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Assessment of antimicrobial activity of leaves extract of *Suaeda fruticosa* against clinically important bacterial strains

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Abstract

Plants have played a significant role in the screening of bioactive compounds for human medications. The aim of present study was to assess the antibacterial activity of *Suaeda fruticosa* leaves fractions against clinically important bacterial strains. The extract of *S. fruticosa* leaves was formed in different solvent of different polarity and their antimicrobial activity was tested against four pathogens i.e. *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella enterica* through Disc diffusion assay and agar well diffusion method. Qualitative phytochemical analysis was performed for the presence of bactericidal active compounds. Results obtained clearly demonstrated the efficacy of methanolic extract, dimethyl sulfoxide filtrate, and flavonoid fraction against pathogenic bacterial strains. The phytochemicals extracted from leaves acted asbroad-spectrum active compounds against these pathogens. These extracts had highest potential against *E. coli* with lowest minimum inhibitory concentration of 60 µl/5ml. The leaves of *S. fruticosa* act as potent origin for numerous phytochemicals that can make headway for antimicrobial drugs.

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Introduction

Bacteria have ability to develop resistance against multiple antibiotics with the passage of time and we are swiftly proceedings towards an era when antibiotics will not work efficiently on bacterial infections (Giourieva *et al*., 2019). New anti-microbial compounds have been required for human related health issues. Resistance to antibiotics is one of the biggest problems that face common health issue (Mah, 2019).

In order to find new antimicrobial agents with a novel mode of action, plants have been discovered as a source for the recognition of new and effective antimicrobials (Sani*et al*., 2019). Herbal medicines are the oldest form of health care.

The herbal medicine interacts with different constituents to increase their activity. In developing countries, the demand of herbal plants is increasing day by day because they do not have any side effects (Mzoughi*et al*., 2018). It has proved to be a great source of benefits to the poor. Now a day there has been a sufficiently great demand in the use of medicinal product as a remedial agent and phytochemicals compounds (Petropoulos *et al*., 2018).

In traditional medicines, the herb *Suaeda fruticosa,* is also known as a shrubby sea blight, is a woody halophyte about 60-90cm tall. *Suaeda* is derived from an Arabic word "Suwaid" which means black (Llanes *et al*., 2018). It has been used for gastro enteritis, stomach pain, wound and skin infection, treatment of sinusitis, diarrhea, infantile eczema and tuberculosis. Many plants have been used until now due to their bacteriostatic and bactericidal nature. They contain secondary metabolites that are very important from an industrial point of view. These active compounds were named as alkaloid, phenol, flavonoid, terpenoid, and steroid(Attia-Ismail, 2016). *S. fruticosa* extract has been reported for hypoglycemia and hypolipidemia activities. It can be used for toothache and chronic rheumatic heart disease. Plant extract exhibits some characteristics that represent an anti-viral agent against hepatitis. It is used as an ointment to remove excess fat and reduces oil salinity (Öztürk *et al*., 2019).

Phytochemicals are able to resist peptidoglycan synthesis, damage the microbial membrane structure, alter the bacterial membrane surface hydrophobicity and also modulate quorum sensing (Januarti *et al*., 2019). Phenols are distinguished for their anti-fungal, anti-cancerous, anti-oxidant and anti-microbial activities. These molecules can easily penetrate due to their low polarity through the cell membrane (Skroza *et al*., 2019). Alkaloids act as allelochemicals, auto-inducers, and siderophores. Their mode of action is to inhibit the nucleic acid synthesis, as they target the enzyme activity of dihydrofolate reductase.

They are respiratory inhibitors as they lower the oxygen consumption in tested bacteria. They also inhibit synthesis of inner and outer cytoplasmic membrane of bacteria by piercing the lipopolysaccharide layer due to the inhibition of efflux pump (Othman *et al*., 2019).

It has been reported that there are about 400 species of stress tolerant plants in Pakistan. Halophytes have been survived in harsh climates including drought, cold, hot, and salinity (Rajpar *et al*., 2018).

The cost of using xenobiotic drugs is 1.5 billion dollars annually (Afsar*et al*., 2019). The objective of this study was to assess the phytochemicals from the leaves of *S. fruticosa* for their antibacterial activity.

Materials and methods

Sample collection

Firm and harmless leaves of salt-tolerant *Suaeda fruticosa* were collected from Kasur, an area located near to Lahore, Pakistan. The leaves were identified by a taxonomist from the Department of Botany, University of the Punjab, Lahore, Pakistan. Afterwards they were washed rigorously under running tap water and sunbaked for two weeks in an incubator at temperature of 50-70℃. They were than grinded to make a fine powder.

Plant leaves powder use for extract and filtrate preparation

The extract and filtrate were prepared with solvents of different polarity namely methanol extract, methanol filtrate, and dimethyl sulfoxide filtrate.

The grinded leaves (2.5g) were used in 25ml of the solvents and were mixed properly. The refined amalgam was left in the shaker for 24 h and it was centrifuged at 4000rpm for 25-30 min. The collected supernatant was then transferred in an empty beaker (500 ml) for the solvent to evaporate.

Collection of bacterial strains

The clinical isolates were obtained from The Services Hospital, Lahore. The bacterial strains used were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Salmonella enterica*.

Preparation of the test cultures

Inoculum of the pathogenic bacterial strains was prepared by culturing in L-Broth for overnight. The bacterial growth was regulated with a thickness equivalent to that 0.5 optical density of McFarland standard. Ampicillin stock solution was prepared as standard for the detection of antimicrobial activity.

Antibacterial assays

Disc diffusion assay

Muller Hilton (MH) agar plates were prepared and clinical isolates were spreader using cotton swab. Filter paper discs of Whatman no 1 (0.5cm) soaked with three extracts were placed in prepared MH agar plates and incubated at 37℃.Diameter of zone of inhibition was measured in cm.

Agar well diffusion method

MH agar was flooded with the inoculum of logarithmic phase samples of bacterial isolates. Wells of (0.8cm) were cut in the agar with the help of Pasteur pipette and plant extracts of 50 µl were added. The plates were incubated at 37℃ for 24h. The antimicrobial activity was evaluated by measuring the diameter of zone of inhibition formed around the wells.

Determination of minimum inhibitory concentration (MIC)

MIC was determined by keeping the bacterial strains in L-broth at a turbidity and optical density of 0.5 MacFarland standards. Methanol extract, methanol filtrate, and DMSO filtrate were added by increasing the concentration of each extract and filter from 20 µl/5ml to 100 µl/5ml of L-broth and incubated for 24 h at 37℃. MIC concentration of extract and filtrate was taken that did not show any visible growth.

Deduction of minimal bactericidal concentration (MBC)

Minimal bactericidal concentration (MBC) was determined with a set of extract and filtrate tubes having no visible growth while determining MIC. They were streaked on MH agar plates and the plates were incubated at 37℃for 24 h.

The accumulation of leaves extract and filtrate at which no visible growth was observed was considered as minimal bactericidal concentration.

Qualitative assessment of phytochemicals

The extract and filtrates were tested for the existence of alkaloids, flavonoids, and phenol using qualitative phytochemical assessment.

Removal of alkaloid from plant leaves

Healthy leaves of *S. fruticosa* (2.5g) were grinded with 10% acetic acid in ethanol (18-20ml)by using mortar and pestle and incubated in a shady place for 4h. After incubation, the extract was filtered and condensed for 20min in a boiling water bath. Ammonia (25%) was added until precipitation and then centrifuged at 2500 rpm for 10 min. The remnant was washed with 1% NH_4OH and filtered. The surplus was then weighed, dissolved in ethanol, and stored at 4℃.

Separation of phenol from leaves

One gram (1g) fine powder of leaves was taken in a conical flask and 20 ml of ethanol (80%) ethanol was added to it. The flask was cotton plugged and placed in a boiling water bath for 15 min with frequent

swing. It was then centrifuged and supernatant was collected.

Flavonoid extraction

Half of the supernatant was transferred to a 50ml clean funnel containing petroleum ether (40-60°C). Formation of an aqueous layer represents flavonoid extract.

These separated phytochemical fractions from leaves were then further tested for their antibacterial action.

Results

Zone of inhibition was recorded maximum with methanolic extract and DMSO filtrate while the filtrated methanol extract showed the least antibacterial activity with disc and agar well diffusion protocols. Methanolic leaves extract exhibited maximum zone of inhibition i.e. 2.5 and 1.4cm against *E. coli* and both *P. aeruginosa* and *K. pneumonia,* respectively (Fig. 1A). The DMSO leaves filtrate was more efficacious against *P. aeruginosa* and *S. enterica* (Fig.1B).

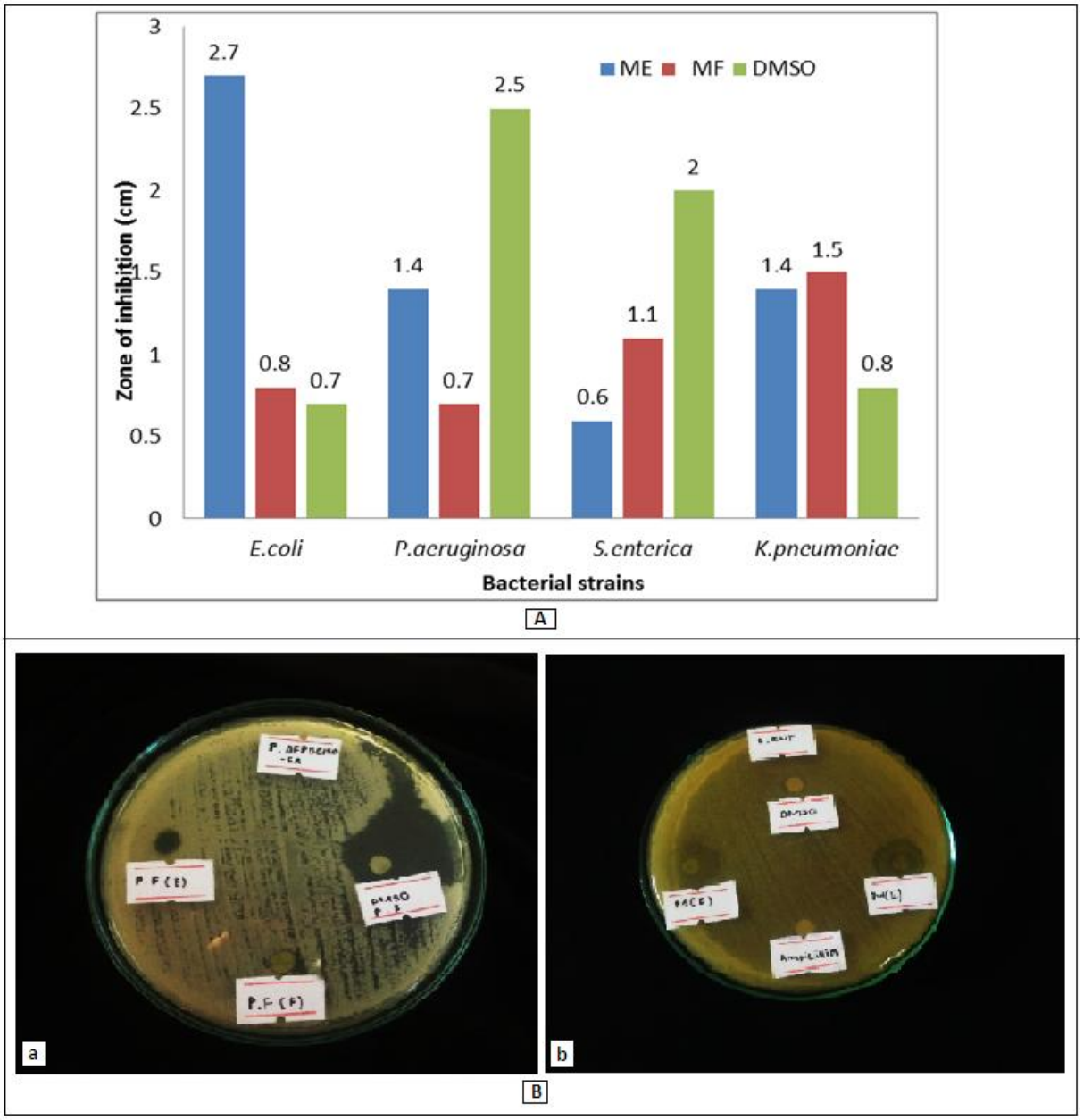

Fig. 1. (A) Antibacterial activities of methanol extract, methanol filtrate, and dimethyl sulfoxide filtrate; (B) Antibacterial activity of *S. fruticosa* leaves extract by using Disc diffusion method against clinical bacterial strains i.e. *P. aeruginosa* (a) and *S. enterica* (b).

The phytochemical compounds i.e. alkaloids, flavonoids, and phenols have been detected for their antibacterial activity by using agar well and disc diffusion methods (Fig. 2). The phenolic fraction from the leaves of *S. fruticosa* showed 2, 2.2, 2.6, and 4cm zone of inhibition respectively against *E. coli*, *K. pneumoniae*, *P. aeruginosa,* and *S. enterica* (Fig. 2).

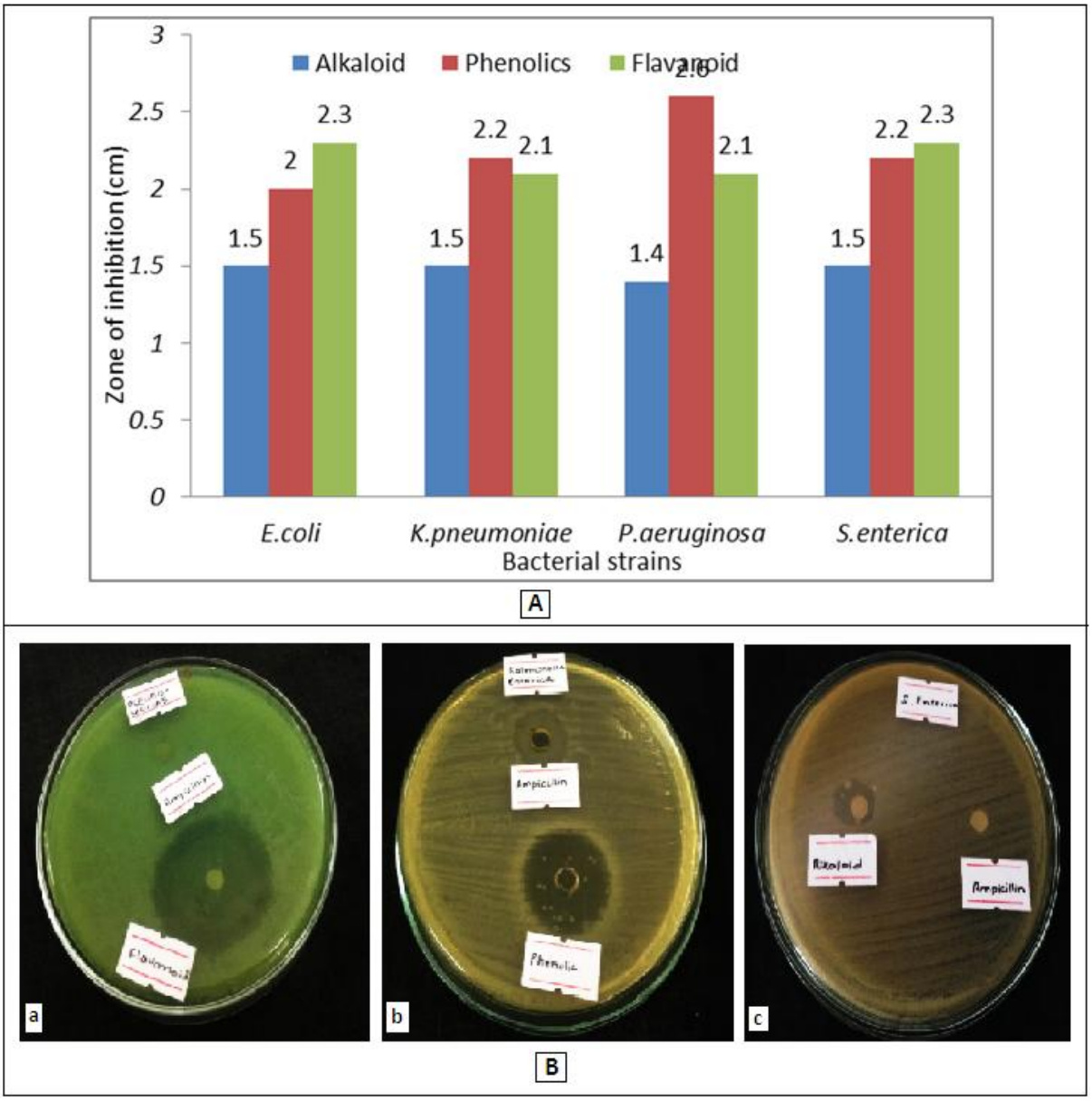

Fig. 2. (A) Antibacterial activities of alkaloid, phenolic, and flavonoid fraction; (B) Antibacterial activity of plant leaves extract by using Disc and Agar well diffusion method against *P. aeruginosa* (a) and *S. enterica* (b) and (c).

The antimicrobial activity of phenolic fraction was greater as compared to the control i.e. ampicillin using disc and agar well diffusion method. The zone of inhibition with ampicillin recorded for *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. enterica* was 1.9, 1.6, 2.6, and 1.9cm, respectively (Fig.4A,B). Alkaloid fraction was potent against *S. enterica* by giving a zone of inhibition of 1.5cm as compared to the control i.e. 1cm (Fig. 5A,B). The flavonoid fraction was potent against bacterial strains by showing zone of inhibition of 2.3cm both for *S. enterica* and *E. coli* and 2.1cm both for *K. pneumoniae*, and *P. aeruginosa* (Fig. 2) which were greater than control which showed 0.9, 1.2, 0.5, and 2.2cm zone of inhibition against *K. pneumoniae, P. aeruginosa*, *S. enterica*, and *E. coli* (Fig. 6A,B).

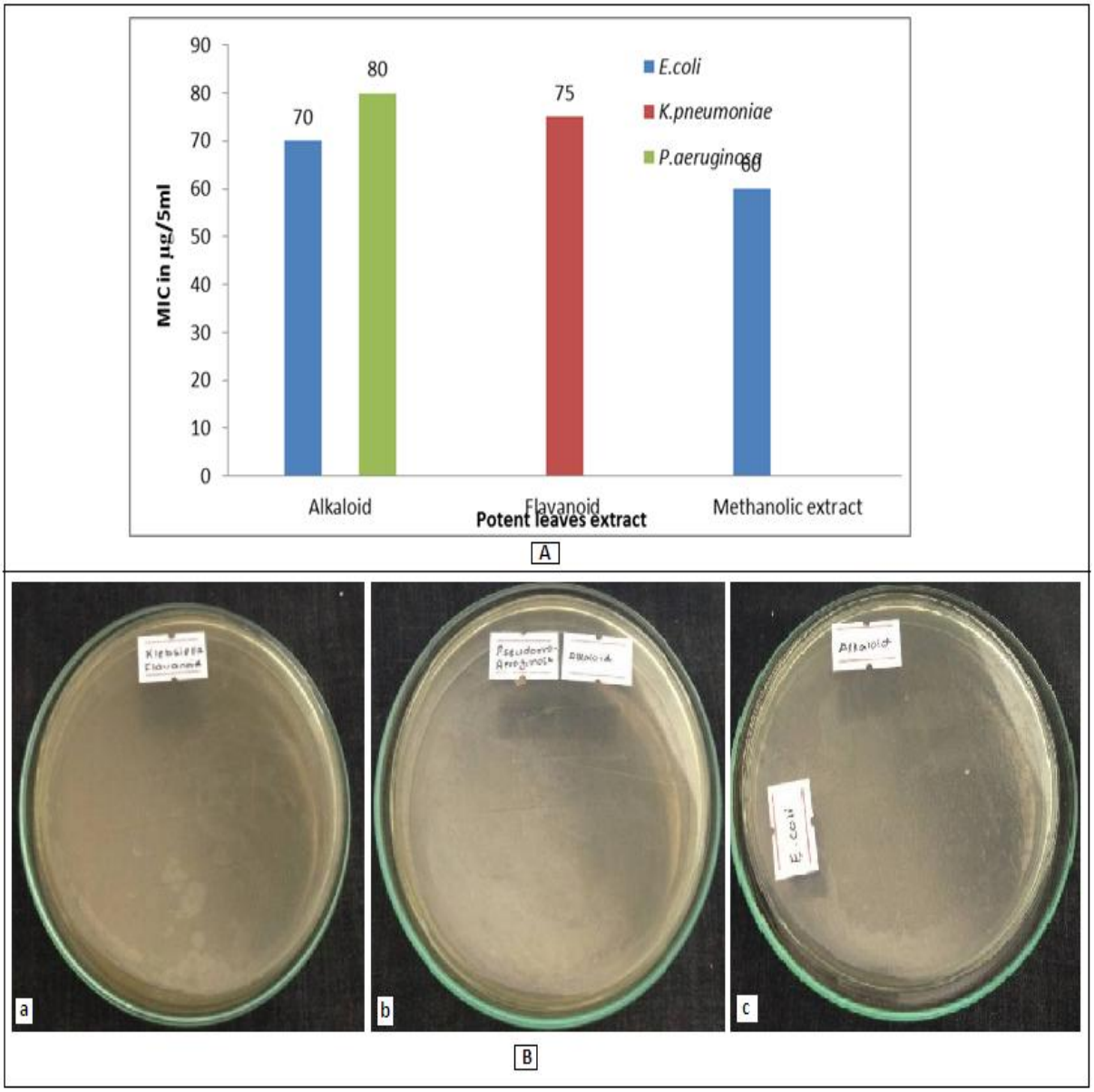

Fig. 3. (A) Minimum inhibitory concentration of *S. fruticosa* leaves extract against clinical bacterial strains at a concentration where no visible growth was observed; (B) Growth susceptibility of *K. pneumoniae* (a), *P. aeruginosa* (b), and *E. coli* (c) with flavonoid and alkaloid of plant leaves extract of *S. fruticosa*.

MIC was determined using the extracts against *E. coli*, *P. aeruginosa*, and *K. pneumoniae* and methanol extract MIC against *E. coli* was within range of 60 µl/5ml while alkaloid fraction MIC against *P. aeruginosa* was 80 µl/5ml (Fig. 3A). MBC was performed with the plant extracts on clinical bacterial isolates and no visible growth was observed on MH agar plates (Fig. 3B).

Discussion

Antimicrobial resistance has been emerging at an alarming due to misuse and overuse of drugs that favor the selection of highly modified organisms hence increasing the cost of health care. New resistance mechanisms have been reported globally in microorganisms including bacteria i.e. *E. coli* resistance has been reported in many parts of the world where the treatment with fluoroquinolone antibiotic is now ineffective. World health organization has now been taking serious measure by providing technical reinforcement to help countries develop their national action plan that might be fruitful enough to combat this global concern in near future (Tornimbene *et al.*, 2018).

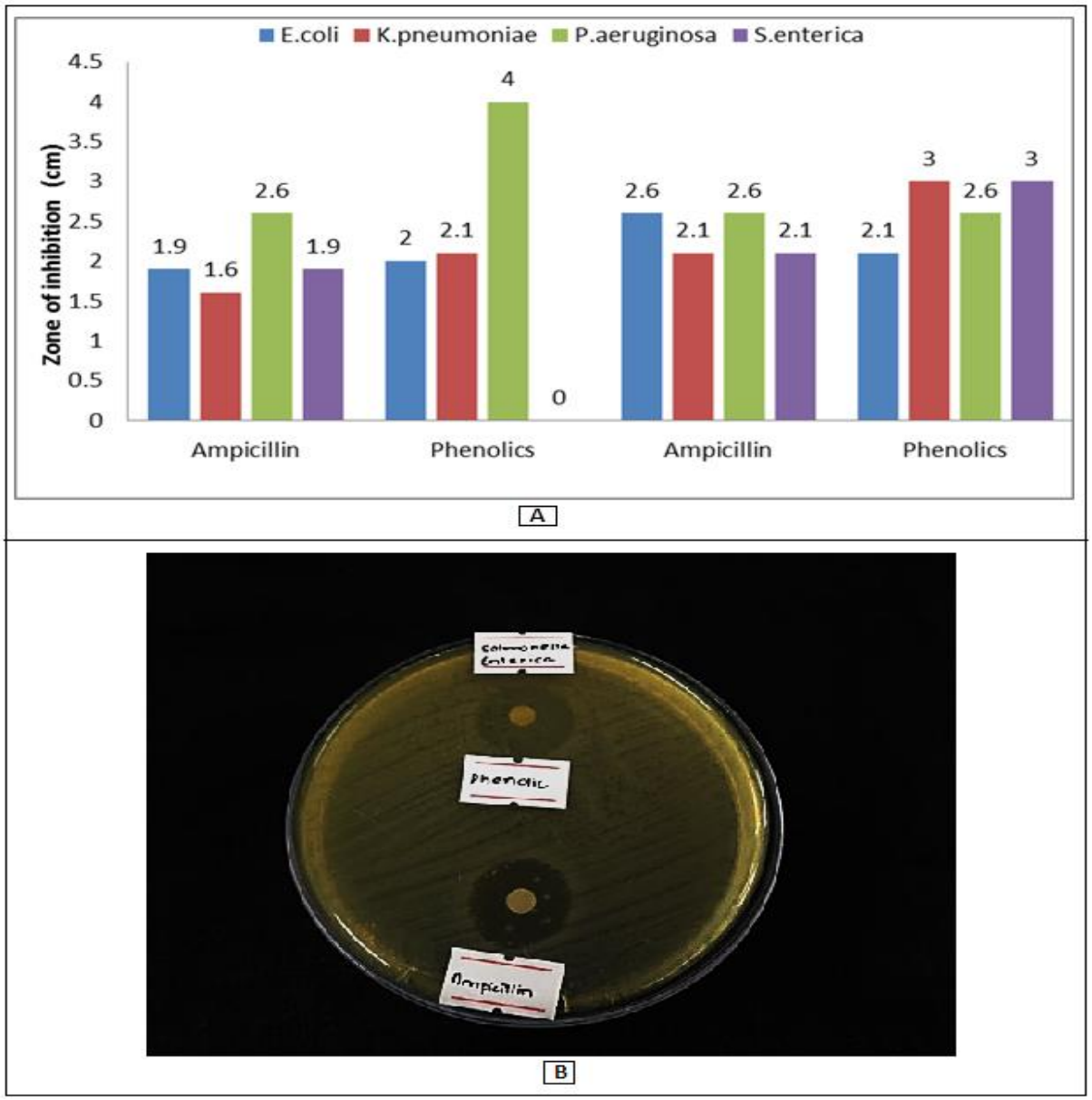

Fig. 4. (**A**) Antibacterial activity of phenolic extract from leaves of *S. fruticosa* by using Disc and Agar well diffusion method. **(B)** Antibacterial activity of phenolic extract from leaves of *S. fruticosa* by using Disc and Agar well diffusion method.

In one of the previous studies, methanolic extract from the leaves of *Trianthema portulacastrum* has been used to assess its activity against *E. coli* and *P. aeruginosa*. The maximum zone of inhibition recorded was 1.6 and 1.3cm, respectively (Falade *et al.*, 2019). There are several reports in the literature that specify the antibacterial potential of the plants. *Cinnamomum cassia*, also called Chinese cassia, is an evergreen tea belongs to the family named as Luraceae (Liang *et al.*, 2019). The methanol leaves extract of *C. cassia* has been reported to inhibit the growth of *E. coli* with a maximum zone of inhibition of 1.8cm by using a disc diffusion method (Chaudhary *et al.*, 2019).The size of inhibitory zone suggests significant antibacterial activity of the *L. camara* leaves extract belonging to the family of Verbenaceae was investigated previously against *S. enterica*, *P. aeruginosa*, and *K. pneumoniae.*

The methanol extract had antibacterial activity against *S. enterica* and *P. aeruginosa* giving a zone of inhibition of 2.1 and 2.0cm, respectively while the extract was failed for its activity against *K. pneumoniae* (Delgado-Altamirano *et al.*, 2019).

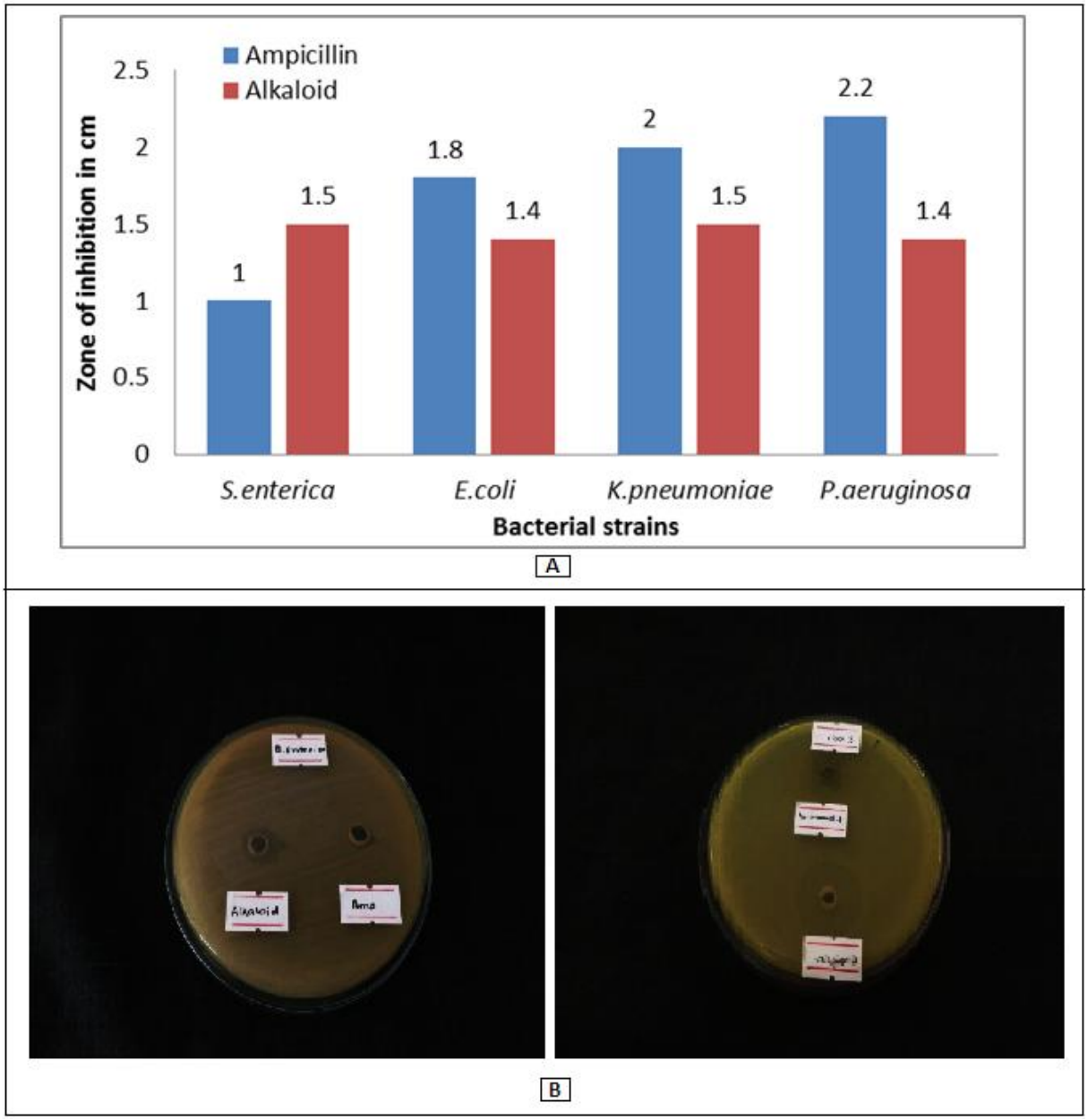

Fig. 5. (**A**) Antibacterial activity of alkaloid extract along with standard by using Agar well diffusion against bacterial strains. (B) Antibacterial activity of alkaloid extract obtained from *S. fruticosa* in comparison with standard by using Agar well diffusion method against *S. enterica* (a) and *E. coli* (b).

One previous study described 4 medicinal plants screened for their antimicrobial properties. The plant substance used was the leaves of *Pipe guineense, Congronema latifolium, Ocium gratissium* and the ripe fruits of *Xylopia aethiopiea*. The phytochemical screening showed the presence of alkaloids, tannins, glycosides and saponens while flavonoids and phenols were absent (Amadi, 2018). Plants of genus *Clerodendron* belong to the family Verbenaceae and have been broadly used for managing various diseases. Qualitative phytochemical examination of this plant confirms the presence of different phytochemicals i.e. sterols, terpenoids, alkaloids, carbohydrates, tannins, and glycosides in its methanolic extract whereas flavonoids, phenols, and saponins were absent. Similarly, the methanolic extract of leaves of *Phyllantus amarus* was reported for the presence of flavonoids and alkaloids absence of phenols (Malayaman *et al*., 2019).

Methanol extracts usually show more inhibition as compared to the other extracts obtained from plants.

In a previous study, the phenolic fraction from leaves of *T. portulacastrum* shows the maximum zone of inhibition with *E. coli* and *P. aeruginosa* had been recorded 0.4 and 0.2cm, respectively (Yamaki *et al.*, 2016) while the flavonoid fraction of *T. portulacastrum* was potent against *P. aeruginosa* and *E. coli* by giving a maximum zone of inhibition 2.1 and 2.3cm, respectively (Geethalakshmi *et al.*, 2018).

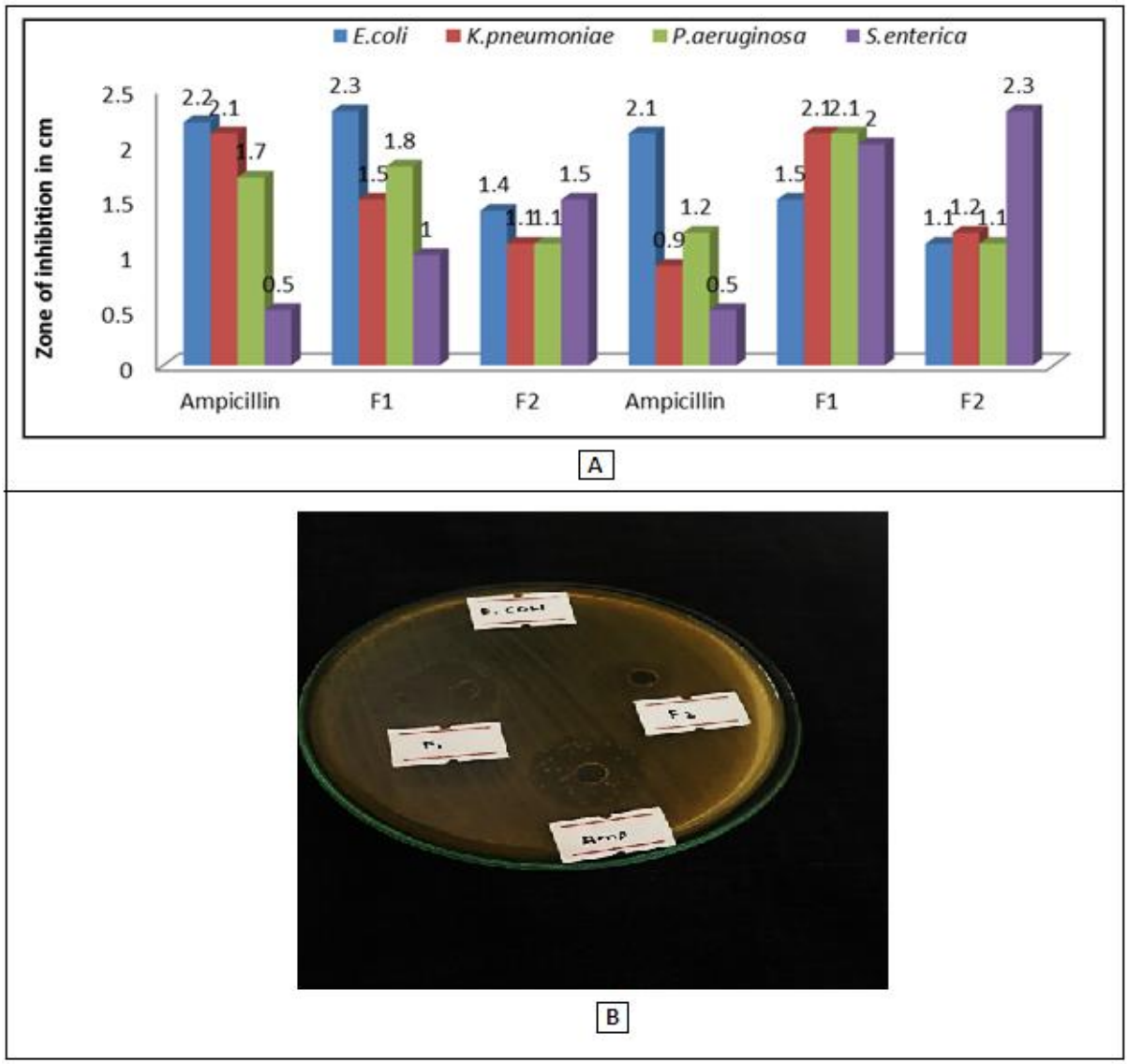

Fig. 6. (A) Antibacterial activity of flavonoid extract obtained from leaves of *S. fruticosa* by using Disc-Agar (F1) and Well diffusion method (F2). (B) Antibacterial activity of flavonoid extract by using Agar well-Disc diffusion method against *P. aeruginosa* and *E. coli.*

MIC values in previously reported studies were found higher that is 150-200 µl/ml with the xerophytes including *Calligonum polygonides, Peganum harmala,* and *Rosa burononii* against *E. coli* (Khan *et al.*, 2018).*C. cassia* methanolic extract depicts MIC against *E. coli* at the highest value of 2640mg/l (Mostafa *et al.*, 2018). The MIC of methanol extract of *L. camara* against *E. coli* has been recorded within the range of 5-8mg/ml (Oduola *et al.*, 2018). The methanol extract from *T. portulacastrum* showed 1.25 mg/ml MIC against *E. coli* (Abd El-Gawad *et al.*, 2016).

Conclusion

The present investigation clearly demonstrates that the methanol leaves extract of *S. fruticosa* has a broad

spectrum of antibacterial activity against the tested clinical bacterial strains. The bioactive phenolic compounds that are present in the extract might be responsible for the antibacterial activities. The clinical trials are highly recommended to confirm potential therapeutic agents.

Acknowledgements
Not applicable.

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