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Full Length Research Paper

Antibacterial activity *in Vitro* of medicinal plants

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In present investigation, the pharmacological importance of selected plants viz. *Cycas revoluta, Cupressus sempervirens, Araucaria columnaris, Ricinus communis, Solanum nigrum, Calotropis procera, Withania coagulans, Cuminum cyminum, Foeniculum vulgare, Nigella sativa* and *Coriandrum sativum* was assessed. Antimicrobial activity of methanol, ethanol and ethyl acetate extracts of selected plants were investigated by well diffusion method. Six strains of bacteria viz. *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Pseudomonas aeruginosa* (ATCC6643) *Escherichia coli* (ATCC15224), *Klebsiella pneumoniae* (MTCC618) and *Salmonella typhimurium* (ATCC13048) were utilized as test organisms. It was revealed in this study that the antimicrobial activity of the extracts was enhanced by increase in the concentration of the extracts. Greater inhibitory activity against *S. aureus, P. aeruginosa, B. subtilis* and *K. pneumoniae* was possessed by ethyl acetate extract of *C. sempervirens*. The methanolic extract of *Nigella sativa* exhibited maximum inhibitory activity against *S. typhimurium*. The ethanolic extract of *Araucaria columnaris* showed higher activity against *E. coli*. It is inferred from present investigation that demonstration of antibacterial activity of selected plants against both gram-positive and gram-negative bacteria may be indicative of the presence of broad-spectrum antimicrobial compounds. These plants can be used in the cure of pathogenic diseases and improvement of crop growth.

Key words: Pharmacology, *Araucaria columnaris, Withania coagulans, Cupressus sempervirens.*

INTRODUCTION

Plants have a long therapeutic history over thousands of years and still considered to be promising source of medicine in the traditional health care system (Hemalatha et al., 2008). In recent year's investigation found several plants of the ethnomedicine posse's interesting biological activities that could be of interest for all parts of the world. The plants possess chemotherapeutic, bacteriostatic and antimicrobial agents (Venkatesan, 2009).

A great variety of ethno medicinal plants are being studied as a source of natural products useful in the development of novel drugs. The antimicrobial properties of drugs from medicinal and other edible plants have been recognized since antiquity (Cowan, 1999). Notwithstanding the wide literature concerning the beneficial effects of plant polyphenols in human health (Cowan, 1999; Krauze-Baranowska et al., 1999; Lin et al., 1999; Cassidy et al., 2000; Wang, 2000).

Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann et al., 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Plant products still remain the principal source of pharmaceutical agents used in traditional medicine (Ibrahim, 1997; Ogundipe et al., 1998). Interest in a large number of traditional natural products has increased (Taylor et al., 1996).

There is at present growing interest, both in the industry

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and in the scientific research, for aromatic and medicinal plants because of their antimicrobial and antioxidant properties. These properties are due to many active phytochemicals including flavanoids, terpenoids, carotenoids, coumarins, curcumines etc. These bioactive principles have also been confirmed using modern analytical techniques. Phenolic components, present in essential oils, have been known to possess antimicrobial activity and some are classified as generally recognized as safe (GRAS) substances and therefore could be used to prevent post-harvest growth of native and contaminant bacteria (Kabara, 1991; Singh et al., 2001).

Pakistan has a varied climate and is quite rich in medicinal herbs scattered over a large area. A total of 1572 genera and 5521 species are identified but only 600 plant species are documented and used for medicinal uses (Waheed et al., 2011).

Joshi et al. (2011) state that plants have provided a good source of antiinfective agents; emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids remain highly effective instruments in the fight against microbial infections (Hussain, 2011). Traditional health care systems as well as international herbal and pharmaceutical markets are dependent on medicines derived from plants. Nearly 80% of the world populations rely on the use of traditional medicines to meet their primary health care needs whereas; up to 90% of the developing world relies on the use of medicinal plants. Out of the total 4, 22,000 flowering plants reported from the world, more than 50,000 are used for medicinal purposes (Waheed et al., 2011).

The major objective of our study was to evaluate the effect of different concentration of *Cycas revoluta, Cupressus sempervirens, Araucaria columnaris, Ricinus comunis, Solanum nigrum, Calotropis pracera, Withania coagulans, Cuminum cyminum, Foeniculum vulgare, Nigella sativa* and *Coriandrum sativum* plant extracts against different strain of bacteria. These plants were selected for different microbial diseases traditionally including diarrhoea and dysentery.

MATERIALS AND METHODS

The present research work was carried out in Plant Taxonomy Laboratory, Department of Plant Sciences, Quaid-I-Azam University Islamabad.

Sample collection

The selected plants viz *C. revoluta, C. sempervirens, A. columnaris, R. comunis, S. nigrum, C. pracera* were collected from Quaid-I-Azam University, Islamabad. The fruit of *Withania coagulans* and seeds of *C. cyminum, F. vulgare, N. sativa and C. sativum* were obtained for testing antibacterial activity. All specimens were identified for their authenticity at Department of Plant Sciences, Quaid-I-Azam University, Islamabad.

Extraction

Fresh and semi dried selected plants were taken, rinsed with distilled water and kept under shade till drying. The dried material was grinded finely in electric grinder. Extraction from aerial parts of plants was carried out by simple maceration process. 50 g powder of leaves of each plant were taken and soaked in 500 ml of solvent (methanol, ethanol and ethyl acetate). The poorly homogenized mixture was kept for two weeks at room temperature (25°C) in extraction bottles with occasional shaking to facilitate extraction. After three weeks maximum amount of solvent was separated from the mixture. The extracts were stored at 4°C in refrigerator.

Antibacterial assay

Requirements

Preparation of samples

Samples prepared for antibiotic assay were of three types:
Methanolic, Ethanolic and Ethyl acetate
150 mg of each extract were dissolved in 10 ml of DMSO (MERCK) to get 15 mg/mL concentration. Penicillin 2 mg/mL in DMSO, were prepared for positive control. Pure DMSO was used as negative control (Table 1).

Test organisms

Six strains of bacteria were used; which were *S. aureus* (ATCC6538), *B. subtilis* (ATCC6633), *P. aeruginosa* (ATCC6643), *E. coli* (ATCC15224), *K. pneumoniae* (MTCC618) and *S. typhimurium (*ATCC13048). The first two are Gram positive and the later four are Gram negative. The strains were obtained from the Department of Microbiology, Quaid-I-Azam University Islamabad.
Pouring, incubation and measurements of zone of inhibition.

After 24 h by using micropipette, 100 µL of test solution was poured in respective well by using cork borer. The solution of antibiotics was made by dissolving 2 mg of penicillin per ml of DMSO. Eight concentration of extract were poured in eight wells, DMSO was used as negative control and antibiotic solution for positive control. Before putting all these extracts, respective wells were sealed by putting a drop of agar in order to avoid any mixing of solutions. These plates were incubated at 37°C. After 24 and 48 h of incubation the diameter of clear zones, showing no bacterial growth, around each well was measured. All the measurements were taken in mm.

Table 1. Dilution prepared for antibacterial assay.

S.No.	Conc. (mg/mL)	Stock solution (mL)	DMSO (mL)	Final (mL)
1	15.0	1.00	0.00	1.0
2	12.5	0.833	0.167	1.0
3	10.0	0.666	0.334	1.0
4	7.50	0.500	0.500	1.0
5	5.0	0.334	0.666	1.0
6	3.0	0.200	0.800	1.0
7	2.0	0.133	0.867	1.0
8	1.0	0.100	0.900	1.0

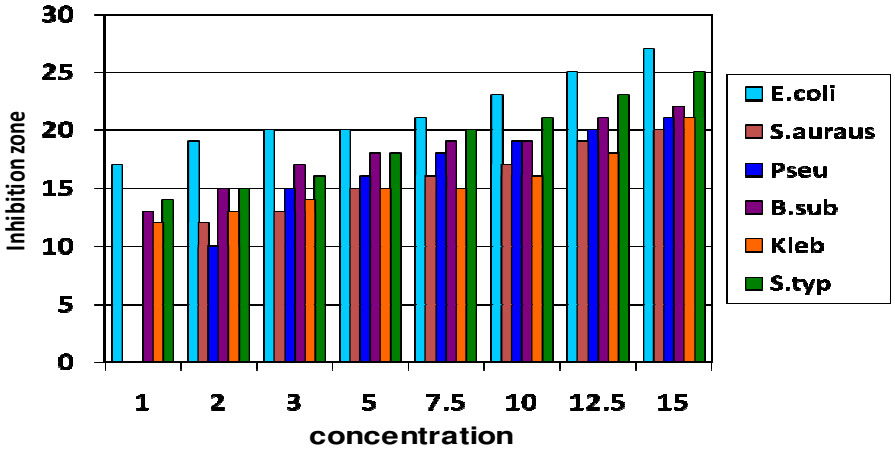

Figure 1. Antibacterial activity of methanolic extract of *Cycas revoluta.*

RESULTS

Antibacterial activity

Three kinds of crude extracts viz. methanolic, ethanolic and ethyl acetate were prepared from selected plants and were screened for their antibacterial activity. Two strains were Gram positive *S. aureus*, *B. subtilis* and the four strains were Gram negative, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. typhimurium* as shown in Figure 1.

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DISCUSSION

Antimicrobial activities

The present research showed that methanol, ethanol and ethyl acetate extracts of selected plants were used. During present investigation, it was found that selected plants exhibited antimicrobial activities. There were found variations among the antibacterial activities of different fractions prepared in different solvents as shown in Figure 1-33. It was also found that the action of the extract of plants on test organisms at different concentration showed that the inhibition zone of these survivors of these test organisms were decreased by increasing concentration of the extract. The antimicrobial activity of the selected plants was evaluated by the inhibition zone method against different microorganisms, *B. subtilis, E. coli, S aureus*, *P. aeruginosa*, *K. pneumoniae* and *S. typhimurium* as shown in Figure1-33. The culture used in current study was nutrient agar, which extensively used by microbiologists for culturing routine pathogens.

For extraction methanol, ethanol and ethyl acetate were selected as solvent (Cagri et al., 2001). The agar diffusion test is a method commonly used to examine antimicrobial activity regarding the diffusion of the compound tested through water-containing agar plate. The diffusion itself is dependent on the size, shape and polarity of the diffusing material. The chemical structure and the cross-linking level of the films also affect this phenomenon (Cagri et al., 2001). In the studies agar well diffusion method was used for determining antimicrobial activity of selected plants. When antimicrobial agents are incorporated, there will be diffusing material through agar gel, and furthermore, resulting clearing zone on the bacterial growth.

The antimicrobial effect of extracts of leaf and latex of *C. procera* was studied against six bacteria (including *E.*

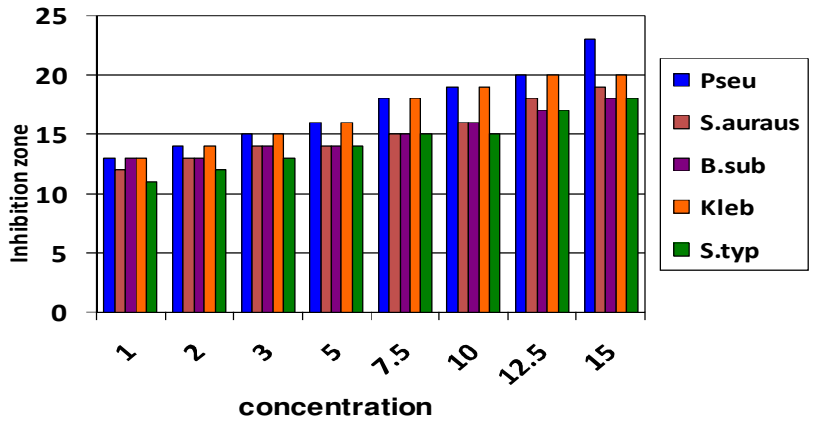

Figure 2. Antibacterial activity of methanolic extract of *Cupressus semipervirens.*

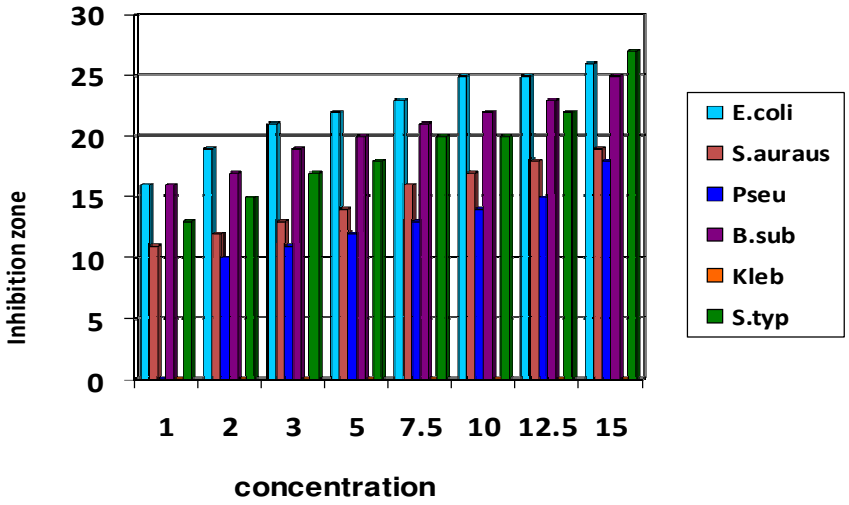

Figure 3. Antibacterial activity of methanolic extract of *Araucaria columnaris.*

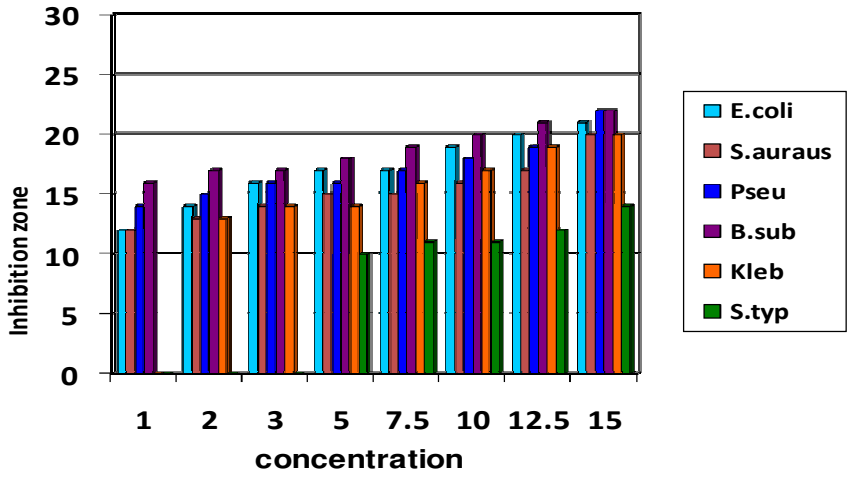

Figure 4. Antibacterial activity of methanolic extract of *Ricinus communnis.*

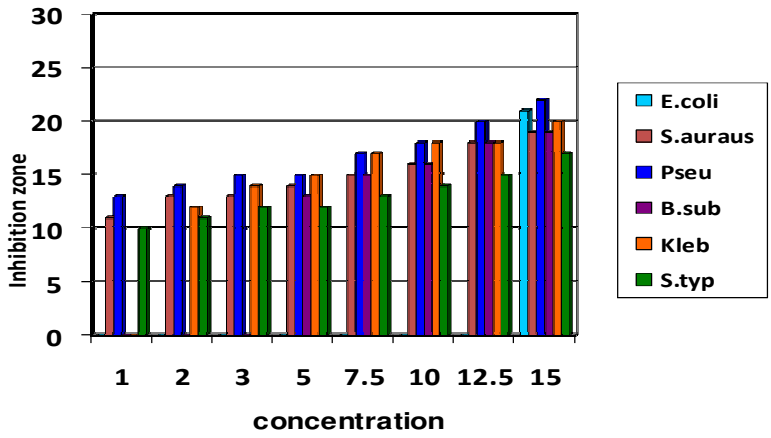

Figure 5. Antibacterial activity of methanolic extract of *Withania coagulans.*

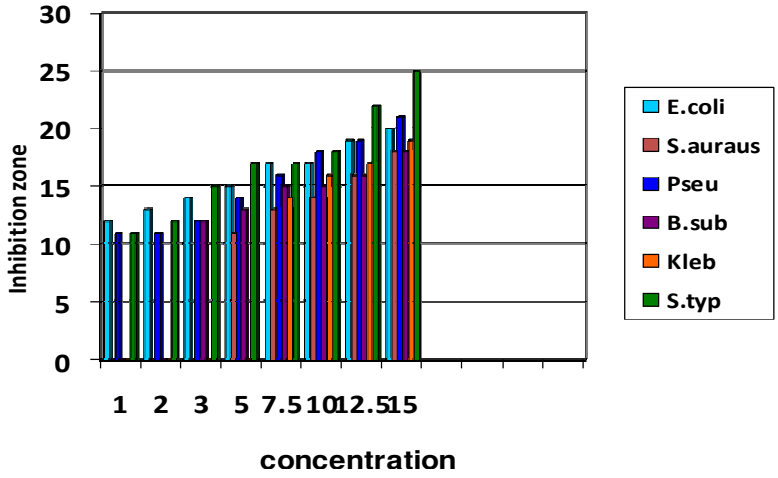

Figure 6. Antibacterial activity of methanolic extract of *Solanum nigrum.*

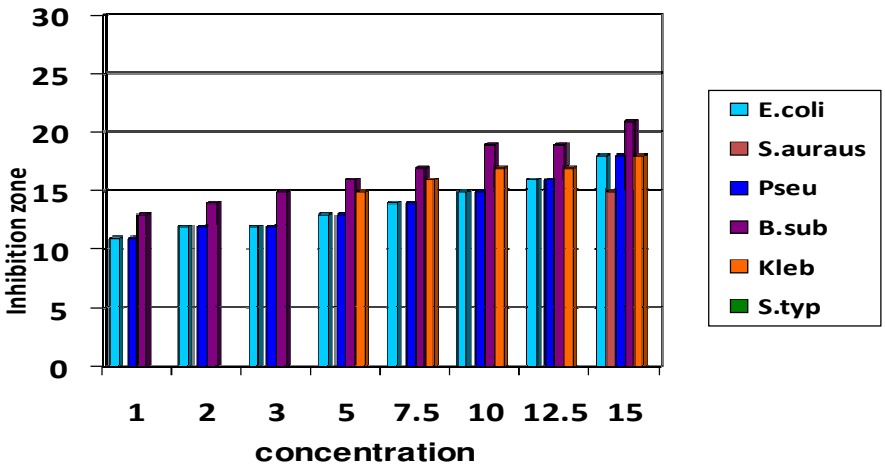

Figure 7. Antibacterial activity of methanolic extract of *Caloropis procera.*

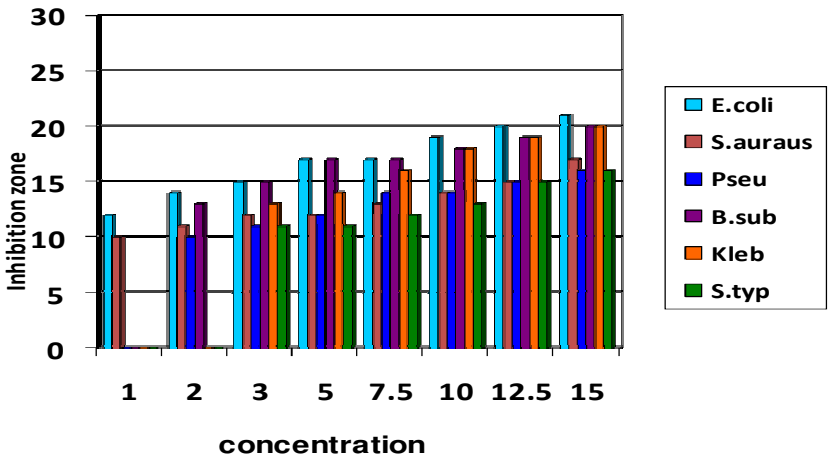

Figure 8. Antibacterial activity of methanolic extract of *Cuminum cyminum..*

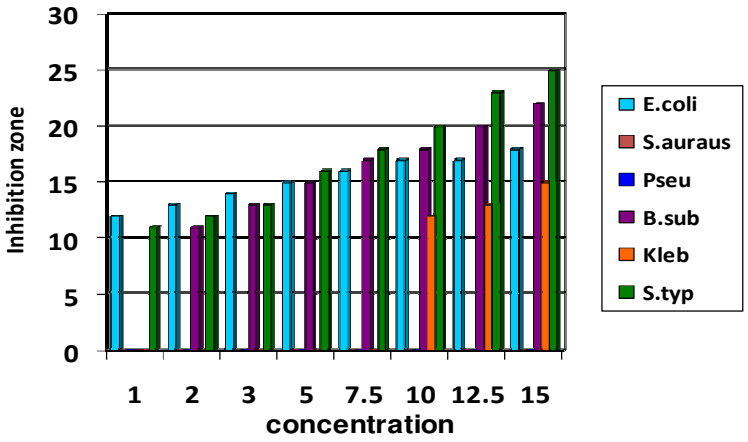

Figure 9. Antibacterial activity of methanolic extract of *Foeniculum vulgare.*

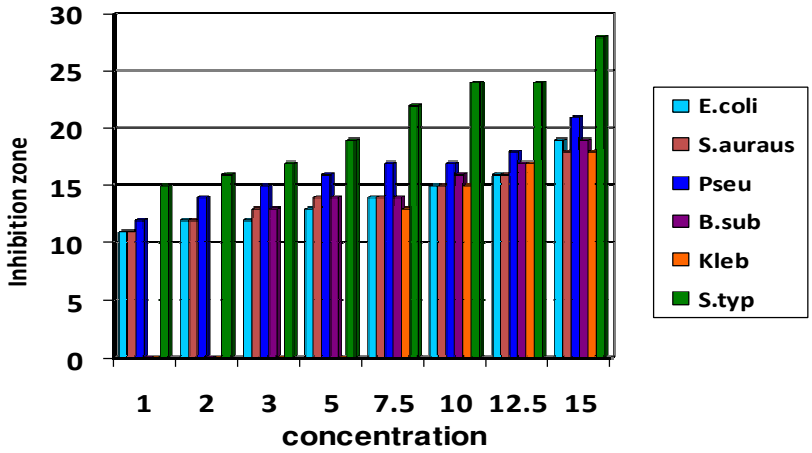

Figure 10. Antibacterial activity of methanolic extract of *Nigella sativa.*

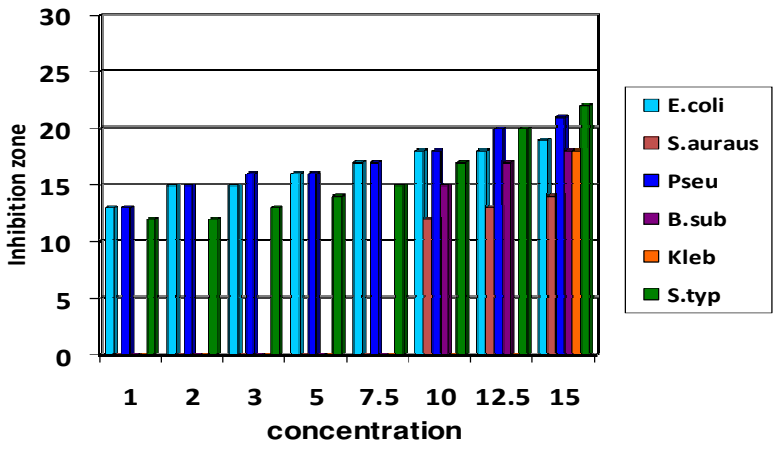

Figure11. Antibacterial activity of methanolic extract of *Coriandrum sativum.*

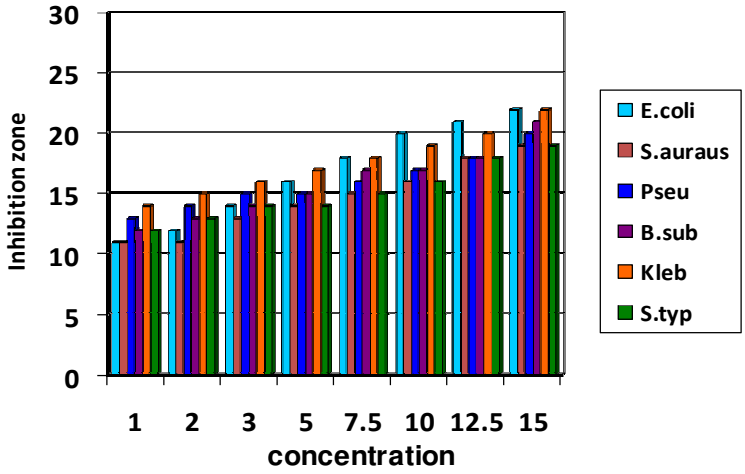

Figure12. Antibacterial activity of ethanolic extract of *Cycas revoluta.*

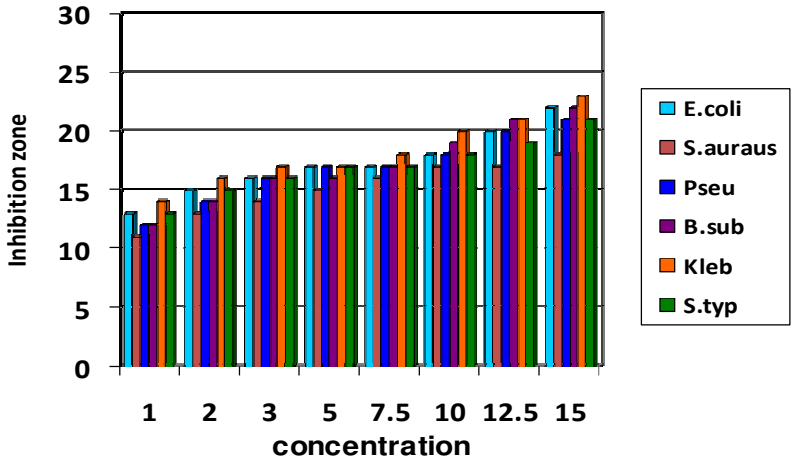

Figure13. Antibacterial activity of ethanolic extract of *Cupressus sempervirens.*

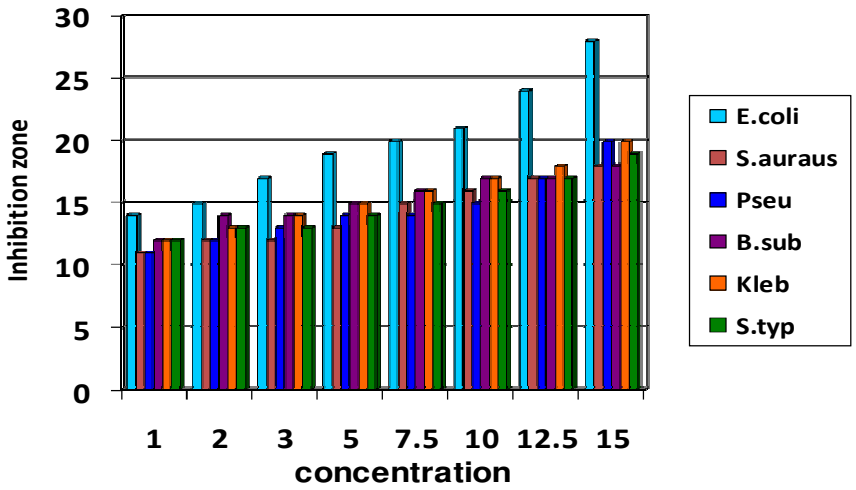

Figure14. Antibacterial activity of ethanolic extract of *Araucaria columnaris.*

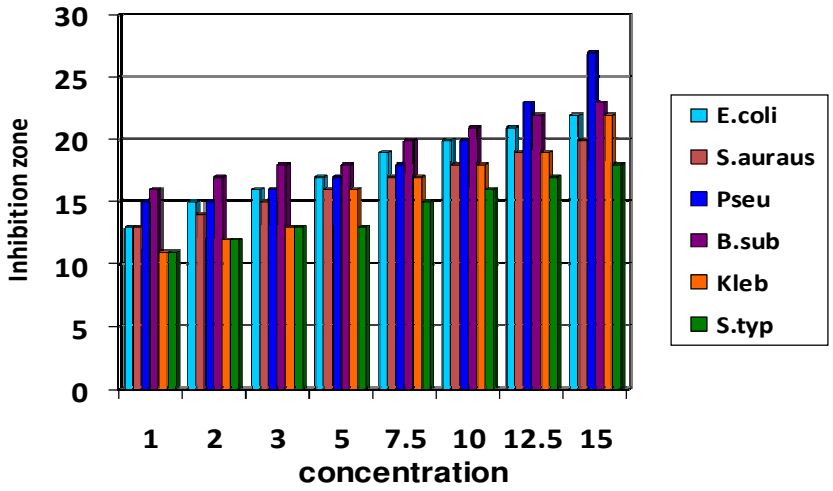

Figure15. Antibacterial activity of ethanolic extract of *Ricinus communis.*

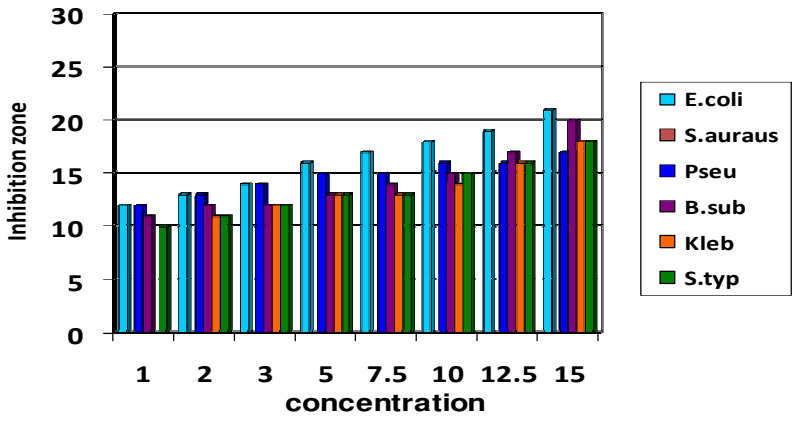

Figure 16. Antibacterial activity of ethanolic extract of *Wihania coagulans.*

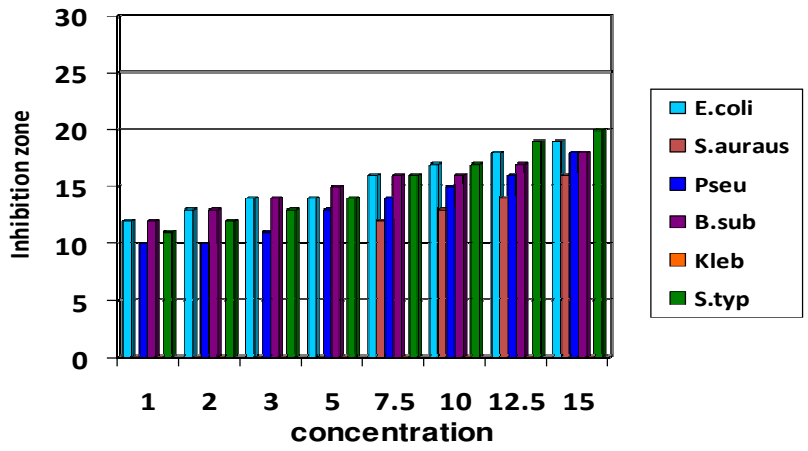

Figure 17. Antibacterial activity of ethanolic extract of *Solanium nigrum*.

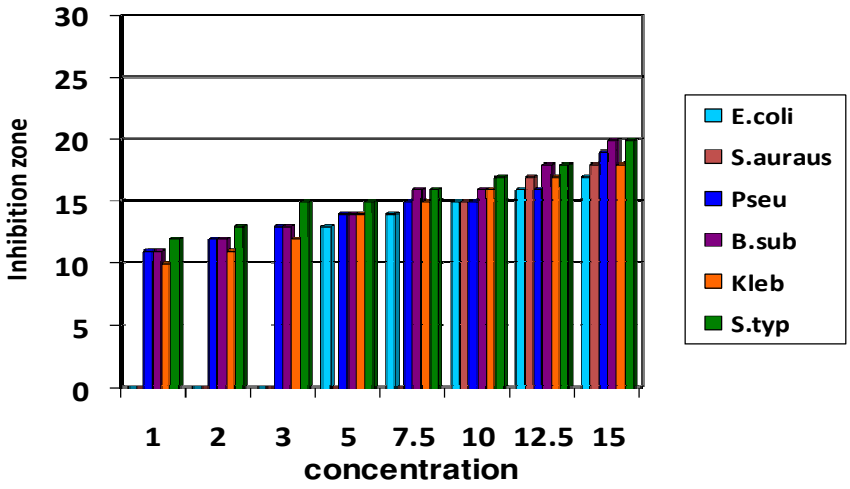

Figure 18. Antibacterial activity of ethanolic extract of *Calotropis procera*.

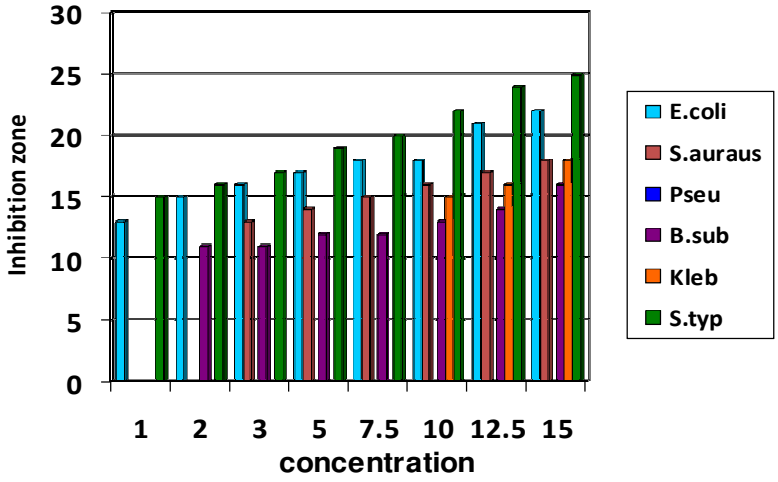

Figure 19. Antibacterial activity of ethanolic extract of *Cuminum cyminum*.

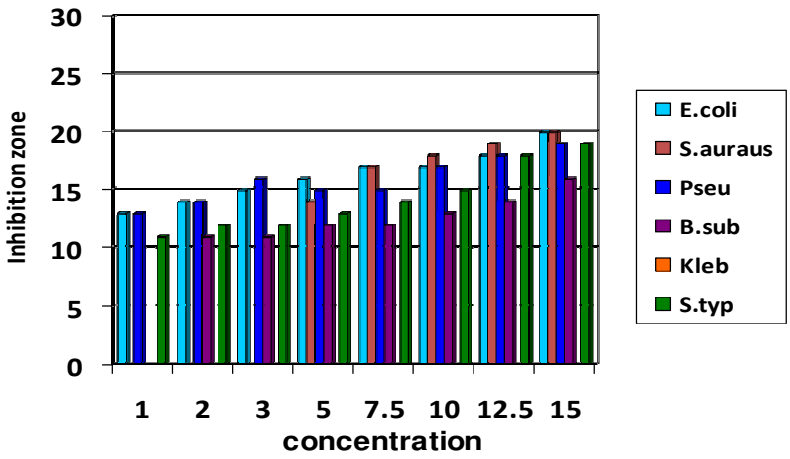

Figure 20. Antibacterial activity of ethanolic extract of *Foeniculum vulgare.*

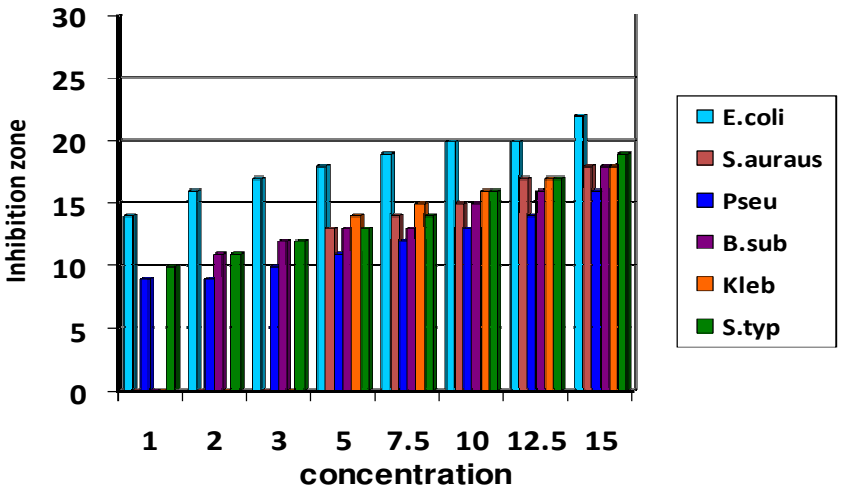

Figure 21. Antibacterial activity of ethanolic extract of *Nigella sativa.*

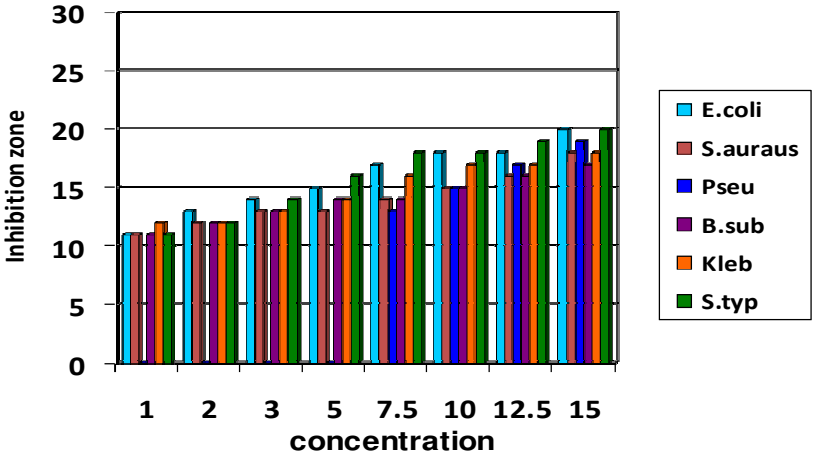

Figure 22. Antibacterial activity of ethanolic extract of *Coriandrum sativum.*

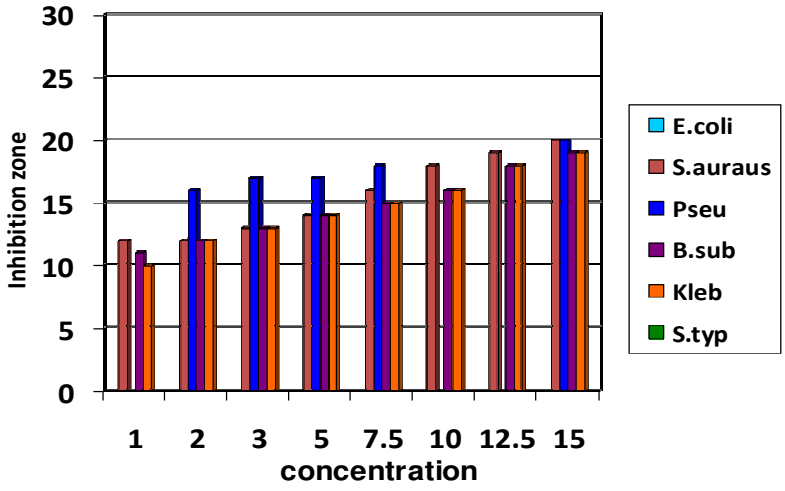

Figure 23. Antibacterial activity of ethyl acetate extract of *Cycas revoluta.*

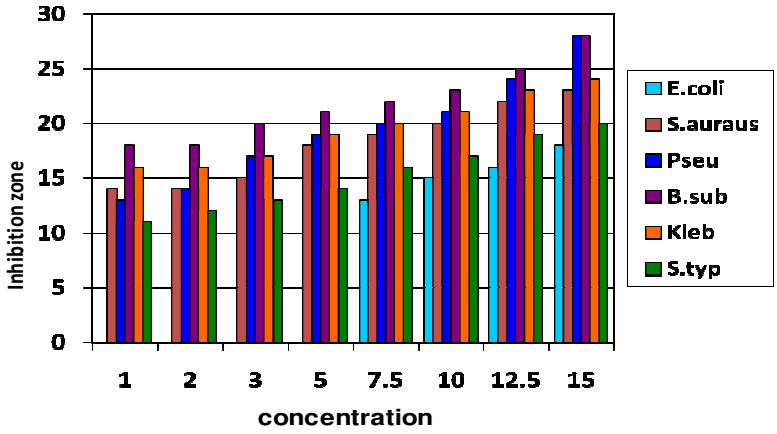

Figure 24. Antibacterial activity of ethyl acetate extract of *Cupressus sempervirens.*

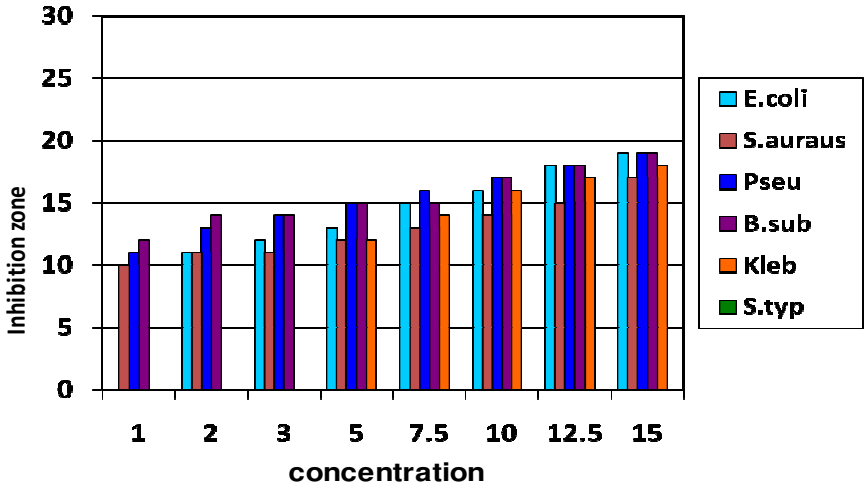

Figure 25. Antibacterial activity of ethyl acetate extract of *Araucaria coumnaris.*

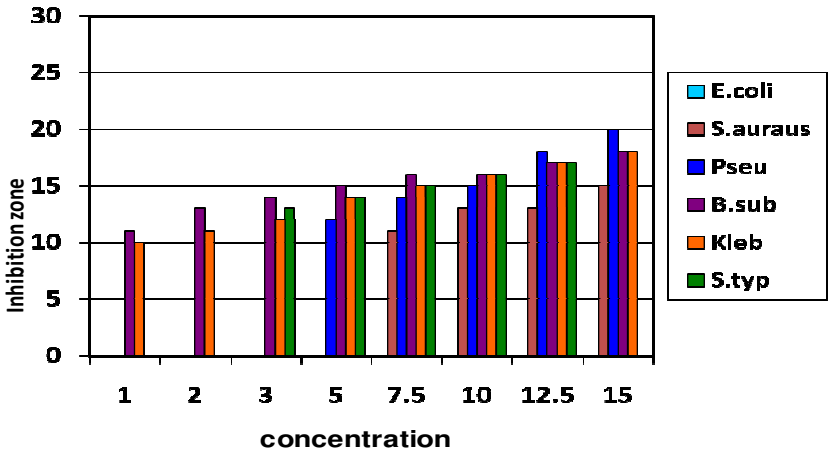

Figure 26. Antibacterial activity of ethyl acetate extract of *Ricinus communis.*

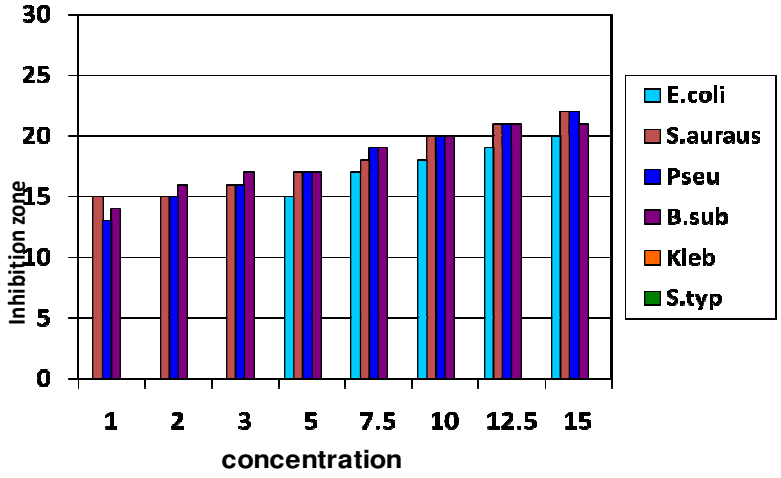

Figure 27. Antibacterial activity of ethyl acetate extract of *Withania coagulans.*

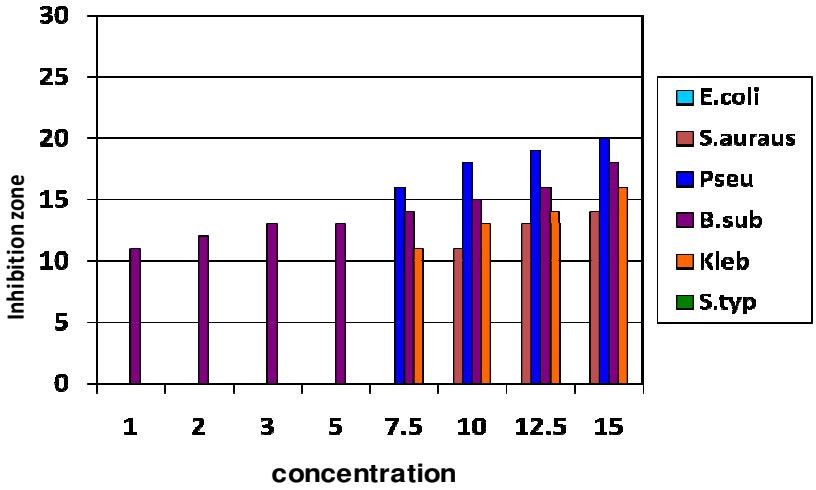

Figure 28. Antibacterial activity of ethyl acetate extract of *Solanum nigrum.*

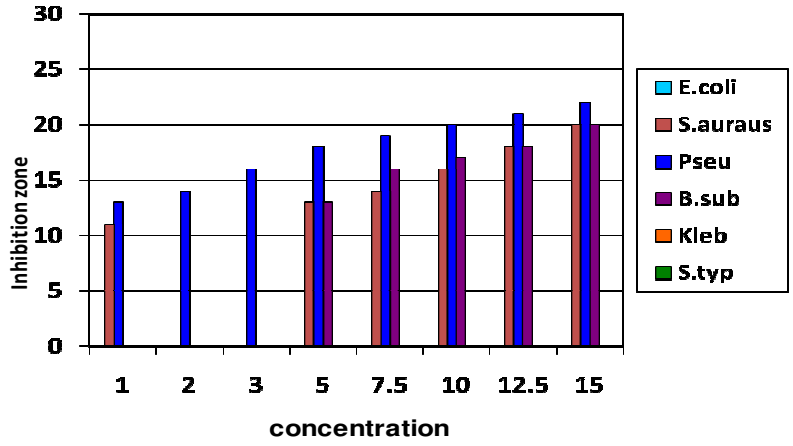

Figure 29. Antibacterial activity of ethyl acetate extract of *Calotropis procera.*

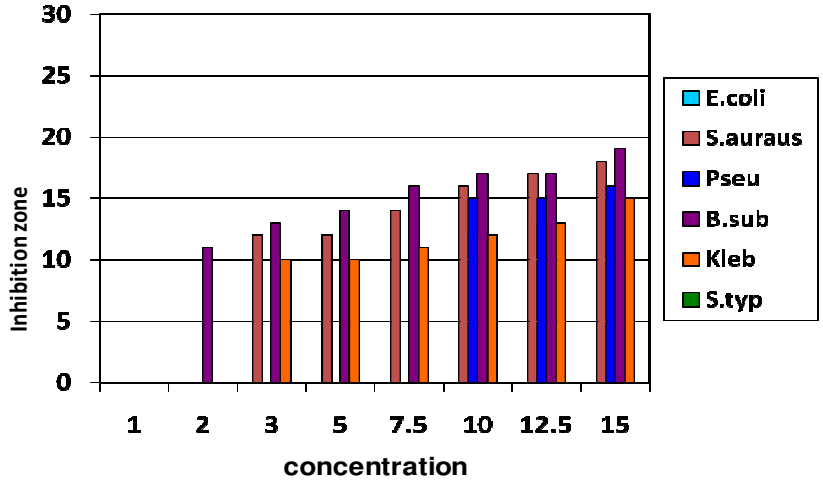

Figure 30. Antibacterial activity of ethyl acetate extract of *Cuminum cyminum.*

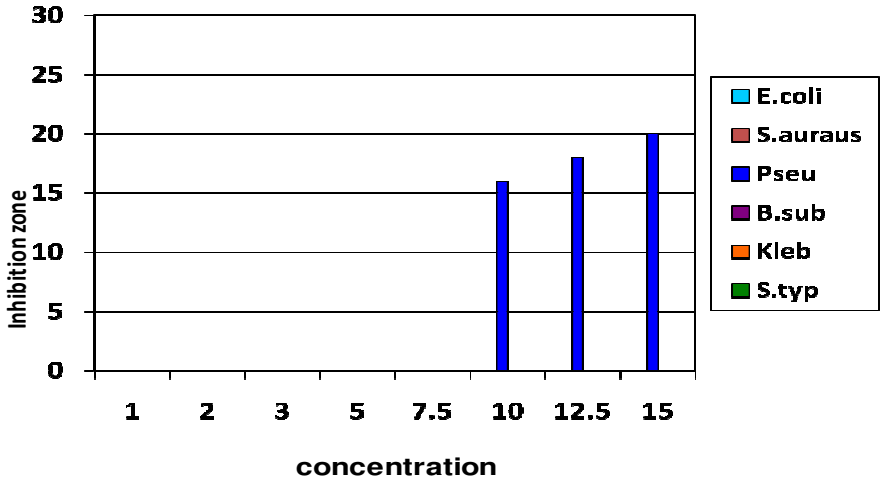

Figure 31. Antibacterial activity of ethyl acetate extract of *Foeniculum vulgare.*

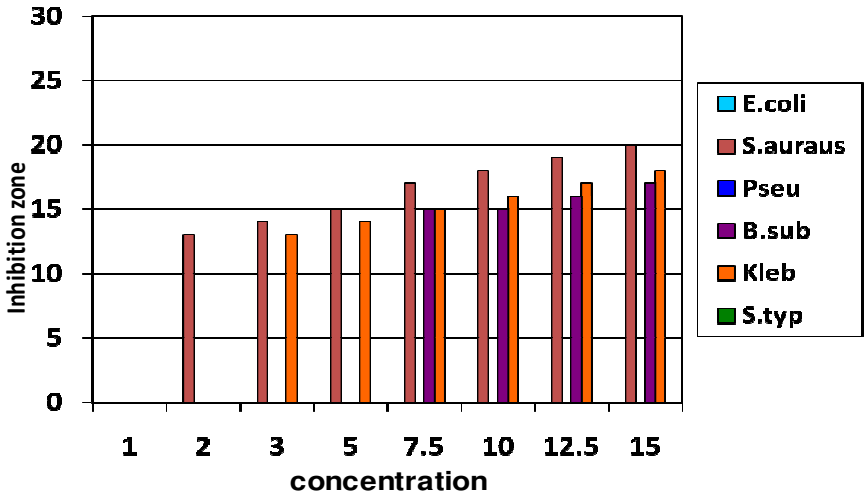

Figure 32. Antibacterial activity of ethyl acetate extract of *Nigella sativa.*

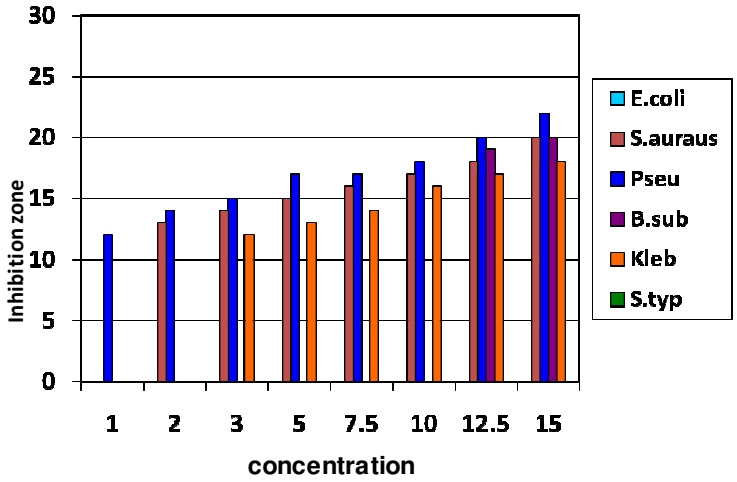

Figure 33. Antibacterial activity of ethyl acetate extract of *Coriandrum sativum.*

coli, S. aureus, and *P. aeruginosa)* by Kareem et al. (2008). Their results indicated that ethanol, aqueous and chloroform exhibited antibacterial activity. Antibacterial activity of *C. procera* using agar well diffusion method was also reported by Mohanraj et al. (2010). Present results showed that ethanol was the best solvent for extracting antimicrobial substances from this plant as compared to methanol and ethyl acetate. The zone of inhibition in all the cases increased with an increase in concentration. Methanol, ethanol and ethyl acetate extracts of *C. sempervirens* had significant effect against all tested strains. Therefore it can be detected that compounds in *C. sempervirens* which were active against all strains were soluble in all used solvent. Mothana et al. (2009) studied 64 methanol and aqueous extracts of 30 Yemeni plants (*Cupressus sempervirens)* in which pronounced antimicrobial activity was observed only against Gram-positive bacteria among them multiresistant bacteria with inhibition zones >15 mm and MIC values <500 µg/ml. *C. sempervirens* essential oil was particularly found to possess moderate antimicrobial activity against a variety of bacteria (Toroglu, 2007).

The antimicrobial activity of the essential oils of *C. sempervirens* could, in part, be associated with theirs major constituents such as α-pinene, β-phellandrene, α-Terpinyl acetate and cedrol. These components have been reported to display antimicrobial effects (Yang et al., 2007; Demirci et al., 2007). The antibacterial activity of essential oil obtained from *C. sempervirens* L. was *in vitro* tested by the agar-well diffusion method.

Results showed that *Cycas revoluta* indicated significant antimicrobial activity against all tested bacterial strains, while ethyl acetate extract of *C. revoluta* had no effect against *E. coli* and *S. typhimurium*. According to this investigation, it could be indicated that antimicrobial activity of the methanol and ethanol extracts of *C. revoluta* is due to the presence of those bioactive compounds. Moawad et al. (2010) reported chromatographic separation of the chloroform extract of *C. revoluta*. Leaflets 12 compounds, the isolated compounds displayed moderate antibacterial activity against *S. aureus*. The antimicrobial activity of *C. revoluta* antimicrobial peptides against Gram-negative and Gram-positive bacteria were evaluated as the concentrations required for 50% growth inhibition, IC_{50}. Cy-AMPs had wide antimicrobial spectra against various plant-pathogenic bacteria. The values of IC_{50} varied from6 to 260 mg/mL, depending on the tested microorganisms (Seiya et al., 2008).

Methanol extracts of *Cuminum cyminum* L. inhibited the growth of all the bacteria studied. The results showed that the methanol extract of *C. cyminum* L. had the best antimicrobial activity. Along with the aforementioned report, results are in agreement with those of Sheikh et al. (2010), reported antibacterial activity of seed extracts ethanol, methanol and acetone seed extracts of *C. cyminum* L. against 10 bacteria (including *E. coli, K. pneumoniae, S. typhi, B. subtilis* and *S. aureus*). The present study showed that *C. cyminum* ethanol extract showed an antibacterial activity against all bacterial strain used in this study except strains of *Pseudomonas aeruginosa*, which were resistant. While ethyl acetate extract of *C. cyminum* showed no sensitivity to *S. typhimurium* and *E. coli*. Iacobellis et al. (2005) reported antibacterial activity of *C. cyminum* L. against Gram-positive and Gram-negative bacterial species. The antibacterial activity of *C. cyminum* essential oil is perhaps attributable to the high level of cumin aldehyde (16.1%), a compound with known antimicrobial properties. The oil of *C. cyminum* showed significant results against all tested bacteria. (Singh et al., 2002). Nostro et al. (2005) showed a significant in vitro effect of *C. cyminum* extracts against *H. pylori*. Spices are frequently used as an active ingredient in certain medicines and reported to possess a number of pharmacological effects to treat different human ailments (Bonjar, 2004) Ultrastructural studies of vegetative cells of *C.cyminum* confirmed the synergistic destructive effects of the essential oil and nisin on membrane and cell wall of the bacteria (Pajohi et al., 2011).

During current studies methanol and ethanol extract of *Coriandrum sativum* showed antibacterial activity against all tested bacterial strains, while ethyl acetate extract of *C. sativum* had no effect against *E. coli* and *S. typhimurium*. However ethanol extract of *C. sativum* had significant effect against all bacteria even at low concentration. Al-Jedah et al. (2000) and Sing et al. (2002) reported that *C. sativum* has strong antibacterial activity against both Gram positive and Gram negative bacteria. Similarly, the compounds aliphatic 2E-alkenals and alkanals, isolated from the fresh leaves of *C. sativum* were found to possess bactericidal activity against *Salmonella choleraesuis* (Isao et al., 2004). The inhibition zone of *C. sativum* essential oil were seen against *E. coli, P. pyocyaneus, Y. enterolitica, B. megaterium, S. faecalis bacteria, S. cerevisiae, K. fragilis* fungi (7 - 16 mm / 2µl) (Sevil Toroglu, 2011).

In the present study, the methanol and ethanol extracts of *Nigella sativa* revealed significant antibacterial potential against all used bacteria. This study is correlated with the study carried out by Tanis et al. (2009) in which *B. subtilis, E. coli, K. pneumoniae* and *S. aureus* inhibited by *N. sativa*. Same result is reported by Mashhadian and Rakhshandeh (2005) in which *N. sativa* has been reported to inhibit *S. aureus* and *P. aeruginosa*. Present study is also in agreement with Morsi's (2000) results who reported that Kalonji extracts showed antibacterial activity against a broad range of microbes and including multiple antibiotic resistant bacteria. The seeds of kalonji have over one hundred different chemical constituents including active ingredient thymoquinone (TQ), which is responsible for antibacterial activity (Ali and Blunden, 2003).Ethanol extract of *F. vulgare* gave better inhibition zones as compared to methanol and ethyl acetate extracts.

Gurinder and Daljit (2009) results who reported that hot water and acetone seed extracts showed considerably good antibacterial activity against all the bacteria except *K. pneumoniae* and one strain of *P. aeruginosa*. While present study revealed that ethyl acetate extract showed antibacterial activity against *Ps. aeruginosa*. Tahira et al. (2010) reported that the *Withania coagulans* seed crude methanol extract showed good antibacterial activity against S*. aureus* and *B. subtilus* but were moderately active against *E. coli* and*. P. aeruginosa.* Methanol and ethanol extracts of *R. communis* had significant effect against all tested strains. While ethyl acetate extract showed antibacterial effect against all bacteria except *E.coli*. Céspedes et al. (2006) determined the antimicrobial activities of five lignans, isolated from methanol extract of *A. araucana* against a variety of bacteria. *B. subtilis* and *S. typhimurium* were more sensitive to methanol extracts of *A. coulmnaris* and *N. sativa.* The ethyl acetate extract of *Cupressus sempervirens* exhibited greater zone of inhibition against *S. aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* as compared to other plant extracts. Whereas, the ethanol extract prepared from *Aracuria coulmnaris* was more effective in inhibiting the growth of *E. coli*.

A reason for these different results compared with this present study may be due to the different methods that were used. Another possible reason could be the different content of active constituents. There was no

chemical analysis in the study, so the contents of the active compounds were not determined.

Conclusion

In vitro studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness varied. Ethyl acetate extract of selected plants showed higher inhibition against tested bacteria at high concentration. While methanol and ethanol extract of selected plants had more significant effect on various tested bacteria as compared to ethyl acetate extract. The antibacterial activity has been attributed to the presence of some active constituents in the extracts. The demonstration of broad spectrum of antimicrobial activities by the plants used in this study may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, wound infections and typhoid fever. The effect of the plants on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

It is clear from the results of the present studies that the plant extracts have great potential as antimicrobial compounds. The development of natural antimicrobial agents will help to decrease negative effects (pollution in environment, resistance) of synthetic chemicals and drugs. It can really contribute to medical and pharmaceutical practices. There are still many more activities waiting for screening the drugs.

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