Research Article

Antimicrobial evaluation of various leaves extracted samples of nettle desert (*Forsskaolea tenacissima* L.)

Tariq Aslam¹, Syed Sadaqat Shah^{1,2}, Sajjad Ahmed^{1,3}, Nazim Hassan⁴, Mu Peng⁵, Saddam Hussain^{1,3} and Zhijian Li^{2*}

1. Department of Botany, Islamia College Peshawar, KPK, Pakistan

2. Key Laboratory of Vegetation Ecology, Ministry of Education, Institute of Grassland Science, School of Life Science, Northeast Normal University, Changchun 130024, China

3. Key Laboratory of Molecular Epigenetics of Ministry of Education, School of Life Science, Northeast Normal University, Jilin, 130024, PR. China

4. Institute of Grassland Science, Northeast Normal University, and Key Laboratory of Vegetation Ecology, Ministry of Education, Changchun, Jilin 130024 China

5. College of Life Science, Northeast Forestry University, Jilin, China

*Corresponding author's email: khankhanafri10@yahoo.com

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Abstract

The present study evaluates the anti-microbial activity of three different crude extracts (ethanol, aqueous and n-hexane) of *Forsskaolea tenacissima* L. leaves against gram negative and gram positive bacteria and fungi using well diffusion method. N-hexane extract showed tremendous inhibition of 12mm (80% ZI) and 10mm (71.42% ZI) against *Staphylococcus aureus* and *Bacillus subtilis* at 1000 µg/ml concentration. Similarly, aqueous extract at the concentration of 1000 µg/ml reduced the growth of *Xanthomonas maltophilia* and *Escherichia coli* as 11mm (68.75% ZI) and 9mm (60.00%), respectively. However, ethanol extract showed good activity of 12mm (75.00%) against *Clavibacter michiganense*. Aqueous extract showed 9mm (75.00%) against *Acromonium alternatum* at 1000 µg/ml. *Rhizopus stolonifer* and *Trichoderma reesei* both were found sensitive to aqueous extract, which showed 11 and 8mm (68.75 and 57.14% ZI) at 1000 µg/ml concentration, respectively. The growth of *Aspergillus niger* was inhibited by ethanol extract through 9mm (56.25% ZI) at the concentration of 1000 µg/ml. The above study determined the medicinal importance of *Forsskaolea tenacissima*.

Keywords: Antimicrobial activity; Bacteria; *Forsskaolea tenacissima*; Well diffusion method

Introduction

In developed countries, about 80% of plants are used as traditional medicines that serve as excellent sources of compounds (drugs). The plants are collected for the properties they exhibit such as the synthesis of secondary

product [1], and inhibitory effect against various growing human [2]. Traditional medicines are being used for practices, knowledge and also need for making of better plants and animal-based approaches [1, 3]. The use of natural medicine and local

practices are common in the treatment of various diseases [4]. An increase in number of infectious agents with strong resistance to commercial antimicrobial compounds has been noticed [5]. Due to this reason, the secondary product produced by medicinal plant is of excellent value due to its antimicrobial constituents [6]. These antimicrobial agents mark the most important discovery of 20 century in the field of medicine [7]. Also, traditional medicines system is now well popular and adopted at global level as a primary health care system [8].

Main resources for modern drugs are from Mother Nature. According to World Health Organization (WHO) reports, the use of traditional medicine in the 1st world countries is at peak. Failure of conventional medicine that can cure chronic diseases, emergence of multi-drug resistance pathogens and parasites, adverse effects of chemical drugs and increasing cost are some of the reasons that made traditional medicines using again by people [9].

The plants serve as an essential source of new chemicals with potential therapeutic effects [10]. In recent times, several infectious diseases mostly caused by fungal and bacterial pathogens are treated by antibiotics [11]. It is believed that chemical classes such as sterols, alkaloids, glycosides, flavonoids, tannins and carbohydrates are more preferred natural antibiotics due to their antimicrobial activity [12]. Developed countries have improved the use of herbal medicine as the alternative solution to health problems and cost of pharmaceutical products. Human pathogens have gained drugs resistance against commonly used antibiotics there by developing immunity to them. However, plants have got cure to infectious as well as chronic diseases [13].

Local communities have been using medicinal plants for centuries [14]. In Pakistan, 6000 species of higher plants are

reported. Out of these reported 6000 species, 12% are used medicinally [14, 15]. Ethno botanical studies have documented local knowledge about the use of plants as a drug in ailment treatment [16]. Ethno botany by providing useful knowledge regarding medicinal plants saves money and time [17]. Through ethno botanical census, knowledge regarding local plants and their use in the treatment of various diseases has been documented as a mean to provide insight for the production of new drugs against infection causing diseases [14]. Dissimilar plant parts are employed for the physiotherapy of several kinds of sicknesses and infections [18]. Medicinal plants can provide alternative treatment minor infectious diseases [19].

Forsskaolea tenacissima is a member of nettle genus. It is found in sandy clay gravelly soils of arid and semi-arid waste lands up to 1,200 meters (3,900 ft) like Mediterranean woodlands and shrub lands, semi-steppe, shrub-steppes, modest to extreme deserts [20]. *Forsskaolea tenacissima* is highly resistance to drought and salinity [21], it is non-cultivated species found in low rain area in sandy and stony soil where no or less water is found.

Forsskaolea tenacissima have already been screened for possible anti-hypertensive, spasmogenic and spasmolytic activity [22]. It belongs to family *Utricaceae* which consist of 45 genera spread in tropical and subtropical region of the world [8]. Medicinal and commercial importance of this family is wide, its plants are known to be galactagogue [24], antiviral [23] and anti-inflammatory [25]. *Urticadioica* is used in benign pro-static hyperplasia and also as libido stimulant, digestive disorder, kidney problems, respiratory, diabetes and excessive menstrual bleeding [26]. Present study aimed at evaluating antimicrobial activity of *Forsskaolea tenacissima* L. against different human pathogens.

Materials and methods

Experiments regarding this study were conducted in research Laboratory of Department of Botany, Islamia College University Peshawar, Pakistan.

Plant materials

Forsskaolea tenacissima leaves were collected from Jarjorey F-R Peshawar Tribal area. The leaves were subjected to room temperature and shade for a period of 3 month to completely dry. An ordinary grinder was used to grind the leaves.

Preparation of crude extract

About 70g of ground plant powder was taken three times in three round bottom flasks, first round bottom flask ethanol to the second n-hexane and to 3rd round bottom flask distal water was added. After 24 hours, the filtrate was filtered with cotton and the process was repeated 3 times. The concentration of the extracts was performed by rotary evaporator at 60°C. The material was again dried through water bath at 55°C and was stored in bottles.

Microorganisms used

Gram -ve (*Escherichia coli* and *Xanthomonas maltophilia*), Gram +ve (*Bacillus subtilis*, *Clavibacter michiganense* and *Staphylococcus aureus*) bacteria and fungus (*Aspergillus niger*, *Trichoderma reesei*, *Rhizopus stolonifer* and *Acromonium alternatum*) were obtain from Institute of Biotechnology and genetic Engineering, University of Agriculture, Peshawar, KPK Pakistan.

Well diffusion susceptibility method

Well diffusion method was used to study antimicrobial activity of different plant extract. Fungal culture was grown on PDA while bacterial cultural were grown on agar media as mentioned by [27]. For the purpose to know antimicrobial activity of the species, petri plates impregnated with microbes were added with the extracts obtained from the plant with dissimilar concentration such as 500 µg and 1000 µg into holes present in

media. The bacterial culture incubated at 37°C for 24 hours and fungal cultural were incubated at 37°C for 3 Days.

Antimicrobial activity bioassay

To determine antibacterial activity, specific amount of nutrient agar was mixed in sterilized distal water contained in bottle. All the experimental apparatus such as petri plates, borer and liquid media were sterilized at the pressure of 1.5 lbs and 121°C for fifteen to twenty minutes. Then the agar was poured in petri plates in sterilized environment and the agar was allowed to solidify. A sterilized borer was used to make holes in the media.

For antifungal activity, the same procedure was used except the nutrient agar media. In antifungal activity, PDA media was used instead of nutrient agar. But the procedure was same as antibacterial activity.

Applying antifungal test

For antifungal activity require amount of Potato Dextrose Agar (PDA) media (14.25 gm in 400 ml of distal water) was prepared for 12 petri plates. The media was prepared in bottle. The media and all the apparatus used in this experiment were sterilized in autoclave for 20 minutes at 1.5 lbs of pressure and 121 °C temperatures. Later, the sterilized media was poured in sterilized petri plates in laminar flow hood and allow it to solidify in petri plates and made 3 holes in each petri plates through sterilized borer. To avoid contamination all the procedure was attempted inside the laminar flow hood.

After formation of holes the fungus was applied by streaking with sterile inoculation loop on the PDA media plates in a laminar flow. When streaking was completed, plant extract was then added and antibiotic at different concentration. An in first hole 1000 µg/ml of plant extract was added and in second hole 500 µg/ml of plant extract was added while in last hole standard antifungal against fungi was added. After that the petri plates were closed and shifted from laminar

flow hood to incubator and kept in incubator at 37°C for 3 days.

Zone of inhibition around each hole for antifungal potential was recorded after three days.

Positive control used against fungal strains
0.05% Flumetazole was used as a positive control.

Statistics

Simple statistics was used as the mean values were converted in percent via the following formula.

$$\text{Per cent inhibition} = \frac{\text{Zone of inhibition of extract (mm)}}{\text{Zone of inhibition of standard (mm)}} \times 100$$

Results

The present work was conducted on three different crude extracts (ethanol, n-hexane and aqueous) of *Forsskaolea tenacissima* (leaves) against gram positive and gram negative bacteria. The result revealed that ethanol extract of *Forsskaolea tenacissima* against *Staphylococcus aureus* showed 8 (53.35%) and 10 (66.66%) mm zone of inhibition (ZI) in concentration of 500 and 1000 µg/ml. The n-hexane extract showed 10mm and 12mm zone of inhibition at concentration of 500 µg/ml and 1000 µg/ml, (66.66 and 80%) respectively. Against *Bacillus subtilis* the ethanol extract reduced the growth by 6mm (42.75% ZI) at concentration of 500 µg/ml and 8mm (57.14% ZI) at concentration of 1000 µg/ml, respectively. The n-hexane extract inhibited the growth of *Bacillus subtilis* by 7mm (50% ZI) at concentration of 500 µg/ml and 10mm (71.42 ZI) at concentration of 1000 µg/ml, respectively.

Against *Xanthomonas maltophilia*, highest zone of inhibition was recorded by aqueous extract at the concentration of 500 and 1000 µg/ml, which showed 9 and 11mm (56.25 and 68.75% ZI), respectively. The ethanol extracted sample showed 7mm (43.75% ZI) at concentration of 500 µg/ml and 9mm (56.25% ZI) at concentration of 1000 µg/ml,

respectively. Against *Escherichia coli* highest zone of inhibition was recorded by aqueous extract, as 9mm (60% ZI) at the concentration of 1000 µg/ml, while lowest zone of inhibition was recorded by N-hexane as 5mm (33.33% ZI), at the concentration of 500 µg/ml. Ethanol extracted sample showed 6 and 7mm (40 and 46.66% ZI) at the concentration of 500 and 1000 µg/ml, respectively. Against *Clavibacter michiganense* all samples were effective and showed reliable results. The highest zone was recorded ethanol extract which was 8 and 12mm (50 and 75% ZI) at the concentration of 500 µg/ml and 1000 µg/ml, respectively. While n-hexane extracted sample displayed lowest ZI of 7 mm and 9 mm (43.75 and 56.25% ZI) at the concentration of 500 and 1000 µg/ml, respectively.

The antifungal activity of ethanol, n-hexane and aqueous extracts of *Forsskaolea tenacissima* leaves against *Acromonium alternatum* have been shown in table 1. The aqueous extract showed 7 and 9mm (58.33 and 75% ZI) at the concentration of 500 and 1000 µg/ml, respectively. The lowest ZI was recorded by n-hexane extract as 5mm (41.66% ZI) at the concentration of 500 µg/ml. Against *Rhizopus stolonifer* the aqueous extract inhibited the growth by 9 and 11mm (56.25 and 68.75% ZI) at the concentration of 500 and 1000 µg/ml, respectively. The ethanol and n-hexane extracts showed 8 and 9mm (50 and 56.25 % ZI) at higher concentration.

Data regarding antifungal activity is shown in table 1. Results revealed that *Trichoderma reesei* was highly inhibited by ethanol and aqueous at higher concentration. The data was recorded as 8mm (57.14% ZI) at the concentration of 1000 µg/ml. The antifungal activity of ethanol, n-hexane and aqueous extracted sample from *Forsskaolea tenacissima* leaves against *Aspergillus niger* is shown in table 1. *Aspergillus niger* was highly sensitive to ethanol extract, which

showed 9 and 7mm (43.75 and 56.25% ZI) at the concentration of 500 and 1000 µg/ml, respectively. Our data also revealed that *Aspergillus niger* was susceptible to the n-

hexane extract, which showed shown 5 and 7mm (31.25 43.75% ZI) at the concentration of 500 and 1000 µg/ml, respectively.

Table 1. Antimicrobial activities of leaves extracts of nettle desert (*Forsskaolea tenacissima* L.) against different human pathogenic microorganisms by well diffusion method

Organisms	Conc. µg/ml	Mean diameter of zones of inhibition (mm)			Percent zone of inhibition (%)			Positive control Ciprofloxacin
		Ethanol	N-hexane	Aqueous	Ethanol	N-hexane	Aqueous	
<i>Staphylococcus aureus</i>	500	8	10	7	53.33	66.66	46.66	15
	1000	10	12	8	66.66	80.00	53.33	
<i>Bacillus subtilis</i>	500	6	7	7	42.85	50.00	50.00	14
	1000	8	10	9	57.14	71.42	64.28	
<i>Xanthomonas maltophilia</i>	500	7	8	9	43.75	50.00	56.25	16
	1000	9	10	11	56.25	62.50	68.75	
<i>Escherichia coli</i>	500	6	5	7	40.00	33.33	46.66	15
	1000	7	6	9	46.66	40.00	60.00	
<i>Clavibacter michiganense</i>	500	8	7	8	50.00	43.75	50.00	16
	1000	12	9	10	75.00	56.25	62.5	
<i>Acromonium alternatum</i>	500	7	5	7	58.33	41.66	58.33	12
	1000	8	6	9	66.66	50.00	75.00	
<i>Rhizopus stolonifer</i>	500	7	7	9	43.75	43.75	56.25	16
	1000	8	9	11	50.00	56.25	68.75	
<i>Trichoderma reesei</i>	500	6	5	7	42.85	35.71	50.00	14
	1000	8	6	8	57.14	42.85	57.14	
<i>Aspergillus niger</i>	500	7	5	6	43.75	31.25	37.5	16
	1000	9	7	8	56.25	43.75	50.00	

Discussion

Three different crude extracts (ethanol, n-hexane and aqueous) of *Forsskaolea tenacissima* (leaves) were used in this study. These extracts were then tested for their antibacterial and antifungal activity against gram positive and gram negative bacteria. The results revealed that; the n-hexane extract of leaves of *Forsskaolea tenacissima* showed tremendous activity against *Staphylococcus aureus* at higher concentration. The data was recorded as 12mm (80% ZI) at the concentration of 1000 µg/ml. The lowest data was recorded by aqueous extract at the concentration of 500 µg/ml. The best result was recorded by n-hexane extract when tested against *Bacillus subtilis*. It showed 10mm (71.42% ZI) at the

concentration of 1000 µg/ml. A study investigated the antimicrobial activity of *Forsskaolea tenacissima* against *Staphylococcus aureus* which showed maximum ZI of 14mm [28]. In another study authors also investigated the antimicrobial activity of ethanol extracted samples of *Forsskaolea tenacissima* (Aril parts) against *Klebsiella pneumonia* by disc diffusion method and measured ZI of 2.0 and 4.3mm [18]. While Owolabi and his co-authors found no antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* using ethanol extract of *Forsskaolea tenacissima* (leaves) at any concentration [21]. In another study, authors investigated the antimicrobial activity of ethanol extracted sample of *Forsskaolea tenacissima* and

observed about 6 mm ZI against *S. auerus*, 8 mm ZI against *Staphylococcus faecolis* and 4mm ZI against *Bacillus subtilis*, respectively [1].

Aqueous extract showed highest inhibition against *Xanthomonas maltophilia* and *E. coli* at both the concentrations, as compared to n-hexane and ethanol. While *Clavibacter michiganense* was highly sensitive to ethanol extract as compared to other extracts. Authors studied the antimicrobial activity of methanol extract sample of *Forsskaolea tenacissima* L. which showed optimum zone of inhibition of 5mm and 6mm against *Pseudomonas aeruginosa* and 12mm against *E. coli*, respectively [22]. In another study authors also investigated the aqueous extracted sample of *Forsskaolea tenacissima* L. and observed effective results against gram positive bacteria [4]. The obtained results of antimicrobial activity of *Urtica dioica* (leaves, stem) and observed that ethanol extract showed significant antimicrobial activity against different human pathogens [29]. In another study, the antimicrobial activity of n-hexane extracted sample of *Forsskaolea tenacissima* L. by disc diffusion method was studied and observed that n-hexane extracted sample was effective against Gram negative bacteria. Antimicrobial activity of *Forsskaolea tenacissima* of n-hexane extracted sample against *Streptococcus* mutants with significant result of 5 µg/ml minimum inhibitory concentration, n-hexane extracted sample of *Forsskaolea tenacissima* L. against clinical isolate which exhibit good activity 5 µg/ml minimum inhibitory concentrations (MIC) against all the tested clinical isolates [30]. In this study authors investigated the antimicrobial activity of n-hexane and aqueous extracted sample of *Urtica dioica* against *Staphylococcus aureus* strain which showed little antimicrobial activity against all tested *Staphylococcus* strain [24].

N-hexane extracted sample of *Forsskaolea tenacissima* (leaves) also showed maximum zone of inhibition against all tested bacteria. Ethanol extract showed best result against *Clavibacter michiganense* As, 12mm (75% ZI) at higher concentration, while against *Acromonium alternatum* aqueous extract showed the highest inhibition of 9mm (75% ZI). In this study the authors investigated the antimicrobial activity of n-hexane extracted sample of *Urtica dioica*, which showed maximum zone of inhibition against gram positive bacteria [31]. Another study was conducted to observe the antimicrobial activity of leaf, stem and root extracts of ethyl acetate against *Xanthomonas malvacearum* bacteria. The ethyl acetate showed activity with 500 µg/ml more than the pure antibiotic [31]. Against *Rhizopus stolonifer* aqueous extract showed the highest ZI at both the concentration, while against *Trichoderma reesei*, highest ZI was recorded by Ethanol and aqueous extracts, which were recorded as 8mm (57.14% ZI) at the concentration of 1000 µg/ml. Ethanol extract showed highest ZI against *Aspergillus niger* at both the concentration as compared to other extracts.

Conclusion

N-hexane extract showed tremendous activity of 12mm (80.00% ZI) against *Staphylococcus aureus*, while ethanol extract showed 12mm (75.00% ZI) against *Clavibacter michiganense* at higher concentration 1000 µg/ml, respectively.

Authors' contributions

Conceived and designed the experiments: Z Li & SS Shah, Performed the experiments: T Aslam & SS Shah, Analyzed the data: S Ahmed, N Hassan & S Hussain, Contributed materials/ analysis/ tools: Z Li & M Peng, Wrote the paper: SS Shah & T Aslam.

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