

Full Length Research Paper

## ***In vitro* antimicrobial activities of *Glycyrrhiza glabra* and *Fagonia arabica***

Fatima Syed<sup>1</sup>, Rifat Jahan<sup>1</sup>, Aftab Ahmed and Shafiullah Khan<sup>2\*</sup>

<sup>1</sup>Institute of Chemical Science, University of Peshawar, KPK, Pakistan.

<sup>2</sup>Department of Chemistry, Gomal University, Dera Ismail Khan, KPK, Pakistan.

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The aim of this study was to test the antimicrobial activities of crude chloroform, hexane, ethyl acetate and ethanol extracts of the leaves *Glycyrrhiza glabra* (GG) and *Fagonia arabica* (FA) against bacteria (*Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis*). Antimicrobial properties of *G. glabra* and *F. arabica* were tested using Agar well diffusion method and Agar disc diffusion method. Streptomycine was used as standard drug with significant activity values, that is, 23 mm against *E. coli*, 36 mm against *S. epidermidis*, 34 mm against *S. aureus* and 26 mm against *B. subtilis*. Analysis of data showed that the crude extract of *G. glabra* and *F. arabica* in dichloromethane exhibited superior activity against *E. coli* and *S. epidermidis*. Results were compared concomitantly to standard drugs; streptomycine. Phytochemical screening of *G. glabra* and *F. arabica* showed the presence of terpenoids, saponins, flavonoids, alkaloids, tannins, glycosides and reducing sugar components. Based on the current conclusion, it can be accomplished that these plants has antimicrobial activity, which is as potent as standard antimicrobial drugs against definite microorganisms.

**Key words:** *Glycyrrhiza glabra* and *Fagonia arabica*, antimicrobial, phytochemical screening, medicinal plants.

### INTRODUCTION

Life and diseases go together where there is life, diseases are bound to exist. Dependency and sustainability of man and animal life has been revolving around plants through their uses as food, clothing and shelter, but also plants have been used to control diseases; therefore, the use of plants as medicines is an ancient and reliable practice (Arshad and Rao, 2001). Recently, it is reported by WHO that more than 80% of the world's population depends on traditional medicine for the treatment of their illnesses (Norman et al., 1985).

The phytochemical research, in which ethno pharmacological properties of plants are evaluated, leads to the discovery of new anti-infective agents from higher plants. Owing to the development of drug resistive strains in

human pathogens against commonly used antibiotics, it is necessary to discover new antimicrobial substances from plants and other sources. *Glycyrrhiza glabra* is one of the oldest used herbs, both as a medicine and a flavoring herb to disguise the unpleasant flavor of other medication (Biondi, 2005). The sweet taste of licorice is due to the active constituent called glycyrrhizina glycol-side in the combined potassium and calcium salt form of glycyrrhizic acid (GA). The constituent is 50 times sweeter than sugar, and thus widely used as a sweetening agent in food industry (Acharya, 1993). *G. glabra* has been used for centuries in the treatment of sore throats, coughs, gastric ulcers, allergic reactions, eczema, rheumatism, arthritis and hepatitis (Aly, 2005;

\*Corresponding author. E-mail: s.khan@gu.edu.pk. Tel: +92 966750359.

Ates, 2003; Rossum, 1998). Nowadays, licorice is widely used for its antimicrobial, antioxidant, anti-inflammatory, anticoagulative, antiulcer, expectorant, antiallergic, antiviral and anxiolytic activities and a number of other pharmacological actions (Shin, 2007; Statti, 2004; Vaya, 1997; Fukai, 2002).

*Fagonia arabica* is a widely used medicinal plant in Pakistan. *F. arabica* is an important Ayurvedic herb that grows throughout arid regions of India, and has been widely used as a folk remedy by the indigenous people for its anti-inflammatory, analgesic and antipyretic effects (Satpute, 2009). *F. arabica/cretica* has been used by various village dwellers in Indo-Pakistan region, for curing fever, dysentery, urinary discharges, erysipelas, typhoid, alexipharmic, reducing tumors and purifying the blood (Sheikh, 2007). It is also used for skin diseases, small pox and for endothermic reaction in the body (Watt, 1972). The twigs of the plant are used as remedy for snake bite and also applied externally as paste on tumors and for the swellings of neck (Hooker, 1982; Rizvi, 1996). *Fagonia* species were extensively studied by many workers regarding their medicinal uses, since these plants were antitumor, antioxidant, analgesic, astringent, febrifuge and prophylactic against small-pox agents. Thus, the purpose of this current investigation is to evaluate the chloroform, hexane ethyl acetate and ethanol fractions of *G. glabra* and *F. arabica* against several bacteria *in vitro*.

## MATERIELS AND METHOD

### Plant

*G. glabra* were collected from Afghanistan and *F. arabica* was picked from District Malakand, Khyber Pakhtunkhwa, Pakistan. The taxonomic identification of both plants was carried out by Dr. Farrukh Hussain at the Department of Botany (DOB), University of Peshawar, Pakistan. A voucher specimen under the scientific name of the plant (bot. 20011 (pup) and bot. 20010 (pup)) was deposited at the herbarium of Department of Botany, University of Peshawar, Pakistan.

### Preparation of crude extracts

Plants materials were dried under shade and powdered. From these powdered materials, weighed amount was taken in separate thimbles. This was suspended above the flask containing the solvent n-Hexane fitted with reflux condenser. The flask was heated (60 to 65°C for 5 to 6 h) on "Heating mantle" the evaporated solvent upon condensation trickled into the extraction chamber containing the plant material.

The extraction chamber was designed so that when the solvent surrounding the sample exceed a certain level, it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the n-Hexane extract was removed. After the removal of n-Hexane extract, the plant material was left in the thimble and next solvent of high polarity was passed like dichloromethane and extract was obtained and then ethyl acetate and at the end highly polar solvent, ethanol was passed and thus different extract were obtained according to increasing polarities of

solvents. The solvents obtained were evaporated under vacuum using Rotary evaporator. The plants extracts were collected in labeled vials. The plant materials taken for extraction were *G. glabra* (65 g) and *F. arabica* (60 g). The crude extracts were subjected for antimicrobial activity and their phytochemical screening.

### Qualitative analysis of chemical constituents

Chemical analysis for the presence of major classes of secondary metabolites (terpenoids, saponins, flavonoids, alkaloids, tannins, glycosides and reducing sugar) in the crude extracts was carried out according to the method described by Pearson (1976). The results are shown in Tables 1 and 2.

#### Alkaloids

About 0.2 g of the extracts was warmed with 2% H<sub>2</sub>SO<sub>4</sub> for 2 min. It was filtered and few drops of Dragendorff reagent were added. Orange red precipitate indicates the presence of alkaloids.

#### Tannins

Small quantity of the extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

#### Anthraquinones

About 0.5 g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH<sub>3</sub> were added to the mixture and heated. Formation of rose-pink color indicates the presence of anthraquinones.

#### Glycosides

The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

#### Reducing sugars

The extract was shaken with distilled water and was filtered. The filtrate was boiled with drops of Fehling's solution A and B for minutes. An orange red precipitate indicates the presence of reducing sugars.

#### Saponins

About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

#### Flavonoids

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

**Table 1.** Secondary metabolites detected in different extracts of *G. glabra*.

Chemical component	n-Hexane	Dichloromethane	Ethylacetate	Ethanol
Alkaloids	++	++	++	+++
Tannins	-	-	-	+
Anthraquinones	-	-	-	-
Glycosides	-	-	-	++
Reducing sugar	-	-	-	+
Saponins	-	+	-	+++
Flavonoids	-	-	-	+
Phlobatanins	-	-	-	-
Terpenoids	++	+++	++	++

+++ = High concentration; ++ = Moderate concentration; + = Low concentration; - = Absent.

**Table 2.** Secondary metabolites detected in different extracts of *F. arabica*.

Chemical component	n-Hexane	Dichloromethane	Ethylacetate	Ethanol
Alkaloids	++	+++	++	+++
Tannins	-	-	-	+
Anthraquinones	-	-	-	-
Glycosides	-	-	-	++
Reducing sugar	-	-	-	+
Saponins	-	++	-	+++
Flavonoids	-	-	-	++
Phlobatanins	-	-	-	-
Terpenoids	++	+++	++	++

+++ = High concentration; ++ = Moderate concentration; + = Low concentration; - = Absent.

### Phlobatanins

The extract 0.5 g was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution; red precipitate shows the presence of phlobatanins.

### Terpenoids (Salkowski test)

0.2 g of the extract was mixed with 2 ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was added carefully to form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

### Antimicrobial assay

#### Preparation of medium

Solid and liquid media were used. Nutrient agar 28 g was dissolved in distilled water and volume was made up to 1 L. It was autoclaved at 121 °C for 15 min. Media was cooled and allowed to solidify in

Petri dishes. Nutrient broth (1.9 g) was dissolved in distilled water and volume was made up to 150 ml. 8 ml of this broth was added to screw capped test tubes, which were placed in autoclave at 121 °C for 15 min, then refrigerated at 25 °C.

#### Preparation of tested materials

The test sample was prepared by dissolving 22 mg of crude extract in 1 ml of dimethyl sulfoxide (DMSO) in Eppendorff tube and was kept for 1 h. To another Eppendorff tube, 1 mg of streptomycin was dissolved in 1 ml DMSO and was kept for 1 h.

### Antibacterial assay

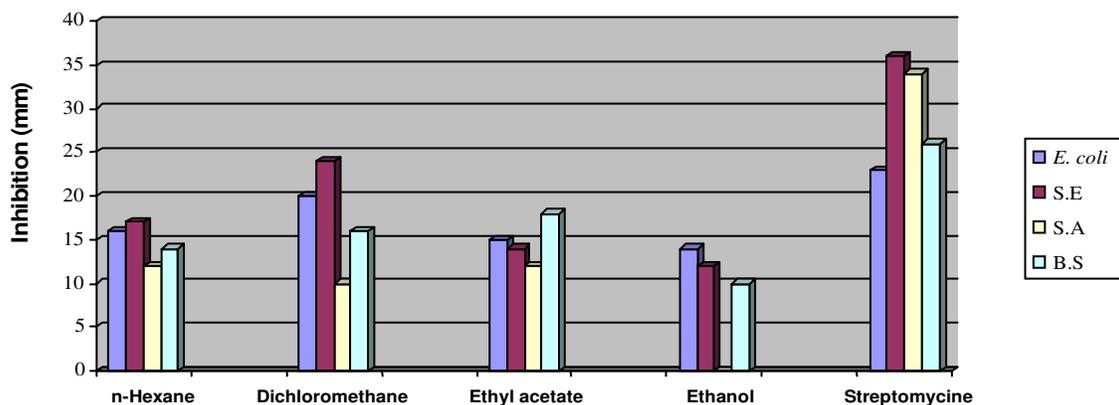
#### Cup-plate diffusion method

Antibacterial activity of plant extracts was carried using cup-plate agar diffusion method (Murray et al., 2009) with some small modifications. One milliliter from each standard bacterial stock suspension was mixed thoroughly with 40 to 45 ml of sterile Molten

**Table 3.** Antibacterial activity of *G. glabra* and *F. Arabica*.

Chemical component	Inhibition zone (mm)*					Inhibition zone (mm)*				
	<i>G. glabra</i>	E.C	S.E	S.A	B.S	<i>F. arabica</i>	E.C	S.E	S.A	B.S
Hexane	Extract	16	20	15	14	Extract	12	14	12	0
Dichloromethane	Extract	17	14	14	12	Extract	12	0	0	14
Ethyl acetate	Extract	12	10	12	0	Extract	0	0	0	10
Ethanol	Extract	14	16	18	10	Extract	12	0	0	10
Streptomycin	1 mg/ml	23	36	34	26	1 mg/ml	23	36	34	26

E.C: *Escherichia coli*; S.E: *Staphylococcus epidermidis*; S.A: *Staphylococcus aureus*; B.S: *Bacillus subtilis*; \*n=3.



**Figure 1.** Antibacterial activities of *G. glabra* in different solvents. E.C: *Escherichia coli*; S.E: *Staphylococcus epidermidis*; S.A: *Staphylococcus aureus*; B.S: *Bacillus subtilis*.

Mueller Hinton agar at 40°C, poured into sterile Petri-dishes and left to solidify. Then, four cup-shape wells (120 mm diameter) were made in each plate using sterile cork-borer. The agar disks were removed and four alternate cups were filled with extract using sterile adjustable pipettes. Four Petri-dishes with two alternate cups were used with the respective solvent instead of the extracts as control. The plates were then incubated in upright position for 22 to 24 h at room temperature. Two replicates were carried out for each extract. After incubation period, the inhibition zones diameters were measured.

#### Disc diffusion method

The antibacterial assay for plant extract was also conducted using disc diffusion method as illustrated by Abdel-Wahab (2009). The nutrient agar solution (16 ml) was poured and kept overnight in a refrigerator. Whatman filter paper (No.1) discs of 6 mm diameter were impregnated with 10 µl of the solution of crude extract (at 4 mg/ml) dissolved in DMSO. Standard disc of streptomycin sulphate (10 µg/disc) was used as positive control, while DMSO was used as a negative control. The Petri-dishes were inverted and incubated for 24 h at 37°C. Clear inhibition zones around the discs indicated the presence of antimicrobial activity.

## RESULTS AND DISCUSSION

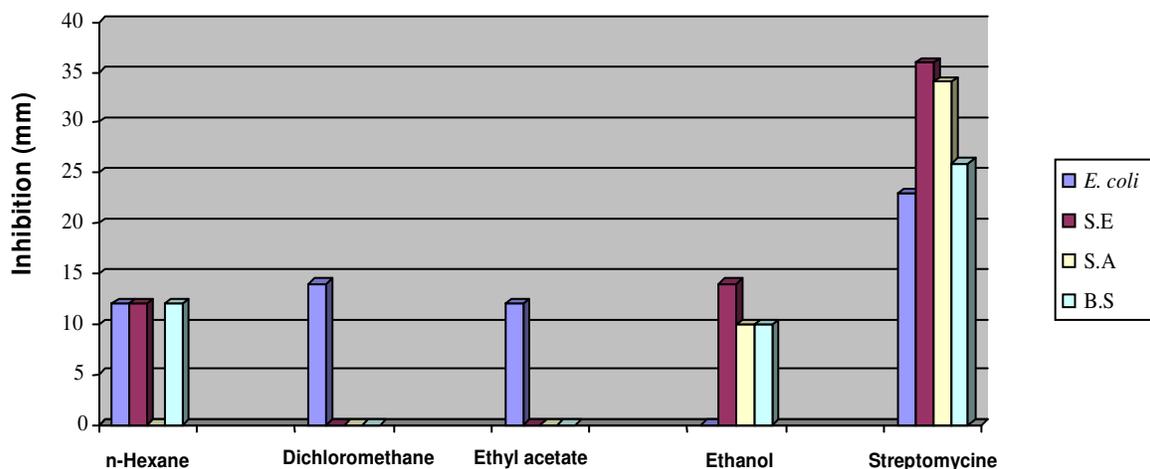
### Antimicrobial screening

Table 3 summarizes the results. However, results were

interpreted in terms of commonly used terms: sensitive, intermediate and resistant. Findings of cup-plate diffusion method for ethanolic extracts of *G. glabra* and *F. arabica* are shown in Figures 1 and 2.

### Antibacterial activities of *G. glabra* and *F. arabica*

The antibacterial activities of crude extract of the selected plants are n-Hexane, dichloromethane, ethylacetate and ethanol. Traditionally, *G. glabra* and *F. arabica* were used to treat bacterial infections. The results from the current study were screened for their microbial activity against four standard bacteria, namely, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Escherichia coli*. The pattern of inhibition varied with the plant's extract; the solvent was used for extraction and the organism was tested. DMSO was used as negative control and no inhibition was shown by it against all the bacteria (Bacterial growth inhibition=0 mm). Streptomycin, an antibiotic was used as a positive control. Streptomycin was used as standard drug with significant activity values, that is, 34 against *S. aureus*, 36 against *S. epidermidis*, 26 against *B. subtilis* and 23 against *E. coli*. The greater activity was shown by *G. glabra* and dichloromethane extract of the plant showed the maximum activity among all the extracts of both plants.



**Figure 2.** Antibacterial activities of *F. arabica* in different solvents. E.C: *Escherichia coli*; S.E: *Staphylococcus epidermidis*; S.A: *Staphylococcus aureus*; B.S: *Bacillus subtilis*.

*S. aureus* showed more susceptibility towards extracts of all studied parts. The n-Hexane extract of *G. glabra* were found to be good against *E. coli* and *S. epidermidis* and zone of inhibition were 16 and 17 mm, respectively; low activity against *B. subtilis* (14 mm) and non-significant against *S. aureus*. n-Hexane extract of *F. arabica* showed no appreciable activity against *S. aureus* and non-significant against *E. coli*, *S. epidermidis* and *B. subtilis*.

The dichloromethane extract of *G. glabra* were found significant against *E. coli* and *S. epidermidis* and its zone of inhibition were 20 and 24 mm, respectively, good against *B. subtilis* with zone of inhibition (16 mm), and non-significant against *S. aureus* (10 mm). Similarly, the dichloromethane extract of *F. arabica* showed low activity against *E. coli* and its zone of inhibition was 14 mm and exhibited no appreciable activity against *S. epidermidis*, *B. subtilis* and *S. aureus*.

The ethylacetate extract of *G. glabra* was examined and was discovered to exhibit low activity against *S. epidermidis* and *E. coli* as its zone of inhibition were 15 and 14 mm, respectively, was good against *B. subtilis* (18 mm) and non-significant against *S. aureus*. No appreciable activity was found by the ethylacetate extract of *F. arabica* against *S. epidermidis*, *B. subtilis* and *S. aureus*, and was non-significant against *E. coli* and its zone of inhibition was 12 mm. Low activity was exhibited by the ethanolic extract of *G. glabra* against *E. coli* with zone of inhibition (14 mm), non-significant against *S. epidermidis* and *B. subtilis* and its zone of inhibition were 12 and 10 mm, respectively and did not show activity against *S. aureus*. Similarly, the ethanolic extract of *F. arabica* was examined and it was found not to have activity against *E. coli*, low activity against *S. epidermidis* and non-significant activities against *B. subtilis* and *S. aureus*. Table 3 summarizes the percentage inhibition of each extract (Streptomycin) activity against different bacteria.

## Conclusion

The antimicrobial effects of *G. glabra* and *F. arabica* extracts against the studied bacteria suggest that, different parts of *G. glabra* and *F. arabica* possess remarkable therapeutic action that can support the traditional usage of this plant in the treatment of bacterial diseases such as gastrointestinal infection, diarrhea, respiratory and skin diseases. These antimicrobial activities are likely due to the presence of secondary metabolites like tannins and flavonoids, alkaloids, saponins, terpenes and glycosides in *G. glabra* and *F. arabica*. The high potency of *G. glabra* and *F. arabica* against these microbes could provide an example of prospecting for new compounds.

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