Research Article

Efficacy of different solvent extracts from selected medicinal plants for the potential of antibacterial activity

Fayaz Asad^{1*}, Hussan Ara Begum², Muhammad Hamayun², Reema Hameed², Tabassum Yaseen¹ and Ajmal Khan^{3,4}

1. Department of Botany, Bacha Khan University Charsadda, KP-Pakistan

2. Department of Botany, Abdul Wali Khan University Mardan, 23200-Pakistan

3. Key Laboratory of Alpine Ecology and Biodiversity, Institute of Tibetan Plateau Research, Chinese Academy of Sciences, Beijing 100101-China

4. University of Chinese Academy of Sciences. No.19A Yuquan Road, Beijing, 100049-China

*Corresponding author's email: fayaz.asad79@yahoo.com

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Abstract

Six medicinal plants were tested, namely *Portulaca oleraceae*, *Cucumis sativus*, *Malus baccata*, *Geranium wallichianum*, *Monothea Buxifolia*, and *Saxifraga flagillaris* for antimicrobial activity against five medically important pathogenic bacterial strains, namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*, and *Salmonella typhi*. The antimicrobial activities of crude ethanolic extracts and fractions were resolved by using agar well diffusion technique. Our results demonstrated that all the extracts have antimicrobial properties against both Gram-negative and Gram-positive bacteria. The fractions of extracts indicated higher activities contrary to bacterial strains tested with the highest activity observed for n-hexane and followed by dimethyl chloromethane fractions. Among the tested pathogenic bacteria, *Staphylococcus aureus* was the most vulnerable, while *Pseudomonas aeruginosa* was less susceptible. In conclusion, ethanolic fractions of *Saxifraga flagillaris* indicated the highest effectiveness against all the bacterial pathogens tested. Hence, this study ratifies the efficiency of certain extracts to be subjected for natural antimicrobials and further pharmaceutical assessment.

Keywords: Antimicrobial activity; Bacteria; Ethanolic extract; Medicinal plants; Pathogens

Introduction

Most human population (around 70-80%) of the developing countries, relies on therapeutic plants for primary health care [1]. Medicinal plants possess an ample source of antimicrobial agents. Therefore, several developing countries use them for medical purposes, because they provide a source for many effective and powerful drugs [2].

However, many plant species have been investigated for their anti-microbial characteristics, whereas the majority of them have not been appropriately assessed [2], particularly in Pakistan. Medicinal plants have been proved to be highly effective to treat an extensive range of disease [3]. Contagious diseases frequently occur in Pakistan, therefore, it is essential to explore

the plant-derived medicines. Mostly, antimicrobial products are imported, due to its less price for the low-income community, which can easily afford them. Thus local medicinal plants can be extensively found and used in Pakistan [4, 5].

From primitive times, different plants with antimicrobial properties have been used for curing various pathogenic diseases. Considerable achievements have been observed in the field of herbal medicine, because of less side effects of plants on health [6]. The natural biochemical ingredients of medicinal plants provide positive physiological effects on the human body [7]. Plants harboring antioxidant and antimicrobial potential [8, 9] contain different secondary metabolites like terpenoids, tannins, flavonoids, alkaloids, glycosides, etc., which was observed *in vitro* to possess antimicrobial properties [10] are valued medicinally. Furthermore, plants with naturally available phytochemicals provide resistance against different diseases [11, 12]. Phytochemical such as proteins, Chlorophyll, and common sugars are considered as primary compounds, and alkaloids, terpenoid, and phenolic are secondary compounds [13].

In developing countries, many studies investigated traditional uses of the medicinal plant [14-22], whereas limited information exists in northwestern Pakistan. Therefore, the present is conducted to identify the antimicrobial activity and efficiency of six different medicinal plant species.

Materials and methods

Sample collection

Plants were sampled from various parts of Khyber Pakhtunkhwa, Pakistan. They were recognized using previous literature [23-25].

Preparation of plant extract

Plants and their parts were washed through distilled water, air dried, and then grinded to fine powder and kept in airtight bottles. Ethanol was used for the extraction of plant

power consecutive three weeks and preserved at 25°C. Every week, ethanol was evaporated to get the extract from the available filtrate using a rotary evaporator, and the extracts were stored in the refrigerator for further analysis. The extracts were fractionated with dichloromethane, n-hexane, and ethyl-acetate on the basis of the polarity of the solvent. After these processes, we got four extracts, one crude extract and three sub-extracts called fractions of each plant powdered material.

Bacterial Strains

Gram-positive bacteria such as *Staphylococcus aureus*, and Gram-negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Salmonella typhi* were analyzed for antibacterial activity.

Media preparation

Nutrient Broth Medium with was prepared for the growth of bacterial inoculum by dissolving 6.25g Luria broth (LB) in 250ml pure water; pH was kept neutral and autoclaved. Bacterial culture was injected into media and reserved at 37°C for 24hrs in shaking incubator at 150 rpm. By dissolving 40g agar in 1000 ml pure water, LB Agar Medium of pH 7.0 was prepared and autoclaved.

Inoculum preparation

24-hour old bacteria culture in media (Miller) were mixed combine with normal brine solution till a McFarland turbidity standard [10^6 colony forming unit (CFU) ml^{-1}] was achieved. Then this inoculum was utilized to seed LB Agar Medium.

Agar plates preparation

LB Agar Medium was transferred into sterilized Petri plates and left to solidify. Following the agar well diffusion technique from a previous publication [26], a sterile cotton swab lawn cultures of the test organisms were done on the agar plates. Per plate, five wells were made by a sterile borer in a sterilized environment.

Extracts preparation

20 mg extracts were dissolved in 10 ml of Dimethylsulfo oxide (DMSO).

Incubation of test solution, and measurement of the zone of inhibition

Test sample solution of 75 µl was introduced into labeled well. In every labeled plate samples of extracts were made available, dimethyl sulphoxide (DMSO) (as negative standard) and cefotaxime (as positive standard) in respective wells. After incubation at 37°C for 24 and 48 hours, the diameter of clear zones showed no bacterial growth around each well. An average zone of inhibition was calculated in millimeter by repeating the assay three times.

Results

Antibacterial activity of crude ethanolic extracts

Table 1. Antibacterial activity of crude ethanolic extracts of selected medicinal plants (zone of inhibition in mm)

Plant Name	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherchia coli</i>	<i>Salmonella typhi</i>
<i>Cucumis sativus</i>	3.57 ± 0.83	4.89 ± 2.47	3.30 ± 0.35	3.58 ± 1.21	2.12 ± 0.12
<i>Portulaca oleraceae</i>	26.17 ± 0.73	14.75 ± 0.69	12.74 ± 0.63	15.00 ± 1.00	20.00 ± 0.58
<i>Malus baccata</i>	11.00 ± 0.58	12.67 ± 0.88	13.41 ± 0.46	12.30 ± 0.70	11.17 ± 1.42
<i>Saxifraga flagillaris</i>	14.00 ± 0.58	11.67 ± 0.67	14.00 ± 1.00	17.67 ± 0.33	14.33 ± 0.44
<i>Geranium wallichianum</i>	15.67 ± 1.45	4.67 ± 1.76	15.63 ± 0.36	12.00 ± 0.58	10.46 ± 0.77
<i>Monothea buxifolia</i>	16.00 ± 0.58	12.67 ± 0.33	14.67 ± 0.33	15.33 ± 0.33	15.67 ± 0.33

Antibacterial activity of selected plants fractions

CEEs of each plant were fractionated into three fractions such as n-hexane, dichloromethane (DCM) and ethyl acetate on polarity bases were analyzed for antibacterial activity (Table 2). The extracted fractions of *S. flagillaris* and *M. buxifolia* indicated maximum antimicrobial activity against all the selected pathogenic bacteria. However, n-hexane fractions of *Malus baccata* (*M. baccata*) showed the highest antimicrobial

The antibacterial activities of crude ethanolic extracts (CEE) of selected medicinal plants have been listed in (Table 1). The CEEs of *Portulaca oleraceae* (*P. oleraceae*) and *Monothea buxifolia* (*M. buxifolia*) showed a high zone of inhibition against all the selected bacterial strains followed by *Saxifraga flagillaris* (*S. flagillaris*). However, CEE of *Portulaca oleraceae* (*P. oleraceae*) showed the highest zone of inhibition such as 26.17 and 20.00 mm against *Staphylococcus aureus* (*S. aureus*) and *Salmonella typhi* (*S. typhi*), respectively. The *S. flagillaris* indicated 17.67 mm zone of inhibition against *Escherchia coli* (*E. coli*), and *M. buxifolia* indicated 16.00 mm zone of inhibition against *S. aureus*. The remaining CEEs were observed less susceptible to selected bacterial strains.

activity of 27.25±0.80 mm against *Shigella flexneri* (*S. flexneri*), followed by *S. flagillaris*, which showed 24.67±1.45, 24.5±1.32, and 24±0.58 mm antimicrobial activity against *E. coli*, *S. aureus*, and *S. flexneri*, respectively.

Among DCM fractions of selected plants, *S. flagillaris* indicated highest antibacterial activity of 25.33±1.45 mm against *S. typhi*, followed by 24.67±1.85, 23.67±1.67, 23.37±1.76 and 23.33 ±1.67 against *S. flexneri*, *E. coli*, *S. aureus*, and *Pseudomonas*

aeruginosa (*P. aeruginosa*), respectively. DCM fractions of *Portulaca olarecae* (*P. olarecae*) and *Geranium wallichianum* (*G. wallichianum*) revealed the antimicrobial activity of 22 mm against each *S. aureus* and *S. flexneri*, respectively. *M. buxifolia* showed the mild activity of almost 20 mm against all the selected pathogens. Ethyl acetate fraction of *P. olarecae* showed the highest

antimicrobial activity of 25.67 ± 0.88 mm against *S. aureus* and followed by *M. baccata* and *G. wallichianum*, which showed the antimicrobial activity of 22.42 ± 0.96 mm and 21.92 ± 0.74 mm against *S. flexneri* and *S. aureus*, respectively. While *M. buxifolia* showed mild antimicrobial activity against all the selected pathogenic bacteria.

Table 2. Antibacterial activity of different fractions of selected medicinal plants (zone of inhibition in mm)

Plant	Fraction	<i>Staphylococcus aureus</i>	<i>Shigella flexneri</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherchia coli</i>	<i>Salmonella typhi</i>
<i>Cucumis sativus</i>	n-hexane	18.67 ± 0.33	11.33 ± 0.88	10.80 ± 1.14	17.58 ± 0.79	23.33 ± 2.40
	DCM	09.95 ± 0.39	11.95 ± 0.53	10.85 ± 2.34	17.25 ± 0.66	16.60 ± 1.31
	Ethyle acetate	12.50 ± 0.76	15.00 ± 1.53	09.50 ± 1.04	14.17 ± 1.17	15.67 ± 0.33
<i>Portulaca olarecae</i>	n-hexane	19.00 ± 0.58	11.00 ± 0.58	08.67 ± 0.88	15.83 ± 1.01	18.00 ± 0.58
	DCM	22.00 ± 0.58	08.00 ± 1.15	11.55 ± 0.33	13.08 ± 2.55	10.67 ± 1.20
	Ethyl acetate	25.67 ± 0.88	08.33 ± 1.76	09.00 ± 1.53	15.50 ± 1.26	09.67 ± 1.20
<i>Malus baccata</i>	n-hexane	20.67 ± 0.67	27.25 ± 0.80	18.67 ± 0.67	17.67 ± 0.88	10.67 ± 0.67
	Ethyle acetate	16.67 ± 0.33	22.42 ± 0.96	09.67 ± 0.33	10.00 ± 0.00	09.67 ± 0.33
	DCM	ND	ND	ND	ND	ND
<i>Saxifraga flagellaris</i>	n-hexane	24.5 ± 1.32	24.0 ± 0.58	23.00 ± 1.53	24.67 ± 1.45	21.67 ± 0.88
	DCM	23.37 ± 1.76	24.67 ± 1.85	23.33 ± 1.67	23.67 ± 1.67	25.33 ± 1.45
<i>Geranium wallichianum</i>	n-hexane	0.67 ± 0.67	23.67 ± 1.03	03.33 ± 3.33	11.00 ± 0.58	6.98 ± 2.08
	DCM	16.00 ± 0.58	22.0 ± 2.52	13.00 ± 2.52	14.33 ± 1.20	07.67 ± 0.33
	Ethyle acetate	21.92 ± 0.74	18.93 ± 4.20	01.33 ± 0.67	11.00 ± 2.802	05.00 ± 1.155
<i>Monothea buxifolia</i>	n-hexane	21.33 ± 0.88	21.33 ± 0.667	20.33 ± 1.20	20.00 ± 1.00	20.67 ± 1.76
	DCM	20.00 ± 0.58	20.33 ± 1.20	20.67 ± 1.45	20.00 ± 0.58	20.00 ± 0.58
	Ethyle acetate	20.67 ± 0.33	18.33 ± 0.88	17.67 ± 1.20	18.00 ± 1.15	20.67 ± 0.68

ND = Not Detected, DCM = dichloromethane

Discussion

This study demonstrated the antibacterial activity of six medicinal plants against five pathogenic bacteria. The six medicinal plants relating to plants were extracted with 70% ethanol, while their fractionation was performed on polarity bases with n-hexane, ethyl acetate, and DCM, using agar well diffusion methods (Table 1 & 2). The type strains tested were a Gram-positive

bacterium *S. aureus* and Gram-negative bacteria *S. flexneri*, *P. aeruginosa*, *E. coli*, and *S. typhi*. Antimicrobial activity of medicinal plants against various pathogens can be estimated on the basis of solvent used in the extraction process. Previously, organic solvent extracts of plants were found more effective against pathogens compared to water solvent extracts. Because, these extracts were rationalized in terms of the

polarity of the compounds being extracted by each solvent, and in addition to their intrinsic bioactivity, they are capable to dissolve or diffuse in the different media used in the essay [27].

The current results indicated that the CEEs of all tested medicinal plants inhabited the bacterial pathogens tested. However, CEEs of *P. oleraceae* exhibited high activity against Gram-positive bacteria i.e. *S. aureus*, while *S. flagellaris* and *M. buxifolia* exhibited high activity against Gram-negative *E. coli* and *S. aureus*, respectively as compared to rest plants. These results are in line with previous findings [28, 29]. Several studies revealed that Gram-positive bacteria were not vulnerable to plant extract when compared to Gram-positive bacteria [30, 31]. In our data, the low efficiency of CEEs of *C. sativus* on all pathogens tested is in accordance with the previous study, where CEE of *Cucumis sativus* (*C. sativus*) have poor activity against *Pseudomonas aeruginosa* (*P. aeruginosa*) [32]. Our results suggested that ethanol extraction is the most successful method for extracting the antibacterial metabolites from medicinal plants, as compared to previous methods such as methanol, acetone, and water extraction [33].

Comparatively, CEEs of medicinal plants have low effects on bacterial strains while its fractions showed high effects (Table 2). These results are in accordance with the previous study, where the seed extracts of *Praecitrullus fistulosus* (Tinda), *Cucumis sativa* (Cucumber), *Lagenaria siceraria* (bottle gourd), and *Cucurbita pepo* (Red pumpkin) have been found very active against *E. coli*, *Serratia marcescens*, *Fusarium oxysporium*, *Streptococcus thermophilus* and *Trichoderma reesei* [34]. The n-hexane and DCM fractions of *S. flagellaris* depicted the most striking antimicrobial effects against all Gram-positive and Gram-negative bacterial strains compared to the rest extracts. The n-hexane fraction of *M. baccata* and

ethyl acetate fraction of *P. oleracea* were highly effective against *S. flexneri* and *S. aureus*, respectively than their other fractions. These findings are in agreement with Fatema and Khanum (2014) [35], who reported high antibacterial activity of ethyl acetate extract against *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *S. aureus*, *E. coli*, *S. typhi*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Shigella boydii*, *Vibrio parahemolyticus* and *Vibrio mimicus*. Mousavi *et al.*, (2015) [36] found that *P. oleracea* leaves and seeds hydroalcoholic extracts, possess significant antibacterial effects on *Streptococcus pneumoniae*, *Hafnia alvei*, *Streptococcus pyogenes*, *Acinetobacter baumannii*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *S. aureus*, *Proteus mirabilis*, and *Serratia marcescens*, and can be a worthy substitute against a drug resistance bacteria. These findings suggested that different CEE fractions of medicinal plants are highly effective against various pathogens.

Conclusion

The current study identified that the medicinal plants CEEs and their fractions (hexane, dichloromethane and ethyl acetate) demonstrated a varying degree of therapeutic potency. The n-hexane fractions indicated the broad spectrum of antibacterial activities against the tested pathogens. The results of the current study highlight the folkloric anticipation of the studied plants as antimicrobial agents and in therapeutic application. These findings suggest that most active extract can be evaluated for therapeutic antibacterial and further pharmacological assessments.

Authors' contributions

Conceived and designed the experiments: F Asad, & HA Begum, Performed the experiments: HA Begum, F Asad, Analyzed the data: F Asad, HA Begum, & M Hamayun, Contributed materials/ analysis/ tools: M

Hamayun, T Yaseen, R Hameed & A Khan. Wrote the paper: F Asad and HA Begum.

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