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Antimicrobial and Antihypercholesterolemic Activities of *Pulicaria gnaphalodes*

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Abstract

Multidrug resistance has increased globally in the communities. Bacterial infections associated with health care have weakened the existing antimicrobial therapy and demand the search for alternative therapies. In the present investigation, the medicinal plant *Pulicaria gnaphalodes* from Quetta, Pakistan, has been screened for antimicrobial potential. In vitro antimicrobial efficacy of *P gnaphalodes* extracts (methanol and ethanol) was quantitatively evaluated on the basis of zone of inhibition against different bacteria and minimum inhibitory concentration (MIC). In vivo, antihypercholesterolemic activity is determined in different rat groups. The results of the study indicated that the ethanol extract of *P gnaphalodes* showed maximum zone of inhibition for *Bacillus subtilis* of 12.1 $\pm$ 1.1 mm from all others. The methanol extract showed maximum zone of inhibition for *Staphylococcus aureus* of 11.9 $\pm$ 1.0 mm and rifampicin showed maximum zone of inhibition of 23.1 $\pm$ 0.9 mm. The results of ethanol and methanol extract of *P gnaphalodes* against different bacteria revealed that this plant has greater antimicrobial activity. However, the plant extract shows nonsignificant antihypercholesterolemic activity. The extract of this plant can be utilized as medicine to inhibit several infections caused by some bacterial pathogens found in human body.

Keywords

Pulicaria gnaphalodes, antimicrobial, antihypercholesterolemic, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pasteurella multocida*

Introduction

The *Pulicaria* genus has its place in the family Compositae (Asteraceae), which comprises more than 100 species that are commonly dispersed throughout Africa, Asia, and Europe.[1,2] The genus contains a number of chemical constituents, including diterpenes and terpenes, such as monoterpenes, xanthanes, germacranes, guaianes, pseudoguaianes, eudesmanes, bisabolenes, caryophyllanes, diterpenoids, triterpenoids, steroids, flavonoids, and essential oils.[3,4] Several biological activities of this genus have been described such as antimicrobial, antibacterial, leishmanicidal, and antifungal.[3,5-7] *Pulicaria* plants are usually consumed as flavoring agent, herbal tea, and medicinal plant.

Pulicaria gnaphalodes is an obstinate plant about 30 $\pm$ 8 cm high and have gold-yellow flowers. The plant mostly grows on sandy, stony, and abandoned areas in the Saudi Arabia, Afghanistan, Pakistan, Iran, India, Iraq, and Turkey.[8] The plant is also known as Kakkosh-Biabani.[9] This plant contains numerous chemical components, including flavonoids such as giperoside and pulicarin, phenol acid such as salicylic acid, clerodane diterpenoids such as salvicin and salvifolin, alpha-pinene, and 1-8-cineole.[10,11] This plant or its components are utilized globally in folk medicine because of its

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antibacterial, antidiarrheal, anti-inflammatory, antioxidant, and leishmanicidal properties.[6,10,12] It cures many infectious diseases such as diarrhea and cutaneous abscesses. It is also utilized conventionally as a flavoring agent in food. Many studies have reported the antibacterial, antimicrobial, and leishmanicidal activity of essential oils of *P gnaphalodes*. The current study has been conducted to assess the antimicrobial property of ethanol and methanol extract of *P gnaphalodes* against different bacteria in vitro and also described its effect on lipid profile in vivo. However, there is no reported study in the past about the effect of *P gnaphalodes* on the cholesterol level.

Materials and Methods

Microbial Strains

Four bacterial species were cultured for these tests comprising gram-positive *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus thuringiensis*, and gram-negative *Escherichia coli*. At diagnostic laboratory of GC University, Faisalabad, the antibacterial activity tests were accomplished. In nutrient agar, bacteria were cultured at 37°C.

Plant Collection

Stems of the *P gnaphalodes* were assembled from Gilgit-baltistan. The plant was identified on macroscopic characteristics relating to authenticated sample from the horticulture at Faisalabad, Pakistan.

Preparation of Extract

The stems of the sample plant were washed with distilled water. Then fine powder was prepared and sieved with 150-mesh sieve after air drying at 35°C in a shaded place. Two solvents, methanol and ethanol, were used for extraction of the plant. Thirty grams of this powder was taken for each solvent in 300 mL, which is placed inside the Soxhlet extractor. After extraction, the filtration of solvent having plant material was carried out using Whatman No. 1 filter. After filtration, the solvents were loaded into Petri dishes for evaporation of solvents to get plant extract, or rotary apparatus was used for rapid evaporation.

In Vitro Determination of Antibacterial Activity

Disk diffusion method. For antimicrobial susceptibility testing, disk diffusion method was accomplished as defined by Anand et al[13] to determine the existence of antimicrobial activity of the plant extracts. The pure cultures of *Staphylococcus aureus, Pasteurella multocida*, *Bacillus subtilis*, and *E coli* were subcultured in nutrient agar. Positive control was prepared using rifampicin. Sterile paper disks was 6 mm in diameter and made from filter paper and impregnated with drug, comprising solution put on the inoculated agar. These Petri dishes were incubated for 24 hours and then antibacterial activity was deliberated by the diameter measurement of the growth inhibition zone.

Table 1. Antibacterial Activity of *Pulicaria Gnaphalodes* Extracts and Diameter of Inhibition Zone (mm) Including Well Diameter of 6 mm.[a]

Bacterial Strains	Ethanol Extract Zone of Inhibition, mm	Methanol Extract Zone of Inhibition, mm	Rifampicin Zone of Inhibition, mm
Escherichia coli	10.1 ± 0.9	10.7 ± 0.8	19.7 ± 0.8
Pasteurella multocida	10.8 ± 0.6	9.7 ± 1.1	21.7 ± 1.1
Bacillus subtilis	12.1 ± 1.1	11.2 ± 0.9	23.1 ± 0.9
Staphylococcus aureus	11.4 ± 0.8	11.9 ± 1.0	22.3 ± 1.2

[a]Values are the means ± standard deviation of triplicate.

Minimum inhibitory concentration assay. Agar well dilution method was utilized for the determination of minimum inhibitory concentration (MIC). In 10% dimethyl sulfoxide, the ethanol and methanol extracts were distinctly dissolved and then diluted to a minimum concentration (500 µg/mL). Nutrient broth (95 µL) was mixed with inoculum (5 µL) to 96-well plate preparation. Plant extract (100 µL) was added in the first well. Then this 100 µL was relocated into 6 consecutive wells. All these plates were incubated for 24 hours and then each plate absorbance was taken at 650 nm by the microplate reader.

In Vivo Determination of Antihypercholesterolemic Activity

Procurement of animals. Thirty male Albino rats of 150 to 225g were purchased from the Animal House of Department Microbiology of Agriculture University, Faisalabad. These rats were kept under the standardized conditions such as stable room temperature of 25°C with alternating 12-hour periods of light and darkness, and the humidity was kept in 50% to 60%. Ethical committee of the Government College University, Faisalabad, approved the in vivo study. World Health Organization guideline has been followed to conduct the animal study.

Hypercholesterolemia induction and treatment protocol. Hypercholesterolemia was introduced into the rats for 30 days by oral administration of cholesterol of 1%. At the end of adaptation period, the rats were separated into 5 groups each having 6 rats.=Group 1 was the control rats group that had no hypercholesterolemia and did not receive any treatment at the chow maintenance diet (48g/kg bw). Group 2 comprises hypercholesterolemic rats administered peanut oil in order to induce cholesterolemia. Group 3 had the hypercholesterolemic rats administered allopathic medicine atorvastatin (10 mg/kg bw/d) along with peanut oil. Groups 4 and 5 had hypercholesterolemic rats administrated *P gnaphalodes* ethanol extract at a dose of 100 and 250 mg/kg bw/d, respectively, along with administration of peanut oil orally.

Blood sample. After 30 days, all the rats were decapitated, and the blood samples were isolated from all experimental rats.

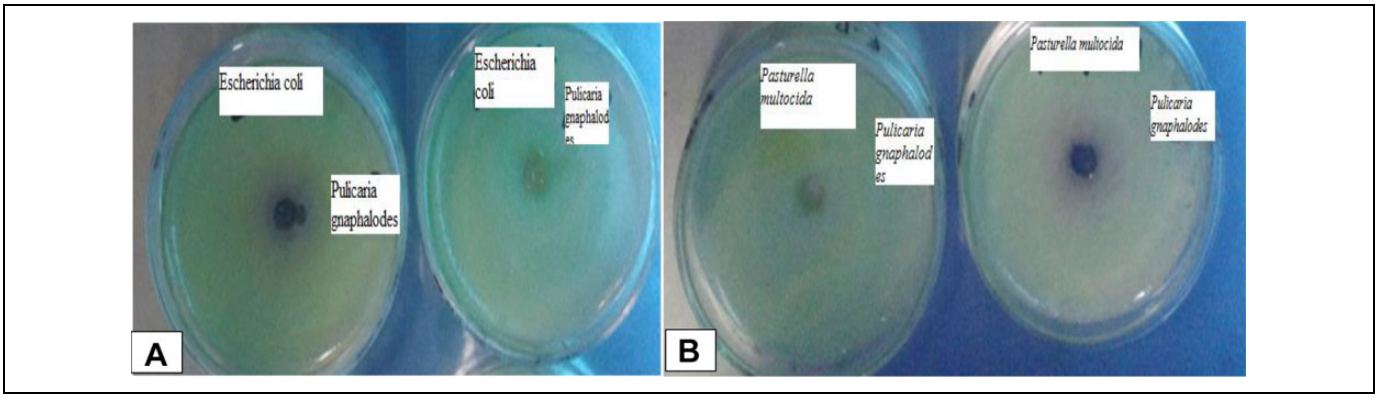

Figure 1. *Escherichia coli* and *Pasteurella multocida* killed by extract of *Pulicaria gnaphalodes*. The ethanol and methanol extracts of P gnaphalodes show the zone of inhibition in Escherichia coli (A) and Pasteurella multocida (B) bacterial culture plate.

Subsequently, the serum was isolated from all rats for the analysis of serum lipid profile parameters and serum liver profile parameters.

Lipid profile. The total lipid profile levels including total cholesterol (TC), high-density lipoprotein (HDL), and triglycerides were found in the blood serum by utilizing the commercially accessible reagent kits (Randox Laboratories, Kearneysville, West Virginia). Low-density lipoprotein level was found by subtraction of the HDL cholesterol concentration from the concentration of the TC.

Liver profile and bilirubin level. Alanine amino transferase (ALT), alkaline phosphate (ALP), bilirubin direct, bilirubin indirect, and bilirubin total were determined by commercially available kits.

Statistical Analysis

The *P gnaphalodes* influence was explicated by the 1-way analysis of variance utilization. For this study, the SPSS 23 software was used. *P* value $\leq .05$ was considered significant.

Results

Antibacterial Activity of P Gnaphalodes

In this study, the zone of inhibition of bacterial pathogens that are found in human body (*E coli, P multocida*, *B subtilis*, and *S aureus*) was determined using ethanol and methanol extracts of *P gnaphalodes* and rifampicin. The ethanol and methanol extract of *P gnaphalodes* and rifampicin showed the zone of inhibition against *E coli* and was 10.1 $\pm$ 0.9 mm and 10.7 $\pm$ 0.8 mm and 19.7 $\pm$ 0.8 mm, respectively (Table 1). In vitro zone of inhibition of ethanol and methanol extract of this plant and rifampicin against *P multocida* was 10.8 $\pm$ 0.6 mm and 9.7 $\pm$ 1.1 mm and 21.7 $\pm$ 1.1 mm, respectively. The ethanol and methanol extract of plant and rifampicin against *B subtilis* showed zone of inhibition of 12.1 $\pm$ 1.1 and 11.2 $\pm$ 0.9 and 23.1 $\pm$ 0.9 mm, respectively. For *Staphylococcus aureus*, the inhibition zone of ethanol, methanol, and rifampicin plant extract are 11.4 $\pm$ 0.8, 11.9 $\pm$ 1.0, and 22.3 $\pm$ 1.2 mm, respectively (Table 1).

Table 2. Minimum Inhibitory Activity of *Pulicaria gnaphalodes*.[a]

Bacterial Strains	Ethanol Extract	Methanol Extract	Rifampicin
Escherichia coli	4.1 $\pm$ 0.2	3.9 $\pm$ 0.2	0.5 $\pm$ 0.0
Pasteurella multocida	3.4 $\pm$ 0.1	3.4 $\pm$ 0.1	0.4 $\pm$ 0.0
Bacillus subtilis	3.1 $\pm$ 0.2	2.9 $\pm$ 0.3	0.2 $\pm$ 0.0
Staphylococcus aureus	2.8 $\pm$ 0.3	3.1 $\pm$ 0.2	0.1 $\pm$ 0.0

Abbreviations: MIC, minimum inhibitory concentration.
[a]Values are the means $\pm$ standard deviation of triplicate.

Determination of MIC

a. Escherichia coli

The ethanol and methanol extract of *P gnaphalodes* and rifampicin kill the *E coil* with zone of inhibition of 4.1 $\pm$ 0.2 mg/mL and 3.9 $\pm$ 0.2 mg/mL and 0.5 $\pm$ 0.0 mg/mL, respectively (Figure 1).

b. Pasteurella multocida

The ethanol and methanol extract of *P gnaphalodes* and rifampicin kill the *P multocida* with MIC value of 3.4 $\pm$ 0.1 mg/mL 3.4 $\pm$ 0.1 mg/mL, and 0.4 $\pm$ 0.0 mg/mL, respectively (Figure 1). The extracts of *P gnaphalodes* give better results in killing *P multocida* than rifampicin, which gives minor results (Table 2).

c. Bacillus subtilis

The ethanol and methanol extract of *P gnaphalodes* and rifampicin kill *B subtilis* with the zone of inhibition of 3.1 $\pm$ 0.2 mg/mL and 2.9 $\pm$ 0.3 mg/mL and 0.2 $\pm$ 0.0 mg/mL, respectively (Figure 2). The results show that the ethanol extract of *P gnaphalodes* gives better results to kill *B subtilis* (Table 2).

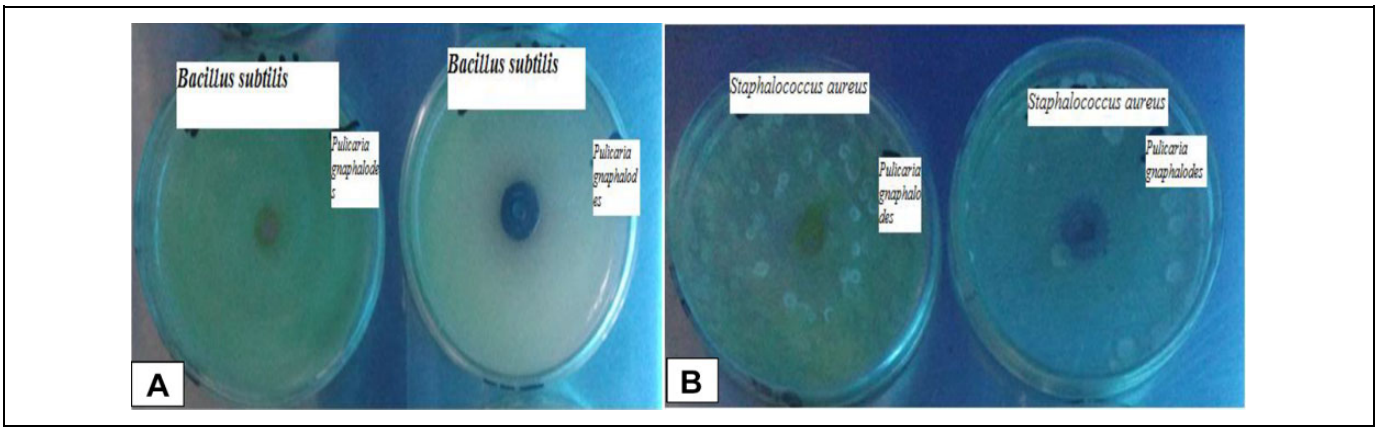

Figure 2. *Bacillus subtilis* and *Staphylococcus aureus* killed by extract of *Pulicaria gnaphalodes*. The ethanol and methanol extracts of P gnaphalodes show the zone of inhibition in Bacillus subtilis (A) and Staphylococcus aureus (B) bacterial culture plate.

Table 3. Lipid Profile (mg/dL) in Different Groups of Experimental Rats at Day 0 and 30.

	TC	HDL	LDL	Triglyceride
Day 0				
Group 1	196 ± 1	59 ± 1.1	137 ± 2	151 ± 1
Group 2	220 ± 2	45 ± 1	175 ± 1.9	156 ± 2
Group 3	224 ± 1.1	43 ± 2	181 ± 1	158 ± 1.1
Group 4	218 ± 1.9	50 ± 1	168 ± 1.5	160 ± 1.9
Group 5	214 ± 1.6	49 ± 1.9	165 ± 1.1	154 ± 1
Day 30				
Group 1	200 ± 0.7	61 ± 0.2	139 ± 0.0	153 ± 0.0
Group 2	239 ± 0.2	40 ± 0.7	199 ± 0.8	159 ± 0.2
Group 3	211 ± 0.7	55 ± 0.0	156 ± 0.7	150 ± 0.8
Group 4	229 ± 0.0	45 ± 0.8	184 ± 0.8	166 ± 0.0
Group 5	223 ± 0.0	44 ± 0.2	179 ± 0.2	160 ± 0.7

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol.

d. Staphylococcus aureus

The ethanol and methanol extract of *P gnaphalodes* and rifampicin kill the *S aureus with* zone of inhibition of 2.8 ± 0.3 mg/mL, 3.1 ± 0.2 mg/mL, and 0.1 ± 0.0 mg/mL, respectively (Figure 2). The results exposed that the methanol extract of *P gnaphalodes* gives better results to kill S *aureus* than the ethanol extract and the rifampicin showed negligible inhibition (Table 2).

Lipid and Liver Profile

The ethanol extract of *P gnaphalodes* did not show any significant antihypercholesterolic activity in the hypercholesterolemic rats (Table 3). The results of ALT and ALP are also nonsignificant in the experimental groups (Table 4). Bilirubin level in the blood was not improved by the induction of extract in hypercholesterolemic rats (Table 5).

Discussion

Numerous types of herbs and plants are stated to be a worthwhile basis of medicines. Diverse medicinal actions of

Table 4. Liver Profile (U/L) in Different Groups of Experimental Rats at Day 0 and 30.

	Day 0	Day 30	Day 0	Day 30
	ALT	ALT	ALP	ALP
Group 1	39 ± 1.0	50.6 ± 0.8	246 ± 3.01	246.1 ± 3.01
Group 2	41 ± 2.0	58.9 ± 3.0	246 ± 3.01	302.9 ± 5.02
Group 3	38 ± 1.5	45.3 ± 2.9	245 ± 3.00	347.3 ± 5.55
Group 4	42 ± 2.5	61.8 ± 3.36	245 ± 3.00	373.6 ± 6.63
Group 5	43 ± 2.6	55.0 ± 4.28	250 ± 3.05	393.1 ± 6.94

Abbreviations: ALT, Alanine amino transferase; ALP, alkaline phosphate.

P gnaphalodes extracts in solvents (methanol and ethanol) was examined. In the present study, the plant was gathered from Quetta, Pakistan, and these stems of the plants were ground to fine powder by grinding the sample. Plant extracts were prepared from the finely ground dry sample of *P gnaphalodes* in 2 solvents, methanol and ethanol. The present study is designed to evaluate the antimicrobial activity of ethanol and methanol extracts of *P gnaphalodes.* The antimicrobial effect was determined by measuring the zone of inhibition. Four bacteria including *E coli*, *P multocida*, *B subtilis*, and *S aureus* strains were selected. Ethanol extract of *P gnaphalodes* showed the zone of inhibition against *E coli* is 10.1 ± 0.9, methanol showed zone of inhibition of 10.7 ± 0.8 mm, and rifampicin showed 19.7 ± 0.8 mm. The in vitro zone of inhibition of ethanol and methanol extract of this plant against *P multocida* is 10.8 ± 0.6 mm and 9.7 ± 1.1 mm, respectively and that of rifampicin is 21.7 ± 1.1 mm. The ethanol and methanol extract of plant and rifampicin against *B subtilis* showed zone of inhibition of 12.1 ± 1.1 and 11.2 ± 0.9 and 23.1 ± 0.9 mm, respectively, and for *S aureus*, the inhibition zone of ethanol, methanol, and rifampicin plant extract is 11.4 ± 0.8, 11.9 ± 1.0 and 22.3 ± 1.2 mm, respectively. The results of study revealed that the ethanol extract of *P gnaphalodes* showed maximum zone of inhibition of 12.1 ± 1.1 mm for *B subtilis* compared to all others. The methanol extract showed

Table 5. Total Bilirubin, Direct Bilirubin, and Indirect Bilirubin (mg/dL) in Different Groups of Experimental Rats.

	Day 0	Day30	Day 0	Day 30	Day 0	Day 30
	Total	Total	Direct	Direct	Indirect	Indirect
Group 1	0.5 ± 0.0	0.53 ± 0.1	0.23 ± 0.0	1.4 ± 0.1	0.75 ± 0.0	1.8 ± 0.3
Group 2	0.4 ± 0.0	0.42 ± 0.1	0.22 ± 0.0	1.2 ± 0.1	0.73 ± 0.0	1.7 ± 0.2
Group 3	0.5 ± 0.0	0.53 ± 0.2	0.25 ± 0.0	1.8 ± 0.3	0.68 ± 0.0	1.4 ± 0.1
Group 4	0.4 ± 0.0	0.50 ± 0.0	0.22 ± 0.0	1.4 ± 0.1	0.70 ± 0.0	1.6 ± 0.1
Group 5	0.5 ± 0.0	0.55 ± 0.2	0.25 ± 0.0	1.8 ± 0.3	0.69 ± 0.0	1.5 ± 0.1

maximum zone of inhibition for *S aureus* of 11.9 ± 1.0 mm and rifampicin showed maximum zone of inhibition of 23.1 ± 0.9 mm. The results of antibacterial activity of *P gnaphalodes* revealed that ethanol and methanol extract of this plant have greater antimicrobial activity. The MIC results indicated that ethanol extract of the plant is more affective against *Staphylococcus aureus*, and methanolic extract of the plant was more effective against the *B subtilis.* Gandomi et al[10] evaluated the antibacterial and antifungal properties of the *P gnaphalodes.* They demonstrated that ethanolic and methanolic extracts exhibited more inhibition zone as compared to the aqueous extract. With MIC of 0.025%, 0.8%, 0.1%, and 0.1% for essential oil, aqueous, ethanolic, and methanolic extracts, respectively, *Bacillus cereus* was the utmost sensitive strain. The essential oils have better ability to inhibit the fungal growth as compared to the extracts. The MIC results of the present study showed that ethanolic extract of the plant was more potent against the *S aureus* with MIC of 2.8 ± 0.3. The methanolic extract of the plant was more effective against the *B subtilis* with MIC of 2.9 ± 0.3. Hozoorbakhsh et al[14] reported the antibacterial activity of *P gnaphalodes* against the *Mycobacterium tuberculosis*. They demonstrated that *P gnaphalodes* essential oil extracts possess strong inhibitory effects on *M tuberculosis*. The mean of inhibition percentage for *P gnaphalodes* in 640 µg/mL was 58.1%. In the present investigation, the in vivo study was also conducted on 30 albino rats. The hypercholesterolemia was induced by giving the cholesterol 1% for 30 days. Then the rats were separated into 5 groups each with 6 rats. Group 1 and group 2 were the negative control and positive control, respectively. Groups 3, 4, and 5 were treated group with allopathic medicine atorvastatin (10 mg/kg bw/d) and *P gnaphalodes* ethanol extract at the dose of 100 and 250 mg/kg bw/d, respectively. After 30 days, blood samples were collected for lipid and liver profile. The extracts of *P gnaphalodes* did not show any significant effect on lipid and liver profiles. However, minor increase in the bilirubin level shows that plant may reduce the cholesterol level in a dose-dependent manner. In the past, no study was reported which describes the effect of *P gnaphalodes* on lipid and liver profiles, and more research is required.

Conclusion

Pulicaria *gnaphalodes* has antibacterial potential against *E coli, P multocida*, *B subtilis*, and *S aureus*. The ethanolic and methanolic extracts of the *P gnaphalodes* were more effective against the *S aureus* with MIC of 2.8 ± 0.3 and *B subtilis* with MIC of 2.9 ± 0.3. However, *P gnaphalodes* does not have not any significant effect on lipid profile. The antimicrobial activity of the plant suggests possibility of utilizing it in the health care to prevent the growth of bacteria that cause infection and improve the safety of human life.

Declaration of Conflicting Interests

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