

Full Length Research Paper

# Assessment of antibacterial activity of three plants used in Pakistan to cure respiratory diseases

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The *in vitro* antimicrobial activity of *Justicia adhatoda*, *Glycyrrhiza glabra* and *Hyssopus officinalis* extracts were studied against selected bacteria by using agar well diffusion assay. Methanol, ethanol, chloroform, diethyl-ether and aqueous extracts were tested in crude form for antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*. Maximum antibacterial activity was exhibited by all the three plant extracts. The results obtained with *P. aeruginosa* were particularly interesting since it was not inhibited by the antibiotic used but the tested plant extracts effectively inhibited the growth of *P. aeruginosa*. The methanolic extract of *J. adhatoda* was effective against *S. typhimurium*. All the plants extract in water were effective against spore forming of *B. subtilis* while *S. aureus* and *E. coli* were not effectively inhibited by extracts of tested plants. The results of analysis of variance have shown significant differences between the species, treatments and interaction between the species and treatments. However, the differences were non-significant between the treatments for *G. glabra*. The results indicate that *J. adhatoda*, *G. glabra* and *H. officinalis* present a noteworthy potential of antibacterial activities.

**Key words:** *Justicia adhatoda*, *Glycyrrhiza glabra*, *Hyssopus officinalis*, antimicrobial assay.

## INTRODUCTION

There are 4,22,127 plant species growing on planet earth, about 35,000 to 70,000 plants species are used as medicinal plants (Hasan et al., 2007). Out of which 20,000 plants species are believed to be used medicinally in the third world (Mukherjee, 2004). Approximately 6000 species of flowering plants occur in Pakistan and 700 of them have medicinal value (Shinwari et al., 2006; Stewart, 1972). Of these species, 500 are known for their active constituents from research conducted in Pakistan and elsewhere and around 250 to 300 species known to have entered the herbal market of Pakistan (Williams and Ahmad, 1999). It is important to mention that over 75% of population in Pakistan is cured by using traditional medicines prescribed by more than 50,000 traditional herb practitioners (Gill, 2003) and the folk knowledge of plant curing pass down from family to family of herb practitioners and within communities (Ahmad, 2004).

The World Health Organization (WHO) estimates that 80% of world population use herbal medicines for some aspects of primary health care (Shinwari et al., 2006). While 70-80% of population in developing countries have often only traditional herbal remedies for their ailments, as they can not afford costly synthetic drugs (Al-Bashan, 2006). The world has witnessed growing scientific and commercial interests in medicinal plants and plant-based products mainly due to their immense economic potential and widespread cultural acceptability (Ali and Jahangir, 2001). But less than 5% species have been analyzed as potential medicine while rests of the 95% of plants are still there to be analyzed (Mukherjee, 2004).

The different system of eastern medicines that is, Unani, Ayurvedic and homeopathy, etc., are entirely based on medicinal properties of these plants. The practice of traditional medicine is widespread in China, India, Japan, Pakistan, Sri Lanka and Thailand (Hasan et al., 2007). Today herbal products and extracts are widely used to control various human diseases (Srinivasan et al., 2006). The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity

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nowadays (Cowan, 1999). Promising bioassay results can be used to perform more exhaustive chemical, biological and pharmacological studies (Saupe, 2005). It has been reported that pharmacological properties of medicinal plants may lead in developing novel therapeutic agents (Hussain and Gorski, 2004). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents (De Smet, 1997; Cowan, 1999; Kelmanson et al., 2000; Srinivasan et al., 2001). Plant extracts have great potential as antimicrobial compounds, especially in the treatment of infectious diseases caused by resistant microorganisms (Nasir and Chanda, 2006).

Respiratory diseases, including allergies, asthma and chronic obstructive pulmonary disease (COPD) are a major public health burden worldwide. The latest WHO statistics (2007) estimate that 300 million people worldwide have asthma, 210 million people have COPD and millions of people are affected by allergies. Each year, 250,000 people die of asthma. The prevalence of these diseases is increasing and there is a continued need for new and improved therapies. The Nature Reviews Immunology focused this issue and highlights the latest advances in our understanding of the immune bases of these respiratory diseases and how this knowledge can be translated into effective treatment strategies (<http://www.nature.com/nri/focus/allergyandasthma/index.html>).

Some respiratory diseases are caused by bacteria. For instance, *Staphylococcus aureus* causes pneumonia (Rubinstein et al., 2008), *Escherichia coli* causes community-acquired pneumonia (Tillotson and Lerner, 1967), *Bacillus subtilis* causes occupational asthma (Chan-Yeung and Lam, 1986) which is a reason for allergic reactions of the lungs (Pepys et al., 1969), *Salmonella typhimurium* causes lobar pneumonia (Mankhambo et al., 2006) and *Pseudomonas aeruginosa* typically infects the pulmonary tract and cause pneumonia (Grant et al., 2000).

The leaves of *Justicia adhatoda* have been in use in Indian systems of medicine for the last more than 2000 years. *J. adhatoda* is well known in the indigenous systems of medicine for its beneficial effects, particularly in bronchitis (Claeson and Malmfors, 2000). The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping-cough (Dhuley, 1999) and chronic bronchitis and asthma as sedative-expectorant, anti-spasmodic and as anthelmintic. The bronchodilatory and expectorant properties of the leaves are attributed to vasicine (Chatterjee, 1999). Warm Decoction of the leaves was expectorant and cured asthma patients (Mahato and Chaudhary, 2005).

The root of the *Glycyrrhiza* has wide range of therapeutic properties from centuries (Tsukiyama, 2002). Glycyrrhizin and glycyrrhizic acid have been shown to inhibit growth and cytopathology of cytomegalovirus (Numazaki et al., 1994). Liquorice root is used in cough medicines

and also in the treatment of catarrhal infections of the urinary tract (Grieve, 1984). It is taken internally in the treatment of asthma, bronchitis, coughs, peptic ulcer, arthritis and allergic complaints (Bown, 1995).

Hyssop derived from *Hyssopus officinalis* has a long history of medicinal use (Chevallier, 1996). Currently it is often used as a household remedy, as an expectorant and stomach tonic (Grieve, 1984) and to treat bronchitis and respiratory infections with excessive mucous production (Chevallier, 1996). A tea made from the leaves is used in the treatment of flatulence, stomachaches, upper respiratory tract infections, coughs in children, etc (Bown, 1995).

The main objective of the present study was to investigate the *in vitro* antimicrobial activity of, *J. adhatoda*, *Glycyrrhiza glabra* and *H. officinalis* extracts on five bacterial species causing various respiratory diseases. These plants have been used for centuries by native people of Pakistan to cure the diseases of pulmonary tract like pneumonia, bronchitis, cough, cold and asthma. The screening of these plants as new anti-infective agents in respiratory diseases will give scientific evidence for the culturally acceptable medicinal plants for respiratory diseases. It will boost the production of plant-based products by pharmaceutical industries and will prosper the economy of third world countries like Pakistan.

## MATERIALS AND METHODS

### Plant collection and extract preparation

Dried medicinal plant materials were collected from natural medicine laboratory at Qarshi Research International (QRI), Qarshi Industries Hattar Haripur, NWFP, Pakistan and identified by Professor Dr. Abdul Rashid, Plant Taxonomist, Botany Department, University of Peshawar, NWFP. The plant parts were placed in a beaker and kept in oven for 10 min at 100°C for drying and then ground to fine powder in blender.

Extract of each plant was prepared by soaking five grams of plant material in 20 ml of five solvents; methanol (T1), ethanol (T2), chloroform (T3), diethyl-ether (T4) and double distilled water (T5) and placed at ambient temperature for 48 h. The extracts were then filtered by using soaked whatman filter paper with the respective solvent. The extracts were centrifuged at 5000 rpm for 5 min; supernatant was collected and stored in screw capped test tubes at room temperature.

### Selection of test organisms

Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, Gram-negative bacteria, *Pseudomonas aeruginosa*, *Escherichia coli* and *S. typhimurium* were obtained from culture library of Microbiology Laboratory of QRI and were checked for purity by colonial morphology and biochemical tests. Pure culture were grown in nutrient broth and preserved at 4°C.

### Antibacterial activity

Antibacterial activity of the extracts was tested on the selected organism of Gram-positive and Gram-negative bacteria by Agar

**Table 1.** Analysis of variance for *Hyssopus officinalis*.

Source	DF	SS	MSS	F-Value	P
Treatments	4	316.83	79.20	20.58***	0.0000
Species	4	102.40	25.60	6.65***	0.0002
Treatment X species	16	161.35	10.08	2.62***	0.0048
Error	50	192.38	3.84	-	-

\* Significant at 5% level, \*\* significant at 1% level, \*\*\* significant at 0.1% level, and <sup>NS</sup>Non-significant.

**Table 2.** Analysis of variance for *Justicia adhatoda*.

Source	DF	SS	MSS	F-Value	P
Treatments	4	210.09	52.52	10.38 ***	0.0000
Species	4	107.42	26.85	5.31***	0.0012
Treatment X species	16	434.58	27.16	5.37***	0.0000
Error	50	252.98	5.06	-	-

\* Significant at 5% level, \*\* significant at 1% level, \*\*\* significant at 0.1% level, and <sup>NS</sup>Non-significant.

**Table 3.** Analysis of variance for *Glycyrrhiza glabra*.

Source	DF	SS	MSS	F-Value	P
Treatments	4	43.64	10.91	2.64 <sup>NS</sup>	0.0447
Species	4	851.69	212.92	51.48***	0.0000
Treatment x species	16	476.24	29.76	7.19***	0.0000
Error	50	206.80	4.14	-	-

\* Significant at 5% level, \*\* significant at 1% level, \*\*\* significant at 0.1% level, and <sup>NS</sup>Non-significant.

well diffusion method. The nutrient agar medium was prepared by using 10 g/l agar technical (Oxoid UK) and 8 g/l nutrient broth (Merck Germany), at pH 7.3. 20 ml of nutrient agar medium was poured into each Petri plate of 20 x 90 mm and allowed to cool up to 45°C to solidify. 50 µl of culture was poured and evenly spread on the medium using sterile steel spreader. Five wells of 8 mm diameter were made in the agar by using a sterile cork borer. 100 µl of plant extract was loaded in each well with the help of micro-pipette. A control antibiotic disc of 30 µg chloramphenicol was also used to compare the activity of plant extract with antibiotics. The plates were incubated at 35°C in three replications in the incubator for 24 h. Zone of inhibition was measured for each extract using digital vernier caliper and the results were recorded.

## RESULTS AND DISCUSSION

The results of antibacterial activity of each extract were recorded after measuring the zone of inhibition in each replication. The data was subjected to two way analysis of variance and LSD test was applied to test the variation in treatments and species.

The antibacterial activity of the solvents was determined in pure form prior to plant extract preparation.

Ethanol and chloroform exhibited antibacterial activity in pure form; therefore the zone of inhibition was adjusted by subtracting the control solvent activity from the zone of corresponding solvent's plant extract. A control antibiotic disc of 30 µg chloramphenicol was used to compare the activity of plant extract. Chloramphenicol also inhibited the tested pathogens except *P. aeruginosa*.

Chloroform in pure form also inhibited the growth of all tested pathogens; the zones of inhibition against *E. coli*, *S. typhimurium*, *S. aureus*, *P. aeruginosa* and *Bacillus subtilis* were 12.21 ± 0, 10.11 ± 0, 13 ± 0, 10 ± 0 and 11.63 ± 0 mm respectively. The zones of inhibition of chloramphenicol against *E. coli*, *S. typhimurium*, *S. aureus* and *B. subtilis* were 26.31 ± 0, 22 ± 0, 29 ± 0 and 28.14 ± 0, respectively.

The data was subjected to two way analysis of variance. The results indicated highly significant variation between the treatments and species and interaction between the treatments x species for *H. officinalis* and *J. adhatoda* (Tables 1 and 2). Whereas the variations were non-significant between the treatments but significant for species and treatments x species for *G. glabra* (Table 3).

**Table 1a.** LSd test for variance.

Treatment	T1	T2	T3	T4	T5
Mean	14.58	12.59	10.45	8.00	8.00
Grouping	A		AB		
		BC			
			C		C

\*Mean with the same letters are not significant at 5% level.

**Table 2a.** LSd test for variance.

Treatment	T3	T1	T5	T2	T4
Mean	17.78	13.78	10.00	9.78	8.00
Grouping	A		B		
			C	C	C

\* Mean with the same letters are not significant at 5% level.

**Table 3a.** LSd test for variance.

Treatment	T4	T5	T1	T2	T3
Mean	8.30	8.20	8.10	8.10	8.00
Grouping	A	A	A	A	A

\* Mean with the same letters are not significant at 5% level.

LSD test also showed significant variations between the treatments for *H. officinalis* and *J. adhatoda* (Tables 1a and 2a). However, the variation in treatment was non-significant in *G. glabra* (Table 3a).

*J. adhatoda* exhibited maximum antibacterial activity against *B. subtilis* producing a maximum zone of inhibition (17.78 mm). This antibacterial activity against gram positive bacteria is due to the presence of flavonoids (Ahmed and Abd, 1999; Shinwari et al., 2006), alkaloids (Brantner and Chakraborty, 1998; Foster and Duke, 1990) or polysaccharides and polypeptides (Cowan, 1999). It may be possible that the extract contain certain compounds which inhibit the cell wall synthesis of bacteria. Methanolic extract of *J. adhatoda* exhibited positive antimicrobial activity for *P. aeruginosa*, *S. aureus* and *B. subtilis*. Similar results were reported by Mahato and Chaudhary (2005) with disk diffusion method.

The wide range of therapeutic properties of the root of *Glycyrrhiza* is well known (Tsukiyama, 2002). *G. glabra* exhibited antibacterial activity against spore forming of *B. subtilis*; the spore formation was inhibited to various degrees in all plant extracts except chloroform extract. Our results correspond with Mowrey (1986) that the alcoholic extracts of *Glycyrrhiza* have displayed antimicrobial activity. Murray (1985) reported the antibacterial activity due to the presence of isoflavonoid-hisplagabridin and B, 4'-O-methylglabridin, glabridin, glabrol and

3-hydroxyglabrol; whereas He et al. (2006) indicated the antibacterial activity due to pterocarpenes - glycyrrhizol A and glycyrrhizol B and Tsukiyama (2002) reported the presence of Licochalcone A.

The results of the present study correspond with the findings of Gruenwald (2000), Jankovsky and Landa (2002) that the methanolic extract of *H. officinalis* showed broad spectrum antibacterial activity against *B. subtilis* and *P. aeruginosa*. Mean zone of inhibition was more than 14.58 mm, which is due to the presence of volatile oil (Hilal et al., 1978) containing pinocamphone and isopinocamphone linalol, 1,8-cineole and limonene (Mazzanti et al., 1998), tannins, marubiin, flavonoids (glycosides of hesperidin and diosmetin) (Gruenwald, 2000). The organic extraction of plant showed greater activity than aqueous extract. Most of the antibacterial principles were either polar or non-polar and were extracted through the organic solvent medium as shown by Britto (2001). Similar results were shown by Krishna et al. (1997) and Singh and Singh, (2000). *P. aeruginosa* showed resistance to chloramphenicol which indicates the plant extract has great potential as antimicrobial compounds, especially in the treatment of infectious diseases caused by resistant microorganism (Nasir and Chanda, 2006).

The tested plant extracts have great potential as antimicrobial compounds having the potential as anti-

microbial compounds, the test plant extracts can be used in the treatment of respiratory infections caused by tested microbes. The screening of these plant extracts, as a source of new anti-infective agents confirms the knowledge of traditional herb practitioners. These findings will not only authenticate the traditional knowledge of folk healers but it will also interest pharmaceutical industries in production of cheap plant based products in third world countries.

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