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Antimicrobial analysis of root and shoot extracts of *Ephedra intermedia* Schrenk & Meyer

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Abstract

This study was done to estimate the antimicrobial activity of root and shoot extracts of *Ephedra intermedia* Schrenk & Meyer. The root and shoot extracts of *Ephedra intermedia* was tested against four Bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluorescens* and *Bacillus subtilis*) and three fungal species (*Fusarium verticillioides*, *Aspergillus flavus* and *Dreschlera turcicia*). In this study the *Ephedra intermedia* (root and shoot) methanol extract showed highest antibacterial actions against *Staphylococcus aureus*, followed by *Escherichia coli* and lowest activity was noted against *Bacillus subtilis*. The alcoholic and distilled water extracts of *E. intermedia* root and shoot also showed significant activity against all examined fungal species. The extreme antifungal activity through methanol extraction shown against *Fusarium verticillioides*, which followed by *Aspergillus flavus* and lowest movement was reported against *Dreschlera turcicia* with minimum inhibition zone. Further that the methanol extract exhibited significant effect against all the investigated bacterial and fungal species as compared to the ethanol and distilled water extract. This study also showed that the roots of *E. intermedia* were more effective as compared to the shoots against under study bacterial and fungal species.

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Introduction

Several medicines usually in use today are of herbal origin. Kirbag *et al.*, (2009) stated that about 50% of all new medicines are from plant invention. Medicinal plants are the greatest source for obtaining the diversity of novel herbal drugs. Therefore, such plants should be examined to better understanding their properties, safety and efficacy (Doss, 2008).

Due to growing population of the world, the requirement of the natural elements are increasing that are utilized as a foodstuff, constituents of useful diets, preventing the different diseases and other uses. Rahman *et al.*, (2011) reported that this is the time to study the “ancient plants” through evaluating their medicinal applications by the using of new methods of examination.

However pharmaceutical industries have created a several novel antibiotics during last three decades, but now the resistance of bacteria to these medicines has increased. In Pakistan the Balochistan province is very important in term of rich biodiversity predominantly with medicinal Flora, which are utilized as a therapeutic resolves. Zaidi & Crow, (2012) described that in Balochistan large number of medicinal plant are used to control the large number of diseases. Due to expansion of human inhabitants, development and the increase of air pollution in the local atmosphere the natural resources of the Province are flattering polluted. Shinwari *et al.*, (2009), informed that medicinal flora have important properties for regulating the different bacterial diseases. In plants many compounds are present, which have several tool of action that comprises some enzymes, protein, and secondary metabolites, such as, carotenoids, vitamins, flavonoids, anthocyanins, and other phenolic components, that is the basis of antioxidant and antimicrobial potential of plant yields (Mazhar *et al.*, (2015).

Balandrin *et al.*, (1985) exhibited that although many pant species have been investigated for their antimicrobial activities but still there are so many plant species which have not been examined.



Fig.1a. *Ephedra intermedia* Schrenk & Meyer.

Ephedra intermedia Schrenk & Meyer is locally known as Parar (Pushto), Umen, Oman (Balochi), Hum, Narom, Naromb (Brahavi). It belongs to the family Ephedraceae. It is native to Pakistan, Afghanistan, Iran and central Asia and in Pakistan it is widely distributed in Balochistani areas such as Harboi, Nichara, Mustung and Quetta districts. It is shrubby plant up to 50 cm to one meter (3.3ft) long, branches are usually many, erect to spreading, striate, minutely tuberculate, internodes straight or slightly bent. The strobili are dioecious, either male or female n any one plant, so both male and female plants are needed for seeds (Fig. 1a).

Locally the whole plant is used in snuff preparation. All part of plant is boiled in water and the decoction is used for asthma and chest infection. The decoction is also used for the treatment of typhoid and mature fruits with hot water used for tuberculosis. Similar observation was also reported by Tareen *et al.*, (2010), as they were studying the indigenous knowledge of folk medicine by the women of Kalat and Khuzdar regions of Balochistan Pakistan. The main purpose of this study was to evaluate the antimicrobial efficacy of different parts (root and shoots) of *E. intermedia* Schrenk & Meyer.

Materials and methods

Collection of Plant Material

The plant materials, the roots and shoots of *E. intermedia* Schrenk & Meyer was collected from district Mustung, Balochistan. The plant material was identified by Dr. Rasool Bakhsh Tareen taxonomist Department of Botany University of Balochistan, Quetta.

These materials were washed away carefully by distilled water and then dehydrated at room temperature for 7 days. With the help of electric blender made it in the powdered form for further uses.

Organism Tested

For antibacterial potential of *E. intermedia* Schrenk & Meyer the bacterial organism selected, that were *E. coli*, *P. fluorescens*, *S. aureus* and *B. subtilis* and for antifungal activity, *A. flavus*, *D. turcicia* and *F. verticilliodes* were used. The bacterial and fungal culture was obtained from the Instituted of Biochemistry University of Balochistan Quetta Pakistan laboratory. For maintaining bacterial culture nutrient broth (NB) at 37°C were used and for fungus Potato dextrose agar (PDA) at 28°C were used as described by (Mahesh and Satish, 2008).

Extraction by Distilled Water

Method described by Parekh and Chanda, (2006) was used for the Plant material extraction by distilled water. 25 grm dehydrated powder of plant material was mixed in 150ml water and then it was heated at low temperature for 2 hours. The boiled material was filtered and then centrifuged for 12min and hold on for 10-12hrs in Soxhlet apparatus at room temperature (25°C). After 12hrs, the mixture were filtered through whatman-41 filter paper and oven dried at 45°C temperature and the extracts were stored in freezer at -4°C for further dilution and processing.

Plant extracts preparation through Methanol and ethanol

For methanol and ethanol extraction, 25grm of desiccated powder plant material root and shoot of *E. intermedia* Schrenk & Meyer were added in 150ml of 96% methanol solvent (Shihabudeen *et al.*, 2010) and 150 ml of 80% ethanol (Jameela, 2011) separately and hold on for 10-12hrs in Soxhlet apparatus at room temperature (25°C). After 12hrs, the mixture were filtered through whatman-41 filter paper and oven dried at 45°C temperature. Methanol and ethanol was completely evaporated by rotary evaporator to get the extract.

The semisolid extracts were kept in open air for 24hrs. After complete evaporation of methanol and ethanol, the extracts were stored in freezer at -4°C for further dilution and processing (Walter, *et al.*, 2010).

Sample dilution preparation

20 mg of extracts were completely dissolved in 1 ml of Dimethylsulfoxide (DMSO). The stock solution of 20 mg/ml was diluted to prepare three concentrations of the extract: 20 mg/ml, 15mg/ml and 10mg/ml. For the discovery of microbial specimen the solution of a standard antibiotic and fungicide (2 mg/ml of ampicillin and 2 mg/ml of griseofulvin) were prepared, respectively. The standard antibiotic and fungicide solution were used for positive control and DMSO solution were used for negative controls (Walter, *et al.*, 2010; Ajaib *et al.*, 2016). The plant (*E. intermedia*) extract dilutions with DMSO are shown in Table 1.

Table 1. Concentration of Plant (*E. intermedia*) extract tested for antimicrobial activity.

S. No.	Final Conc. (mg/ml)	Plant (<i>E. intermedia</i>) extract (ml)	DMSO (ml)	Final volume (ml)
1	20	1.00	0.00	1
2	15	0.80	0.20	1
3	10	0.60	0.40	1

Media preparation

Nutrients broth medium was prepared by dissolving 0.65grm nutrient broth in 50 ml of distilled water for the growth of bacterial inocula; pH was adjusted 7.0 and the solution was autoclaved. Nutrient ager medium was prepared by dissolving 2.0grm of nutrient agar in 100ml of distilled water; pH was adjusted 7.0 and the solution was autoclaved.

Inoculums preparation

For the preparation of inoculums nutrient broth and nutrient agar the spectrophotometer were used as described by Mahesh and Satish, (2008). The gram negative bacteria viz, *E. coli* and *P. fluorescens* and gram positive such as *S. aureus* and *B. subtilis* were pre-cultured overnight in nutrient broth in a rotary shaker at 37°C. Then centrifuged at 10,000 rpm for 5 min.

Pellet was suspended in double distilled water and the cell density was standardized spectrophotometric ally ($A_{610\text{ nm}}$). Fungal inoculums such as *A. flavus*, *D. turcicia* and *F. verticilliodes* were prepared on Potato dextrose agar medium that was 10 to 15 day old culture. 8 to 10ml of distilled water was flooded in petri dish and by using the sterile spatula the conidia were scraped. The spore density of each fungus was adjusted with spectrophotometer ($A_{595\text{ nm}}$) to obtain a final concentration of approximately 10 spores/ml.

Pouring of test solution; incubation and measurement of Zone of inhibition

Method clarified by Obeidat *et al.*, (2012) was used for plant extracts activity assay. For Bacteria, 20 ml agar of Mueller- Hinton (AMH) was hardened by an inoculums suspension and the inoculums were permitted to dehydrate for 5min. By using the glass pipettes 6mm well in diameter were made in the seeded agar. From each plant crude extract 20 μ l was stroked into the each hole on seeded agar and for suitable dispersion permitted to stand point for 1 hour. Thereafter at 37°C protected for 24 hours. After 24 hours of incubation, the diameter of the inhibition area made as the results of plant extracts against bacterial activity were measured in millimeters (mm) by ruler. Antibacterial activity of three dilutions of each plant extract was determined against four bacterial strains.

Antifungal activity examination

Antifungal activity was examined by paper disc diffusion method described by Kumara *et al.*, (2009). The testing fungal suspension was spread on AMH medium and the filter paper of 5mm in diameter was placed on the medium plates. The plates with the tested fungal were saturated with 20 μ l of plant extract. Then these plates were placed in incubator for 24 hours at 37°C temperature. After 24 hours of incubation period inhibition areas were measured in mm by ruler.

Results and discussion

Antibacterial activity

The plant parts roots and shoots of *E. intermedia* Schrenk & Meyer were used in this study and the four

bacterial strains comprising of *E. coli*, *P. fluorescens*, *S. aureus* and *B. subtilis* were used for the estimation of antibacterial activity. Additionally, three fungal strains such as *A. flavus*, *D. turcicia* and *F. verticilliodes* were designated for antifungal potential. The antimicrobial activity of *E. intermedia* Schrenk & Meyer against selected bacterial and fungal strains was analyzed by the presence and diameter (mm) of inhibition zones, as noted by Ajaib *et al.*, (2014), when investigating the Antioxidant and Antimicrobial activities of an ethno botanically important plant *Notholirion thomsonianum* from District Kotli, Azad Jammu & Kashmir. Antibacterial activity at different concentrations of *E. intermedia* Schrenk & Meyer extracts was represented in Figs.1-9. Extracts from the root of *E. intermedia* exhibited strongest antibacterial activity against *S. aureus* (22, 18 and 14.7mm) at 20, 15 and 10 mg/ml in methanol solvent, respectively (Figs. 3, 6 & 9) which fallowed by ethanol extract (Figs. 2, 5 and 8) and the least antibacterial activity was noted (4, 2 and 1.4mm) in distilled water extract against *B. subtilis* at 20, 15 and 10mg/ml concentration, respectively (Figs. 1, 4 and 7). The dried shoots of *E. intermedia* Schrenk & Meyer used to prepared extracts in different solvents showed low antibacterial activity against all the investigated bacterial species at all the concentration (20, 15 and 10mg/ml) as compared to the roots. The maximum antibacterial activity from shoots of *E. intermedia* was also exhibited against *S. aureus* (19, 14.3 and 12mm) in methanol solvent (Figs. 3, 6 and 9) and lowest was again noted in distilled water extracts. The ranking of antibacterial activity of *E. intermedia* Schrenk & Meyer (root & shoot) against the four bacterial strains was *S. aureus* > *E. coli* > *P. fluorescens* > *B. subtilis* at all the concentration and solvents (Figs. 1-9). Present results indicated that the methanol extract of root had maximum potential as compared to shoot of methanol extract. The variation in antibacterial and antifungal activities potential for different plant parts was also reported by Ajaib *et al.*, (2016). They reported that the fruit extract of methanol exhibited maximum potential as compared to bark, when they were examining *Chenopodium ambrosioides*.

The examination don by Mahesh and Satish, (2008) obviously designated that the antimicrobial action differs with the plant materials (leaf, root & shoot) used. They also reported that the methanol extract of the root of *Z. mauritiana* prevent the harshness of diarrhea brought by castor oil. The minimum potential against bacterial and fungal species was showed by distilled water (aqueous) extracts in this study for both parts of the *E. intermedia* at different concentration (20, 15 and 10 mg/ml) (Figs 1-18). Similar observation was reported by Ajaib *et al.*, (2016) when investigating *Chenopodium ambrosioides* and other researchers (Seddik *et al.*, (2010) and Mohamed *et al.*, (2010).

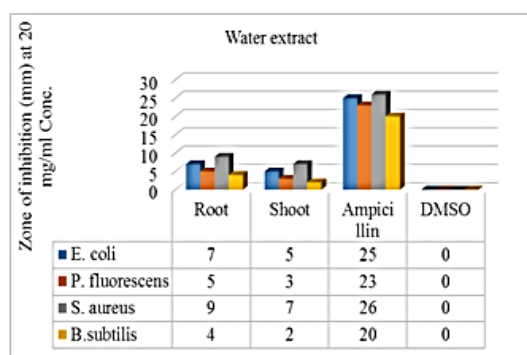


Fig. 1.

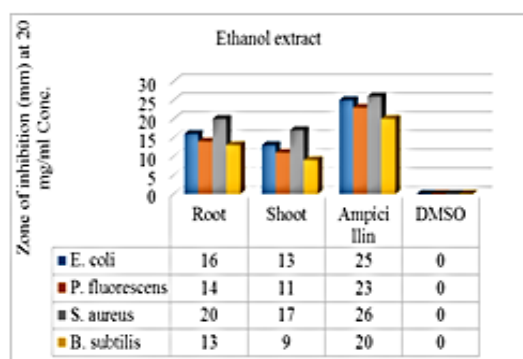


Fig. 2.

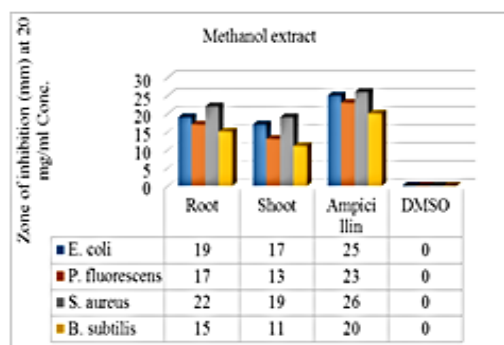


Fig. 3.

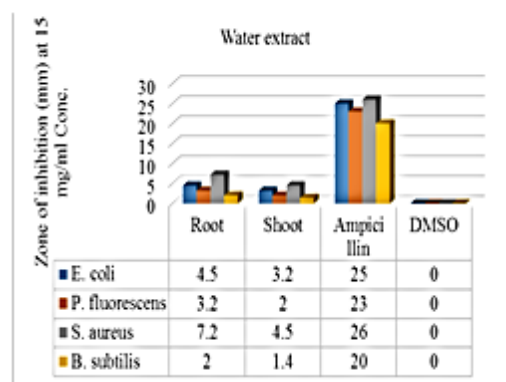


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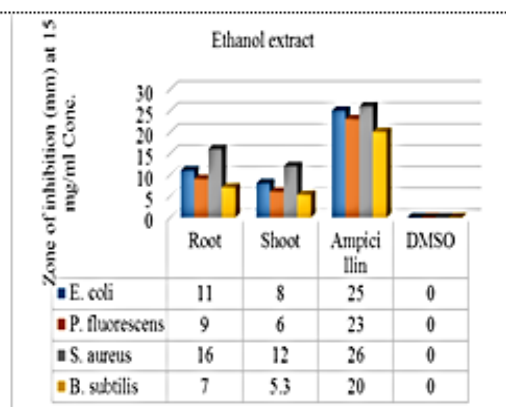


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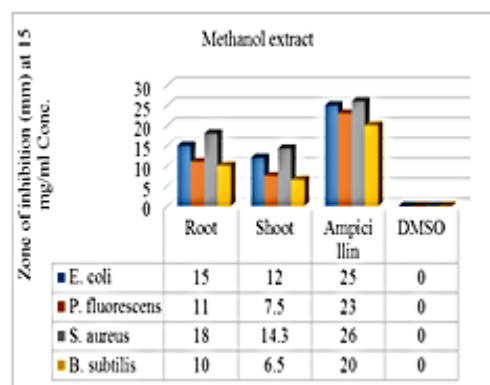


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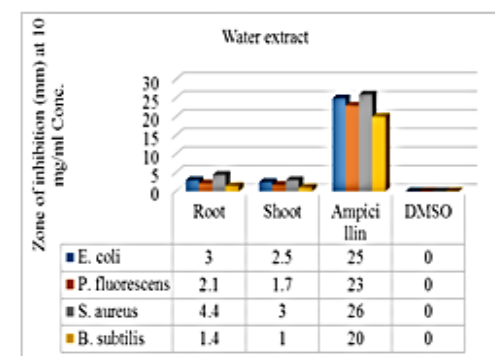


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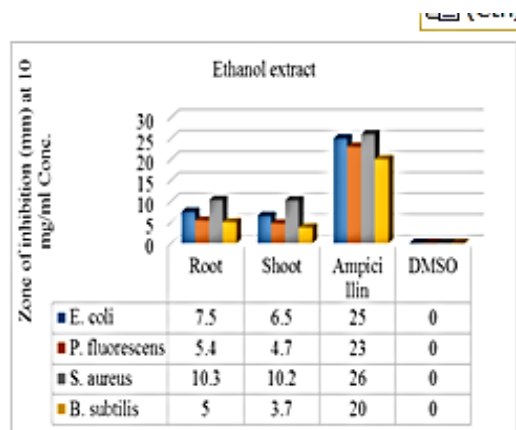


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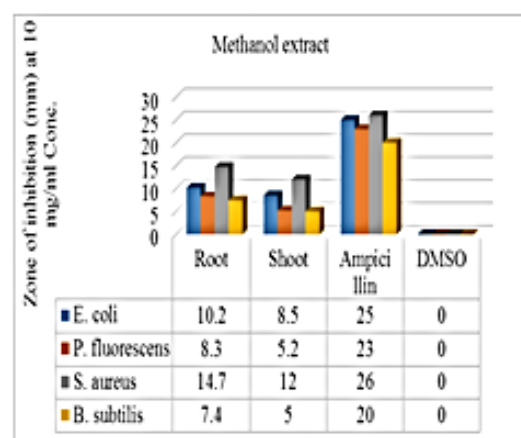


Fig. 9.

Figs. 1-9. Zone of inhibition (mm) after 24 hours showing antibacterial activity at the concentration of 20, 15 & 10mg/ml in different solvents. Where Ampicillin = Positive control and DMSO = Negative control.

Antifungal activity

Antifungal activity at the different concentrations (20, 15 and 10mg/ml) of *E. intermedia* Schrenk & Meyer extracts are represented in Figs.10-18. Extracts from root of *E. intermedia* showed the maximum antifungal activity against *F. verticillioides* (21, 17 and 14mm) at 20, 15 and 10mg/ml in methanol solvent, respectively (Figs. 12, 15 and 18), which followed by ethanol extracts (Figs. 11, 14 and 17) and the minimum antifungal activity was found (4.6 mm) against *D. turcica* in distilled water extract at 10 mg/ml (Fig. 16). The ranking of antifungal activity of *E. intermedia* (root and shoot) against the three fungal strains was as follow; *F. verticillioides* > *A. flavus* > *D. turcica* at all the concentration and solvents (Figs. 10-18).

This study exhibited that all extracts had good to satisfactory results against bacterial and fungal strains. Methanolic extracts showed extreme potential as compared to other extracts against both Bacterial and fungal species (Figs. 1-18). This might be for the reason that of many phytochemical compounds such as terpenoids, flavonoids, polyphenolic compounds as well as tannins expected to be extracted in methanol. The maximum methanol antimicrobial activity was also noted by Mahesh and Satish, (2008), they exhibited that the methanol (leaf, root/bark) extraction of *Ziziphus mauritiana*, *Acacia nilotica*, *Withania somnifera*, *Tinospora cordifolia* and *Sida cordifolia* had the maximum activity against *F. verticillioides*, *D. turcica*, *A. flavus*, *Pv. malvacearum*, *X. axonopodis*, *S. aureus*, *P. fluorescens*, *S. coli* and *B. subtilis*.

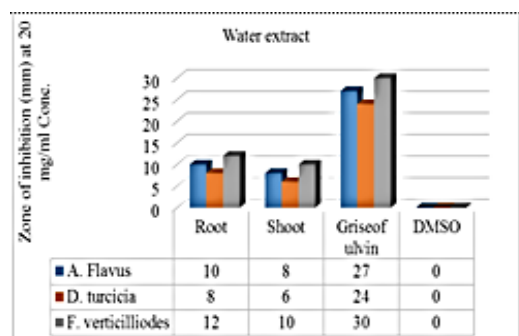


Fig. 10.

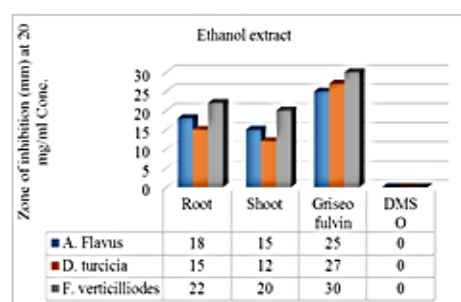


Fig. 11.

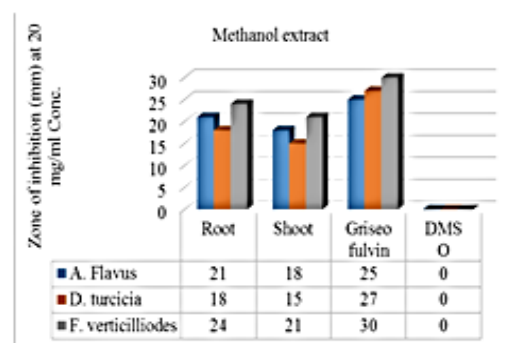


Fig. 12.

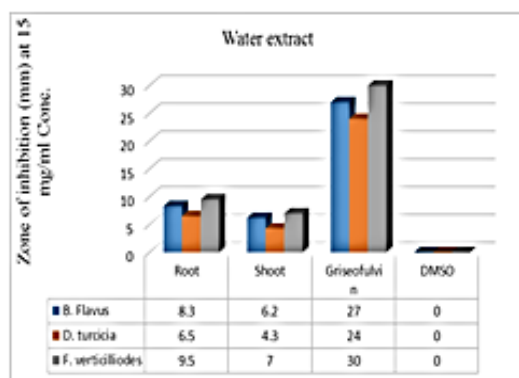


Fig. 13.

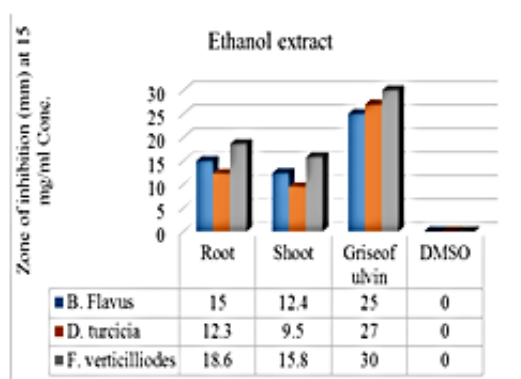


Fig. 14.

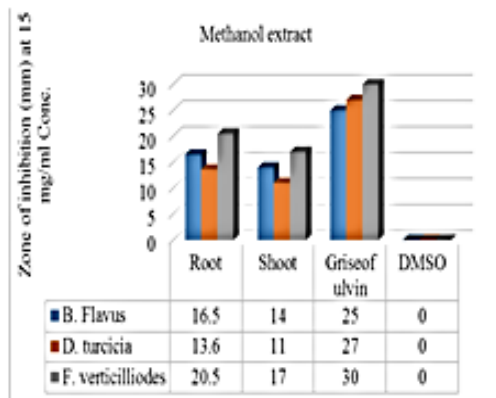


Fig. 15.

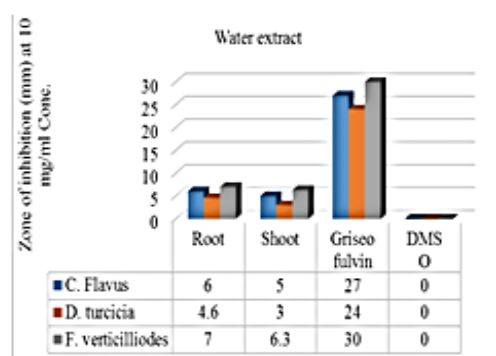


Fig. 16.

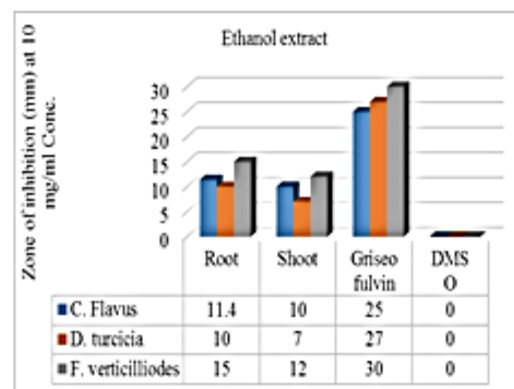


Fig. 17.

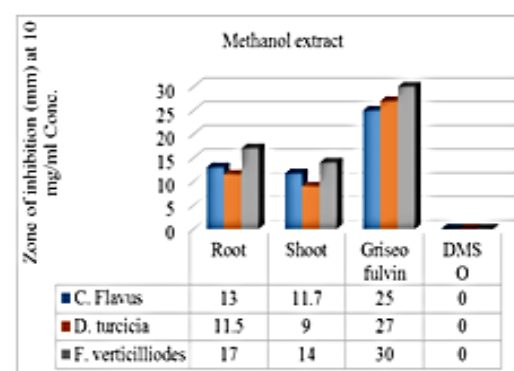


Fig. 18.

Figs. 10-18. Zone of inhibition (mm) after 24 hours showing antifungal activity at the concentration of 20, 15 & 10 mg/ml in different solvents. Where Ampicillin = Positive control and DMSO = Negative control.

Observation reported by Cheruiyot *et al.*, (2009) were also similar to our results, once they were working on the antimicrobial activities of methanol plant extracts of *Psidium guajava* leaves against *P. aeruginosa*, *E. coli* and *S. aureus*. Similarly Ramzi *et al.*, (2005).

Found the highest antibacterial activity in methanolic extracts, while investigating the antimicrobial activity of many plants, such as *Boswellia elongate* etc. against *S. aureus*.

Although Ajaib *et al.*, (2013a and 2013b) also found the alike consequences during the investigation of antimicrobial and antioxidant activities of *Rivina humilis* L., *Echinochloa colona* (Linn.) Link, and *Sporobolus coromandelianus* (Retz.) Kunth.

Conclusion

This study clearly designated that the *Ephedra intermedia* Schrenk & Meyer extracts had significant effects against the growth of studied bacterial and fungal species. Therefore these results point out that it is possibility of using these extracts for the treatment of fungal and bacterial infections. This study also concludes that the roots of *E. intermedia* Schrenk & Meyer are more effective against microbial activities. Further that alcoholic extraction is better than the distilled water extraction for medicinal plant parts extracts.

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