Antibacterial, Anti-fungal and Phytotoxic activities of *Ferula narthex* Boiss.

Shumaila Bashir*[1], Mahboob Alam[1,2], Bashir Ahmad[3] and Akhtar Aman[1,2]
[1]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan
[2]Department of pharmacy, Hazara University, Havelian Campus, Abbottabad, Pakistan
[3]Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan

Abstract: Crude methanolic extract of roots, aerial parts and its subsequent fractions of *Ferula narthex* Boiss were tested for antibacterial, anti-fungal and phytotoxic activities. Crude methanolic extract of roots and its fractions showed significant antibacterial effect against *P.aeruginosa* (86.95%, 73.91, 69.59, 78.26 & 73.91%) represented by percent inhibition except ethyl acetate (EtoAc) fraction. The EtoAc fraction of roots and aerial parts showed significant activity against *E. coli* (80%), *S. typhi* (81.2 & 81.25%) and *S. pneumoniae* (80%). The *n*-hexane, chloroform and aqueous fractions of aerial parts showed significant activity against P. aeruginosa (78.26, 69.56 & 73.91%). Following fungal strains (*T. longifusus, C. albicans, A. flavus, M. canis, F. solani, C. glabrata*) were also used for anti-fungal activity. Among tested samples only crude methanol extract of roots, *n*-hexane and chloroform fraction showed moderate anti-fungal activity against *M. canis* (40, 35 & 30%) represented by percent inhibition. The remaining fractions showed no effect on tested fungi. Different oils fractions were also tested against above fungal strains. Fraction I, II & V showed mild to moderate activity against *M. canis* (40, 40 & 25%). Phytotoxic effect of tested samples of roots, aerial part and its fractions showed concentration dependent growth inhibition. Maximum phytotoxic effect was noted for *n*-hexane and aqueous fraction (50% growth inhibition). The remaining tested samples showed mild effect on growth of *Lemna minor* plant.

Keywords: *Ferula narthex* Boiss, antibacterial, anti-fungal, phytotoxic, *Lemna minor.*

INTRODUCTION

In developing countries the major cause of morbidity and mortality is associated with pathogenic microorganisms (Khan *et al*., 2007). Different chemical agents have been identified and were used against these microorganisms, but the major problem faced by drugs is microbial resistance. To overcome this problem the researcher are now trying to obtain the anti-microbial agents from natural (plants) sources (Naz *et al*., 2010).

Genus *Ferula* belongs to family umbelliferae, which comprises 275 genera and 2850 species. The *Ferula* distributed throughout the world, especially in Afghanistan, Iran, India and Pakistan (Indrayan *et al*., 2009). *Ferula* is a rich source of biologically active compounds. More than 278 active compounds are reported from this genus. Different species of *Ferula* are used in various ailments like bronchitis, asthma, whooping cough, gastrointestinal disorders, also used in epilepsy, hysteria and cholera. Some of *Ferula* species also used in veterinary products(Ahmad *et al*., 1998, Parvez *et al*., 1992).

Oils obtained from *Ferula asafetida* is effective in rheumatism and also used for purification of blood. Gum resin of this plant is also uses in intestinal problems as a stomachic, as an antiseptic, as a carminative agent. Gum resin also used for toothache, in diseases of threadworm and in scorpion sting (Appendino *et al*., 1990). All these biological activities are due to secondary metabolites which are responsible for all these different activities (Aqel *et al*., 1991). Antihistaminic effect posses by aqueous extract of *Ferula ovina* (Al-Khalil *et al*., 1990).

A large number of active compounds isolated from *Ferula* are mainly sesquiterpene coumarins and sulfur containing compounds (Iranshahi *et al*., 2010, Bandyopadhyay *et al*., 2006).

Ferula narthex Boiss, locally known as raw in Chitral and found in various localities of Pakistan like Gilgit, Chitral (Shinwari and Gilani, 2003). Locally it is used for cough, asthma, toothache, gastric problems and also in constipation, angina pectoris. Gum resins of *F. narthex* Boiss. is used in hysteria, treatment of habitual abortion, whooping cough and scorpion sting (Shinwari and Gilani, 2003, Khan *et al*., 2011, Srinivasan, 2005, Mahendra and Bisht, 2012). Extract and pure compounds from this plant showed anticancer (Saleem *et al*., 2001), anti-diabetic (Iranshahy and Iranshahi, 2011) and anti-fertility effect (Kalita *et al*., 2011).

**Corresponding author:* e-mail: saadb23@yahoo.com

MATERIAL AND METHODS

Plant material, preparation of crude extract and fractionation

In July 2010 *F. narthex* Boiss plant was collected from Chitral, Khyber Pakhtunkhwa Pakistan and was identified by Taxonomy section Department of Botany University of Peshawar. A specimen was kept in the herbarium with voucher number bot. 20002 (PUP).

The roots and aerial parts were shade dried, crushed and then powdered. Dry powder of roots (8.0 Kg) was extracted by maceration method using methanol as an extraction solvent at room temperature for 14 days with daily shaking. The above procedure was carried out with dry powder (03kg) of aerial parts. Crude extract of roots and aerial parts was obtained and concentrated under vacuum at low temperature (40^{o}C). It was fractionated into *n*-hexane, chloroform, ethyl acetate, butanol and aqueous fractions. Crude extract as well as their subsequent fractions of roots and aerial parts were tested for antibacterial, anti-fungal and phytotoxic activities.

Antibacterial activity

The crude methanolic extract of roots, aerial parts and its fractions were tested against *P. aeruginosa, Staphylococcus epidermidis, S. aureus, Salmonella typhi, K. pneumoniae, Streptococcus pneumonia* and *E. coli* using Agar well diffusion procedure (Bibi *et al*., 2006, Ahmad *et al*., 2011). From nutrient broth 18 h old bacterial culture was taken and spread on sterile agar plates for the preparation of bacterial lawn. Metallic cork borer (6 mm) was used for formation of wells in agar plates. In DMSO stock solution of test samples were prepared at a concentration of 3mg/mL. From each stock solution 100 µL was taken and transferred to their respective well in agar plates. At 37^{o}C all agar plates were incubated for 24h.

Amoxicillin was used as a standard drug and served as positive control, while DMSO was used as negative control. Zone of inhibition was measured for standard as well as for crude methanolic extract of roots, aerial parts and its fractions. Antibacterial activity was determined by zone of inhibition of test samples and it was compared with standard drug.(ALAM and QURESHI, 2010, Cotter and Adley, 2001).

Antifungal bioassay

Anti-fungal activity of crude methanolic extract of roots, aerial parts and its subsequent fractions were analyzed using Agar tube dilution procedure (Atta-ur-Rehman., 1991, Hussain *et al*., 2010). 24mg/mL of test samples were dissolved in sterile DMSO to prepare stock solution. In petri plates Sabouraud Dextrose Agar (SDA) was taken for refreshment of fungal strains. For preparation of slants about 5mL of SDA medium was taken in each test tube and autoclaved. Then 66.6µL of sample from stock solution was transferred to test tube and also a seven days old culture of fungal culture was introduced in these pre-labeled test tubes. In growth chamber at 25±1^{o}C for seven days these were incubated. The linear growth of the fungal culture was noted in slanted test tubes and compared with negative control.

Percent growth inhibition was determined with formula given below:

$$\% \text{ Inhibition} = 100 - \frac{\text{Growth in sample tube (mm)}}{\text{Growth in control tube (mm)}} \times 100$$

Amphotericin-B drug was used as a standard drug for Candida albicans while for other fungi Miconazole was used (Nisar *et al*., 2010, Khan and Khan, 2012).

Phytotoxic activity

The crude methanolic extract of roots, aerial parts and various fractions of *F. narthex* Boiss plant was screened for phytotoxic activity using Lemna bioassay protocol (Atta-ur-Rehman, 1991, Rashid *et al*., 2009).In distilled water the medium was prepared by dissolving different ingredients and was autoclaved for 15mins at 121^{o}C. The pH (5.5-6.5) of the medium was adjusted with potassium hydroxide (KOH). In methanol stock solution was prepared by dissolving (20mg/mL) of samples. From stock solution 500, 100 and 10µg/mL was taken in flasks and these flasks were kept under sterilized condition for the evaporation of solvent. In each flask 20mL of the medium was added and healthy *Lemna minor* plants (10) with three fronds were also added to flasks. The flask with only stock medium and *Lemna minor* plant served as negative control while the flask with standard (Paraquat) served as positive control. In growth chamber all these flasks were kept for seven days at room temperature. After seven days the phytotoxic activity of test samples was determined by recording number of dead fronds in each flask (Nisar *et al*., 2010, Itokawa *et al*., 1982).

The percent growth regulation was determined with the formula given below:

$$\% \text{ Inhibition} = 100 \frac{\text{Number of fronds in test sample}}{\text{Number of fronds in negative control}} \times 100$$

RESULTS

Antibacterial activity

Keeping in view the ethno pharmacological uses of this valuable medicinal plant for the treatment of various gram positive and gram negative pathogenic infections, we tested the roots as well as the aerial part of the plant against different bacteria. The clinical significance of *P. aeruginosa, S. epidermidis*, *S. aureus*, *S. typhi*, *K. pneumoniae*, *S. pneumonia* and E. coli is that they are responsible for various infectious disorders (Khan *et al*., 2012).

Antibacterial effect of roots

The roots crude extract and subsequent fractions were tested against gram positive and gram-negative bacteria. These bacteria include gram-positive *S. epidermidis, S. aureus, S. pneumoniae, while gram-negative E. coli, S. typhi, P. aeruginosa* and *K. pneumonia*. Zone of inhibition was compared with Amoxicillin (10µg/disc) and percent growth inhibition was calculated. Against *S. epidermidis* the following percent zone of inhibition was observed: *In*-hexane (64), fraction followed by chloroform, crude methanol, butanol and ethyl acetate fraction 16, 12, 11, 11 and 10 mm respectively and percent inhibition of stated fractions were 64, 48, 44, 44 and 40 % correspondingly. Against *S. pneumoniae* all tested fractions were showed antibacterial effect but maximum was recorded for crude methanol, *n*-hexane, aqueous, butanol, ethyl acetate and chloroform with zone of inhibition 18, 15, 15, 14, 11 and 10 mm respectively, the percent antibacterial effect were 72, 60, 60, 56, 44 and 40% respectively. The maximum antibacterial activity against *S. aureus* was recorded for *n*-hexane followed by the remaining fractions with zone of inhibition 14, 12, 12, 12 and 12 mm while butanol fraction was inactive against *S. aureus*. The percent inhibition effects for all tested samples were recorded as 60, 52, 52, 52, and 52% respectively.

All the tested samples showed significant antibacterial effect against gram-negative *P. aeruginosa*. The crude methanolic extract showed maximum antibacterial effect followed by butanol, *n*-hexane and aqueous showed same effect and least by chloroform fraction with zone of inhibition 20, 18, 17, 17 and 16 mm respectively, while ethylacetate fraction was inactive against this tested organism. The percent effect for tested samples was 86.95, 78, 73.91, 73.91 and 69% respectively. Ethyl acetate fraction showed maximum antibacterial effect against *E. coli* followed by aqueous and crude methanolic extract with zone of inhibition 12, 11 and 07 mm, while *n*-hexane, chloroform and butanol fractions were inactive against *E. coli*. The percent inhibition for tested samples was recorded as 80, 73 and 46% respectively.

The maximum antibacterial activity against *S. typhi* was noted for ethyl acetate fraction followed by butanol and crude methanolic extract with zone of inhibition 13, 12 and 09 mm respectively, while *n*-hexane, chloroform and aqueous fractions were inactive against *S. typhi*. The percent inhibition was 81, 75 and 56% respectively for tested samples. All the tested samples were showed antibacterial effect against gram-negative *K. pneumonia*. Maximum antibacterial effect was showed by *n*-hexane followed by ethyl acetate, chloroform, crude methanolic extract, butanol and aqueous fraction with zone of inhibition 16, 15, 13, 10 and 10 mm respectively. For all tested samples the percent effect was recorded as 53, 50, 43, 33 and 33% respectively.

Antibacterial effect of Aerial part

Antibacterial effect of aerial crude methanolic extract and its subsequent fractions were also tested against gram positive and gram-negative bacteria as tested for root part. These bacteria include gram-positive *S. epidermidis, S. aureus, S. pneumoniae,* while *gram-negative E. coli, S. typhi, P. aeruginosa* and *K. pneumonia*. Zone of inhibition of crude extract and of various fractions was compared with Amoxicillin (10µg/disc) standard antibacterial agent and percent growth inhibition was calculated as mentioned in fig. 2. All tested samples showed antibacterial activity against *S. epidermidis* except ethyl acetate fraction. Maximum antibacterial effect was recorded for chloroform fraction followed by *n*-hexane, crude methanolic extract and aqueous fraction with zone of inhibition 16, 13, 11 and 11mm respectively. The percent inhibition effect was 64, 52, 44 and 44 %. Against *S. pneumoniae* all tested samples were active, high antibacterial effect was recorded for ethyl acetate followed by crude methanolic extract, chloroform and aqueous with same results and minimum for *n*-hexane fraction with zone of inhibition 20,15, 15, 15 and 12 mm respectively. The percent inhibitory effect for all tested samples was 80, 60, 60, 60 and 48 % respectively. The *n*-hexane and aqueous fraction showed maximum effect against *S. aureus* followed by crude methanolic extract and chloroform fraction with zone of inhibition 13, 13, 11 and 11 mm respectively while ethyl acetate test sample was inactive against *S. aureus*. The percent inhibition for all tested samples was 56, 56, 47 and 47% respectively. The effect of tested samples against *E. coli* showed that only ethyl acetate and crude methanolic extract fraction are active with zone of inhibition 13 and 10 mm. The remaining fractions were inactive against *E. coli*. The percent inhibition was 86 and 66 % respectively. Against *S. typhi* only ethyl acetate and crude methanolic extract fractions were showed antibacterial effect with zone of inhibition 13 and 08 mm and percent inhibition was 81 and 50% respectively. The remaining fractions were inactive against this tested organism. The crude methanolic extract was more active against *P. aeruginosa* among all tested fractions followed by ethyl acetate, aqueous and chloroform fraction with zone of inhibition 18, 17, 17 and 16 mm respectively and with percent inhibitory effect presented in decreasing disorder78, 73.91, 73.91 and 69% respectively. Against *K. pneumonia* highly antibacterial effect was showed by *n*-hexane followed by chloroform and crude methanolic fraction with zone of inhibition 20, 12 and 10 mm and percent inhibition effect was 66.66, 40 and 33% respectively, while ethyl acetate and aqueous fraction was inactive against *K. pneumonia*.

Anti-fungal activity

Anti-fungal activity of roots

The crude methanolic extract and its subsequent fraction were tested against various strains of fungus, which were

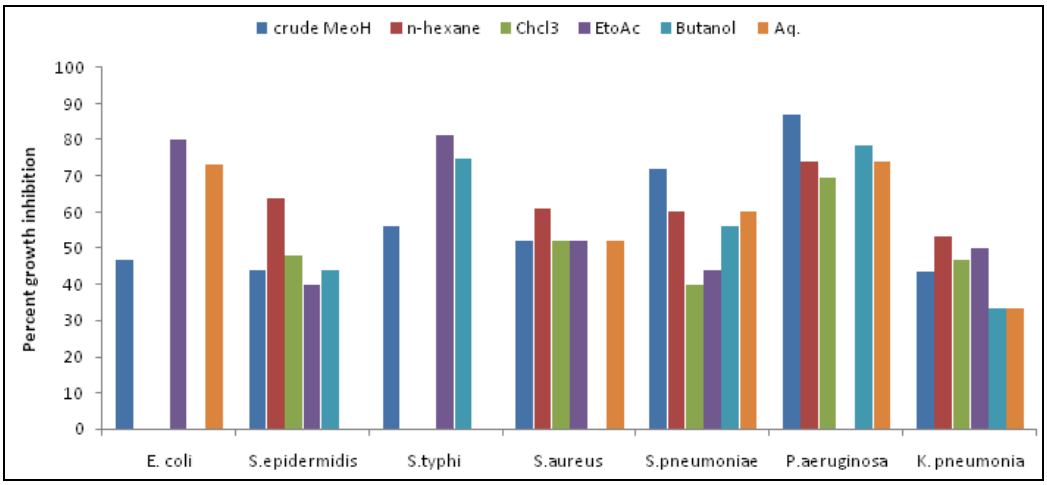

Fig. 1: Antibacterial activity of crude methanolic extract of roots and its various fractions

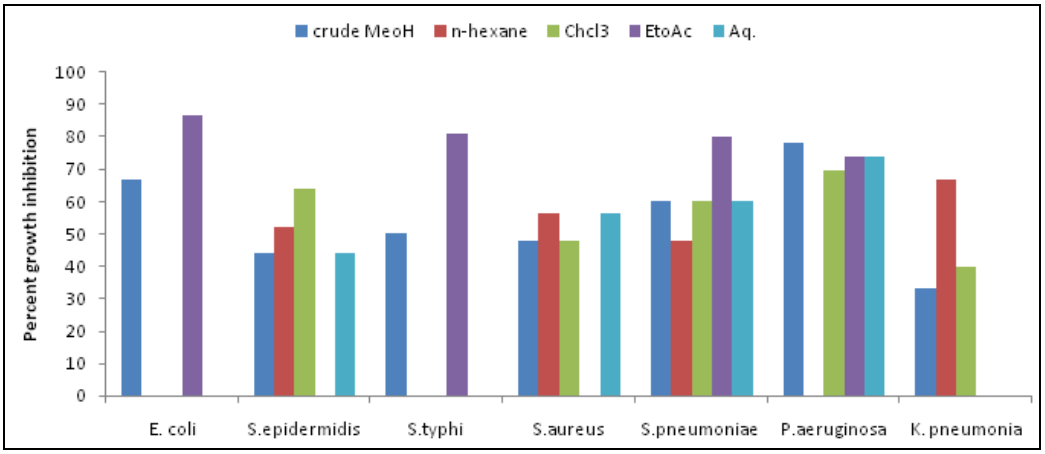

Fig. 2: Anti-bacterial activity of crude methanolic extract of aerial part and its various fractions

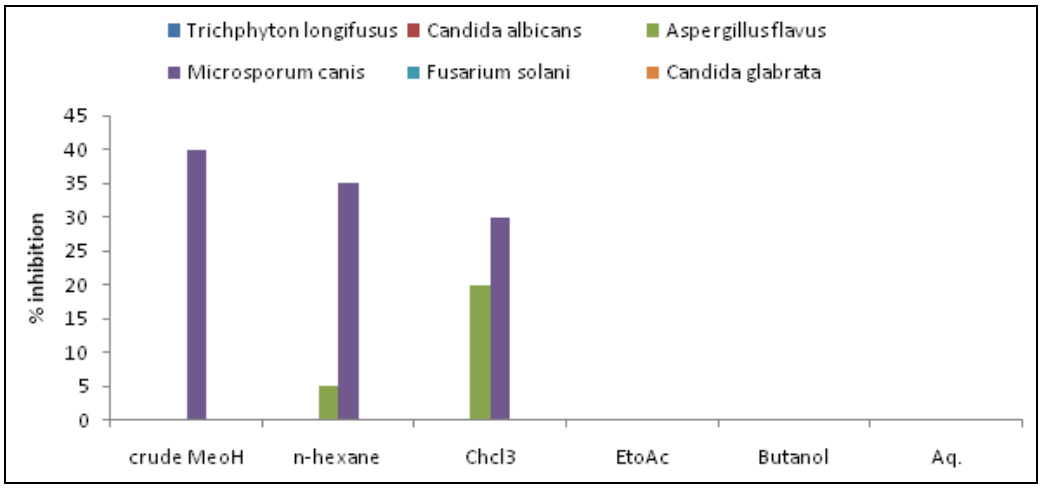

Fig. 3: Anti-fungal activity of crude methanolic extract of roots and its various fractions

T. longifusus, C. albicans, A. flavus, M. canis, F. solani and *C. glabrata.* The result of anti-fungal activity is presented in fig. 3. Among all tested samples only crude methanolic extract, *n*-hexane and chloroform fractions demonstrated fungicidal activity against *M. canis and A. flavus* only, while against remaining fungus strains all tested samples were inactive. The crude methanolic extract, *n*-hexane and chloroform fractions showed moderate fungicidal effect against *M. canis* fungus strain with percent inhibition 40, 35 and 30% respectively. Against *A. flavus* only *n*-hexane and chloroform fraction showed mild fungicidal effect and percent inhibition was 05 and 20% respectively.

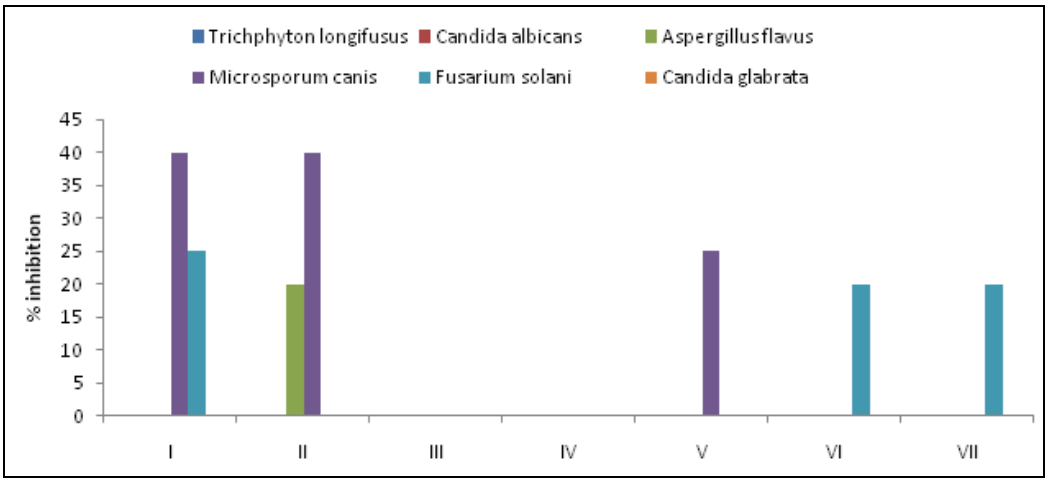

Fig. 4: Anti-fungal activity of various oil fractions

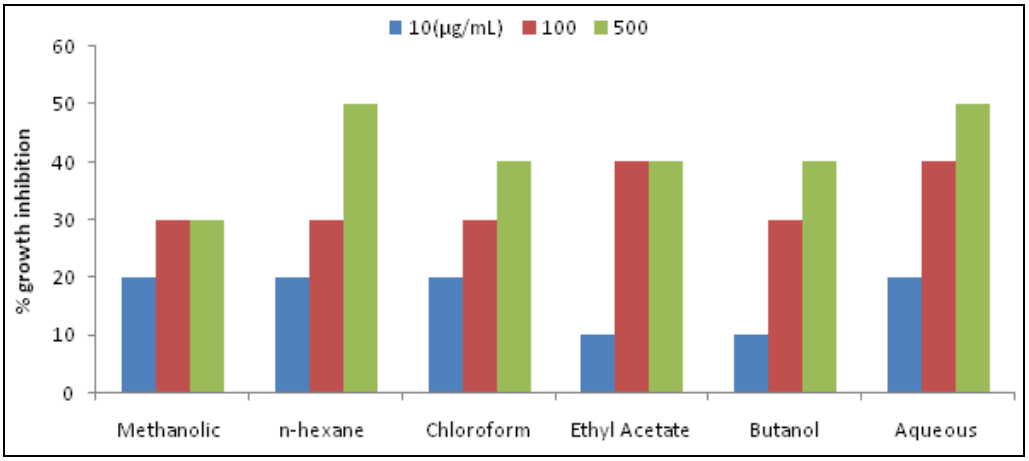

Fig. 5: Phytotoxic activity of crude methanolic extract of roots and its various fractions

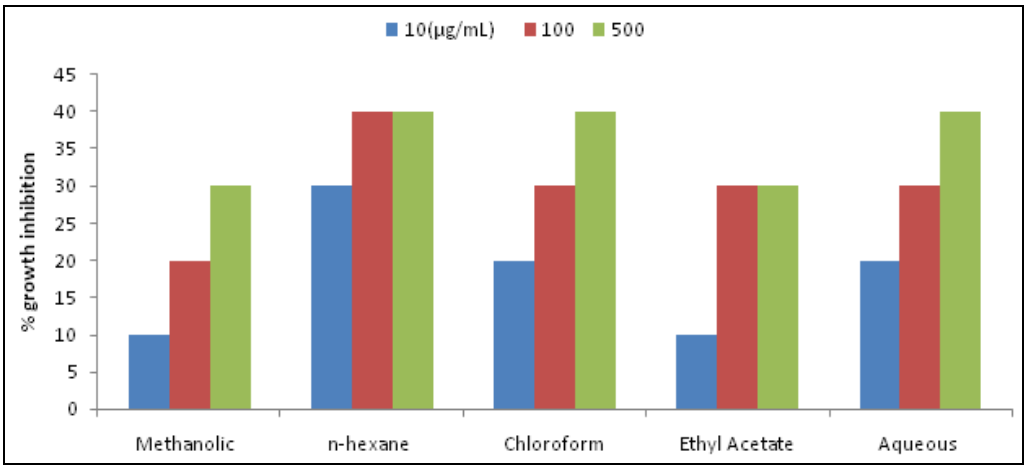

Fig. 6: Phytotoxic activity of crude methanolic extract of aerial part and its various fractions.

Anti-fungal activity of oils

Various oil fractions (I, II, III, IV, V, VI, VII and VIII) were also tested against above stated different fungal strains. The result showed by tested samples was also moderate in this case presented in fig. 4.Only oil fraction II showed mild anti-fungal effect against *A. flavus* and percent inhibitory effect was 20 %. Different oil fractions I, II and V also showed moderate fungicidal effect against *M. canis* with percent inhibition 40, 40 and 25% respectively. Against *F. solani* only oil fractions I, VII and VIII were active with percent inhibition of 25, 20 and 20 % respectively. The remaining oil fractions were inactive against all tested strains of fungus.

Phytotoxic activity of roots and aerial parts

Crude methanolic extract as well as their subsequent fractions of both roots and aerial part were tested for phytotoxic activity at 500, 500 and 10µg/mL concentrations respectively. The result of this activity is presented in figs. 5 and 6. As given in fig. The percent inhibition by these tested samples is concentration dependent. The maximum percent growth inhibition was showed by *n*-hexane and aqueous fractions of roots at 500µg/mL concentration i.e. 50%. In the remaining fractions of tested samples the percent inhibition ranges from 10-40%.

DISCUSSION

The antibacterial results show that our tested samples are considered to be a rich source for antibacterial agents and can be used in various pathological conditions due to these different bacteria. This result strongly supports the folkloric use of this plant in various ailments like bronchitis, sinusitis and various others (Shinwari and Gilani, 2003). Our antibacterial results are strongly supported by the antibacterial properties of related species like *F. communis* (Al-Yahya *et al*., 1998), *F. szowitsiana* (Özek *et al*., 2008) and *F. hermonis* (Ibraheim *et al*., 2012).

Fixed oils are actually the *n*-hexane or non-polar fraction of any plant. The chemical composition of all fractions is different from each other (Muhammad *et al*., 2013a). Due to this chemical difference we evaluated all the fractions of the plant. So the anti-fungal effect of fixed oils means that the non-polar fraction of the plant also shares their anti-fungal property. Large number of fixed oils / *n*-hexane fraction has been reported with significant fungicidal effect reflecting the ethno medicinal use (Muhammad *et al*., 2013b, Khan and Khan, 2012).As these different fungal strains which were tested can produce diseases in human beings specially *M. canis* and *C. albicans*. In human the *M. canis* can produce *T. capitis* and in pets can produce ringworm, while *C. albican s*can produce skin, ear and *Bronchial candidiasis* (Ginter□Hanselmayer *et al*., 2004). *A. flavus* is another fungus which can deteriorate cotton seed, it also contaminate peanuts during their harvesting and storage (Ahmad *et al*., 2012). The researchers are trying to isolate such type of fungicidal chemicals from medicinal plants which should be resistant to such disease producing fungi (Eloff *et al*., 2005). Both tested samples of roots as well as oil fraction showed moderate anti-fungal effect so it can be used in these diseased states, which further support the folkloric use of this plant. Further the anti-mycotic actions of our tested samples are accordance with the anti-fungal effect of other spp of genus ferula (Mahendra and Bisht, 2012).

From agricultural point of view the phytotoxic potential of plants origin is very beneficial and helpful to screen substances possessing weedicidal effect (Khuda F *et al*., 2012). The *Lemna* bioassay is an economical and simple assay to screen phytotoxic effect of plants. This assay provides guidance regarding the growth stimulation activity of tested samples. For the control of weeds, different chemicals are available in the market, but many problems associated with these chemicals like health hazards and environmental pollution, so for these reasons discovery of effective and safe weedicidal agents is needed. The production of crops in terms of quality and crop can be increased by controlling the weeds with the help of safe and effective weedicidal agents.

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