Research Article

Antimicrobial activities of different solvent extracted sample of *Hippophae rhamnoides* L. (Sea Buckthorn)

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Citation

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Abstract

The current study was aimed to investigate the antibacterial and antifungal activities of various extracted samples of fruit of *Hippophae rhamnoides* L. The antibacterial and antifungal activities of various solvent extracted samples (crude ethanolic, chloroform, butanolic, ethyl acetate and aqueous) of *Hippophae rhamnoides* L. fruit were investigated through Agar Well Diffusion Method against five bacterial strain (two were Gram positive and three were Gram negative) and three fungal strains. The data was taken in comparison with reference drugs like azithromycin and clotrimazole. Three different concentrations i.e., 1mg/well, 2mg/well and 3mg/well each of five extracts were subjected against five pathogenic bacterial isolates i.e. *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* and three fungal isolates i.e. *Curvulria*, *Alternaria* and *Rhizopus spp.* The antibacterial effects of crude ethanolic and aqueous extracts were highest, followed by butanol, chloroform and ethyl acetate fraction respectively. The crude aqueous and ethanolic extracts showed highest inhibitory activity against *Salmonella typhi* (66.87±1.15%, 66.9±0.577%), *Staphylococcus aureus* (97.5±1%, 88.5±0.577%), *Escherichia coli* (80.58±0.577%, 69.70±1.52%), *Bacillus subtilis* (93.54±3.60%, 77.41±1.73%) and *Pseudomonas aeruginosa* (95.67±1.15%, 91.34±2.30%) at concentration 3mg/well. Similarly, maximum antifungal activity was shown by ethanolic extract i.e. 96.37±0.577% zone of inhibition against *Curvularia sp.* 79.28±1.52% against *Alternaria* and 89±1% against *Rhizopus sp.* at concentration of 3mg/well.

Keywords: Antibacterial; Antifungal; *Hippophae rhamnoides* L.; Sea Buckthorn

Introduction

For decades, bacterial and fungal infections have been a major problem causing various diseases in humans, plants, animals and spoilage of food products leading to the

losses in the crop productivity and major health problems. The use of higher plants and preparation made from them to treat various infections is an old age practice in many parts of the world [1, 2]. Therefore, antimicrobial

properties of plants have revived as a consequence of present diseases associated with use of antibiotics. Moreover, the medicinal plants have been investigated to be biologically active because of occurrence of many compounds with antimicrobial, antioxidant, allopathic and bio-regulatory properties [2-4]. *Hippophae rhamnoides* L. belongs to the family Elaeagnaceae. It is a shrub and unisexual plant commonly recognized as sea-buckthorn. It is widely distributed throughout Europe and Asia. Three species were reported in Europe, Central Asia and the Himalayas and one species present in Pakistan [5, 6]. According to the flora of Pakistan, *Hippophae rhamnoides* L. have oblong, shiny seed with scanty endosperm, female solitary flowers, pedicellate, perianth, and tubular male flowers in axillary clusters, having four stamens. Fruit are utricle, deciduous shrubs or small trees. Leaves are linear-lanceolate, petals 2, opposite, longer than the perianth tube [7, 8]. Sea buckthorn plants, particularly berries and leaves are considered as an excellent source of important vital compounds like flavonoids, carotenoids, sterols and vitamins (C, A, K and E), which contains maximum nutritional and medicinal properties [9]. Elemental analysis of sea buckthorn seeds shows that it contains K, Mg, P, Ca, Na, Fe, Zn and Ag. Sea buckthorn seed oil and leaves have an efficacy on wound healing area while sea buckthorn have a large spectrum of antiviral, antifungal or antibacterial activities [10]. Pulp of *Hippophae rhamnoides* L. is not only medicinally used but also utilized in a variety of products such as marmalade and juices [11]. The antibacterial and antifungal activities of sea buckthorn plant extracts may reside in a variety of different components, including phenolic and aldehyde compounds. The root and stem extracts of *Hippophae rhamnoides* L. express strong antifungal and antibacterial behavior [12]. More than 200

bioactive components, carotenoids, phenolics, lipids, citric acid, flavonoids and above 15 microelements (B, K, F, Al, Mn, Fe etc.) and many vitamins found in Sea buckthorn berries [13]. A number of nutraceutical and pharmacological companies for various products development, attracted to the minerals along with antioxidant attributes of sea buckthorn [14, 15]. To cure various ailments like, lung problems, skin diseases, ulcers, liver disorders, gastro-intestinal problems, sexual dysfunction, malnutrition, jaundice, cancer and chronic hepatitis-B *Hippophae rhamnoides* L. is used as a remedy [16, 17]. The presence of many bioactive substances including fatty acids, vitamins, lipids, flavonoids, phenols, tannins and steroids etc. in *Hippophae rhamnoides* L. plant contributes greatly to its different properties like antifungal, antibacterial, anti-inflammatory, immunostimulant, anti-radiation, antioxidant, cytoprotective, anti-cancerous, hepatoprotective, antiatherosclerosis and wound healing action etc. The present investigation was aimed to examine the antifungal and antibacterial potential of *Hippophae rhamnoides* L.

Materials and Methods

Sample collection

Plant material

Fruits of *Hippophae rhamnoides* L. (sea buckthorn) were collected from river bank of Warijun, Tehsil Mulkhow, District Chitral, province KPK, Pakistan. The fruits were collected after identification process, the plant was given the voucher no. ICP/000532 and was placed in Department of Botany, Islamia college, Peshawar. Sea buckthorn berries were collected and washed carefully with distilled water to remove the sticking dust particles. The berries were shade dried at room temperature for 21 days and crushed to obtain a fine powder which was preserved in plastic bag for chemical evaluation.

Microbial samples

Pure cultures of bacterial (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Bacillus subtilis*) and Fungal isolates (*Rhizopus*, *Alternaria*, *Curvulria*) were obtained from Microbiology Department, University of Agriculture, Peshawar, Pakistan. Nutrient agar and potato dextrose agar slants were used for preserving bacterial and fungal cultures, respectively.

Extracts preparation

The powdered material of sea buckthorn berries was soaked in 1000ml methanol solvent and was kept under shaking for overnight. The mixture was filtered after 24 hours by Whatman Filter paper No. 1. The same process was repeated three times. Through rotary evaporator, the extracts were concentrated at 50°C with 60 rpm. The solvent was recollected and extracts was further concentrated through water bath at 50°C.

Fractionation of crude extracts

From crude ethanolic extract of *Hippophae rhamnoides* L. fruit, different fractions (Butanol, chloroform, aqueous and ethyl acetate) were prepared. 8.5g of crude ethanolic extract was dissolved in 100ml of distilled water. The aqueous ethanolic solution was washed with butanol (350ml), chloroform (500ml) and ethyl acetate (350ml). The process was repeated three times. The extracts were then filtered through filter paper, concentrated with rotary evaporator at 50 °C for chloroform, aqueous, ethyl acetate, butanol while 62 °C for ethanol extract. To obtain the complete chloroform, aqueous, ethyl acetate, butanol, ethanol extract transfer into different china dishes separately and was place in water bath at a temperature of 50 °C for three weeks.

Antimicrobial activity

Antimicrobial potential of various extracted samples of *Hippophae rhamnoides* L. fruits was conducted by Agar Well Diffusion

Method. Microbial inoculums were prepared by inoculating 20ml of sterilized nutrient broth which was incubated at 37°C for 18-24 hrs. After incubation, cultured broth was standardization by comparing it with 0.5 McFarland standard. With the help of sterile cotton puff, the bacterial culture on nutrient agar media and fungal culture on potato dextrose agar media plates were thoroughly swabbed. 8 mm diameter wells were punched in each agar plate by mean of a sterile cork borer. 0.5g of each extract were diluted to 1mg/6µl, 2mg/12µl and 3mg/18µl in dimethyl sulfoxide (DMSO). By using sterile micropipettes, different concentrations of extracts (1mg/well, 2mg/well and 3mg/well) were added to the wells. Azithromycin, Ciprofloxacin and Clotrimazole were used as a positive control at a concentration of 1mg/well against tested microbes. The plates were incubated at 37 °C for 18-24hrs. After incubation, the diameter of zone of inhibition was observed and measured. From all the three replicates, the reading was taken and the average values were tabularized. With the help of following formula percentage inhibition growth was calculated.

Percentage inhibition of microbial growth = (zone of inhibition of extracts in mm) / (zone of inhibition of positive control in mm) × 100.

Results

Antibacterial activity

Salmonella typhi

All extracts showed effective growth inhibition of *Salmonella typhi*. The ethyl acetate and aqueous extracts inhibited bacterial growth with maximum zone of inhibition of about 77.18±3.05% and 66.87±1.15% at a concentration 3mg/well. Crude ethyl acetate extract showed zone of inhibition of 60.62±0.577% at 2mg/well while aqueous extracts exhibited 62.5±1.73% and 58.43±0.577% zone of inhibition in concentration 2mg/well and 1mg/well respectively. Similarly, inhibitory zone of

ethanol was $59.37 \pm 1\%$ at 2mg/well and $66.9 \pm 0.577\%$ at 3mg/well concentrations. Chloroform extracts showed least zone of inhibition at the rate of $28.12 \pm 0\%$,

$34.37 \pm 1.73\%$ and $39.68 \pm 2.51\%$ in concentration of 1, 2 and 3mg/well respectively. The results are summarized in (Fig. 1).

Figure 1. Antibacterial activity of ethyl acetate, chloroform, aqueous, ethanol and butanol extracted sample of *Hippophae rhamnoides* L. fruit against *Salmonella typhi*

Escherichia coli

Data presented in (Fig. 2) indicates the antibacterial activity of different extracted samples of *Hippophae rhamnoides* L. fruit against *Escherichia coli*. Among all extracts, aqueous extract possess larger antibacterial potential followed by butanol, chloroform, ethanol and ethyl acetate extracts. The maximum inhibition was indicated for aqueous extract towards *E. coli* were $58.82 \pm 0\%$, $65.88 \pm 1.52\%$ and $80.58 \pm 0.577\%$ at 1mg/well, 2mg/well and 3mg/well concentrations. Similarly, butanol extract showed $64.70 \pm 2\%$ activity at 1mg/well, $66.77 \pm 0.577\%$ at 2mg/well and $66.77 \pm 0.577\%$ 3mg/well concentrations. Correspondingly, the ethanol extracted sample showed $21.47 \pm 0.577\%$, $42.35 \pm 0.577\%$ and $69.70 \pm 1.52\%$ activity at 1, 2 and 3 mg/well while chloroform extracted sample showed activity of $20.59 \pm 1.73\%$, $36.47 \pm 0.577\%$ and

$46.17 \pm 0.577\%$ in concentrations of 1mg/well, 2mg/well and 3mg/well against the same microbe. The activities are comparable with the reference drug ciprofloxacin applied in concentration of 50 $\mu\text{g}/\mu\text{l}$.

Bacillus subtilis

The result outlined in (Fig. 3) elaborated that all the fruit extracts of *Hippophae rhamnoides* L. decreased the growth of pathogenic *Bacillus subtilis*. The investigation showed that *Bacillus subtilis* growth was highly suppressed by the extracts. Maximum zone of inhibition $77.41 \pm 1.73\%$ of ethanolic and $93.54 \pm 3.60\%$ of aqueous extracted samples obtained against the tested bacteria showed that 3mg/well as the effective drug concentration. Ethanol and aqueous solvent extracts also produced a higher percentage of zone of inhibition at 1 and 2 mg/well i.e. ethanol: $70.96 \pm 1\%$, $70.96 \pm 1\%$ and aqueous:

77.41±2%, 93.54±0% as compared to other solvent extracts. Similarly, butanol solvent extract inhibited the growth of same bacteria by 59.35±0.577%, 64.51±1% and 65.80±0.577% at 1mg/well, 2mg/well and 3mg/well concentrations, even though it showed good inhibitory action than

chloroform and ethyl acetate extracts. Chloroform extracted sample offered minimum zone of inhibition of 29.03±0% at 1 mg/well, 35.48±0% at 2mg/well and 47.41±2.88% at 3mg/well concentration respectively.

Figure 2. Antibacterial activity of ethyl acetate, chloroform, aqueous, ethanol and butanol extracted sample of *Hippophae rhamnoides* L. fruit against *Escherichia coli*

Figure 3. Antibacterial activity of ethyl acetate, chloroform, aqueous, ethanol and butanol extracted sample of *Hippophae rhamnoides* L. fruit against *Bacillus subtilis*

Pseudomonas aeruginosa

The data shown in (Fig. 4) indicates that different extracted sample of *Hippophae rhamnoides* L. fruit might contain different inhibitory potential towards *Pseudomonas aeruginosa*. It was determined from the result that aqueous extract proved itself as good antibacterial agent. It showed higher inhibition ($95.67 \pm 1.15\%$ at 3mg/well) of test bacteria. The second highest inhibitory activity was expressed by ethanol extract, which showed $91.34 \pm 2.30\%$ at a concentration of 3mg/well. At concentration 2mg/well, aqueous extract inhibited the growth of *Pseudomonas aeruginosa* with same zone of inhibition ($95.67 \pm 3.05\%$). But at concentration of 1mg/well it exhibited less inhibition ($69 \pm 0.577\%$). Similarly, the butanol extracted sample showed $56.67 \pm 0\%$, $71.34 \pm 1.15\%$ and $74.47 \pm 2.08\%$ activity at 1, 2 and 3 mg/well, while least activity was showed by ethyl acetate and chloroform against same bacteria with smallest inhibition zone. Ethyl acetate showed $27.67 \pm 0.577\%$ at 1mg/well, $35.67 \pm 1\%$ at 2mg/well and $39 \pm 0.577\%$ at 3mg/well. On other the hand chloroform showed $33.34 \pm 0\%$ at 1mg/well, $41.34 \pm 0.577\%$ at 2mg/well and $46.67 \pm 0\%$ at

3mg/well. All the data were compared with standard drug ciprofloxacin which showed 30mm zone of inhibition at 1mg/6 μ l against the tested bacteria.

Staphylococcus aureus

Among all extracts, aqueous extract possesses larger antibacterial potential followed by ethanol, chloroform, butanol and ethyl acetate extracts. The maximum inhibition was indicated for aqueous extract towards *Staphylococcus aureus* were $81 \pm 2.51\%$, $90 \pm 1\%$ and $97.5 \pm 1\%$ at 1mg/well, 2mg/well and 3mg/well concentrations. Similarly, ethanol extract showed $71 \pm 0.577\%$ activity at 1mg/well, $83.5 \pm 1.52\%$ at 2mg/well and $88.5 \pm 0.577\%$ at 3mg/well concentrations. Correspondingly, the butanol extracted sample showed $36 \pm 0.577\%$, $55 \pm 1\%$ and $56 \pm 0.577\%$ activity at 1, 2 and 3 mg/well while chloroform extracted sample showed activity of $26.75 \pm 1.15\%$, $57.5 \pm 2\%$ and $76.67 \pm 12.50\%$ in concentrations of 1mg/well, 2mg/well and 3mg/well against the same microbe. The activities are comparable with the reference drug Azithromycin applied in concentration of 50 μ g/ μ l. all the results are summarized in (Fig. 5).

Figure 4. Antibacterial activity of ethyl acetate, chloroform, aqueous, ethanol and butanol extracted sample of *Hippophae rhamnoides* L. fruit against *Pseudomonas aeruginosa*

Figure 5. Antibacterial activity of ethyl acetate, chloroform, aqueous, ethanol and extracted sample of *Hippophae rhamnoides* L. fruit against *Staphylococcus aureus*

Antifungal activity

Curvulria

Various extracted samples from *Hippophae rhamnoides* L. fruit were also screened for their antifungal activity against *Curvulria* as shown in (Fig. 6). The summarized findings states that the ethanol extracted sample of *Hippophae rhamnoides* L shows maximum antifungal activity against the tested microbes with zone of inhibition lying in the range of $92.73 \pm 1\%$ at 1mg/well, $93.82 \pm 0.577\%$ at 2mg/well and $96.37 \pm 0.577\%$ at 3mg/well. The aqueous and butanol extracts of *Hippophae rhamnoides* L also showed significant antifungal activity with the zone of inhibition lying in the range of $75 \pm 1\%$ (1mg/well), $91.46 \pm 1.52\%$ (2mg/well) and $96.98 \pm 1.52\%$ (3mg/well), while in butanol the zone of inhibition lying in the range of $76.92 \pm 5.50\%$ at 1mg/well, $76.92 \pm 1.15\%$ at 2mg/well and $77.46 \pm 1.52\%$ at 3mg/well respectively. Correspondingly, the ethyl acetate extracted sample showed $51.46 \pm 1.52\%$, $52.73 \pm 1.52\%$ and $55.09 \pm 0.577\%$ activity at 1, 2 and 3 mg/well while chloroform extracted sample showed activity of $55.09 \pm 4.50\%$, $58.19 \pm 1\%$ and $66 \pm 5.50\%$ in concentrations of

1mg/well, 2mg/well and 3mg/well against the same pathogen. Clotrimazole showed 55 mm zone of inhibition at 1mg/well.

Alternaria

All the extracted sample of *Hippophae rhamnoides* L. fruit i.e. crude butanol, chloroform, ethanol, water and ethyl acetate were screened against pathogenic fungus *Alternaria* and results of experiments are summarized in (Fig. 7). The data reveals that aqueous extracted sample had a profound inhibitory effect against *Alternaria sp.* In aqueous extracted sample, maximum growth reduction occurred at higher concentration of 3mg/well as $96 \pm 0.577\%$. Ethanol showed lowest inhibition at concentration of 1mg/well as $44.28 \pm 0.577\%$. Aqueous extracted sample showed $71.43 \pm 1\%$ in concentration 2 mg/well and $56.19 \pm 1.52\%$ at concentration 1mg/well. The butanol extracted sample exhibited inhibition i.e. $69.76 \pm 0.577\%$, $71.42 \pm 1\%$ and $80.95 \pm 1\%$ at the concentration of 1, 2 and 3mg/well respectively. Similarly, chloroform extracted sample showed $75.23 \pm 0.577\%$, $75.23 \pm 0.577\%$ and $76.90 \pm 0.577\%$ at 1mg/well, 2mg/well and 3mg/well concentrations respectively.

Correspondingly, the ethyl acetate extracted sample showed $67 \pm 0.577\%$, $70.47 \pm 0.577\%$ and $72.14 \pm 0.577\%$ activity at 1, 2 and 3 mg/well while ethanol extracted sample showed activity of $61.90 \pm 1\%$ and

$79.28 \pm 1.52\%$ in concentrations of 2mg/well and 3mg/well against the same microbe. The results were compared with standard drug Clotrimazole.

Figure 6. Antifungal activity of ethyl acetate, chloroform, aqueous, ethanol and extracted sample of *Hippophae rhamnoides* L. fruit against *Curvulria sp*

Figure 7. Antifungal activity of ethyl acetate, chloroform, aqueous, ethanol and extracted sample of *Hippophae rhamnoides* L. fruit against *Alternaria*

Rhizopus

The data presented in (Fig. 8) revealed that the activity of crude butanol, chloroform, ethanolic, aqueous and ethyl acetate extracted samples obtained from fruit of *Hippophae rhamnoides* L. against *Rhizopus*. The detail data showed that only the ethanol

extracted sample of *Hippophae rhamnoides* L. fruit showed the best activity against *Rhizopus* i.e. $53.14 \pm 0.577\%$ at concentration 1mg/well, $81 \pm 0.577\%$ at concentration 2mg/well and $89 \pm 1\%$ at concentration 3mg/well respectfully. The results were compared with standard drug Clotrimazole.

Figure 8. Antifungal activity of ethyl acetate, chloroform, aqueous, ethanol and extracted sample of *Hippophae rhamnoides* L. fruit against *Rhizopus*

Discussion

From decades, microbial infection causes various diseases in insects, plants, humans and spoilage food products, which leads to losses in crop productivity and health problems [10]. The study was aimed to investigate the antimicrobial strength of sea buckthorn, a commonly used traditional medicinal plant around the world. Among the test organisms *Salmonella typhi* and *Curvularia* were the most sensitive to the water extract and established highest inhibition as compared to other extracts and showed good control.

In current research work, antibacterial activity of the crude ethanolic as well as aqueous was highest, followed by butanol, chloroform and ethyl acetate extracts respectively. Crude aqueous and ethanolic extracts showed more significant activity against *Bacillus subtilis* (aqueous: $93.54 \pm 3.60\%$, ethanol: $77.41 \pm 1.73\%$), *Pseudomonas aeruginosa* (aqueous: $95.67 \pm 1.15\%$, ethanol: $91.34 \pm 2.30\%$) and *Staphylococcus aureus* (aqueous: $97.5 \pm 1\%$, ethanol: $88.5 \pm 0.577\%$) at concentration of 3mg/well. towards *Staphylococcus aureus* (chloroform: $76.67 \pm 12.50\%$, butanol:

56±0.577%), and *Pseudomonas aeruginosa* (chloroform: 46.67±0%, butanol: 74.47±2.08%) at concentration of 3mg/well dose. Ethyl acetate extract also destroyed all the pathogen with smallest inhibition zones nearly 77.18±3.05% in case of *Salmonella typhi*, 33.52±0.577% (*E. coli*), 61.29±1% (*Bacillus subtilis*), 39±0.577% (*Pseudomonas aeruginosa*) and 64.25±14.57% (*Staphylococcus aureus*) at 3mg/well concentration. Similarly, Upadhyay [18] showed the inhibiting growth effects of aqueous and hydro alcoholic leaf extracts of *Hippophae rhamnoides* L against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis*. According to Gill [19], the methanolic leaf extracts of *Hippophae rhamnoides* L. showed a good inhibiting effect against some human pathogenic microbes such as *Escherichia coli* (MTCC 739), *Micrococcus luteus* (MTCC 2682), *Arthrobacter protophormial* (MTCC 106) and concluded that methanolic leaf extracts of *Hippophae rhamnoides* L. was found most effective against *Escherichia coli* (MTCC 739), which exhibited maximum inhibition zone (24mm) at a concentration of 25 mg/mL. In earlier studies, Sea buckthorn berries extracts exhibited the similar trends for inhibition growth observed against bacteria [20]. The phenolic compounds obtained from the berries of sea buckthorn were used for their antibacterial activities and recorded that they were effective against some gram negative bacteria and inhibited their growth but were not effective against the gram positive bacterial strains. Due to the difference between the cell wall composition of gram-positive bacteria and gram-negative bacteria, gram-positive bacteria have been higher sensitivity than gram negative bacteria. As in the outer layer of cell wall of gram positive bacteria contains peptidoglycan, which is not effective permeability barrier. The antifungal potential

of all extracted samples of *Hippophae rhamnoides* L. fruit exerted more significant activities against tested fungi (*Curvularia sp.*, *Alternaria sp.* and *Rhizopus*) at different concentrations. The ethanolic extracted sample showed significant activity against *Curvularia sp.* and *Rhizopus sp* while moderate activity against *Alternaria sp.* at all concentration (1, 2 and 3mg/well). Similarly, crude butanol and aqueous extracts showed significant inhibition against *Curvularia sp.* and *Alternaria sp.* The chloroform and ethyl acetate extracted sample showed the highest sensitivity of 76.90% (chloroform) and 72.14% (ethyl acetate) against *Alternaria sp.* while the inhibition growth towards *Curvularia sp.* was 66% (chloroform) and 55.09% (ethyl acetate) at 3mg/well. In the case of *Rhizopus*, no significant activity was observed against all fruit extracts samples except butanol. Lu [21] indicated that concentrated seed extract and crude seed extract of *Hippophae rhamnoides* L. showed significant antifungal activity against *Tilletia* and *Mucor* fungus, while in case of *Rhizopus* no activity was observed against all test extracts.

Conclusion and Recommendations

The antimicrobial activities of butanol, ethanol, ethyl acetate, aqueous and chloroform extracts of the sea buckthorn fruit berries matched to Azithromycin (standard antibiotics). These antibiotics give the impression to be wide range in its activities, independent of gram reaction. It is concluded that *Hippophae rhamnoides* L. fruit contains active constituents, which are helpful against microbial diseases. In order to get better results, more solvent extracts should be applied. Advanced research should be done against different parts of the plant. The study of medicinal plants as antibacterial or antifungal agents is necessary for gaining insight in to the medicinal plants and their real values. A standard method for investigation is essential to use. Similarly, the

concentration or dilution used must be appropriate. The antimicrobial activities may be due to strong occurrence of active compounds i.e. saponins, tannins, alkaloids, steroids, phenols and flavonoids. However, these medicinal plant species may be subjected to detailed phytochemical and pharmacological studies in order to find out new drugs against pathogenic bacterial and fungal strains.

Authors' contributions

Conceived and designed the experiments: WM Khan & A Iqbal, Performed the experiments: Saima, H Shumail, S Khalid & A Iqbal, Analyzed the data: N Akhtar & N Ghaffar, Contributed materials/ analysis/ tools: Saima, A Iqbal & N Akhtar, Wrote the paper: SIU Haq, S Khalid & WM Khan.

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