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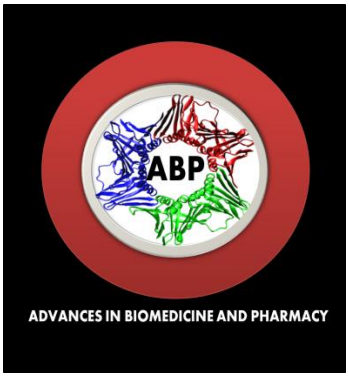

Original Article

Evaluation of antimicrobial activity of *Primula denticulata*

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Abstract

The aim of the present study was to evaluate the antibacterial and antifungal activities of *Primula denticulata* commonly called as "Primrose". Different solvents viz. ethyl acetate, chloroform, butanol, ethanol, methanol were used for the preparation of the extracts. Antibacterial and antifungal studies were carried out using agar disc diffusion and broth dilution methods. During the study it was found that the ethyl acetate extract of *P. denticulata* exhibited potent growth inhibitory effect against three bacterial strains including *Shigella flexneri, Pseudomonas aeruginosa and Escherichia coli*. The ethyl acetate extract exhibited no significant activity against *Salmonella typhimurium and Staphylococcus aureus* at 500 µg/ml and 750 µg/ml concentrations. 250 µg/ml and 500 µg/ml concentrations of the ethyl acetate extract exhibited potent inhibition of three fungal strains including *Aspergillus versicolor, Aspergillus Flavus and Candida albicans*. The choloroform extract of *P. denticulat*a was found to be effective against *Shigella flexneri, Escherichia coli and Salmonella typhimurium strains*. However, the extract showed no growth inhibitory effects against *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The methanol extract of *P. denticulata* exhibited highest growth inhibition against *Pseudomonas aeruginosa*. We therefore propose that the plant may prove as an important source of antimicrobial molecules.

Keywords: Antibacterial, Antifungal, ethyl acetate, *Primula denticulata*

Introduction

Medicinal and aromatic plants have played a vital role in alleviating human sufferings from times immemorial. The use of plant extracts with known antimicrobial properties, are of great significance to therapeutic treatments. The therapeutic properties of medicinal plants are conditioned by the presence of active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and phenolic compounds, which are biologically active in relation to the causative agents of various diseases [1]. Keeping in view, the importance of medicinal plants, one of the medically important plant namely *P. denticulata*, commonly called as *'primrose'* was taken for present study. *Primula* plants are found to be medically very important. *Primula denticulata* is a perennial plant, deciduous, clump-forming plant with compact heads of many flowers, and overwinters as large, above-ground buds with thick roots, is among the first plant to open in spring season and prefers to grow in a sunny to half-shady situation on moist soil and tolerate temperatures down to -29ºC. The saponins from Primula plants are found effective orally or by injection in the treatment of edemas. The roots of some *primula* species act as an expectorant in bronchial cataract and pneumonia [2]. Efficacy of primrose extracts which are rich in

saponins have been demonstrated in a number of pharmacological studies, which has potent anti-asthmatic, anti-inflammatory and anti-viral properties [3]. The present study was carried out with the main objective to assess the antibacterial and antifungal properties of activity of *P. denticulata* plant extract. The effect of plant extracts on bacteria has been studied by a number of researchers in different parts of the world [4-6]. Owing to the presence of a diversity of pharmacologically important molecules in *Primula* species and ethnopharmacological relevance, the present study aimed at evaluating the antimicrobial activity of *P. denticulata.*

Material and Methods

Chemicals and reagents

Sodium hydroxide (CDH), HCl (Qualigens), Nutrient Agar (Hi Media), Nutrient Broth (Hi Media), Barium sulfate (McFarlands opacity tube), normal saline, Muller Hinton Agar (Hi Media), Muller Hinton Broth (HiMedia), Mackonkey's Agar (Hi Media) and Sabouraud Dextrose Agar (Hi Media) Potato Dextrose Agar (Hi media)

Plant material

Primula denticulata was collected as a whole plant from Shopian area of Kashmir Himalaya, J&K, India. Sampling was carried out immediately after flowering and plants were collected manually in bulk from the area. All the parts were separated, washed and dried under shade for few days and then cut into small pieces. These small pieces were then made in powdered form in a wood grinder under sterilized conditions. Plant was identified at Kashmir University Herbarium (KASH), Centre of Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar.

Microbial Strains

The bacteria and fungi used in this study were clinical isolates (*Escherchia coli, Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhimurium, Staphylococcus aureus* and fungal strains viz, *Aspergillus versicolor*, *Candida albicans*, *Candida kruesie, C. Parapsilosis*, *Aspergillus flavus* and *Accremonium* sp) from patients of different epidemiological cases obtained from the Bacteriological and Mycological section (Department of Microbiology) SKIMS, Soura, Srinagar, J&K.

Extraction of parts of medicinal plant

50g of dried powder of plant material was loaded in Soxhlet extractor and was then defatted with ethyl acetate in Soxhlet extractor. The extract was collected, dried, weighed and kept for further usage and the defatted material was dried and then re-loaded in Soxhlet extractor for extraction by other solvent (chloroform, butanol, ethanol, methanol and aqueous). Extracts collected were later dried and weighed and kept for further usage in sterilized caped vials at 4ºC. The solvent-free dried extract residues were re-suspended in di-methylsulphoxide (Aq. 10 % DMSO) for subsequent analysis.

Antimicrobial Susceptibility Testing

The method of Sette *et al.* (2006) [7] was followed for MIC & MBC determination using Muller-Hinton Broth on a tissue culture test plate (96 wells) for bacterial cultures.

Evaluation of antibacterial and antifungal activity

For the evaluation of antimicrobial activity of *P. denticulata* extracts, Agar Disc diffusion assay of Kirby-Bauer method [8] (Bauer *et al*., 1966) was followed in the present study. In this method sterile discs with antimicrobial agents were poured on microbial cultures and inhibition zone diameters around the discs were measured as the potency of crude plant extracts.

Result

Antibacterial activity

The antimicrobial (antibacterial and antifungal) activities of crude extracts, including ethyl acetate, chloroform, butanol, ethanol, methanol, acetone and aqueous, of *P. denticulata* were determined against four Gram negative bacterial strains; *S. flexneri*, *P. aeruginosa*, *E. coli*, *S. typhimurium***;** one Gram positive bacterial strain - *S. aureus* and six fungal strains including *A. versicolor*, *A. flavus*, *Acremonium* sp., *C. albicans*, *C. kruesie* and *C. parapsilosis*. Different concentrations of the extracts ranging from 500 µg/ml to 750 µg/ml for the bacterial strains and 250 µg/ml to 500 µg/ml for fungal strains were analyzed. Streptomycin antibiotic was used as positive control for bacterial strains and nystatin for fungal strains. Moreover, 10% aqueous DMSO (dimethyl sulfoxide) was used as negative control. The inhibitory activities of all the extracts were found to be concentration-dependent. The antimicrobial activity was recorded as Inhibition Zone Diameter (IZD), measured in 'mm'

The ethyl acetate extract of *P. denticulata* showed an inhibitory effect against three bacterial strains (*S. flexneri*, *P. aeruginosa and E. coli*) with the highest IZD of 16mm at the concentration of 750 µg/ml against *S. flexineri* in comparison to the positive control streptomycin as shown in table 1.The lowest IZD of 5mm at concentration 500 µg/ml was recorded against *P. aeruginosa*. Two bacterial strains *S. typhimurium* and *S. aureus* were found resistant to both the concentrations of the extract. Two fungal

strains tested (*Acremonium* sp. & *C. parapsilosis*) were found to be resistant for ethyl acetate extract at both the concentrations. The fungal strains, *A. versicolor, A. flavus* and *C. albicans* were inhibited at both the concentrations (250 µg/ml & 500 µg/ml) with a maximum IZD of 16mm recorded against *A. flavus* in comparison to the positive control nystatin.

The chloroform extract of *P. denticulata* was found effective against three bacterial strains (*S.flexneri, E.coli and S. typhimurium*) with highest IZD of 12mm exhibited by *S. flexneri* at higher concentration (750 µg/ml) as depicted in Table 2. *E. coli* was susceptible to the effects of this extract at higher concentration. However the extract was ineffective against two tested bacterial strains *P. aeruginosa and S. aureus*. The lowest IZD of 5mm at concentration 500 µg/ml was recorded against *S. typhimurium*. The chloroform extract showed significant inhibitory activity against *A. flavus* and *C. kruesie* at both the tested concentrations (250 µg/ml and 500 µg/ml). The IZD recorded for *A. flavus* were 8mm and 14mm at lower and higher concentrations respectively, and that for *C. kruesie* was 10mm and 18mm in comparison to the positive control nystatin.

The butanol extract of *P. denticulata* exhibited higher activity against *P. aeruginosa* at both the concentrations (500 µg/ml and 750 µg/ml) with the highest IZD of 19mm at 750 µg/ml as shown in Table 3. All the other strains showed complete resistance against both the concentrations of the extract. Of all the six fungal strains tested, only three strains (*A. versicolor*, *Acremonium* sp.,*C. kruesie*) were found to be inhibited by both the concentrations of the butanol extract with a maximum IZD of 18mm recorded for *Acremonium* sp. at higher extract concentration (500µg/ml) with respect to the positive control nystatin.

The ethanol extract of *P. denticulata* showed antibacterial effects against the three strains (*S. flexneri, P. aeruginosa and S. typhimurium*) as depicted in table 4.The highest IZD of 28mm was recorded for *P. aeruginosa* at 750 µg/ml concentration of the extract and the lowest 11mm against *S. flexneri* and *S. typhimurium* at 500 µg/ml concentration. The ethanol extract exhibited the highest IZD among all the other extracts. The ethanolic extract exhibited significant antifungal activity towards four fungal strains (*A. versicolor, Acremonium* sp., *C. albicans and C. krusie*. The highest IZD of 19mm was observed against *Acremonium* sp. at higher concentration in comparison to the control nystatin. A minimum IZD of 8mm was recorded against (*A. versicolor & C. krusie*) at lower concentration. All the other tested fungal strains were found resistant towards ethanolic extract.

The methanol extract of *P. denticulata* showed highest inhibitory activity against *P. aeruginosa* with IZD of 20mm at higher concentration. The lower IZD of 8mm was recorded for *S. flexneri* against the positive control (streptomycin) as shown in table 5. *E.coli* and *S.aureus* were found resistant towards the methanolic extract at both the concentrations and showed no inhibitory activity. The higher methanolic concentration exhibited antifungal activity against four tested fungal strains, however all the fungal strains except *A. flavus* were found resistant at lower concentration (250 µg/ml). Among these fungal strains, the maximum IZD (16mm) was shown by *A. flavus* followed by *A. versicolor* (14mm) at higher concentration.

The aqueous extract of *P. denticulata* showed inhibitory activity against three bacterial strains (S. *flexineri, P .aeruginosa and S. typhimurium*) as shown in table 6. The highest inhibition zone of 20mm was recorded for *P. aeruginosa* and lowest inhibition zone of 8mm was recorded for three species (*S. flexneri, P. aeruginosa and S. typhimurium*).

Table 1: Antibacterial activity of ethyl acetate extract of *P .denticulata*.

Bacterial Strains	Inhibition zone diameter (mm)		
	ETHYL ACETATE EXTRACT		
	500µg/ml	750µg/ml	Streptomycin
Shigella flexneri	6±0.65	16±0.57	40±0.9
Pseudomonas aeruginosa	5±0.35	12±0.57	18±1.73
Escherichia coli	8±1.0	14±0.57	20±1.0
Salmonella typhimurium	NA	NA	18±1.0
S. aureus	NA	NA	18±1.52

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains

Table 2: Antibacterial activity of chloroform extract of *P. denticulata*

Bacterial Strains	Inhibition zone diameter (mm)		
	CHLOROFORM EXTRACT		Antibiotic
	500µg/ml	750µg/ml	Streptomycin
Shigella flexneri	8±0.98	12±0.57	20±1.0
Pseudomonas aeruginosa	NA	NA	18±1.73
Escherichia coli	NA	8±1.0	20±0.9
Salmonella typhimurium	5±0.12	11±1.0	17±0.57
S. aureus	NA	NA	17±0.57

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains

Table 3: Antibacterial activity of butanol extract of *P. denticulata*

Bacterial Strains	Inhibition zone diameter (mm)		
	BUTANOL EXTRACT		Antibiotic
	500µg/ml	750µg/ml	Streptomycin
Shigella flexneri	NA	NA	25±1.0
Pseudomonas aeruginosa	15±0.34	19±1.0	35±1.52
Escherichia coli	NA	NA	30±0.57
Salmonella typhimurium	NA	NA	20±0.12
S. aureus	NA	NA	20±1.0

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Table 4: Antibacterial activity of ethanol extract of *P. denticulata*

Bacterial Strains	Inhibition zone diameter (mm)		
	ETHANOL EXTRACT		Antibiotic streptomycin
	500µg/ml	750µg/ml	
Shigella flexneri	11±1.0	17±0.57	37±1.0
Pseudomonas aeruginosa	12±0.57	28±1.52	35±0.57
Escherichia coli	NA	NA	32±1.0
Salmonella typhimurium	11±0.57	17±0.08	38±1.0
S.aureus	NA	NA	25±1.52

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Table 5: Antibacterial activity of methanol extract of *P. denticulata*

Bacterial Strains	Inhibition zone diameter(IZD) mm		
	METHANOL EXTRACT		Antibiotic streptomycin
	500µg/ml	750µg/ml	
Shigellaflexneri	8±0.57	12±0.46	25±1.52
Pseudomonas aeruginosa	11±1.0	20±1.52	30±0.57
Escherichia coli	NA	NA	30±0.57
Salmonella typhimurium	12±1.0	14±0.34	25±1.52
S. aureus	NA	NA	20±1.52

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Table 6: Antibacterial activity of aqueous extract of *P. denticulata*

Bacterial Strains	Inhibition zone diameter (mm)		
	AQUEOUS EXTRACT		Antibiotic streptomycin
	500µg/ml	750µg/ml	
Shigella flexneri	8±0.57	12±1.0	30±1.521.0
Pseudomonas aeruginosa	8±0.43	20±0.57	35±0.57
Escherichia coli	NA	NA	25±0.2
Salmonella typhimurium	8±1.0	16±0.57	25 ±1.8
Staphylococcus aureus	NA	NA	20±1.0

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Antifungal activity

No activity was observed for the lowest concentration of the aqueous extract (250µg/ml). The maximum IZD of 12 mm was recorded for *C. albicans* & *C. parapsilosis* at higher concentration (500 µg/ml) (Table 7-Table 11).

Table 7: Antifungal activity of ethyl acetate extract of *P. denticulata*

Fungal Strains	Inhibition zone diameter (mm)		
	ETHYL ACETATE EXTRACT		Antibiotic
	250µg/ml	500 µg/ml	Nystatin
Aspergillus versicolor	8±0.4	14±0.5	20±0.9
Aspergillus flavus	8±0.54	16±0.32	20±1.0
***Acremonium* sp.**	NA	NA	11±0.68
Candida albicans	6±0.34	12±0.98	18±0.12
Candida kruesie	8±0.10	14±0.5	18±0.17
Candida parapsilosis	NA	NA	15±0.12

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Table 8: Antifungal activity of chloroform extract of *P. denticulata*

Fungal Strains	Inhibition zone diameter (mm)		
	CHLOROFORM EXTRACT		Antibiotic
	250µg/ml	500µg/ml	Nystatin
Aspergillus versicolor	NA	NA	20±0.33
Aspergillus flavus	8±0.54	14±1.0	15±0.1
***Acremonium* sp.**	NA	NA	22±0.9
Candida albicans	NA	NA	20±0.03
Candida kruesie	10±0.33	18±2.08	21±0.9
Candida parapsilosis	NA	NA	17±0.1

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Table 9: Antifungal activity of butanol extract of *P. denticulata*

Fungal Strains	Inhibition zone diameter (mm)		
	BUTANOL EXTRACT		Antibiotic
	250µg/ml	500µg/ml	Nystatin
Aspergillus versicolor	8±0.5	15±1.4	11±1.15
Aspergillus flavus	NA	NA	17±1.52
***Acremonium* sp.**	10±0.8	18±2.08	19±0.8
Candida albicans	NA	NA	17±1.6
Candida kruesie	8±0.5	14±1.0	21±0.57
Candida parapsilosis	NA	NA	18±1.0

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains

Table 10: Antifungal activity of ethanol extract of *P. denticulata*

	Inhibition zone diameter (mm)		
Fungal Strains	METHANOL EXTRACT		Antibiotic
	250µg/ml	500µg/ml	Nystatin
Aspergillus versicolor	NA	14±0.5	20±0.5
Aspergillus flavus	8±0.5	16±0.32	25±0.32
***Acremonium* sp.**	NA	11±0.32	20±0.5
Candida albicans	NA	13±0.5	16±0.32
Candida kruesie	NA	NA	21±0.5
Candida parapsilosis	NA	NA	18±0.5

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Table 11: Antifungal activity of aqueous extract of *P. denticulata*

Fungal Strains	Inhibition zone diameter (mm)		
	AQUEOUS EXTRACT		Antibiotic
	250µg/ml	500µg/ml	Nystatin
Aspergillus versicolor	NA	10±0.5	25±0.32
Aspergillus flavus	NA	NA	20±0.5
***Acremonium* sp.**	NA	NA	21±0.5
Candida albicans	NA	12±0.32	18±2.0
Candida kruesie	NA	NA	21±0.32
Candida parapsilosis	NA	12±0.32	22±0.32

* Values are represented as mean ± SD; All experiments were carried thrice; NA= no activity; 10% Aq. DMSO was used as negative control and was found resistant in all strains.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC of *P. denticulata* was determined by using the Broth dilution assay. Agents with low activity against a particular organism usually gives high MIC and MBC values, while a highly reactive agent gives low MIC and MBC values. As shown in Table 12, methanol, butanol and ethanol and aqueous extracts of *P. denticulata*, MIC values were 225 µg/ml, 500 µg/ml and 300 µg/ml and 725 µg/ml against *P. aeruginosa*; 250 µg/ml, 500 µg/ml, 500 µg/ml and 1000 µg/ml against *S. typhimurium* respectively, in comparison to the positive control, Erythromycin. MBC values were 425 µg/m, 725 µl/ml, and 1000µg/ml against *P. aeruginosa;* 725 µg/ml, 1000 µg/ml, and 500 µg/ml against *S. typhimurium* respectively, in comparison to the positive control, erythromycin.

Table 12: MIC and MBC values of 4 different extracts of *P. denticulata* against *P. aeruginosa* and *S. typhimurium.*

Plant Extracts	*P. aeruginosa*			*S. typhimurium*		
	MIC	**MBC**	**MIC index**	**MIC**	**MBC**	**MIC index**
Methanol	225	425	1.88	250	725	2.9
Butanol	500	725	1.45	500	1000	2
Ethanol	300	1000	3.33	500	500	1
Aqueous	725	-	-	1000	-	-
Erythromycin	100	50	0.5	75	125	1.66

Discussion

Natural compounds from plants represent a major source of molecules with medicinal properties. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the world. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds. In the present investigation, different extracts of *P. denticulata* was evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria and some fungal strains. Secondary metabolites of plant origin appear to be one of the alternatives for the control of antibiotic resistant human pathogens. Thus, antibacterial activity may be due to the presence of secondary metabolites [9]. The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Pharmaceutical and scientific communities have recently received the attention of the medicinal plants and various publications have documented the therapeutic worth of natural compounds to validate the claims of their biological activity. Medicinal plants have their intrinsic ability to resist pathogenic microorganisms and this has led the researchers to investigate their mechanisms of action and isolation of active compounds. This has enabled exploitation of medicinal plants for the treatment of microbial infections of both plants and humans by developing new antimicrobial agents. The use of natural products for treatment of a number of diseases is due to their less harmful effects as compared to drugs synthesized in the laboratory and are safe with very little side effects if used in proper dosage [10]. Susceptibility of each plant extract was tested by disk diffusion method. The antimicrobial activity of all the extracts of the plant at different concentrations; 500 µg/ml and 750 µg/ml against different bacterial strains and 250 µg/ml and 500 µg/ml against fungal strains was confirmed by broth dilution method. The disk diffusion test is a commonly used method to examine the antimicrobial activity based on the principle of diffusion of the compound tested through agar medium. Dimethylsulfoxide (DMSO) was used for re-dissolving the solvent-free dried plant extract residues as has been done in many other studies [11, 12]. The sterile paper discs previously impregnated with the test substances are placed on the surface of the Mueller-Hinton agar medium [13] inoculated with the target organisms, for the diffusion of antimicrobial agents. The plates are incubated and the zones of inhibition around each disc are measured.

The antimicrobial activity of the plant extracts against the different clinical strains of bacteria (*S. flexneri*, *P. aeruginosa*, *E. coli*, *S. typhimurium*, *S. aureus*) and fungi (*A. versicolor*, *A. flavus*, *Acremonium* sp, *C. albicans*, *C. kruesie* and *C. parapsilosis*) supported the scientific validity of the plant being used traditionally as a medicine. Butanol yielded the highest average concentration of plant material as 4.5 g, followed by methanol, ethanol, ethyl acetate, chloroform and a minimum of 2.9 g for aqueous extracts. The increased extract yielded may be due to the fine plant particles (increased surface area). This correlates with observations of [14] Eloff, (1998) who reported that finely processed plant material (increased surface area) facilitates the production of concentrated plant material from extraction solvents.

The preliminary investigation showed that ethanol, methanol, and aqueous extracts of *P. denticulata* were strongly active against the pathogens like *S. flexneri*, *P. aeruginosa*, *S. typhimurium*. The antibacterial activity was evaluated by measuring the diameter of inhibition zone.

The biggest inhibition zone (28mm) was observed with ethanol fraction. Similar findings were also reported by [15] (Gamze *et al*., 2008). Except ethyl acetate extract, none of the other extracts showed inhibitory zone against *E. coli*. The results reveal that except chloroform and butanol, all the other extracts yielded good antimicrobial activity inhibiting greater number of bacterial strains, thereby confirming the potential of *Primula* herb for the presence of bioactive compounds. Because of water extract found as active against many bacterial strains results of this study support traditional use of this herb as tea prepared from *Primula* extracts .The increased antimicrobial activity may be attributed to the presence of soluble phenolic and polyphenolic compounds [16]. The lack of antibacterial activity in some of the concentrations of the extract is not surprising as a number of plant extracts have been found ineffective against certain test organisms at lower concentrations and may be attributed to the presence of lesser amounts of the antimicrobial compounds. Antifungal activity was mostly observed at 500 µg/ml. All the extracts showed antifungal properties against one or the other strains but excellent antifungal activity was found in the extracts of ethyl acetate and ethanol against *A. versicolor*, *C. albicans*, and *C. kruesie*).The antifungal activity of ethyl acetate extract of *Primula* showing excellent results . The antifungal activity of this plant may be attributed to its biologically active constituents. Several studies have attributed the antifungal activity of plant extracts to the presence of saponins [17, 18]. Several studies have been conducted to understand the mechanism of action of plant extracts, but it is still unclear [19]. However, some researchers attributed the antifungal activity to the phenolic compounds. It is evident from the results of the current study that susceptibility of pathogens to plant extracts depends upon solvent used for extraction and extract concentration [20], as well as the organism tested [19, 21]. The antibacterial activity of ethanol, methanol and aqueous extracts compared with that of the standard antibiotics appeared to be effective.

The results demonstrated a wide variation in antimicrobial activity of extracts of *P. denticulata* against the tested organisms. One of the measures of assaying the effectiveness of antimicrobial agents is to determine their Minimum Inhibition Concentration (MIC) and Minimum Bacterial Concentration (MBC) values, MIC and MBC of *Primula denticulata* was determined using the broth dilution assay against the specific bacterial strains. Two bacterial strains *P. aeruginosa* and *S. typhimurium* were found viable for testing with the specific plant extracts. The bactericidal and bacteriostatic effects of three extracts were determined using the ratio MBC/MIC, called MIC index. Many factors affect the MIC and MBC values including temperature, inoculum size and type of organism [22]. As shown in Table 12, the MIC values for methanol, butanol, ethanol and aqueous plant extracts were 225 µg/ml, 500 µg/ml ,300 µg/ml and 725 µg/ml against *P. aeruginosa* ; 250 µg/ml, 500 µg/ml, 500µg/ml and 1000 µg/ml against *S. typhimurium* respectively. In this study, the MIC and MBC values tend to support the results obtained in the antibacterial screening, showing clearly that the methanol extract was most potent than either ethanol or butanol extracts. In this study, the MIC values were lower than the MBC values, similar to the results of Karou *et al*. (2006) [23]. George *et al*.(2002) [24] explained that the observed differences to be due to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activities. Hugo and Russell, (1984) [25] have reported that the MBC values can either be the same or higher than the MIC values.

Conclusion

The results suggest that *P. denticulata* is a potential source of antibacterial and anti-fungal molecules. The plant extract can be used as natural preservative in food and non-food systems. However, further research endeavors are required for the isolation of bioactive molecules from the plant that may be further tested for in-depth studies.

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Conflict of interest

The authors declare that there is no conflict of interest.

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