *Staphylococcus saprophyticus*, the inhibitory effect of the extract was not significant. In the case of *Streptococcus faecalis, Shigella dysenteriae, Enterobacter aerogenes, Staphylococcus saprophyticus,* Methicillin-resistant *Staphylococcus aureus (MRSA*), and *Klebsiella pneumoniae*, no inhibitory effect was observed at any concentration of the extract.

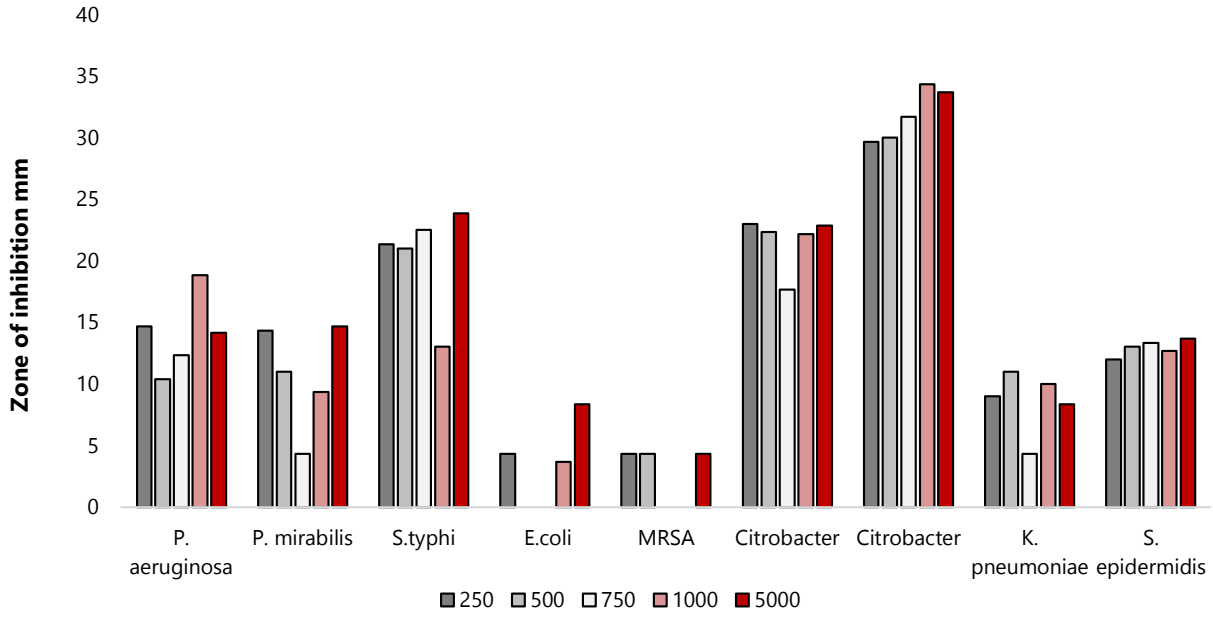

Figure 2: Antibacterial effect of different concentrations of methanolic extract

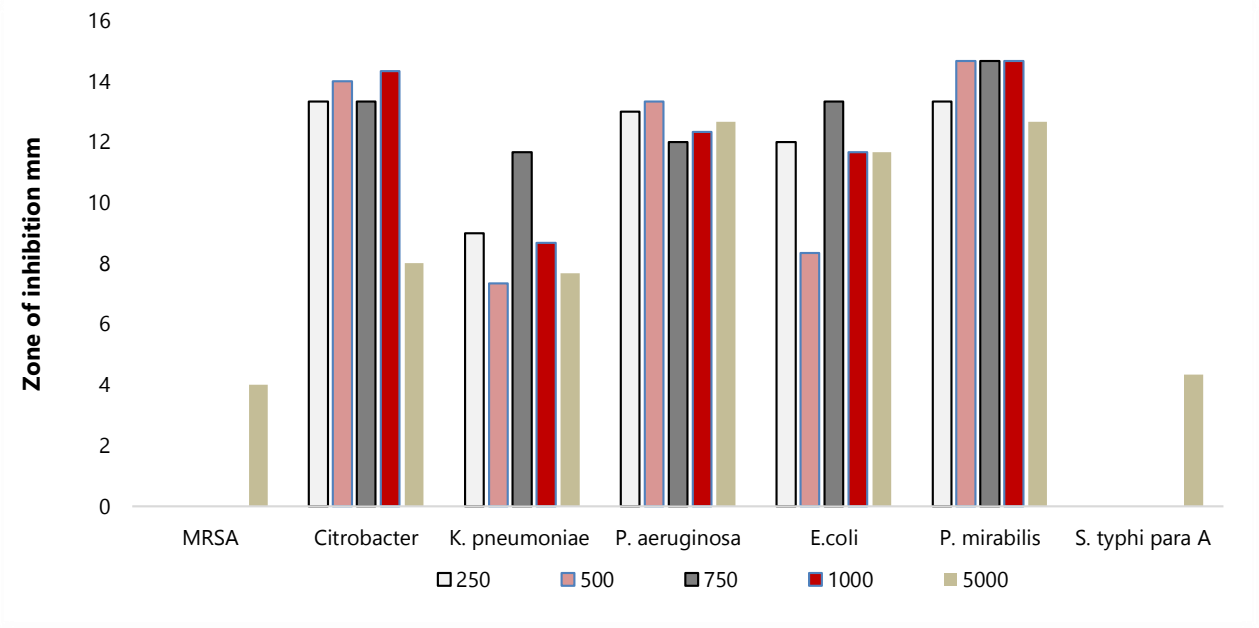

Figure 3: Antibacterial effect of different concentrations of ethyl acetate extract

Chloroform extract of *Arthrocnemum macrostachyum* was significantly effective against *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus* (p <0.001***). In the case of *Citrobacter freundii* and *Enterobacter aerogenes,* the extract's inhibitory effect was not significant. In the case of Methicillin-resistant *Staphylococcus aureus (MRSA*), *Staphylococcus epidermidis, Streptococcus faecalis, Shigella dysenteriae, Salmonella paratyphi A, Escherichia coli,* and *Klebsiella pneumonia,* no inhibitory effect was observed at any concentration of the extract.

Discussion

Development of multidrug resistance is a major health problem, emphasizing the exploration of new alternative treatments[29]. Plant extracts are used traditionally as alternative treatment options to cure many infectious diseases[30]. A number of reports are available showing in vitro and in vivo efficacy of many plant extracts against various plant and human pathogens[31]. The inhibitory effect of many plant extracts is due to their hydrophobic property as they react with different microbial proteins[32].

Arthrocnemum macrostachyum contains a number of bioactive compounds which are effective for the treatment of various diseases. It is reported that this plant has anti-inflammatory, antioxidant, antidiabetic activities[33]. *Arthrocnemum macrostachyum* also contains a high concentration of polyunsaturated fatty acids, fatty acid methyl esters, phenolic compounds, alkaloids, flavonoids, and tannins[34]. These might be the possible compounds and constituents that hold various biological activities. In this study, we used four different solvents as different solvents have varied potential to dissolve different compounds. The solvent hexane dissolves non-polar compounds while chloroform, ethyl acetate, and methanol have increasing solubility of polar compounds. The anti-inflammatory activity of various extracts was analyzed and compared to the standard anti-inflammatory drug aspirin[18]. Methanol and chloroform extracts of *Arthrocnemum macrostachyum* hold potential anti-inflammatory activity as both showed the maximum inhibition of albumin denaturation. At the same time, other extracts did not show any considerable inhibition that had less and insignificant anti-inflammatory activity.

In the last few years, the incidence of fungal infections has increased. The common ones are *Aspergillus niger, Mucor, Penicillium, Saccharomyces, Candida, Microsporum gypseum, Trichophyton mentagrophyte*s, etc. In this study, we used different human pathogenic fungi, and our results showed that *Penicillium, Aspergillus niger, Aspergillus flavus, Mucor,* and *Saccharomyces* significantly inhibited at all the concentrations of the hexane extract. In the case of *Candida,* no zone was observed at 250 µg concentration, while at all other concentrations less significant zone was observed. The extract showed no activity against *Microsporum gypseum* and *Trichophyton mentagrophytes.* The chloroform extract affected the growth of *Penicillium, Mucor, Aspergillus niger, Aspergillus flavus, Saccharomyces* and *Candida* over a wide range of concentrations of extract, i.e., 250 µg, 750 µg, 1500 µg, 3000 µg, 4000 µg, and 5000 µg. *Microsporum gypseum* and *Trichophyton mentagrophytes* did not show any zone of inhibition.

When the fungal isolates were treated with the ethyl acetate extract, it was observed that *Penicillium, Mucor, Aspergillus flavus, Saccharomyces, Candida,* and *Microsporum gypseum* were significantly sensitive to all the concentrations of the extract. No zone of inhibition was observed in the case of *Aspergillus niger* and *Trichophyton mentagrophytes.* The results of methanol extract showed that this extract affected the growth of *Penicillium, Mucor, Aspergillus niger, Aspergillus flavus, Saccharomyces* and *Candida* as the significant zone was observed over a wide concentration of extract, i.e., 250 µg, 750 µg, 1500 µg, 3000 µg, 4000 µg, and 5000 µg. *Microsporum gypseum* and *Trichophyton mentagrophytes* remained insensitive to all the concentrations of the extract.

Collectively, the results demonstrate that the order of inhibitory potential of *Arthrocnemum macrostachyum* against different fungal isolates as hexane extract > methanol extract > chloroform extract > ethyl acetate. Hexane, methanol, and chloroform extracts showed inhibitory activity against six test fungal isolates. However, hexane extract showed significant large zones as compared to other extracts. Ethyl acetate extract was less effective as it showed activity against five test fungi. The most sensitive organisms found were *Mucor*, *Aspergillus flavus, Saccharomyces,* and *Candida* because they were inhibited by all the extracts. *Penicillium, Mucor,* and *Aspergillus niger* were also sensitive to all the extracts except one extract. *Microsporum gypseum* inhibited by only ethyl acetate extract and *Trichophyton mentagrophytes* found resistant to all the extracts of *Arthrocnemum macrostachyum.* It is evident by our study that different extracts of *Arthrocnemum macrostachyum* can inhibit the growth of different fungi significantly.

All the clinical bacterial isolates were treated with five different concentrations of chloroform extract of *Arthrocnemum macrostachyum,* i.e., 250 µg, 500 µg, 750 µg, 1000 µg, and 5000 µg. The results showed that in the case of *Proteus mirabilis, Salmonella typhi,* and *Pseudomonas aeruginosa, a* statistically significant and large zone of inhibition was produced at all the concentrations of the extract. On the other hand, *Staphylococcus saprophyticus* showed significant zone only at 5000 µg and 250 µg concentrations. No zone of inhibition was found at any concentration of the extract when Methicillin-resistant *Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Streptococcus faecalis, Shigella dysenteriae, Salmonella paratyphi A, Escherichia coli,* and *Klebsiella pneumonia* were exposed to this extract.

In the same manner, we treated the clinical isolates with different concentrations of ethyl acetate extract, i.e., 250 µg, 500 µg, 750 µg, 1000 µg, and 5000 µg. The results demonstrated that in the case of *Proteus mirabilis* and *Citrobacter freundii* significant and large zone of inhibition was produced at all the concentrations of extract compared to the control. However, *Escherichia coli* showed significant zones at four concentrations of the extract. In the case of *Salmonella typhi, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae,* all concentrations showed the inhibitory effect, but the effect was not statistically significant. In the case of Methicillin-resistant *Staphylococcus aureus (MRSA)* and *Salmonella paratyphi A*, the inhibitory effect was only observed at the highest concentration, i.e., 5000 µg. In *Staphylococcus epidermidis, Streptococcus faecalis, Shigella dysenteriae, Enterobacter aerogenes, and Staphylococcus saprophyticus,* no zone of inhibition was found observed at any concentration of the extract.

Bacterial isolates were also treated with the various concentrations of hexane extract, i.e., 250 µg, 500 µg, 750 µg, 1000 µg, and 5000 µg. In this extract, *Pseudomonas aeruginosa* was the only organism that showed significant zones at all test concentrations of the extract. *Proteus mirabilis, Salmonella paratyphi A,* and *Staphylococcus epidermidis* showed sensitivity towards all concentrations, but the effect was not statistically significant as compared to control. In the case of *Citrobacter freundii* and *Escherichia coli,* the sensitivity was observed on three concentrations of the extract, i.e., 250 µg, 500 µg, and 750 µg, but *Salmonella typhi* showed sensitivity towards only two concentrations, i.e., 1000 µg and 5000 µg but the effect was not statistically significant as compared to the control. *Streptococcus faecalis, Shigella dysenteriae, Enterobacter aerogenes, Staphylococcus saprophyticus,* Methicillin-resistant *Staphylococcus aureus (MRSA), and Klebsiella pneumoniae*, no zone of inhibition was observed at any concentration of the extract.

When the clinical isolates were treated with different concentrations of methanol extract, i.e., 250 µg, 500 µg, 750 µg, 1000 µg, and 5000 µg, the results showed that in the case of *Staphylococcus saprophyticus* and *Staphylococcus epidermidis*, statistically significant zones appeared at all the concentrations of the extract. *Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Citrobacter freundii, Klebsiella pneumonia,* and

Escherichia coli showed the inhibitory effect at all concentrations. However, the effect was not significant as compared to the control. Methicillin-resistant *Staphylococcus aureus (MRSA)* showed the sensitivity towards three concentrations of the extract, i.e., 250 µg, 500 µg, and 5000 µg, and at other two concentrations, i.e., 750 µg and 1000 µg; no zone of inhibition was observed. In the case of *Streptococcus faecalis, Shigella dysenteriae, Enterobacter aerogenes,* and *Salmonella paratyphi A*, no zone of inhibition was observed at any extract concentration.

This study supports previous findings in the literature that the antimicrobial activities have a direct relationship to the increased concentration of extracts[35]. As plant extracts are used for the treatment of infectious diseases, our study also showed the antimicrobial effect of *Arthrocnemum macrostachyum* extract[36,37]. Studies reported the inhibitory effect *Arthrocnemum macrostachyum* against few bacteria. Our study reported the inhibitory effect of methanol, hexane, ethyl acetate and chloroform extracts[38].

Altogether, our results show that the methanol extract was most effective as it showed the inhibitory activity against nine test organisms. Hexane and ethyl acetate extracts showed activity against seven and eight test organisms, respectively. The chloroform extract was the least effective as it showed activity against only six test organisms. The most sensitive organisms found were *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa,* and *Citrobacter freundii* because all the extracts of *Arthrocnemum macrostachyum inhibited them.* The most resistant organisms were *Streptococcus fecalis* and *Shigella dysenteriae*, as any of these extracts did not inhibit them.

Conclusion

The demand for plant-based alternative medicines has increased significantly because of the increasing drug resistance among microorganisms. *Arthrocnemum macrostachyum* showed considerable anti-inflammatory, antifungal, and antibacterial activity. The hexane and methanol extracts of *Arthrocnemum macrostachyum* showed the maximum inhibition of the tested fungal and bacterial isolates, respectively. The organisms that are resistant to various antibiotics showed sensitivity to the extracts of this plant. This plant can be used as a good candidate for the treatment of various fungal and bacterial infections. Further studies are needed to evaluate the proper mechanism of action of this plant for the development of potential anti-inflammatory, antifungal, and antibacterial drugs.

Conflicts of Interest

The authors have declared that no competing interests exist.

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