Saudi Journal of Biological Sciences 30 (2023) 103750

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Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Analysis of antibacterial and cytotoxic potential of medicinal plants from Cholistan desert, Pakistan

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ARTICLE INFO

Article history:
Received 26 February 2023
Revised 17 July 2023
Accepted 22 July 2023
Available online 28 July 2023

Keywords:
Calligonum polygonides
Farsetia hamiltoni
Pulicaria crispa
Antibacterial activity
Cytotoxicity
Brine shrimp
Bacterial strains

ABSTRACT

This study aimed to investigate the antibacterial and cytotoxic activity of 03 medicinal plants, *Calligonum polygonides, Farsetia hamiltonii*, and *Pulcaria crispa,* from Cholistan desert, Pakistan. The active constituents of plants species were extracted in 05 different solvents and the extracts were tested against various bacterial strains and brine shrimps. Although all *Calligonum polygonides*'s extracts except chloroform were active against *Staphylococcus aureus* the most active was the acetone extract (21 ± 0.00 mm at 200 µg/disc) and activity was better than Caricef (p-value 0.03). While its water extract was more potent (18 ± 1.45 mm at 200 µg/disc) than Augmentin and Caricef (p-value < 0.005). The methanol extract's activity (15 ± 0.39 mm in 200 µg/disc) was comparable to Fucidin against *Proteus vulgaris* (p-value > 0.99) and activity of diethyl ether extract against *Escherichia coli* (10 ± 1.16 mm in 200 µg/disc) was same as of Urixin (p-value 0.91). *Farsetia hamiltonii*'s acetone extract against *Pseudomonas aeruginosa* (10 ± 0.15 mm in 1 µg/disc) was more active than Augmentin Caricef and Cefotax (p-value < 0.02) and against *Staphylococcus aureus* (15 ± 1.15 mm in 200 µg/disc) activity was higher than Caricef (p-value 0.03). All *Pulicaria crispa*'s extracts except water extract were found active against *Staphylococcus aureus.* However, the diethyl ether extract was most effective (25 + 0.00 mm at 150 µg /disc) and activity was more than Augmentin, Oxy-tetracycline, Fucidin, Urixin, Ceftriaxone (p-value < 0.05). Although all extracts were exhibited cytotoxic activity, the *Calligonum polygonides*'s acetone extract (100%), *Farsetia hamiltonii*'s diethyl ether extract (90%) and *Pulicaria crispa*'s methanol extract (100%) were most active at 1000 µg/ml concentration. This study validated the medicinal significance of the studied plants and thus opens the way for their therapeutic applications.

1. Introduction

Medicinal plants are significantly used for disease preclusion and play a vital role in human health improvement. The natural products derived from these plants are crucial source of anticancer drugs and antibiotics. Nearly 78% drugs are synthesized and obtained from different medicinal plants (Khan et al., 2015). Today, the exploration of biologically active components in plants possessing antimicrobial potential has been extensively in practice to reduce the infectious diseases caused by pathogenic bacteria, viruses, fungi, and parasites. Plant extracts are proved to be the essential sources of curative agents (Mohamed et al., 2020). The plants extract are also valuable to restrain the microbes and are beneficial for the prevention and cure of various disease in humans and animals (El-Sharkawy et al., 2017).

The plants of Cholistan desert exhibit valuable medicinal properties and are largely utilized by the herbs specialist and the inhabitants for treating various infectious diseases. The Cholistan desert is situated in the south-west of Punjab, Pakistan covering an area of

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2023.103750
1319-562X/

26000 km^2. It is situated between 27° 42′ and 29° 45′ north latitudes and 69° 52′ and 75° 24′ east longitudes (Mustafa et al., 2016). Din Garh area of Cholistan which is located between 28°56′33″ north latitude and 71°50′13″ east longitude enrich with medicinal plants (Zubair et al., 2018). The climate of Cholistan is arid, dry, and xeric with little i.e., 100 mm (4 in) and 255 mm (10 in) or no rainfall leading to prolonged drought. Summers are very hot with 34 to 38 °C temperature while in winter temperature ranges between 15 and 20 °C. Din Garh, Cholistan climate conditions's vary from very warm in summer with temperature ranges between 34 °C (during May to August) and 51 °C (during July and August) to low temperature in winter. The annual precipitation ranges between 100 mm (4 in for the west) to 200 mm (8 in for the east) mainly in monsoon (Zubair et al., 2018).

This desert is enriched with 138 species of plants with 64 medicinal plants. Among them, worthy medicinal plants are *Capparis decidua*, *Farsetia hamiltonii (F. hamiltonii)*, *Corchorus depressus*, *Calotropis procera*, *Citrullus colocynthis*, *Panicum turgidum*, *Alhagi maurorum*, *Fagonia cretica* and *Capparis decidua* (Hayat et al., 2014). Besides these, many species belonging to Polygonaceae family including *Calligonum* are great source of bioactive components and possesses medicinal characters. Almost 80 species of *Calligonum L.* (Polygonaceae) genus are situated in Western Asia, Southern Europe and Northern Africa while *C. comosum* located mostly in Pakistan, Central and Eastern Arabia, Middle East desert and North African deserts (Soliman et al., 2018). *Calligonum polygonoides (C.polygonides)* commonly known as Phog is also a member of Polygonaceae family (Ahmad and Akram 2019). *C. polygonides* is a perennial shrub found on sandy deserts and acclaimed for their usage as fuel (wood), forage (leaves), bud(flowers). It can withstand the harsh conditions of weather and are stress tolerant (Berwal et al., 2021). Different parts of this plant serve different medicinal purposes i.e. aerial part helpful in stomach diseases and toothache, for gum sores its roots decoction used and as flowers are rich in sugar and nitrogen components therefore used as food Zouari et al., 2012. Along with these other therapeutical applications includes the cure of ulcer, inflammation, low blood glucose level and possess antioxidant and cytotoxicity activities by aerial portion. The fruits of this plant can be used to treat helminthiasis. Moreover, various plants parts also possess antimicrobial activity (Ahmed et al., 2016). Several studies (Khan et al., 2017, Alehaideb et al., 2020) have reported the cytotoxicity activity of *C.polygonides* against cancer cells lines (TNBC MDA-MB-231 cell line) and brine shrimp representing their cytotoxic effect and enhancing their importance as medicinal plant. Another important plant from medical point of view is *F. hamiltonii* (*F. hamiltonii*) of desert Cholistan, Pakistan generally used for the therapy of arthritis, oxidative stress, diabetes, fever, respiratory and gastrointestinal diseases (Hayat et al., 2022). It is woody shrub of brassicaceae family and commonly known as ‘‘Al-fareed” (Hayat et al., 2014). These different plants of genus Farsetia is well known for its cytotoxic activity as reported by literature. The *F. aegyptia* ethanolic extract display cytotoxic effect against HCT116, HepG2 and MCF-7cell lines and contain cytotoxic components due to which represents cytotoxic potential against MCF-7 and Hela cell lines (El-Sharkawy et al., 2017). *Pulicaria crispa* (*P. crispa*) from Asteraceae family are the annual herbs and are the most common plants of desert found in Pakistan, Saudi Arabia, Iran, Iraq, India, Southern Egypt, north and west tropical regions and Kuwait. It used in folk medicine preparation is in practice in various countries. Due to its antioxidant property, it has been used in the treatment of heart disease, inflammation, fever, coughs, used to repel insects (Mohamed et al., 2020). These medicinal plants also possess the antimicrobial activity against *Mycobacterium*, Gram-negative bacteria, *Candida albicans,* Leishmania, hepatitis B virus and Schistosoma mansoni (Albrahim et al., 2020). Although different studies have reported the medicinal importance and ethnobotanical value of these plants, no study was found to report the antibacterial activity of these plants in different solvents like water, diethyl ether, chloroform and acetone against *Proteus vulgaris (P. vulgaris)*, *Staphylococcus aureus (S. aureus)*, *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa (P. aeruginosa)* in relevance to standard antibiotics which are required to validate the antibacterial potential of these plant extracts.

Keeping in view the significance of these medicinal plants this study was initiated with aim to explore the antimicrobial potential of three plants (*Calligonum polygonoides*, *Farsetia hamiltonii* and *Pulicaria crispa)* against four different bacterial strains (*Staphylococcus aureus, Proteus vulgaris, Pseudomonas-aeroginosa* and *Escherichia coli)* in combination with investigation of the cytotoxicity of these plants against brine shrimp which will be advantageous to develop antibiotics and other therapeutics drugs.

2. Methodology

2.1. Study area

All the three plants samples were collected from Din Garh area of Cholistan which is located between 28°56′33″ north latitude and 71°50′13″ east longitude (Zubair et al., 2018). Map of sampling area is given in Fig. 1.

2.2. Sample collection

Samples of *C. polygonoides, F. hamiltonii* and *P. crispa* were collected from Cholistan desert during summer season (July and August) in 2019.

The whole plant sample with their stem, roots and leaves were washed 3 times with sterile water to remove any dust. Then plant samples were washed 1 time with 1% KMnO4 solution to disinfect samples following a previously published method (Subramanya et al., 2018). In this method samples were soaked in KMnO4 solution and shaken 20 times/per minutes for total time of 10 min at room temperature. The solution was poured off and plant samples were washed with sterile water for 1 time to remove $KMnO_4$′s traces. After washing the plant samples were cut into small pieces (1 cm) and dried under shade for 15–20 days. The dried samples then ground using mechanical method of grinding i.e. mortar and pestle to get fine powder of 125–180 μm particle size (El-Sharkawy et al., 2017).

2.3. Preparation of plant extracts

Plant samples' extracts were prepared using Soxhlet apparatus (24/29) of Pyrex brand separately in five solvents: methanol, chloroform, diethyl ether, acetone, and water. The working principle of using Soxhlet described by Redfern et al., (2014) was followed for extract preparation. First the powdered plant samples were added in cellulose thimble (15 cm), packed and thimble was inserted in the Soxhlet extraction chamber (30 cm). Solvent was added (1 solvent at 1 time) in the round bottom flask and heated on heat mantle (at the boiling temperature of solvent). The vapors form and move through the tube of extraction unit to the condenser and thus condense and then drop back into the extraction unit with inserted thimble. On reaching the solvent level to siphon tube it pours again in the flask and the cycle is repeated again. The extraction process was carried out for about 24 h until the color (yellow/-green) of the circulating solvent turned colourless. Plant extract in each solvent was prepared separately one by one using this

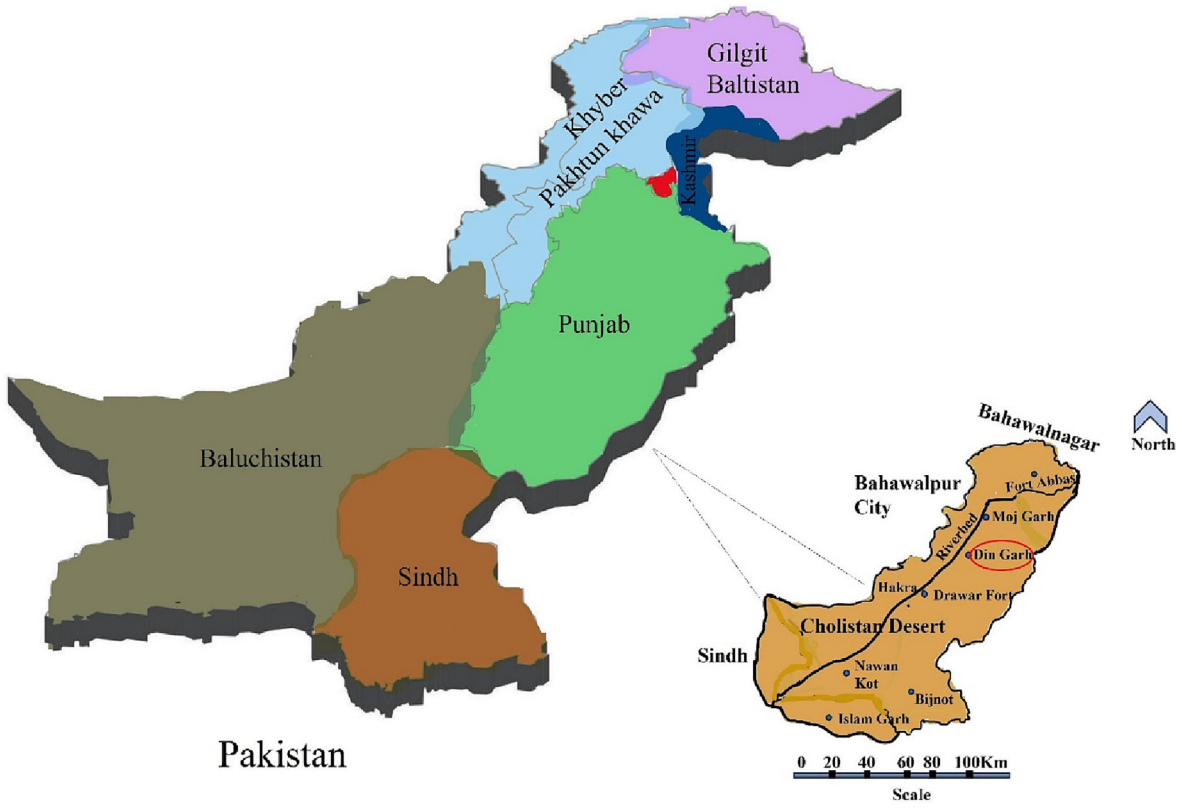

Fig. 1. Map of Cholistan Desert. A representation of the study area is provided in the map. Red encircled area is the place from where plants were collected.

method. The extracts obtained after several hours of extraction, were evaporated to dryness. The residue left after the complete evaporation of solvent from the extract was then used to prepare the solutions of different concentrations in respective solvents.

2.4. Detection of antibacterial activity

Disc diffusion method given in Atta-ur-Rehman et al., (1999) was used for the determination of antibacterial activity against four different strains of bacteria i.e. *S. aureus* (Gram + ive), *P. vulgaris* (Gram -ive), *P.aeroginosa* (Gram -ive) and *Escherichia coli* (Gram -ive) that were obtained from the Microbiology section, Department of pathology, Quaid–e–Azam medical college Bahawalpur. The standard antibiotic discs were used as positive controls. These antibiotics include oxy-Tetracycline, Chloramphenicol, Augmentin, Caricef, Amikacin, Ciprofloxacin, Fucidin, Enoxacin, Sparaxin, Urixin, Tazobactum, Cefotax and Ceftriaxone. The Augmentin (30 µg) contained amoxicillin and clavulanic acid in 2:1 i.e 20 µg amoxicillin and 10 µg clavulanic acid. Some of the standard antibiotic discs were purchased from the manufacturer and others were supplied by Microbiology section, Department of pathology, Quaid-e-Azam medical college Bahawalpur. Solutions of different concentrations i.e 2.5%, 5% & 10%, were prepared using the respective solvent in which extraction was done. Discs of 5 mm diameter and 0.65 mm thickness were cut of Whatmann filter paper (No. 44) and sterilized in hot air oven. To prepare discs of different potencies, a known volume of extract was applied on the discs, air dried and used. A disc prepared in the same way by applying solvent only, was used as negative control. All the plates were incubated in incubator (Model IN30, *Memmert,* Germany) for 16–18 h at 37 °C. Extracts of the studied plant species having antibacterial potential were further examined to specify whether they are bacteriostatic or bactericidal in action. The diameter of the zone of inhibition was measured in millimeter (mm) using vernier caliper (Jaidka et al., 2017).

2.5. Cytotoxicity analysis

The different concentration (µg/ml) i.e., 10, 100 & 1000 of each plant extract in respective solvents were prepared and processed for the cytotoxicity analysis using Brine shrimp lethality assay. The method given in Atta-ur-Rehman et al., (1999) and the protocol established by McLaughlin, 1991 was employed for the assay. Brine shrimp (*Artemia salina*) eggs hatched in a hatching tray filled with seawater (pH 7.4) under light for 1–2 days at 30 ± 3 °C. Different volumes of test solutions, 0.5, 0.05 and 0.005 ml were disposed off in three test tubes, each corresponding to a different concentration. The solvent evaporated to dryness overnight. 2–4 days old 10 larvae were taken with the help of Pasteur pipette and moved into the sample containing test tubes. Seawater was added into these test tubes to a total volume of 5 ml incubation was done at 30 ± 3 °C for 24 h under illumination. The number of survivors after 24 h were counted and recorded. Other test tubes were supplemented with solvent (methanol, chloroform, acetone, diethyl ether) serving as positive control and water was used as negative control. The results were expressed in terms of percentage dead larvae.

2.6. Statistical analysis

All the experiments were carried out in triplicate. Statistical analysis was done using Statistical Package of Social Sciences (SPSS) software version 22 software. Antibacterial activity results were presented in terms of mean and standard deviation. Significance was calculated by performing un-paired *t*-test. A p-

value < 0.05 was considered significant. Cytotoxicity results were calculated in terms of % mortality.

3. Results

3.1. Antibacterial activity results

All the three studied plants extract showed different antibacterial potential in different solvents at different concentrations. The water extract of only *C. polygonoides* was found to be active. Similarly, methanolic and diethyl ether extracts of *C. polygonoides* and *P. crispa* were detected to be active. The chloroform extracts of *F. hamiltonii* was active only. Acetone extracts of all three plants were found to be active (Fig. 2).

3.2. Calligonum polygonoides antibacterial activity

The water extract of the plant was observed to be totally inactive against *E. coli*. Against *P. vulgaris*, water extract of *C. polygonoides* (at the concentration of 1 mg/disc) was non-significantly more effective, with 14 ± 1.15 mm zone of inhibition, than standard antibiotics Augmentin (12.88 ± 5.53 mm, p-value 0.76), Caricef (3.57 ± 5.70, p-value 0.08) and Ceftriaxone (14.00 ± 7 .00 mm, p-value > 0.99) and was almost equally effective as Cefotax (14.75 ± 9.89 mm, p-value 0.90) and Fucidin (15.00 ± 9.58 mm, p-value 0.87). Whereas other tested antibiotics were found to show high but non-significant activity. Against *S. aureus*, water extract of *C. polygonoides* was found to be most active (18 ± 1.45 mm) at 200 µg/disc and 18 ± 0.52 mm at 1 mg/disc which in comparison to antibiotics was found to be significantly more active than Augmentin (11.90 ± 1.14 mm, p-value 0.005), Caricef (0, p-value 0.002), while non-significantly more active than Ceftriaxone (9.36 ± 5.94 mm, p-value 0.39), Oxy-Tetracyclin (16.29 ± 2.96 mm, p-value 0.43), Amikacin (15.09 ± 5.40 mm, p-value 0.45), Fucidin (17.33 ± 2.56, p-value 0.71) and Urixin (17.88 ± 2.85 mm, p-value 0.95). Against *P. aeruginosa*, water extract of the plant at the concentration of 200 µg/disc exhibited maximum activity with the zone of inhibition 15 ± 0.59 mm, that was even significantly greater than Caricef (1.00 ± 2.83 mm, p-value 0.01) and non-significantly higher than Augmentin (9.80 ± 7.53) and Ceftriaxone (14.22 ± 4.47 mm, p-value 0.79). It was almost equal to that of Fucidin (15.60 ± 8.11 mm, p-value 0.90) and Cefotax (15.25 ± 4.2 mm, p-value 0.92). While other antibiotics showed non-significantly higher activity than *C.polygonides*'s water extract besides Ciprofloxacin (23.11 ± 3.84 mm, p-value 0.02), Enoxacin (25.13 ± 4.75, p-value 0.02) and Saparaxin (24.70 ± 5.71 mm, p-value 0.03) which displayed activity significantly more than the extract.

Methanolic extract of *C. polygonoides* was active against all the four tested strains. Against *P. vulgaris*, maximum zone of inhibition (15 ± 0.39 mm) was seen at the concentration of 200 µg /disc plant extract, that was equal to Fucidin activity (15.00 ± 9.58 mm, p-value > 0.99) and was greater than the zone of inhibition as observed in case of Augmentin (12.88 ± 5.53 mm, p-value 0.57), Caricef (3.57 ± 5.70 mm, p-value 0.07), Cefotax (14.75 ± 9.89 mm, p-value 0.96) and Ceftriaxone (14.00 ± 7.00 mm, p-value 0.82) but not to the significant level. All the other antibiotics represented non-significant higher activity than *C. polygonoides*'s methanol extract. The antibacterial potential of *C. polygonoides*'s methanol extract against *S. aureus*, showed maximum antibacterial potential (14 ± 0.79 mm) at the concentration of 1 mg/disc, representing it to be significantly more effective than Caricef (0 mm, p-value 0.001) and non-significantly more active than Augmentin (11.90 ± 1.14 mm, p-value 0.06), Ceftriaxone (9.36 ± 5.94 mm, p-value 0.83) and Cefotax (13.08 ± 6.75 mm, p-value 0.39). Other tested antibiotics remained non-significantly more active than *C. polygonoides*'s methanol extract except Chloramphenicol (22.7 ± 3.64 mm, p-value 0.04), Ciprofloxacin (25.50 ± 2.35 mm, p-value 0.008), and Sparaxin (25.22 ± 2.48 mm, p-value 0.01). The *C. polygonoides*'s methanol extract's activity against *E. coli* was maximum (9 ± 0.00 mm) at 200 µg/disc and 1 mg/disc concentration. In relevance to the standard antibiotics the activity was non-significantly greater than Augmentin (7.70 ± 5.88 mm, p-value 0.73), Oxy-Tetracyclin (7.51 ± 9.71 mm, p-value 0.81), Caricef (2.80 ± 6.03 mm, p-value 0.21), Fucidin (8.40 ± 9.97 mm, p-value 0.91) and Ceftriaxone (7. 67 ± 7.93, p-value 0.77). While other standard antibiotics were proven to be relatively more potent ones against *E.coli* but not significantly except Amikacin (14.38 ± 1.22 mm, p-value 0.01) and Tazobactam (15.30 ± 6.86 mm, p-value 0.04). Against *P. aerugi-*

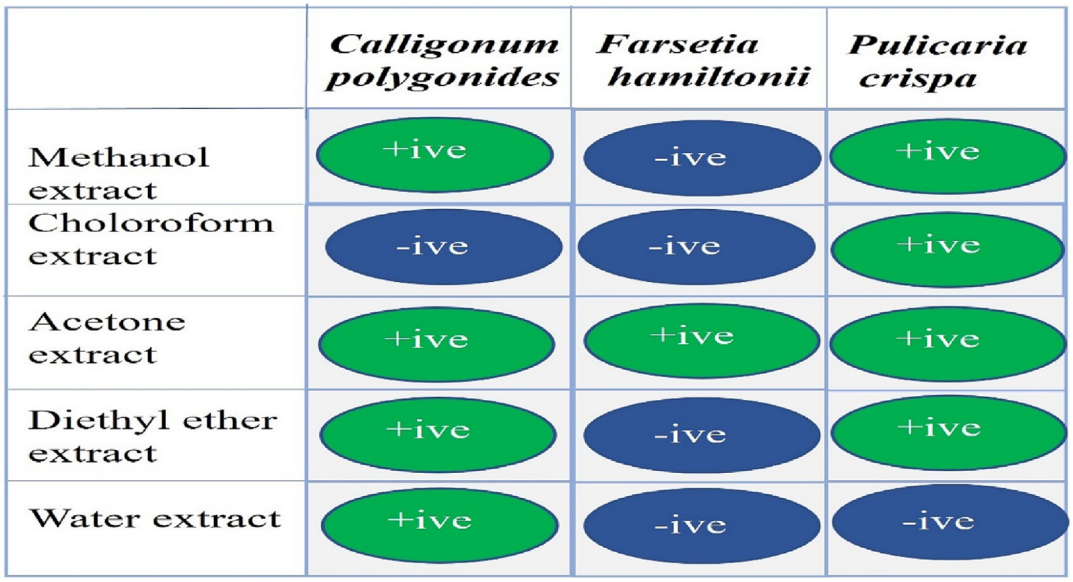

Fig. 2. Results of antibacterial activity of *Calligonum polygonoides*, *Farsetia hamiltonii* & *Pulicaria crispa* in different solvents. Green colour represents positive results and blue colour indicates negative result.

nosa's methanol extract was more active (13 ± 0.41 mm zone of inhibition) at 200 µg /disc and 1 mg/disc concentration that was significantly higher than Caricef (1.00 ± 2.83 mm, p-value 0.01) and non-significantly more than Augmentin (9.80 ± 7.53 mm, p-value 0.53). While in contrast all other standard antibiotics remained more effective among which Ciprofloxacin (23.11 ± 3.8 4 mm, p-value 0.04), Enoxacin (25.13 ± 4.75 mm, p-value 0.04) and Saparaxin (24.70 ± 5.71 mm, p-value 0.04) represented significantly higher activity.

The acetone extract was found to possess activity against *S. aureus*, *P. vulgaris* and *E. coli*. It was totally inactive against *P.aeruginosa*. Among the three strains, it was least active against *E. coli* and most active against *S. aureus*. Against *P. vulgaris*, the acetone extract at the concentration of 1 mg /disc was found to be more effective with 19 ± 1.21 mm zone of inhibition. In comparison to antibiotics, it represented significantly high activity than Caricef (3.57 ± 5.70 mm, p-value 0.03). While exhibited non-significant high activity than Augmentin (12.88 ± 5.53 mm, p-value 0.19), Amikacin (16.50 ± 2.74 mm, p-value 0.25), Fucidin (15.00 ± 9.58 mm, p-value 0.54), Urixin (17.71 ± 8.07 mm, p-value0.80), Cefotax (14.75 ± 9.89 mm, p-value 0.53) and Ceftriaxone (14.00 ± 7.00 mm, p-value 0.34). Moreover, other antibiotics including Chloramphenicol (20.83 ± 9.48 mm, p-value 0.77), Ciprofloxacin (22.20 ± 9. 37 mm, p-value 0.61), Enoxacin (22.12 ± 9.98 mm, p-value 0.64), Saparaxin (22.00 ± 9.53 mm, p-value 0.64) and Tazobactam (20.6 3 ± 4.09 mm, p-value 0.56) were noticed to be more active than plant extract but not up to significant level. *C. polygonoides*'s acetone extract against *S. aureus* represented maximum growth inhibition (21 ± 0.00 mm) 200 µg/disc which seemed to be significantly more than the Augmentin (11.90 ± 1.14 mm, p-value 0.005) and non-significantly high than control drugs Oxy-Tetracyclin (16.29 ± 2.96 mm, p-value 0.11), Amikacin (15.09 ± 5. 40 mm, p-value 0.19), Fucidin (17.33 ± 2.56 mm, p-value 0.13), Urixin (17.88 ± 2.85 mm, p-value 0.19), Tazobactum (18.60 ± 4.4 mm, p-value 0.44) Cefotax (13.08 ± 6.75 mm, p-value 0.17) and Ceftriaxone (9.36 ± 5.94 mm, p-value 0.07). Whereas its activity at 200 µg/disc was non-significantly less than Chloramphenicol (22.7 ± 3.64 mm, p-value 0.50), Ciprofloxacin (25.50 ± 2.35 mm, p-value 0.08), Enoxacin (24.90 ± 4.89 mm, p-value 0.30), and Sparaxin (25.22 ± 2.48 mm, p-value 0.09) antibiotics. Against *E. coli*, the *C.polygonides* acetone extract exhibited maximum activity (12 ± 1. 15 mm) at the concentration of 200 µg /disc which is nearly equal to the zone of inhibition observed in case of Ciprofloxacin (12.57 ± 9.35 mm). However, it was found to be non-significantly more potent than Chloramphenicol (11.13 ± 9.65 mm, p-value 0.89), Augmentin (7.70 ± 5.88 mm, p-value 0.33), Oxy-Tetracyclin (7.51 ± 9.71 mm, p-value 0.50), Caricef (2.80 ± 6.03 mm, 0.11), Fucidin (8.40 ± 9.97 mm, p-value 0.56), Urixin (10.63 ± 9.19 mm, p-value 0.63), Cefotax (11.70 ± 8.28 mm, p-value 0.95) and Ceftriaxone (7.67 ± 7.93 mm, 0.39). Whereas other studied antibiotics exhibited more activity than extract but not up to significant extent.

The diethyl ether extract of *C.polygonoides* was only active against *E.coli* and it was examined that the discs of plant extract at the concentration of 200 µg /disc and 1 mg/disc showed highest diameter of zone of inhibition (10 ± 1.16 mm, 10 ± 1.72 mm), respectively. In comparison to maximum activity of diethyl ether extract, Urixin showed nearly equal zone of inhibition (10.63 ± 9. 19 mm, p-value 0.91). A non-significant less activity was depicted by Augmentin (7.70 ± 5.88 mm, p-value 0.57), Oxy-Tetracycline (7. 51 ± 9.71 mm, p-value 0.70), Caricef (2.80 ± 6.03 mm, p-value 0.16), Fucidin (8.40 ± 9.97 mm, p-value 0.7) and Ceftriaxone (7.6 7 ± 7.93 mm, p-value 0.62). Only Amikacin represented significantly high activity (16.20 ± 3.31 mm, p-value 0.01) than ether extract. While all other tested antibiotics were found to be non-significantly more active than this plant extract i.e., Chloramphenicol (30 µg/disc) (11.13 ± 9.65 mm, p-value 0.85), Ciprofloxacin (23. 11 ± 3.84 mm, p-value 0.67), Enoxacin (17.30 ± 9.93 mm, p-value 0.29), Saparaxin (15.00 ± 9.56 mm, p-value 0.43), Tazobactum (15.30 ± 6.86 mm, p-value 0.26) and Cefotax (11.70 ± 8.28 mm, p-value 0.34). Data has been summarized in Figs. 3 and 6 and Supplementary Data Tables 1 and 4.

3.3. Farsetia hamiltonii antibacterial activity

Among all the extracts of *F. hamiltonii*, only acetone extract was found to possess antibacterial activity and it was only active against *S. aureus* and *P.aeruginosa*. Against *S.aureus* acetone extract showed maximum activity and zone of inhibition was measured to be 15 ± 1.15 mm at 200 µg /disc that was observed to be significantly more than Augmentin (11.90 ± 1.14 mm, p-value 0.02), and Caricef (0 mm, p-value 0.001) and non-significantly greater than Cefotax (13.08 ± 6.75 mm, p-value0.67) and Ceftriaxone's zone of inhibition (9.36 ± 5.94 mm, 0.23). While observed almost equal to Amikacin (15.09 ± 5.40 mm, p-value 0.97). All the other tested antibiotics were observed to be non-significantly more effective than *F. hamiltonii actone*'s extract except Ciprofloxacin (25.50 ± 2.35 mm, p-value 0.006), Saparaxin (25.22 ± 2.48 mm, p-value 0.008) which showed significantly larger zone of inhibition than that observed in case of plant extract at 200 µg /disc concentration.

Against *P. aeruginosa*, the plant extract exhibited maximum activity at the concentration of 200 µg /disc. The diameter of the zone of inhibition was '10 ± 0.15 mm' that was significantly higher than Caricef (1.00 ± 2.83 mm, p-value 0.03), and non-significantly greater than Augmentin (9.80 ± 7.53 mm, p-value 0.96). Whereas, Ciprofloxacin (23.11 ± 3.84 mm, p-value 0.02), Enoxacin (25.13 ± 4. 75 mm, p-value 0.03) and Saparaxin (24.70 ± 5.71 mm, p-value 0.04) in comparison showed significantly more activity and remaining antibiotics represented non-significant high activity (Figs. 4 and 6, and Supplementary Tables 2 and 4).

3.4. Pulicaria crispa antibacterial activity

All the *P. crispa*'s extracts displayed activity only against *S.aureus*. The methanol extract of *P. crispa* exhibited maximum activity at the concentration of 200 µg /disc. The zone of inhibition was observed to be 15 ± 1.11 mm in diameter thus the extract was found to be significantly more active than Augmentin (11.90 ± 1. 14 mm, p-value 0.02), Caricef (0 mm, p-value 0.001). While non-significantly more active than Cefotax (13.08 ± 6.75 mm, p-value 0.67) and Ceftriaxone (9.36 ± 5.94 mm, p-value 0.23) and equally active as Amikacin (15.09 ± 5.40 mm, p-value 0.97). The other antibiotics were significantly more active than the plant extract.

Acetone extract of *P.crispa* showed maximum activity (21 ± 2. 13 mm at 75 µg /disc, 21 ± 1.45 mm at 100 µg /disc, 21 ± 0.00 m m at 150 µg /disc and 21 ± 0.65 mm at 200 µg /disc) against *S.aureus*. In comparison to antibiotics, it showed significantly high activity than Augmentin (11.90 ± 1.14 mm, p-value 0.006) and Caricef (0 mm, p-value 0.001). While noted to be non-significantly more active than all the tested standard antibiotics except Chloramphenicol, Ciprofloxacin, Enoxacin and Sparaxin which showed more activity.

Chloroform extract exhibited maximum activity (21 + 0.95 mm at 75 µg /disc, 21 + 0.00 mm at 200 µg /disc) against *Staphylococcus aureus* and it was found to be significantly more effective than Augmentin (11.90 ± 1.14 mm, p-value 0.0005) and Caricef (0 mm, p-value 0.0006). While non-significantly more active than all the other tested antibiotics except Chloramphenicol (22.7 ± 3.64 mm, p-value 0.5), Ciprofloxacin (25.50 ± 2.35 mm, p-value 0.08), Enoxacin (24.90 ± 4.89 mm, p-value 0.30) and Sparaxin (25.22 ± 2.48 mm, p-value 0.09) which showed greater but non-significant zone of inhibition.

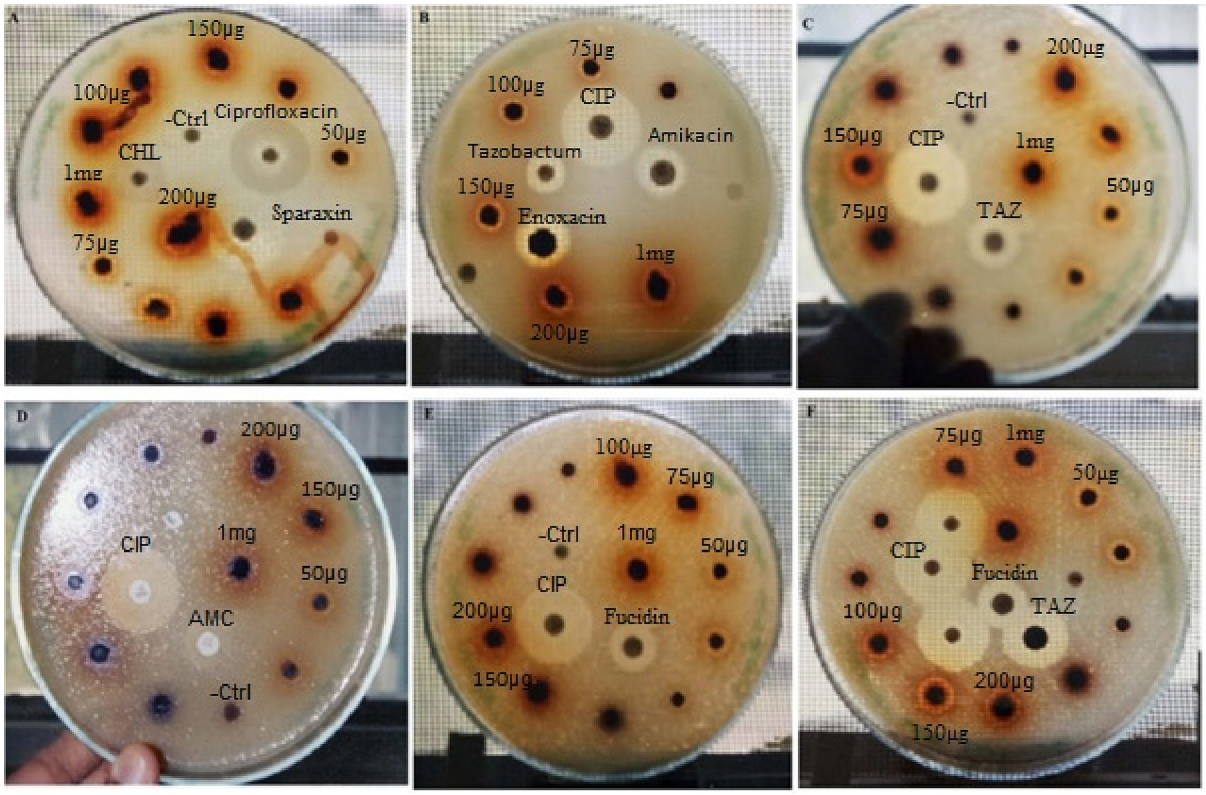

Fig. 3. Antibacterial activity of *Calligonum polygonides* against different bacterial strains (A) Acetone extract activity against *Staphylococcus aureus* (B) Methanol extract activity against *Staphylococcus aureus* (C) Water extract activity against *Staphylococcus aureus* (D) Acetone extract activity against *Proteus vulgaris* (E) Methanol extract activity *Proteus vulgaris* (F) Water extract activity against *Pseudomonas aeruginosa.* Values above zone represents the concentration (µg/disc, mg/disc) of *Calligonum polygonides* extract at which zone of inhibition appear in comparison to standard antibiotics. TAZ = Tazobactam, CHL = Chloramphenicol, AMC = Augmentin, CIP = Ciprofloxacin, -Ctrl = Negative control.

Diethyl ether extract of *P.crispa* activity against *S.aureus* was noted to be maximum at 150 µg /disc concentration with 25 + 0.00 mm diameter of the zone of inhibition that was almost equal to that as observed in case of Ciprofloxacin (25.50 ± 2.35 m m, p-value 0.74) and Sparaxin (25.22 ± 2.48 mm, p-value 0.89) and significantly greater than Augmentin (11.90 ± 1.14 mm, p-value 0.002), Oxy-tetracycline (16.29 ± 2.96 mm, p-value 0.03), Fucidin (17.33 ± 2.56 mm, p-value 0.03), Urixin (17.88 ± 2.85 mm, p-value 0.04), Ceftriaxone (9.36 ± 5.94 mm, p-value 0.04). The data is shown in Figs. 5 and 6 and Supplementary Data Tables 3 and 4.

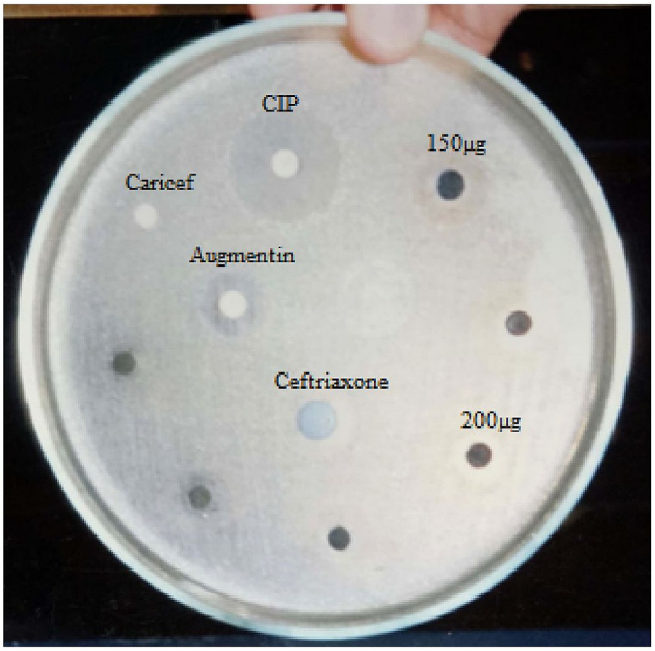

Fig. 4. Antibacterial activity of *Farsetia hamiltonii*. Zone of inhibition by acetone extract of *F.hamiltonii* against *Staphylococcus aureus* has been shown in the image. Values above zone represents the concentration (µg/disc, mg/disc) of *F.hamiltonii* extract at which zone of inhibition appear in comparison to standard antibiotics.

Moreover, further evaluation of bacteriostastic and bacteriocidal effect indicates that the water, methanol and acetone extract of *C. polygonides* was observed to exhibit bacteriostatic activity against *S. aureus, P. vulgaris* and *P. aeruginosa,* while bacteriostatic activity against *E. coli* was found in methanol, acetone and diethyl ether extract of *C. polygonides. F. hamiltoniia* acetone extract represents bacteriostatic activity against *S. aureus.* Further, *P. crispa* was observed to be active against only *S.aureus*. Its acetone extract exhibit bactericidal action against *S. aureus*. Whereas its methanol, chloroform and diethyl ether extracts were found to show bacteriostatic role (Fig. 7).

3.5. Cytotoxicity results

The extracts of *C polygonoides*, *F. hamiltonii* and *P. crispa* in five different solvents (i.e. methanol, chloroform, acetone, diethyl ether and water) were used for cytotoxicity assay at different concentrations (i.e. 10, 100 and 1000 µg/ml).

In case of *C. polygonoides* diethyl ether extract 50% and 60% mortality was noted in test sample at the concentration of 1000 µg/ml. At the same concentration the % mortality was 60% for methanol control (solvent) and 70% for test sample. Using chloroform extract the larvae death rate was noted to be (70%) in solvent (90%) in test sample at 1000 µg/ml. The % mortality was

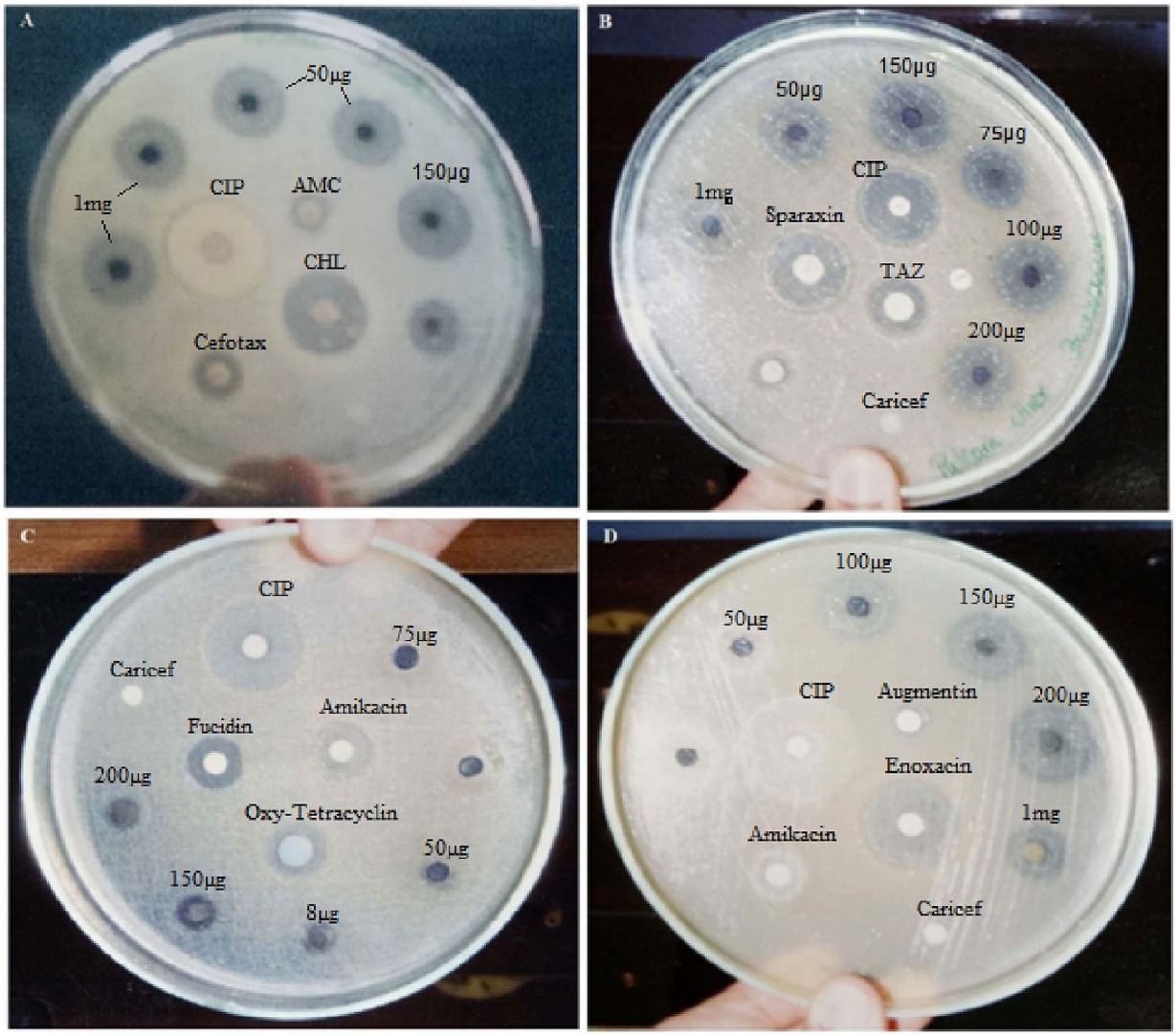

Fig. 5. Antibacterial activity of *Pulicaria crispa* in different solvents extract (A) Acetone extract activity against *Staphylococcus aureus* (B) Diethyl ether extract activity against *Staphylococcus aureus* (C) Methanol extract activity against *Staphylococcus aureus* (D) Chloroform extract activity against *Staphylococcus aureus.* Values above zone represents the concentration (µg/disc, mg/disc) of *P. crispa* extract at which zone of inhibition appear in comparison to standard antibiotics. TAZ = Tazobactam, CHL = Chloramphenicol, AMC = Augmentin, CIP = Ciprofloxacin.

increased to 30% using acetone extract (i.e. 70% solvent: 100% test sample at 1000 µg/ml). Water was proved to be non-toxic for the larvae. But the water extract of *C. polygonoides* showed larvae death rate increased from 20 to 70% as the concentration increased from 10 to 1000 µg/ml (Fig. 8A and Supplementary Data Table 5).

In case of *F. hamiltonii*, methanol and chloroform showed maximum toxicity as solvent and just 10% more larvae died in the extract at higher concentrations as compared to the solvent. In case of ethanol death rate was 70% in sample and 60% in solvent at 1000 µg/ml. While using chloroform at the same concentration the larvae death rate was found to be 80% with test sample and 70% with solvent. The larvae death rate was increased to 20% in case of acetone extract (70% solvent: 90% sample at 1000 µg/ml). Diethyl ether extract at higher concentration was estimated to possess maximum cytotoxicity as compared to the solvent i.e. 20%, 40%, 50% larvae died with solvent at the concentration of 10, 100, 1000 µg/ml, respectively. Water was shown to possess no cytotoxic activity but the water extract of *F. hamiltonii* indicated that the components are cytotoxic. As the concentration was increased from 10 to 1000 µg/ml, larvae death rate increased from 50% to 80% (Fig. 8B and Supplementary Data Table 6).

In case of *P. crispa* Chloroform as a solvent showed maximum toxicity and just 20% more larvae died in the extract at higher concentrations as compared with the solvent (70% in chloroform and 90% in test sample at 1000 µg/ml). Using methanol extract, the larvae death rate was increased to 40% as compared to methanol control (60% larvae in sample and 100% in test sample at 1000 µg/ml). On the other hand, in case of acetone and ether extract the larvae death rate was increased by 30% as compared to their respective solvent controls. It was found to be 70% in acetone and 100% in test sample at the concentration of 1000 µg/ml. While at the same concentration, the larvae death rate was 50% in diethyl ether and 80% in test sample. The most effective of all was water extract. Water remained non-toxic solvent for the larvae but as the concentration of the test sample was increased from 10 to 1000 µg/ml, larvae death rate increased from 30 to 80% (Fig. 8C and Supplementary Data Table 7).

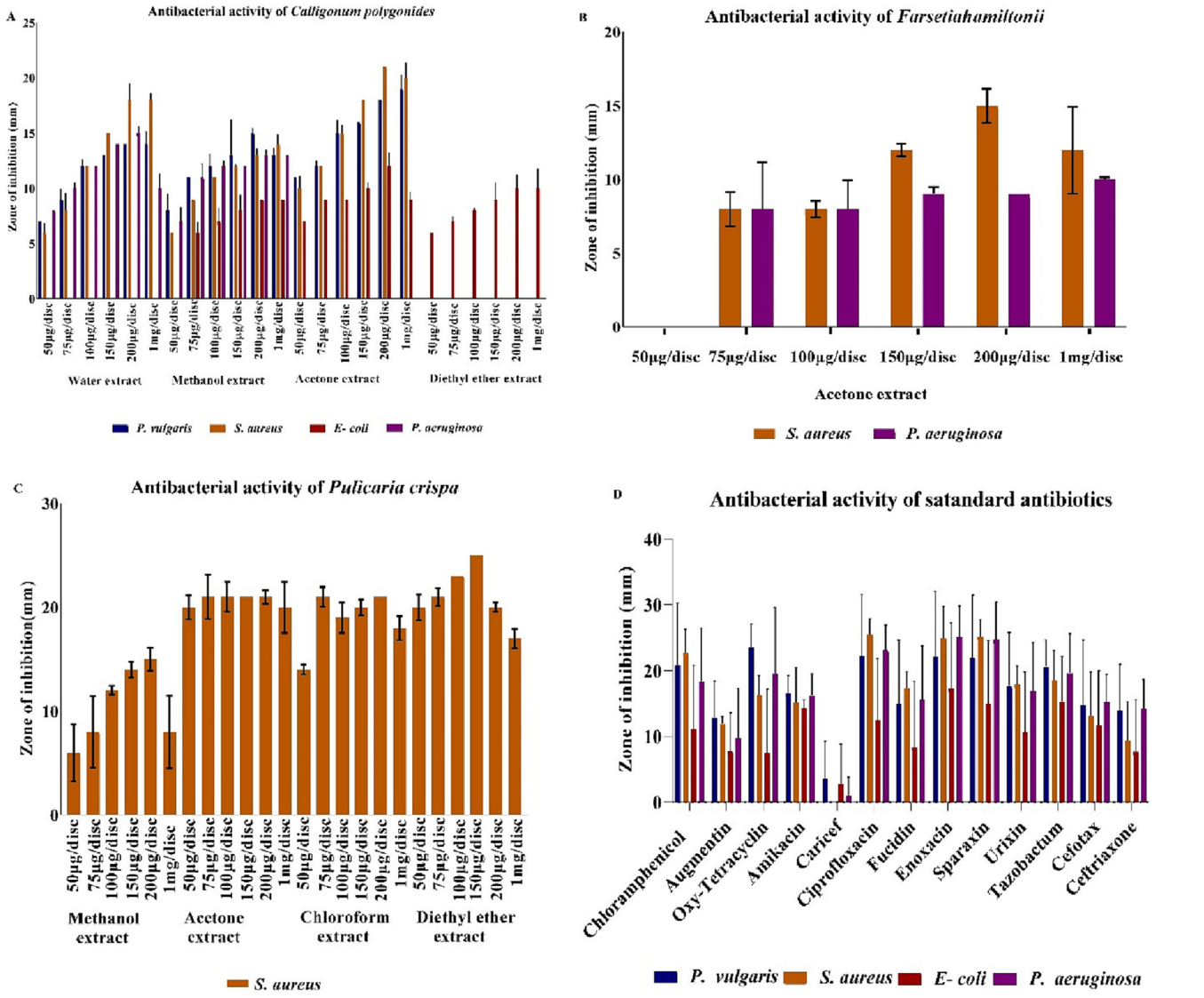

Fig. 6. Antibacterial activity results detected in different solvents against *Staphylococcus aureus (S.aureus)*, *Proteus vulgaris (P.vulgaris)*, *Escherichia coli (E.coli)* and *Pseudomonas aeruginosa (P.aeruginosa)* bacterial strains. (A) Antibacterial activity of Calligonum *polygonides* (B) Antibacterial activity of *Farsetia hamiltonii* (C) Antibacterial activity of *Pulicaria crispa* (D) Antibacterial activity of standard antibiotics.

4. Discussion

Now a days plants are extensively studied and used for medicinal purposes and can serve as good source of antibiotics/drugs (Sultana 2011). Keeping in view the medicinal importance of plants the presented study was conducted to explore antibacterial activity, bacteriocidal and bacteriostatic effect, cytotoxicity effect of three different plants species namely *C. polygonides*, *F. hamilton* and *P. crispa* from Cholistan desert, Pakistan. Plants possessing antibacterial potential are of extreme importance, being the source of antibiotics. Many human, animal and plant diseases are caused by pathogenic microbes. Historically it is evident that many of the new antibiotics were isolated from natural sources (plants and soil microbes etc.). Many more were later synthesized and introduced in clinical practices. In the describe study the extracts of *C. polygonoides, F. hamiltonii* and *P. crispa* in five different solvents (i.e. water, methanol, chloroform, acetone and ether) were examined to evaluate their antibacterial potential against four different strains of bacteria i.e. *S. aureus, P. vulgaris, P.aeroginosa* and *E. coli.*

All the extract of *C. polygonoides* were observed to show antibacterial potential against all tested strains of bacteria. Its water extract showed bacteriostatic inhibitory effect against *P. vulgaris*, *S.aureus* and *P. aeruginosa* while found ineffective against *E.-coli* which is in agreement to earlier work reporting no antibacterial activity of water extract against *E.coli* (Mukhtar et al., 2018). It was observed that with increasing concentration the antibacterial potential of *C.polygonides* against *P. vulgaris* also increased from 7 ± 0.00 mm to 14 ± 1.15 mm. In comparison to antibiotics, it was noted that extract is as much effective as Cefotax and Fucidin as they displayed equal zone of inhibition. Its activity was found higher than Augmentin, Caricef, and Ceftriaxone but not much difference between the activities of water extract and these antibiotics's activity was seen which indicates that the extract was not more effective than drugs. Further analysis revealed that Chloramphenicol, Oxy-tetracycline, Ciprofloxacin, Fucidin, Enoxacin, Sparaxin, Urixin and Tazobactam exhibited non-significantly higher zone of inhibition against *P. vulgaris* as not much difference was noted between these drugs and extract's activity which reflects the similar potential of drugs and *C. polygonides* water

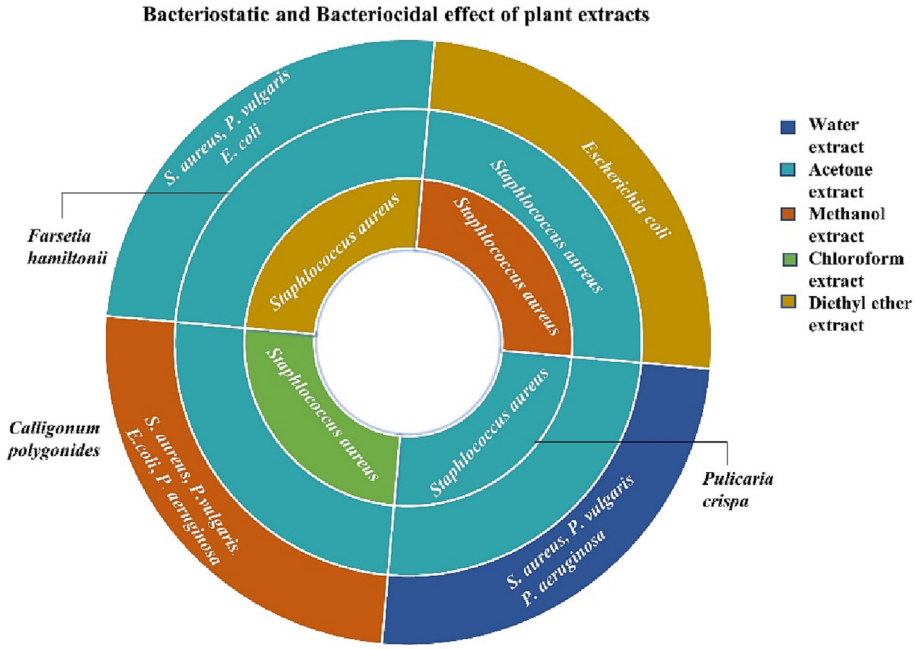

Fig. 7. Bacteriostatic effect determination of biologically active extracts of *Calligonum polygonoides*, *Farsetia hamiltonii* & *Pulicaria crispa*. Legends shows the plant extract in which activity was observed against respective bacterial strain mention inside the circle. Different colours represents the different plants extracts which shows antibacterial activity. Bacteriocidal effect was only observed by acetone extract of *Pulicaria crispa*. While others plant extracts exhibit bacteriostatic effect against various strains.

extract. This observation thus supports the presence of antibacterial potential in the water extract of *C.polygonides*. The activity of *C.polygonides's* water extract against *S. aureus* increased from 6 ± 0.75 mm at 50 µg/disc reaching maximum to 18 ± 0.52 mm at 1 mg/disc. This indicates that higher concentration of extract should be preferred for antibacterial activity. Moreover, the antibacterial potential was significantly greater than standard antibiotics, Augmentin and Caricef, hence, it is deduced that the *C.polygonides* water extract can be more effectively used to combat *S. aureus* infections. It was also investigated that at low concentration of extract the activity was significantly less than antibiotics but on increasing the concentration the difference between their activities was reduced and extract behaved similar to antibiotics as seen in case of Chloramphenicol. Against *P. aeruginosa*, *C.polygonides's* water extract represented increasing trend of activity (8 ± 0.00 mm to 15 ± 0.59 mm) at increasing concentration i.e., 50 µg/disc to 200 µg/disc. But further increase in the concentration of extract to 1 mg resulted in decreased antibacterial activity (10 ± 1.29 mm). This may be due to the fact that the extract is the mixture of many components including active components and other molecules which are their antagonists. Hence, at 1 mg /disc concentration definitely active components's concentration is elevated but the crude extract mixture will also contain higher proportion of the antagonistic molecules which reduces the antibacterial potential.

The comparison with standard antibiotics showed *C.polygonides*'s water extract more effective than Caricef and equally effective as Cefotax and Fucidin. Similarly the extract's activity was greater than Augmentin and Ceftriaxone but there was no significant difference between their results thus we cannot suggest that extract has more antibacterial potential than these antibiotics. Besides this, Chloramphenicol, Oxy-tetracycline, Amikacin, Urixin and Tazobactum presented no significant higher difference between the zone of inhibition when compared with water extract representing extract to have similar antibacterial potential as these drugs. It was also found that at low concentration extract's activity was less than Ciprofloxacin, Enoxacin and Sparaxin but on high extract concentration i.e 200 µg/disc, 150 µg/disc and 100 µg/disc respectively the water extract began to show equivalent effectiveness against *P. aeruginosa* as shown by these antibiotics. This analysis of antibacterial potential of *C. polygonoides* water extract revealed that water extract contains active components that play role in the antibacterial activity against these three bacterial strains thus the studied plant extract can be used to control the infections. These finding contrast with previous study where no activity (0.0 mm at 50 mg/ml) was noted in water extract of *C. polygonides* (Mukhtar et al., 2018). This might be due to the high concentration used for extract making as high concentration may increase the availability of antagonistic components which can reduce the activity. Further no study has reported the antibacterial activity of *C. polygonoides*'s water extract, against *P. vulgaris*, *S. aureus* and *P. aeruginosa.* But above description provides first evidence of greater antibacterial potential of *C. polygonides*'s water extract, comparable to antibiotics, against the studied bacterial strains.

The methanol extract of *C. polygonides* was tested positive against all the four bacterial strains. Against *P. vulgaris* it displayed increasing trend of zone of inhibition on increasing concentration up to 200 µg /disc and then activity was reduces at 1 mg/disc. In comparison to antibiotics, this extract has the same efficiency as possess by Fucidin against *P. vulgaris*. All the other studied drugs except Augmentin, Caricef, Cefotax and Ceftriaxone have antibacterial potential that is comparable with the extract. This demonstrated that methanol extract behaves similarly as antibiotics against *P. vulgaris*. No study was found in the literature which investigated antibacterial activity of the *C. polygonides*'s methanol extract against *P. vulgaris*. Against *S. aureus* the maximum activity of methanol extract was found to be more than Caricef suggesting its higher effect and more potency. In this case it was also observed that the difference between activity at high extract's concentration and of drugs namely Fucidin, Urixin, Tazobactam, became non-significant representing that these drugs have similar antibacterial activity. Against *E. coli* methanol extract displayed increasing activity trend which in comparison to studied antibiotics found not much effective than drugs except in case of Chloramphenicol, Ciprofloxacin, Enoxacin, Urixin, Sparaxin, Tazobactam and Cefotax

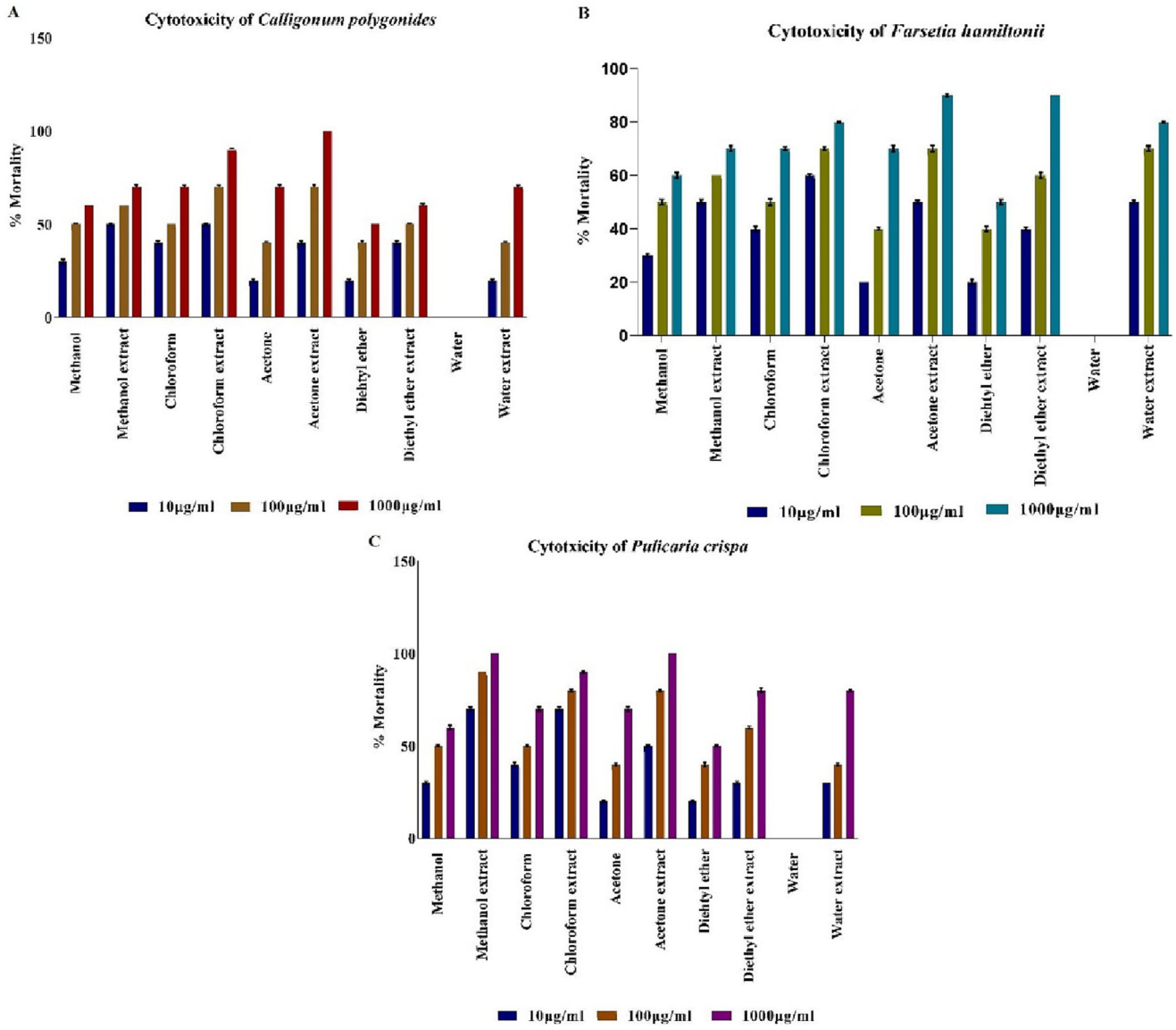

Fig. 8. Cytotoxic activity of different solvent extracts of *Calligonum polygonoides, Farsetia hamiltonii* and *Pulicaria crispa* against the larvae of brine-shrimp. (A) Cytotoxicity of *Calligonum polygonoides* (B) Cytotoxicity of *Farsetia hamiltonii* (C) Cytotoxicity of *Pulicaria crispa.*

which display non-significant difference between the zone of inhibition in relevance to that observed in case of extract. This study is in agreement with previous findings where *C. polygonides* was found to have activity against S. aureus and E.coli. In an earlier study from KPK province, Pakistan methanol extract of *C. polygonoides* was described to have antibacterial effect of 7.1 ± 0 mm, 3.5 ± 0.3 mm against *Escherichia coli* at respective concentration of 10 mg/1ml and 5 mg/1ml which is equivalent to Tetracycline 7. 01 ± 0.5 mm used as control (Khan et al., 2017). Another study reported the maximum inhibitory effect of methanol extract against studied gram negative bacteria i.e *E. coli* (15.2 ± 1.5 mm) and gram positive bacteria *S. aureus* (16.5 ± 0.5) at concentration of 30 mg/ml (Khan et al., 2015). A previous investigation reported this plant to have less inhibition effect against *E. coli* and *Bacillus subtilis* in relevance to *Calligonum arich* leaves extract (Yahia et al., 2019). Earlier conducted research indicated maximum antibacterial activity of *C. polygonoides* ethanolic extract stem against *E. Coli* 9.40 mm and *S. Aureus* 14.60 mm (Mukhtar et al., 2018). While shoot extract of it exhibited no activity against *E. Coli and* 11.00 ± 0 .90 mm inhibition zone against Staphylococcus aureus (Heshmat et al., 2018). It was also found in case of another member of Polygonaceae family, *Calligonum* arich L. where the plant's methanol extract demonstrated high inhibition activity 15.00 ± 0.20 mm and 10.80 ± 0.16 against *S. aureus* and , respectively (Yahia et al., 2019). These studies ascertain the presence of microbial growth's inhibitory components in *C.polygonides's* methanol extract which can therefore be exploited for the isolation and purification of antibacterial drugs with possibly less harmful side effects.

In our study we have also observed that methanol extract has activity against *P.aeruginosa* showing more growth of inhibition activity than Caricef. While Chloramphenicol, Oxy-tetracycline, Amikacin, Fucidin, Sparaxin, Urixin, Tazobactum, Cefotax and Ceftriaxone although show greater zone of inhibition but not much significant difference was noted in relevance to that observed by methanol extract which proves that the extract has same effect as these drugs. This is in accord to earlier report by Arsalan et al., (2022) in which noted activity (7 ± 0.58 mm 50 µg/disc) against *P. aeruginosa* was equal to that observed in our study at the same concentration. This observation supports our findings that activity against *P. aeruginosa* is present in *C. polygonides.*

Acetone extract of *C.polygonides* was found active against *S. aureus*, *P. vulgaris* and *E. coli*. Its activity against *P. vulgaris* increases with elevating concentration and was comparable with the antibiotics representing more growth inhibition as compared to Caricef which means it is more effective than Caricef against *P. vulgaris* and behave like Chloramphenicol, Ciprofloxacin, Enoxacin, Saparaxin and Tazobactam. Even though these antibiotics have higher inhibition zone but that was not much different to the one as observed in case of extract. Against *S. aureus* acetone extract was seen to be more effective than Augmentin while it showed similar effect as that of Chloramphenicol Ciprofloxacin, Enoxacin, and Sparaxin. Acetone extract's activity against *E. coli* was noted to be equivalent to Ciprofloxacin which shows that it is as effective as this antibiotic. Whereas other drugs including Enoxacin, Sparaxin and Tazobactam shows higher but not much different growth of inhibition in comparison to extract which reveal that they have same effect. Previously no study described *Calligonum*'s acetone extract's role against tested strains. However, crude extract of another plant species in acetone has been proved to have greater antibacterial potential (Famuyide et al., 2019) which favours our findings of acetone extract as potential inhibitor of bacterial strains.

C. polygonoides's diethyl ether extract possesses growth inhibition activity against *E. coli* and was found as effective as Urixin. This extract was noted to have more antibacterial potential than Augmentin, Oxy-tetracycline, Caricef, Fucidin and Ceftriaxone. Whereas it was also noted that despite high activity of Chloramphenicol, Ciprofloxacin, Enoxacin, Saparaxin, Tazobactum and Cefotax these antibiotics and extract show same effect as no significant difference in their zone of inhibition was noted. This analysis reveals that *C. polygonides* have antibacterial potential that are comparable to standard antibiotics which helps to document this plant to be possess medically significant active component that play role in antibacterial activity against the studied bacterial strains. No previous study has explored the antibacterial activity of *C. polygonoides*'s diethyl ether extract whereas diethyl ether extract of *Spirulina platensis* has been found to possess high activity against *E. coli* and *P. aeruginosa* (El-Sheekh et al., 2014).

Our study is in agreement with a previous study narrating that *C. polygonides*'s different extract have greater antibacterial potential against pathogenic bacterial strains like *E. Coli* and *S. aureus* (Mukhtar et al., 2018). But here we also document that *C. polygonides*'s *extracts* also have growth inhibition potential against *P. vulgaris* and *P. aeruginosa.*

F.hamitonii analysis reveal that only acetone extract has the antibacterial activity against *S.aureus* and *P.aeruginosa* while no activity of it was noted against *P.vulgaris* and *E.coli.* This investigation is in agreement with a previous study indicating no sensitivity of *F. hamitonii*'s methanol extract against *E. coli* and *S. aureus* (El-Sharkawy et al., 2017). Testing acetone extract against *S. aureus* indicated increase in activity with increasing concentration up to 200 µg/disc reaching maximum to 15 ± 1.15 mm. But it was observed that further enhancing the concentration to 1 mg/disc will result in loss of activity (12 ± 2.95 mm). In relevance to standard antibiotics the activity (15 ± 1.15 mm) against *S.aureus* was higher than Augmentin, Caricef, Cefotax. While its activity was found equal to Amikacin. Other drugs showing non-significant higher zone of inhibition than that of acetone extract suggest that the extract and drug behave similar. It was also observed that at higher concentration extract became as effective as Oxytetracycline, Fucidin, Enoxacin, Urixin and Tazobactam. However, at low extract's concentration, Oxytetracycline is more effective than extract. This indicated that different concentrations of acetone extract have antibacterial potential which are comparable with standard antibiotics. Acetone extract also exhibited increasing antibacterial potential with an increase in concentration and it was found more potent than Caricef against *P. aeruginosa*. In relevance to other drugs, it appears that on high concentration of *Farsitia's* extract activity enhances and its antibacterial potential becomes alike Chloramphenicol, Tazobactam, Cefotax, Ceftriaxone as difference among their zone of inhibition is lessened with increasing concentration. No work has been done earlier on *F. hamiltonii* to investigate its antibacterial potential.

P. crispa's extracts in all the solvents were found to be active only against *S.aureus* which is in contrast to previous findings where the methanol extract of *P. crispa* was reported to be active against *P.Aurogenosa* exhibiting 2 ± 0.58 mm to 16.33 ± 0.67 mm zone of inhibition (Arsalan et al., 2022). The methanol extract was examined to be effective in inhibiting growth of *S. aureus* and found more effective than Augmentin and Caricef whereas displayed same antibacterial potential as Amikacin. Moreover, extract's activity observed at high concentration is comparable with Tazobactam, Urixin, Fucidin and Oxy tetracycline which manifested that they have antibacterial effect against *S. aureus.* Our results are supported by former study which described potent antibacterial activity (>16 mm) in methanol extract of *P. crispa* against *S.aureus* (Abdelah Bogdadi et al., 2007).

Acetone extract of *P. crispa* manifested consistent activity against *S.aureus* at high concentration but at 1 mg/disc activity was reduced which confers that principal active component of the *P. crispa* losses activity at high concentration. It has more antibacterial potential than Augmentin and Caricef while has same effect as that of Chloramphenicol, Ciprofloxacin, Enoxacin and Sparaxin. Although the antibiotics's zone of inhibition was larger than extract, but no significant difference was present between them which proves that the antibiotics and extracts have similar effect against *S. aureus.* Disparity exists between our results of acetone extract and previous study conducted in Saudi Arabia, by Foudah et al., (2015) in that we have observed no activity in acetone extract of *P.crispa* against *E.coli* but formerly good inhibitory effect with 21 mm zone of inhibition was detected against *E.coli* in aerial part of *P.crispa* suggesting that it has enough antibacterial potential against *E.coli* (Mirghani et al., 2020). This might be due to the fact that some active components that are responsible for activity against *E.coli* in *P.crispa* might be absent in our plant due to difference in the plant habitat as the condition of soil differ country wise. The *P. crispa*'s Chloroform extract also exhibited potential inhibitory activity that was lessened on high concentration of extract and was found to be more potent than Augmentin and Caricef and similar in effect to Chloramphenicol, Ciprofloxacin, Enoxacin and Sparaxin. Diethyl ether extract's activity was also decreased on enhancing the concentration beyond 150 µg/dics. In comparison to antibiotics, this extract is as effective as Ciprofloxacin and Sparaxin while more effective than Augmentin, Oxytetracycline, Fucidin, Urixin and Ceftriaxone. This analysis helps to document that *P. crispa* contains such essential components which play roles in the antibacterial activity against *S.aureus* thus *P.crispa* extract can be beneficial in the curing the *S.aureus* infectious. Our findings are also supported by earlier study which documented greater antibacterial activity (>16 mm) in chloroform extract of *P. crispa* against *S.aureus* (Abdelah Bogdadi et al., 2007). This study contrasts with a previous study where *P. crispa* essential oils exhibited less antibacterial activity against *E. Coli* (3.04 ± 0.55) and *S. aureus* (4.27 ± 0.12) in comparison to standard antibiotic Gentamicin (26.0 ± 0.0) and Ampicillin (25.0 ± 0.9) respectively. Minimal inhibitory concentration (MIC) was described to be $\leq$ 0.5 for *E.Coli* and 2 for S. aureus (AlMotwaa and Al-Otaibi 2022). Antibacterial assay results demonstrated that all the plants possess antibacterial activity even more than drugs thus can be used to find the active components and using them in antibiotics development.

In the present study we have observed that some solvents are inactive against any of the studied strain as water extracts of *F. hamiltonii* and *P. crispa* were found inactive while examined to be active in case of *C. polygonides*. One of the possible reasons will be the polarity of active components present in a specific plant source. The increasing polarity trend of solvents, used during study, is water > methanol > acetone > chloroform > diethyl ether. Active principal components of plants, responsible for antibacterial activity, will be soluble in solvents that match with the nature of components i.e., polar components will be soluble/extracted in polar solvents and vice versa.

Due to the difference in the polarity of different solvents we may expect the difference in the extraction profile of the active components. Moreover, along with the active components many other components present in plant will be solubilized in water and many of the other components may act antagonistically to active components hence, water extract gives negative test results for antibacterial activity. Water is highly polar among all solvents. The principal components of *F. hamiltonii* and *P. crispa* might be soluble in less polar solvent due to which their water extract gives negative test results. Another reason can be that in water extract a very small quantity of components were extracted showing no activity or at high concentration of solvent extraction antagonist component will be extracted in high amount which will mask the effect of active components. These conditions should be considered for further analyzing the principal component responsible for antibacterial activity.

Plants with cytotoxic potential are of significant importance. It is now known that a positive correlation exists between brine shrimp lethality and 9 KB (human nasopharyngeal carcinoma) cytotoxicity therefore, brine shrimps are being used in many prescreens for potential anti-tumor activity. In addition, brine shrimp bioassays effectively predict pesticidal activities and respond to a broad range of chemical and pharmacologically diverse compounds McLaughlin, 1991

Various plants are known to have different biologically active components which add in their medicinal properties and can be considered in drug designing. Inspite of this, some plants possess diverse components which can have harmful impact depending on the dosage/concentration i.e high concentration toxic for biological system (Hamidi et al., 2014). Therefore, it is required to accurately measure the concentration and toxicity effect of these plants. The extracts of studied medicinal plants i.e. *C polygonoides*, *F. hamiltonii* and *P. crispa* in five different solvents (i.e. water, methanol, chloroform, acetone and ether) were investigated to evaluate their cytotoxic potential against *Artemia salina* (leach) larvae. Results indicated that in case of *C. polygonoides* the solvents, chloroform and diethyl ether were more toxic and only 10% increase in the rate of toxicity in the *C. polygonoides* extract in these solvents was noted. It was also observed that by increased in the concentration of extract, the rate of toxicity (larvae death rate) also increased. The maximum toxicity of *C. polygonoides* was observed in acetone extract at maximum concentration than in other slovents. These findings were in agreement to a previous study where the maximum toxicity (80%/1000 μg/mL death rate) was noted in methanol extract of *C. polygonoides* in District Banu,Pakistan (Khan et al., 2015). Another study from District Banu, Pakistan reported the maximum cytotoxicity of *C. polygonoides* in methanol extract presenting 100% death rate at concentration of 70 μg/mL (Khan et al., 2017) representing presence of biological components in this plant. Moreover, it is the first study to report the cytotoxicity in comparison to five different solvents. Previously, no study presented the changing level of toxicity with increasing concentration or using different solvents. The trend of cytotoxicity in decreasing order at maximum concentration can be stated as Acetone > Chloroform > Methanol, Water > Diethyl-ether. This shows that this plant possess bioactive components which have potential toxic effects. A previous study narrated the cytotoxic effect of *C. polygonoides* due to the presence of flavonoids (Quercetin) against the Human hepatocarcinoma cell line (HepG2) and human Caucasian breast adenocarcinoma cell line (MCF-7) with IC50 values of 4.88 and 0.87 μg/mL respectively (Ahmed et al., 2016).

F. hamilton cytotoxicity analysis revealed that cytotoxicity is directly proportional to the concentration of plant extract in different solvents. Although in all the solvents the cytotoxicity increases but the maximum cytotoxicity of *F. hamiltonii* was noted in diethyl ether extract representing less toxicity of ether and high toxicity of studied plants. It is the first study to report the cytotoxicity of *F. hamiltonii* extract in five solvents against brine shrimp. Although the medicinal importance of these plants has been reported but no study was conducted to notice its toxic effect. The cytotoxicity increases in the following order in studied plant extract: methanol < chloroform < acetone < diethyl ether < water at maximum concentration. This indicates that although the solvents are toxic enough but the extract of these plants in these solvents enhances the cytotoxic effect.

P. crispa possess cytotoxic potential as narrated by this study, among all the solvents extract maximum toxicity rate was observed in methanol. While among solvents, diethyl ether was less toxic. A previous study also reported the *P. crispa* cytotoxicity in terms of IC50 to be 37.9 (Elshiekh, 2015). Earlier its cytotoxicity was described to be 620.8 ppm (LD50) in 70% methanol, 18.7 ppm in petroleum ether and 10.8 ppm in ethyl acetate extract at concentration of 1000 μg/mL (Elshiekh and Mageed 2020). Another study in contrast to our results presents the antimicrobial effect of aqueous and alcohol plants extract against the gram negative and positive bacteria at low concentration (Ahmed and Ibrahim 2018). The decreasing trend of cytotoxicity was methanol > acetone > diethyl ether > chloroform. This analysis shows that even though the studied plants were known to be significant from a medicinal point of view, they also contain some biologically toxic components which need to be studied to explore their active use in medicine.

5. Conclusion

In the described study we have identified the potential effect of studied medicinal plants against different bacterial strains. We have also observed the effect of varying solvents and concentrations of plant extract over the different strains of bacteria which display remarkable results. Among the three plants, *C.polygonides* extract possess activity against *P.vulgaris and E. coli* maximum activity was noted in acetone extract of all three plants against *S. aureus, C.polygonides and F.hamiltonii have antibacterial activity against P. aeruginosa* that are in relevance to antibiotics. The brine shrimp lethality assay defined the cytotoxic effect of these plants which will be helpful in describing the toxicity of these plants while used for drugs or antibiotics preparation. This research exhibited that the *C. polygonides, F. hamiltonii* and *P. crispa* possess antibacterial and cytotoxic activity which shows that these contain the biological active components responsible for these activities. Further the discovery of active components present in these plants will pave the way for better and less harmful drug discovery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

Authors are thankful to Dr. Muhammad Arshad (Late), Ex-Director Cholistan Institue of Desert Studies (CIDS), The Islamia University of Bahawalpure for his valuable support regarding identification and collection of plant samples and study design.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103750.

Multimedia 1 file is the raw data used for the analysis. Multimedia 2 file is the statistical analysis result detail conducted for antibacterial analysis

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