International Journal of Phytomedicine 5 (2013) 113-118
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Antimicrobial activity of *Fagoniaindica* from Thal desert, Punjab, Pakistan

Rehana Kouser[1], Rahmatullah Qureshi[1*]

***Corresponding author:**

Rahmatullah Qureshi

[1]Department of Botany, PirMehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan

Abstract

Fagoniaindicais a member of Zygophyllaceae family. The plant is used in indigenous medicine to treatvarious ailmentssuch as tumors, abscesses, wounds, scrofulous glands and other swellings of neck. The present study was designed to evaluate antimicrobial activity of this plant. For this purpose, polarity based extraction of the powdered sampleswere carried out with n-hexane, chloroform, acetone, ethyl acetate, butanol, ethanol and methanol. The extracts were tested on fungal pathogens viz., *A.niger, F.oxysporum, A.fumigatus, C.albicansand N.sitophillaand bacterial strains i.e., S. aureus, S.epidermidis, E. coli, K.pneumoniae, P.aeruginosa, S.typi, L.bulgaricus and M.luteus.* All the extracts isolated from *F.indicahave* demonstrated pronounced antibacterial and antifungal activity. Maximum activities were shown by acetone and ethyl acetate extracts which exhibited activity against all the tested bacterial strains.Maximum antifungal activity was expressed by ethyl acetate extract which inhibited all fungal pathoges.The present study supports the use of this plant in traditional medicines as an effective drug used to treat various skin diseases.

Keywords: *Fagoniaindica*, antimicrobial activity, Thal desert, traditional medicine, skin ailments.

Introduction

*Fagoniaindica*L. is a member of Zygophyllaceae family, distributed in India, Iran, Pakistan, Aden, Eritrea, Ethiopia, Sudan, Somalia and Kenya [1]. This is a dwarf spiny shrub with stiff branches often more or less prostrate [2]. In Unani system of medicine, this plant is considered as bitter, antiseptic, astringent, febrifuge, stimulant, alterative, analgesic, tonic, antiemetic and diuretic. It is generally prescribed in fever & typhoid, asthma, constipation, vomiting, dysentery, urinary discharge, leucoderma and biliousness[3,4,5].Its paste is externally applied on tumors, abscesses, wounds, scrofulous glands and other swellings of neck. Leaves and twigs are used in snakebite [3, 6]. The other reports indicated that the decoction of plant is used in skin diseases [7].

Due to multifarious uses in treating various diseases, it was worthwhile to investigate its antimicrobial activity. Meanwhile, there is sporadic information available from Pakistan with reference to its antibacterial and antifungal activity; hence it is supposed that the taken plant may possess antimicrobial properties.

Materials and Methods

Plant material

The plant sample was collected from Thal desert Pakistan during the month of its maturation. The voucher specimens were prepared (No. 13) and identified with help of flora [1] and deposited in the herbarium of the department of Botany PirMehr Ali Shah Arid Agriculture University Rawalpindi.The plant sample was air dried followed by oven drying and ground to fine powder. The Samples were saved in plastic sealed bags for further processing.

Extraction of plant material

The powdered samples were extracted with different solvents (n-hexane, chloroform, acetone, ethyl acetate, butanol, ethanol and methanol) based on polarity. Initially the sample was extracted by shaking with n-hexane (1: 10) for 24 hours at 28 C and 200 rpm in shaker incubator, followed by centrifuge at 10,000 rpm and 30 C for 10 minutes. Supernatant was filtered and transferred to a pre-weighed falcon tube and evaporated to dryness at 40 C in incubator. The residue was re-extracted with the next solvent which was slightly more polar than n-hexane. The same procedure was repeated with all the solvents. The dried extracts were stored at 4 C in refrigerator. For antimicrobial assay the extracts were dissolved in 30% dimethylsulfoxide (DMSO).

Microorganisms

Different extracts of *Fagoniaindica* were assessed for its antifungal activity against pathogens i.e. *Aspergillusfumigatus* (ATCC#1022), *Aspergillusniger*(ATCC#1015),*Fusariumoxysporum*(ATCC#7601), *Candida albicans*(ATCC#10231) and*Neurosporasitophilla* (ATCC#36935). Safradine and fluconazole were used as standard antibiotic.Bacterial strains viz.,*Staphylococcus aureus* (ATCC#6528), *Staphylococcus epidermidis*

(ATCC#12228),*Escherichia coli* (ATCC#15224),*Klebsiella pneumoniae*,*Pseusdomonas aeruginosa* (ATCC#9027), *Salmonella typhi*(ATCC#13048), *Lactobacillus bulgaricus* (ATCC#11842) and *Micrococcus luteus*(ATCC#10240) were used in the antibacterial assay. Chloramphenicol was used as standard antibiotic

Antibacterial Activity

Antibacterial activity was determined by Agar-well diffusion assay[8]LauriaBertini agar media was prepared [9], and autoclaved at 121 C for l5 minutes which was then cooled and poured in autoclaved Petri plates under sterilized conditions of the safety chamber. Wells of 6mm diameter were bored in each plate by a sterile borer. Bacterial inoculums were prepared from overnight grown cultures (24 h) in Luria broth and the turbidity was adjusted equivalent to approximately $1.2x10^8$ CFU/ml [10]. Each bacterial suspension (30 µl) was spread over the surface of LauriaBertini agar plates containing 4 wells of 6 mm diameter [9]. The wells were filled with 75 µl each of the extracts. The plates were incubated at 37 C for 24 h. The results were expressed in terms of the diameter of the inhibition zone [8]. DMSO was used as negative control and chloramphenicol as positive control.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of the effective plant extracts was worked out by the same agar well diffusion method described by Hemaiswarya[8]. For MIC four different concentrations (7.5, 5, 2.5and 1mg/ml) of each extract were prepared. Each bacterial suspension (30 µl) was spread over the surface of LauriaBertini agar. Each well was filled with 75 µl of each concentration of the extract. The plates were incubated at 37 C for 24 h. the lowest concentration of the extract causing complete inhibition of the bacterial growth was taken as MIC [11].

Antifungal Activity

Antifungal activity was determined by agar tube dilution method [12]. Plant extracts dissolved in DMSO were diluted in 1.5 ml of sterile Sabouraud dextrose agar, and allowed to solidify in slanting positions. Controls containing the solvent alone and positive control with safradine were applied. Test fungal cultures were inoculated on the slanting position of the media in the test tubes and the test tubes were incubated between 28-30 C. Fungal growth was examined for 48 h [8]**.** The diameter of the fungal growth was compared with that of the control. Percentage inhibition was calculated with reference to negative and positive controls.

Determination of Antifungal Index (%)

Antifungal assays were performed based on the modified method described by Wanchaitanawong*et al.* [13]for testing the elected plant extracts with high inhibition activity. Sterile SDA (3 ml) was individually diluted with 300 µl of five concentrations (10, 7.5, 5, 2.5 and 1 mg/ ml) of each crude extract, whereas SDA without crude extract served as a control. The spores of 5 days test fungi cultured on Potato Dextrose Agar were placed on SDA slant. The test tubes were incubated at room temperature for 5 days. The antifungal index (%) was calculated as follows [13]:

Antifungal index (%)= $\frac{D_{Control} - D_{Extract}}{D_{Control}} \times 100$

Where $D_{Control}$ = the diameter of growth in the control plate and $D_{Extract}$ = diameter of mycelia growth in the test plate.

Results and Discussion

Antibacterial Activity of Different Crude Extracts

Antibacterial activity of different extracts (n-hexane, chloroform, acetone, ethyl acetate, butanol, ethanol and methanol) of *F. indica* was tested against four gram+ve (*S. aureus*, *S. epidermidis*, *L. bulgaricus* and *M. luteus*) and four gram–ve bacterial strains (*E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. typhi*). Zone of inhibition of all tested samples for gram+ve and gram–ve bacterial strains were compared to standard antibiotic chloramphenicol. Results are provided in Table 3.1.

All extracts have shown activity against the tested bacterial strains. The activity of the extracts against bacterial strains was in the order of Acetone > Ethyl acetate >Butanol> Ethanol > Chloroform > N-hexane and > Methanol. N-hexane extract was found more affective against *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *L. bulgaricus*than the standard and zones of inhibition were 11-14 mm; while no activity was shown against *S. aureus*, *S. typhi and M. luteus*(Table 1). Chloroform extract was observed effective against all tested strains except *S.typhi* and *M. luteus*.

The zone of inhibition was in the order of 14mmagainst *E. coli a*nd *K. pneumoniae*> 13mm against *S. epidermidis* and > 12mm against *S. aureus*, *P. aeruginosa* and *L. bulgaricus* each. Acetone extract showed high inhibition zones against *P. aeruginosa* (16 mm), *S. typhi* & *E. coli* (15 mm each),*S. aureus* and *S. epidermidis* (14 mm each). The ethyl acetate extract showed high inhibition zones against *S. typhi* (17 mm) and *P. aeruginosa* (14.5 mm) which were found higher than the standard antibiotic. Acetone and ethyl acetate were the most successful extracts as they were effective against all selected bacterial strains tested and their inhibition zones were higher enough than that of the antibiotic, except in case of *S. aureus* and *S. epidermidis* (Table 1).

Butanol extracts showed highest activity against *P. aeruginosa* (21.7 mm), *E. coli* (20 mm), *S. epidermidis* (17.6 mm), *K.pneumoniae* (15 mm), *L.bulgaricus* (13 mm)and *M.luteus* (9 mm), while no activity was found against *S. Typhi* and *S. aureus*.

Butanol extracts showed higher inhibition zones against *S. epidermidis*, *E. coli*, *P. aeruginosa* and *L. bulgaricus* than the antibiotic. Growth of *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. typhi* was inhibited by ethanolic extract of *F. indica*, with zones of inhibition 10-16.7mm; while *L. bulgaricus* and *M. luteus*were resistant to it. Ethanolic extract

showed better activity against *E. coli* and *P. aeruginosa* than the antibiotic (Table 1). The ethanolic extract and antibiotic showed equal zone of inhibition against *K.pneumoniae* and *S.typhi*. Effectiveness of the ethanolic extracts might be due to its ability to dissolve most of the aromatic or saturated organic compounds of the plants [14]. Methanolic extract showed activity only against *S.typhi*(12 mm) and *E. coli* (8 mm)and rest of the strains were resistant to the extract. This extract exhibited higher zone of inhibition against *S.typhi* than the chloramphenicol (10.7 mm).

The results revealed that all extracts exhibited higher zone of inhibition than the standard antibiotic, since their activity was greater or equal to inhibition zones against six out of eight bacterial strains. But the antibiotic showed higher zone of inhibition than all the extracts in case of *S. aureus* and *S.epidermidis* (Table 1). Minimum inhibition zones were observed in case of *S.aureus* being a multi-resistant bacterium to drugs [15]; while *E. coli* and *P.aeruginosa* were found more susceptible to the extracts. The results revealed that the plant extracts have great potential as antimicrobial compounds against microorganisms.

A number of factors influence the diameter of the inhibition zone such as drug infusibility, nature, composition, thickness and pH of the medium, presence of stimulatory or inhibitory substances and the incubation time [16]. According to the results, *S.typhi* was susceptible to almost all the extracts. Gehlot&Bohra[17] confirmed that leaf extract of *F.indica* was found most effective against *Salmonella typhi*. *E. coli* is known as multi-resistant to drugs however it was found susceptible to all the plant extracts tested[15]. Similarly *P.aeruginosa*, which is also resistant to different antibiotics [15], had growth inhibited by the extracts from *Fagoniaindica*.

Table1: Zone of Inhibition of *Fagoniaindica* extracts at concentration of 15mg/ml against selected bacterial strains.

Extracts	Zone of Inhibition (mm)							
	Bacterial strains							
	S.aureus	*S.epidermidis*	*E. coli*	*P.aeruginosa*	*K.pneumoniae*	*S.typhi*	*L.bulgaricus*	*M.luteus*
n-hexane	-	14±1.06	11±0.00	12±0.00	13±0.35	-	13±0.00	-
Chloroform	12±0.3	13±0.35	14±0.00	12±0.00	14±0.70	-	12±0.30	-
Acetone	14±0.1	14±0.17	15±0.57	16±0.58	13.6±0.00	15±1	9±0.70	13±0.0
Ethyl acetate	13±1	13.7±0.58	13±1.03	14.5±0.58	11±2	17±1.5	10±1.75	11±0.5
Butanol	-	17.6±0.30	20±1	21.7±1	15±0.86	-	13±0.70	9±1.7
Ethanol	14±0.7	15±0.00	13.6±2	16.7±0.00	12±1.4	10±2.8	-	-
Methanol	-	-	8±0.57	-	-	12±0.0	-	-
Chloram-phenicol	18.3±0.0	17±0.00	10±0.00	10.5±0.00	12±0.00	10.7±0.0	9±0.00	11±0.0

Values are expressed as mean ±S.D. after triplicate analysis, - No activity

Minimum Inhibitory Concentration (MIC)

The strains which exhibited sensitivity to plant extracts were selected further to determine minimum inhibitory concentration (MIC) of the plant extracts. The MIC for crude extracts is shown in Table 3.2. Minimum inhibitory concentration for n-hexane, chloroform, acetone, ethyl acetate and ethanol extracts ranged from 1-2.5 mg/ml. The MIC of n-hexane extract was 2.5mg/ml for *S.epidermidis*, *E.coli* and *K.pneumoniae*and1mg/ml for *P.aeruginosa*.

The MIC of chloroform extract was 2.5mg/ml for *S.epidermidis*, *E. coli* and *S.aureus* while *K. pneumonia* and *P.aeruginosa* had 1mg/ml. Ethyl acetate extract showed inhibitory growth against *S.aureus* and *S.epidermidis* at 1mg/ml MIC;while *E. coli*, *K.pneumoniae* and *P.aeruginosa* was inhibited at 2.5mg/ml (Table 3.2).

Acetone extract showed activity at 1 mg/ml MIC against *S.epidermidis*, *S.aureus*, *P.aeruginosa* and *K.pneumonia*e; while *E. coli* was inhibited at 2.5 mg/ ml, Similarly, ethanol extract exhibited activity at 1mg/ml against *P.aeruginosa*, *E. coli* and *K.pneumoniae*, while growth of *S.aureus* and *S.epidermidis* was inhibited at 2.5 mg/ml (Table 2).Growth of all the bacterial strains was completely inhibited at 1mg/ml by butanol extract.

E. coli being an antibiotic resistant bacterium was inhibited by relatively higher concentration of about all the extracts i.e., 2.5 mg/ml. Growth of *P.aeruginosa* and *K.pneumoniae* was effectively inhibited by most of the extracts at 1 mg/ml. Minimum inhibitory concentration of the antibiotic also ranged from 1-2.5 mg/ml. Thus antimicrobial activity of the *F.indica* is similar to the standard antibiotic (Table 2).

Butanol extract exhibited highest zones of inhibition, wherever it showed activity. The largest inhibition zone was exhibited by it against *P.aeruginosa* (21.7mm) and *E. coli* (20mm). *M.luteus* was the resistant bacterium, as has shown resistance to four out of seven extracts. The minimum MIC values (1mg/ml) were exhibited by the butanol extract.

Table 2: MIC of effective *F.indica* extracts against selected bacterial strains.

Microorganisms	MIC (mg/ml)						
	Extracts						Standard
	n-hexane	Chloroform	Acetone	Ethyl acetate	Butanol	Ethanol	Chloramphenicol
S.aureus	-	2.5	1	1	-	2.5	1
S.epidermidis	2.5	2.5	1	1	1	2.5	1
E. coli	2.5	2.5	2.5	2.5	1	1	2.5
P.aeruginosa	1	1	1	2.5	1	1	2.5
K.pneumoniae	2.5	1	1	2.5	1	1	1

- No activity

Antifungal Activity of Different Crude Extracts

Antifungal activity of different extracts (n-hexane, chloroform, acetone, ethyl acetate, butanol, ethanol and methanol) of *F.indica* was tested against *Candida albicans*,*Aspergillusniger*,*Fusariumoxysporum*,*Aspergillusfumigatus*and*Neurosporasitophilla*.According to the results, all the extracts have shown moderate inhibitory effects against the tested fungal strains. The results are presented in Table 3. The growth inhibition of n-hexane extract was in the order of *N.sitophilla*(15mm) >*F.oxysporum* (11mm) and >*A.niger* (10mm), while it was found ineffective against *C.albicans* and *A.fumigatus*.

Table 3: Antifungal activity of *F.indica*extracts against selected fungal strains.

Extracts	Zone of Inhibition (mm)				
	Microorganisms				
	A.niger	*A.fumigatus*	*F.oxysporum*	*N.sitophilla*	*C.albicans*
n-hexane	10	-	11	15	-
Chloroform	-	10	-	-	10
Acetone	-	11	12	-	11
Ethyl acetate	13	11	10	9	11
Butanol	9	12	8	-	10
Ethanol	-	10	16	-	9
Methanol	12	11	10	-	-
Antibiotic	17	21	18	18	16

- No activity

Aspergillusniger, *Fusariumoxysporum* and *Neurosporasitophilla* were found resistant to chloroform extract, while the growth of *Aspergillusfumigatus* and *C.albicans* (10mm each) was inhibited by it (Table 3). Acetone and ethanolic extracts demonstrated good activity against *Fusariumoxysporum* (12mm), followed by*Aspergillusfumigatus* and *C.albicans* (10mm each),while it failed to express any activity against *Neurosporasitophilla* and *Aspergillusniger*. Ethyl acetate extract exhibited antifungal activity against all the tested fungi. Butanol and methanolic extracts were active against all the fungal strains tested except *Neurosporasitophilla*. Highest inhibition zone was shown by ethanol extract against *Fusariumoxysporum*at 16mm, followed by 15mm by n-hexane against *N.sitophilla*.

All the extracts showed less activity than the antibiotic as their inhibition zones were less than that of the antibiotic used (Table 3). While, these extracts have shown distinct feature that all these completely checked the fungal spores and interestingly no growth was observed even after a month of incubation.

Antifungal Index (%)

Results of antifungal index of different extracts against selected strains are presented in Table 4. Various concentrations of the extracts of *F.indica* inhibited the fungal growth of *A. fumigatus*, *A. niger* and *F.oxysporum* and *C.albicans*. Results of the antifungal index indicated that the inhibition of fungal growth was directly proportional to the concentration of extracts (Table 4). Butanol and ethyl acetate extracts showed complete inhibition (i.e., 100% fungal index) on the growth of *A.niger* and *A. fumigates* at 5 and 10 mg/ml respectively. Ethyl acetate extract completely inhibited growth of *F.oxysporum* and *C. albicans* at 7.5 mg/ml; while butanol extract showed inhibition of *F.oxysporum* at 2.5 mg/ml and *C. albicans* at 5

mg/ml. Acetone extract showed 100% antifungal index at a concentration of 10 mg/ml against *A. fumigates* and *F.oxysporum* and 2.5 mg/ml against *C. albicans*.

The ethanol extract exhibited 100% antifungal index at 7.5 mg/ml concentration against *F.oxysporum*, while *A.fumigatus* and *C. albicans* were inhibited at 10 mg/ml concentartion. The antibiotic showed 100% antifungal activity at 1 mg/ml (Table 4). The results have shown that the extracts of *F. indica* completely inhibited growth of the fungi however, complete inhibition was observed relatively at higher concentration. The results of this work suggested that the suppression of fungal growth supports the use of *F. indica* for different types of skin infections by indigenous people of Thal desert [6]. These results showed a fairly high correlation between antifungal activity of *F. indica* and its traditional medicinal use.

Table 4: Antifungal Index (%) of fungal strainsinhibited by various extracts

Extracts Concentrations mg/ml		Antifungal index of fungal strains (%)			
		A.niger	*A.fumigatus*	*F.oxysporum*	*C.albicans*
Acetone	10	-	100	100	100
	7.5	-	80	88	100
	5	-	70	40	100
	2.5	-	57	20	100
	1	-	50	20	28.57
Ethyl acetate	10	100	100	100	100
	7.5	100	82.5	100	100
	5	100	80	80	94
	2.5	86.67	75	72	89
	1	66.67	37.5	64	57
Ethanol	10	-	100	100	100
	7.5	-	90	100	91
	5	-	62.5	84	89
	2.5	-	37.5	76	80
	1	-	35	76	65
Butanol	10	100	100	100	100
	7.5	100	85	100	100
	5	100	75	100	100
	2.5	50	50	100	80
	1	33	50	76	14.28
Chloroform	10	-	-	-	100
	7.5	-	-	-	100
	5	-	-	-	100
	2.5	-	-	-	100
	1	-	-	-	83
Antibiotic	10	100	100	100	100
	7.5	100	100	100	100
	5	100	100	100	100
	2.5	100	100	100	100
	1	100	100	100	100

- Due to less/no activity, MIC value was not determined

Minimum Inhibitory Concentration

The fungal strains which showed good sensitivity to plant extracts were selected further to determine minimum inhibitory concentration (MIC). MIC values are given in Table 5. Minimum inhibitory concentration for ethyl acetate and ethanol extracts was found 1mg/ml against all the fungal strains tested. The total minimum inhibitory concentration range of butanol extract was 1-2.5 mg/ml.

Table 5: MIC of *F.indica*extracts (mg/ml) against selected fungal strains.

Microorganisms	Extracts					Standard
	Ethyl acetate	Butanol	Ethanol	Acetone	Chloroform	
A.niger	1	1	-	-	-	1
A.fumigatus	1	1	1	1	-	1
F.oxysporum	1	1	1	5	-	1
C.albicans	1	5	1	2.5	1	1

- Due to less/no activity MIC was not determined.

The butanol extract showed minimum inhibitory concentration of 1 mg/ml against *F.oxysporum*, *A. fumigates* and *A.niger* followed by 2.5 mg/ml for *C.albicans* (Table 5). The MIC value of acetone extract was recorded at 2.5 mg/ml for *A. fumigates* followed by 2.5 mg/ml for *C.albicans*, and 5 mg/ml for *F.oxysporum*. Chloroform extract had shown activity against *C.albicans*; therefore MIC of the extract was recorded at 1 mg/ml. The antibiotic showed activity at 1mg/ml. Antifungal activity was shown at all concentrations of each extracts; this indicates that *F. indica* is highly active against the selected fungal strains.

Acknowledgement

Authors are thankful to Pakistan Science Foundation, Islamabad for the collection of plant material under the research project titled, “Ethnobotanical survey of Thal Desert, Punjab, Pakistan No. RES/P-PMASAAUR/Bio(418).

References

[1]. Ghafoor A. Zygophylaceae. In Nasir, E, & Ali SI, editors.The Flora of West Pakistan, Department of Botany, University of Karachi; 1974; vol. 74.

[2]. Ansari AA, Kenne L. Isolation Procedure and Structure of New Saponins from *Fagoniaindica*. Planta Medica.1982; 45(7):143.

[3]. Baquar SR. Medicinal and Poisonous Plants of Pakistan, Printas, Karachi; 1989.

[4]. Wazir SM, Saima S, Dasti AA, Subhan M. Ethnobotanical Importance of Salt Range Species of District Karak, Pakistan.Pakistan Journal of Plant Sciences.2007;13 (1): 29-31.

[5]. Qureshi R, Bhatti GR. Ethnobotany of plants used by the Thari people of Nara Desert, Pakistan. Fitoterapia.2008;79: 468–473.

[6]. Panhwar AA, Abro H. Ethnobotanical Studies of MahalKohistan (Khirthar National Park).Pakistan Journal of Botany. 2007; 39(7): 2301-2315.

[7]. Qureshi R, Humaira S. First Annual Technical Report of the project titled, "Ethnobotanical survey of Thal Desert, Punjab, Pakistan. No. RES/P-PMASAAUR/Bio(418).2011; p. 47.

[8]. Hemaiswarya S, Raja R, Anbazhagan C, Thiagarajan V. Antimicrobial and mutagenic properties of the root tubers of *Gloriosasuperba* Linn. (*Kalihari*).Pakistan Journal of Botany. 2009; 41(1): 293-299.

[9]. Bertani G. Lysogeny at mid-twentieth century: P1, P2, and other experimental systems. Bacteriology.2004; 186: 595-600.

[10]. Luqman S, Srivastava S, Darokar MP, Khanuja SPS. Detection of antibacterial activity in spent roots of two genotypes of aromatic grass *Vetiveriazizanioides*. PharmaceuticalBiology.2005; 43(8): 732–736.

[11]. Kaur GJ, Arora DS. Antibacterial and phytochemical screening of *Anethumgraveolens*, *Foeniculumvulgare* and *Trachyspermumammi*. BMC Complementary and Alternative Medicine 2009; 9: 30.

[12]. Sultanova N, Makhmoor T, Abilov ZA, Parween Z, Omurkamzinova VB, Rahman A, Choudhary MI. Antioxidant and antimicrobial activities of *Tamarixramosissima*.Journal of Ethnopharmacology.2001; 78: 201–205.

[13]. Wanchaitanawong P, Chaungwanit P, Poovarodom N, Nitisinprasert S.*In vitro* Antifungal Activity of Thai Herb and Spice Extracts against Food Spoilage Fungi.Kasetsart Journal(Natural Science).2005;39: 400–405.

[14]. Durmaz H, Sagun E, Tarakci Z, Ozgokce F. Antibacterial activities of *Allium vineale*, *Chaerophyllummacropodum* and *Prangosferulacea*. African Journal of Biotechnology.2006, 5(19): 1795-1798.

[15]. Nascimento GGF, locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant Bacteria.Brazilian Journal of Microbiology.2000; 31: 247-256.

[16]. Selvakumari PAS, Britto AJD. Bactericidal activity of *Legenandraovata*(Linn.)Thw. rhizome oil.Natural Product Radiance. 2007; 6(5): 382-385.

[17]. Gehlot D, Bohra A. Antibacterial effect of some leaf extracts on *Salmonella typhi*. Indian Journal of Medical Science. 2000; 54(3): 102-105.