

Full Length Research Paper

# Antibacterial activity of some selected plants of family Zygophyllaceae and Euphorbiaceae

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The antibacterial activity of the methanolic and n-hexane extracts of *Fagonia cretica* L., *Peganum harmala* L., *Tribulus terrestris* L., *Chrozophora tinctoria* (L.) Raf and *Ricinus communis* L., were investigated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi*. Phytochemical screening of the methanolic extract of *F. cretica*, *P. harmala* and *C. tinctoria* revealed the presence of saponins, phlobatannins, anthraquinones and alkaloids. Tannins and flavonoids were found in *C. tinctoria* and *R. communis*. Glycosides were found in *F. cretica* and *P. harmala*, while terpenoids and phenolics occurred in *P. harmala*. Group tests revealed that the methanolic and n-hexane extract of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* had OH, carboxylic and phenolic groups. The methanolic and n-hexane extracts of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* showed the absence of aldehyde and ketone group. The ability of the methanolic and n-hexane extracts of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* to inhibit the growth of different bacteria has shown their broad spectrum antibacterial potential, which may be used in the management of microbial infections.

**Key words:** Zygophyllaceae, Euphorbiaceae, antibacterial activity, extract, phytochemical screening.

## INTRODUCTION

In the past, plants provided a source of inspiration for novel drug compounds, as plant-derived medicines made large contributions to human health and well being. Their role is two-fold in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (Igbino et al., 2009). Globalization interferes with infectious disease control at the national level while microbes move freely around the world. Human response to infectious diseases is conditioned by jurisdictional boundaries (Stepanovic et al., 2003). In recent years, about 43% of the total deaths that occurred in the developing countries are due to infectious diseases. The search for new effective antimicrobial agents is necessary

due to the appearance of microbial resistance and occurrence of fatal opportunistic infections. In epidemic areas, resistance against antimicrobial agents has emerged due to recurrent infections (Carballo et al., 2002). Antibacterial activity of plants has been tested by various researchers (Tauseef et al., 2007; Chaudhry and Tariq, 2006; Saeed and Tariq, 2007). The antibacterial activity of medicinal plants of Khyber Pakhtunkhwa is needed to be done for the identification of candidate plants.

Zygophyllaceae is a family of about 25 genera and 240 species and widespread in tropical, subtropical and warm temperate, often in drier areas. In Pakistan, it is represented by 8 genera and 22 species. Plants are annual or perennial herbs. Flowers are perfect and regular; sepals imbricate or valvate, free, persistent or deciduous; petals usually free and imbricate. Disk or nectary glands are either present or absent. The ovary is superior, 2 to 5 or 10-lobed, and fruit capsule is often spiny or tuberculate. The family is important for *Lignum vitae* wood (*Guaiaecum officinale*), spices, and few ornamentals (Perveen and Qaiser, 2006). Examples of

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this family include the followings:

***Fagonia indica* var. *schweinfurthii* Hadidi in Rech. (*F. arabica* L. Edgeworth and Hook. f., *F. cretica* L. Parker):** It is an annual to biennial to glabrous shrublet. It flowers throughout the year. It is distributed in India, Pakistan, Iran, Sudan, Somalia and Kenya. It is commonly known as Azghakhi, Damiya and Dhaman in Khyber Pakhtunkhwa, Pakistan. It is used in the treatment of piles, urinary disorders, dysentery, stomach ache, typhoid, cancer and as a blood purifier (Marwat et al., 2008; Akhtar and Begum, 2009), to relieve constipation and as a laxative (Wazir et al., 2007).

***Peganum harmala* L.:** It is a perennial with corymbosely branched herb. Locally, it is called Harmal (Saraiki), Spalani (Pashto). Vern. Harmal, Ispand, Isband. It flowers in April to October and is distributed in India, Tibet, Pakistan, westwards to North Africa, Europe and Russia. The plant's seed powder is used for asthma, colic, and anthelmintic. Furthermore, the seeds are antiseptic, gastrointestinal, urinary, antispasmodic and antiperiodic. The plant is also used for healing wounds, inflamed body parts, diarrhea and in indigestion (Shah et al., 2006; Marwat, et al., 2008; Wazir et al., 2004). Plant is used in lumbago and the plants alkaloids are protozoacidal agents, coronary dilators, embolic as well as for the nervous diseases. It is known to contain harmine, which induces a fall in blood pressure, and harmaline, which possess abortifacient, narcotic, aphrodisiac, stimulant, sedative, emmanagogue, vermifuge and soporific properties (Bukhari et al., 2008). Javale and Sabnis (2010) have also reported its antibacterial activity.

***Tribulus terrestris*:** It is an annual or biennial, prostrate herb. Locally, it is known as Gokhru, Khar-e-khashak, Bhakra. It flowers throughout the year and is distributed in tropical and subtropical countries in Asia, Africa, S. Europe and North Australia. It is common throughout Pakistan from sea level to 3500 m, in sandy soils of barren lands and cultivated fields as a weed. It has highly variable species in leaf and flower size and fruit characters. The fruits are used in urinary bladder, while leaves are used in colic and chronic cough (Marwat et al., 2008; Khan, 2009). In Sindh: nutlets are used against retention and itching in urine and improving male sexual strength. It has hypoglycaemic activity and has been used in the traditional medicine of China and India for centuries. Moreover, the plant has antimicrobial, antihypertensive, diuretic properties, and stimulates spermatogenesis and libido. Presently, preparations and the dietary supplements containing extracts of *T. terrestris* are used for sexual impotence, edema, skin diseases, vermifuge, rheumatoid arthritis and others (Semerdjieva, 2011).

On the other hand, the Euphorbiaceae family has about 300 genera and 7950 species and in Pakistan, it is represented by 24 genera and 90 species. It is one of the

most diverse families, ranging from herbs, shrubs and tall trees (Perveen and Qaiser, 2005). The castor bean plant, probably indigenous of Eastern Africa is nowadays growing on a wide scale on marginal and wastelands of South Asia. It is widely grown as a commercial crop in Ethiopia for its oil. It is a non-hardy fast growing perennial shrub and is cultivated in the Indo-Pakistan region as an annual crop on marginal lands and coastal sandy belts under warm climates and it can stand moderate arid/saline environments. Its fruit is produced in typical clusters; each pod contains well developed seeds bearing sufficient oil (47 to 49%) (Chakrabarti and Ahmed, 2008). Examples include the followings:

***Chrozophora tinctoria* (L.) Raf.:** It is an annual prostrate herb common in arid soils. It is a common weed of fallow fields and dry waste places on sandy clay loam (Nasir and Ali, 1973-1995). It flowers in January to September and April to September. Plant prefers light (sandy), medium (loamy), heavy (clay), acid, neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or having no shade. It also requires moist soil (Chopra et al., 1956). It is known as 'dyer's-croton', 'giradol' or 'turnsole' and is native to Africa, (Algeria, Egypt, Libya, Morocco, Tunisia and Yemen) temperate and tropical Asia (Kuwait, Saudi Arabia, Afghanistan, Iran, Iraq, Israel, Lebanon, Syria, Turkey, Kazakhstan, Turkmenistan, India and Pakistan) and Europe (Ukraine, Albania, Bulgaria, Greece, Italy, Malta, France, former Yugoslavia, Portugal and Spain). It is found at 900 - 7500'/275 - 2300 m. In Iran, the plant is used to treat warts, emetic, cathartic and fever (Delazar et al., 2006). Root ashes are given to children for cough. The seeds are purgative or cathartic, while its bark is used for tanning and dyeing (Mathew, 1991).

***Ricinus communis*:** It is an erect, single-stemmed or much-branched shrubby or tree like somewhat glaucous herb. Locally, it is known as Arand, Erand (Hindko, Gujri), Arand (Punjabi, Urdu). Sindhi name: Haran; Urdu name: Arand, Arandae, Murpad, Harnoli, Murghpad, Bed Anjeer. It flowers throughout the year. It is native to Africa, and has been introduced and is cultivated in many tropical and subtropical areas of the world. It is found throughout India, and is being cultivated and found wild up to 2400 m (Verma et al., 2011). It is widely found at 4000'/1220 in the Sub-Himalayan tract and in the plains, and naturalized near villages. The plant is used in polyherbal formulation in Ayurveda and homeopathy (Verma et al., 2011). Leaves are used as pain killers and in bronchial pneumonia (Attaur Rehman, 1983). It is also used as expectorant, purgative, sedative and narcotic. The plant's (castor) oil is used in rheumatism, paralysis, asthma, cough and constipation. Seeds are used against warts and freckles. Seeds oil is used in constipation (Barkatullah and Hussain, 2009). Furthermore, castor oil is given in constipation before and after child birth to

mothers (Qureshi et al., 2009). The oil is a purgative, leather-preservative and a lubricant, may be used in delicate machinery; the oil-cake is used as fertilizer and fuel (Afzal et al., 2009). The oil and seeds are used for warts, cold tumors, indurations of the abdominal organs, whitlows, lacteal tumors, indurations of the mammary gland, corns and moles. Castor-oil is a cathartic and has labor-inducing properties. Ricinoleic acid has served in contraceptive jellies. Ricin, a toxic protein in the seeds, acts as a blood coagulant. The oil is used externally for dermatitis and eye ailments (Kensa and Yasmin, 2011).

## MATERIALS AND METHODS

The fresh specimens of *F. cretica* L., *P. harmala* L., *T. terrestris* L., *C. tinctoria* (L.) Raf. and *R. communis* L., were collected between June and August from Peshawar and Attock Hills. The plants were identified following the Flora of Pakistan (Ali and Qaiser, 2001). The plants were dried in shade at temperature of 28 to 30°C for 14 days.

### Test bacteria

Both Gram negative and Gram positive strains *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* were tested. Nutrient agar medium was used for the growth of bacteria for agar diffusion method (Mariam et al., 1993) and nutrient broth medium for serial dilution method (Spooner and Sykes, 1972).

### Preparation of extracts

*F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* (the whole plant) was ground to 60 mesh diameter powder using an electric grinder, and then 50 g of each sample was soaked in 250 ml 70 % methanol for 72 h. Each plant extract was filtered through Whatman filter paper No. 1823. This process was repeated 3 times. Each extract was concentrated using rotary evaporator. The concentrates of each extracts were stored at 4°C prior to use. The standard drug (imepinem) was also used (Mariam et al. 1993). The antibacterial study of the crude extracts was carried out by agar well diffusion method (Carron et al. 1987). The antibacterial activity data were subjected to T- test following Cox (1967).

### Phytochemical screening (micro-chemical tests)

The fresh specimens of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* were collected from Peshawar and Attock Hills for the screening of the chemical constituents. The collected plant samples were washed, cleaned, dried and packed in the kraft paper. Plant parts were crushed using grinding machine and powdered samples were treated onwards. Chemical tests were carried out on the aqueous, methanolic and n-hexane extracts using standard procedures to identify the constituents as described by Sofowora (1993), Trease and Evans (1989) and Harborne (1973).

### Test for alkaloids

The detection of alkaloids was carried out by the method adopted by Trease and Evans (1989). By precipitation with Dragondroff's reagent (solution of potassium bismuth iodide), the reddish brown

pinkish purple indicated the presence of alkaloids (Trease and Evans 1989). The Dragondroff's reagent was made of two portions: first, the reagent (1- 0.85 g of bismuth substrate) was dissolved in a solution of 10 ml acetic acid and 40 ml water; secondly, the reagent (2 – 8 g of potassium iodide (KI)) was dissolved in 20 ml of water (stock solution mixture of equal parts of solution 1 and 2 (Harborne, 1973). The spray reagent was prepared by mixing 1 ml of the stock solution with 2 ml of fresh acetic acid and 10 ml of water. Detection of alkaloids and other nitrogen compounds is by orange-brown spots on yellow background (Harborne, 1973).

### Test for mucilage

A small quantity of powder drug was mixed with a few drops of water and allowed to stand; if the powder was not dissolved completely and became gummy, mucilage were detected (Trease and Evans, 1989).

### Test for anthraquinone

A small quantity of powder was macerated with ether, and the presence of pink, red, or violet colour, in aqueous layer after shaking indicated the presence of anthraquinone derivatives (Trease and Evans, 1989).

### Test for saponins

About 0.2 g of powdered sample extract was boiled in 2 ml of distilled water on a water bath and filtered. A fraction of aqueous filtrate measuring 1 ml was mixed with 2 ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with about three drops of olive oil and shaken vigorously. Formation of an emulsion confirmed presence of saponins (Ngoci et al., 2011).

### Test for oils

A small quantity of powdered drug was pressed between filter papers; the appearance of an oily stain indicated the presence of fats and oils (Trease and Evans 1989).

### Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Edeoga et al., 2005).

### Test for flavonoids

Three methods were used to determine the presence of flavonoids in the plant sample. First, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Again, few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow coloration observed indicates the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow coloration observed indicates a positive

test for flavonoids (Edeoga et al., 2005).

#### **Test for steroids**

Briefly, 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H<sub>2</sub>SO<sub>4</sub>. A colour change from violet to blue or green in some samples indicates the presence of steroids (Edeoga et al., 2005).

#### **Test for terpenoids (Salkowski's test)**

Five milliliters of each extract was mixed in 2 ml of chloroform, and concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids (Edeoga et al., 2005).

#### **Test for cardiac glycosides (Keller-Kiliani test)**

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Edeoga et al., 2005).

#### **Test for reducing sugars (Fehling's test)**

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube and colour reaction was observed (Khan et al., 2011).

#### **Chemical group tests**

The fractions were tested for some of the functional groups as follows:

##### **Phenolic group test**

To test for the presence of phenolic groups, 3 to 5 drops of 1 M NaOH (aqueous) were added to 2 ml of the sample. The solubility of the sample was an indication of presence of phenolic groups (Ngoci et al., 2011).

##### **Carboxylic acid group test**

To test for the presence of carboxylic acid groups, 3 to 5 drops of 1 M NaHCO<sub>3</sub> (aq) were added to 2 ml of sample extract. Solubility and effervescence of the sample was a confirmation of a presence of carboxylic groups (Ngoci et al., 2011).

##### **Lucas test for alcohol groups**

To test for the presence of alcohol/hydroxyl groups, 3 to 5 drops of Lucas reagent were added to 2 ml of the sample. The formation of green precipitate was a confirmation of positive results. If the reaction took place very fast, tertiary alcohols were present, if moderate reaction took place, secondary alcohol were present and where reaction was unobservable, it indicated presence of primary

alcohols (Ngoci et al., 2011).

#### **Potassium permanganate test for unsaturation or hydroxyl group**

To test for the presence of double and/or triple bonds or OH groups, 3 to 5 drops of 1 M potassium permanganate was added drop wise and shaken. Decolorization of potassium permanganate was a confirmation of a positive test (Ngoci et al., 2011).

#### **Tollen's test for aldehyde and/or ketone groups**

To test for the presence of aldehydes and/or ketones, 3 to 5 drops of Tollen's reagent were added to 2 ml of the sample. The presence of silver or a black precipitate was a confirmation of a positive test (Ngoci et al., 2011).

## **RESULTS AND DISCUSSION**

The methanolic and n-hexane extracts of five plants were tested against pathogenic microbes namely *S. typhi*, *P. aeruginosa*, *E. coli*, *S. aureus* and *K. pneumoniae*. *E. coli*, is one of the most common bacteria of which virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis. *K. pneumoniae* is the causative organism of pneumonia; *S. aureus* is a wound infecting pathogen which can cause septicemia, endocarditis, and toxic shock syndrome; *P. aeruginosa* infects the pulmonary tract, urinary tract, burns and wounds; *S. typhi* it is a causative agent for typhoid.

The results of antibacterial activity of plant extracts are shown in Table 1. *F. cretica* showed highest antibacterial activity against *S. aureus* and lowest inhibition against *S. typhi*. *P. harmala* showed the highest inhibition against *S. aureus* and *K. pneumoniae*. Hayet et al. (2010) reported that the methanolic extract of *P. harmala* leaves showed strong antibacterial activity against Gram positive than Gram negative bacteria. Meanwhile, *P. harmala* showed low antibacterial activity against *E. coli* and *S. aureus* in the present study. This low activity might be due to the negligible amount of active principles that were present in the plant. Amadi et al. (2010) reported that the bio-efficacy of plant extracts is affected by the extraction method and concentration.

Furthermore, *T. terrestris* showed highest inhibition against *E. coli*. Mohanty et al. (2010) also reported similar results. The methanolic leaf extract of *T. terrestris* had alkaloids, tannins, saponins and glycosides. This agrees with Hussain et al. (2011) who reported the reducing sugar, anthraquinone, flavonoids, saponins, tannins, alkaloids and glycosides in stem, leaves and fruits of *T. terrestris*. Similar results were also observed by Kianbakht and Jahaniianian (2003) who reported that methanolic extracts (fruits, stems, leaves and roots) of *T. terrestris* growing in Iran inhibited the growth of *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa* and plant showed considerable activity against all bacteria. In

**Table 1.** Antibacterial activity of plants of family Zygophyllaceae and Euphorbiaceae.

Plant extract	Bacteria	Zone of inhibition of sample (mm)	Percentage inhibition	Mean (no. of samples = 5)
Control		33		
<b>Methanol</b>				
1. <i>Fagonia cretica</i> L	<i>Salmonella typhi</i>	25	24.2	(48.44) NS
	<i>Pseudomonas aeruginosa</i>	15	54.5	
	<i>Escherichia coli</i>	20	39.3	
	<i>Staphylococcus aureus</i>	12	63.6	
	<i>Klebsiella pneumoni</i>	13	60.6	
2. <i>Peganum harmala</i> L.	<i>Salmonella typhi</i>	15	54.5	(39.36) NS
	<i>Pseudomonas aeruginosa</i>	16	51.5	
	<i>Escherichia coli</i>	24	27.2	
	<i>Staphylococcus aureus</i>	30	9.09	
	<i>Klebsiella pneumoni</i>	15	54.5	
3. <i>Tribulus terrestris</i> L	<i>Salmonella typhi</i>	12	63.6	(50.18 6) NS
	<i>Pseudomonas aeruginosa</i>	17	48.4	
	<i>Escherichia coli</i>	10	69.6	
	<i>Staphylococcus aureus</i>	19	42.4	
	<i>Klebsiella pneumoni</i>	23	30.3	
4. <i>Chrozophora tinctoria</i> (L.) Raf	<i>Salmonella typhi</i>	15	54.5	(46.00) NS
	<i>Pseudomonas aeruginosa</i>	10	69.6	
	<i>Escherichia coli</i>	19	42.4	
	<i>Staphylococcus aureus</i>	25	24.2	
	<i>Klebsiella pneumoni</i>	20	39.3	
5. <i>Ricinus communis</i> L	<i>Salmonella typhi</i>	14	57.5	(52.06) NS
	<i>Pseudomonas aeruginosa</i>	15	54.5	
	<i>Escherichia coli</i>	16	51.5	
	<i>Staphylococcus aureus</i>	10	69.6	
	<i>Klebsiella pneumoni</i>	24	27.2	
<b>n-Hexane</b>				
6. <i>Fagonia cretica</i> L	<i>Salmonella typhi</i>	18	45.4	41.76NS
	<i>Pseudomonas aeruginosa</i>	10	69.6	
	<i>Escherichia coli</i>	23	30.3	
	<i>Staphylococcus aureus</i>	25	24.2	
	<i>Klebsiella pneumoni</i>	20	39.3	
7. <i>Peganum harmala</i> L	<i>Salmonella typhi</i>	18	45.4	(44.80) NS
	<i>Pseudomonas aeruginosa</i>	13	60.6	
	<i>Escherichia coli</i>	21	36.3	
	<i>Staphylococcus aureus</i>	29	12.1	
	<i>Klebsiella pneumoni</i>	10	69.6	
8. <i>Tribulus terrestris</i> L	<i>Salmonella typhi</i>	25	24.2	(38.70) NS
	<i>Pseudomonas aeruginosa</i>	29	12.1	
	<i>Escherichia coli</i>	30	9.09	
	<i>Staphylococcus aureus</i>	12	63.6	
	<i>Klebsiella pneumoni</i>	15	54.5	

Table 1. Contd.

	<i>Salmonella typhi</i>	18	45.4	
	<i>Pseudomonas aeruginosa</i>	24	27.2	
9. <i>Chrozophora tinctoria</i> (L.) Raf	<i>Escherichia coli</i>	30	9.09	(41.16) NS
	<i>Staphylococcus aureus</i>	10	69.6	
	<i>Klebsiella pneumoni</i>	15	54.5	
	<i>Salmonella typhi</i>	10	69.6	
	<i>Pseudomonas aeruginosa</i>	16	51.5	
10. <i>Ricinus communis</i> L	<i>Escherichia coli</i>	14	57.5	(54.50) NS
	<i>Staphylococcus aureus</i>	12	63.6	
	<i>Klebsiella pneumoni</i>	23	30.3	

NS, Non-significant.

addition, one of the uses of *T. terrestris* is in urinary infections. Since the present study showed activity against the most prevalent Gram positive bacteria in urinary infections namely *E. coli*, the use of the plant as a urinary anti-infective is validated.

On the other hand, *C. tinctoria* showed highest inhibition against *P. aeruginosa* and low antibacterial activity against *S. aureus* and *K. pneumoni*. *R. communis* showed highest inhibition against *S. aureus*. Kensa and Yasmin (2011) reported similar results. *F. cretica*, *P. harmala* and *R. communis* showed highest % inhibition of *S. aureus*. This maximum inhibition of *S. aureus* may be due to the solubility of some active principles in the methanol. Moreover, the n-hexane extracts of *F. cretica* showed highest inhibition against *P. aeruginosa*. Javale and Sabnis (2010) stated that inhibition of *K. pneumoni* might be due to the presence of capsular polysaccharide surrounding *K. pneumoni* which might have interfered with the antimicrobial activity of the leaf and fruit extract of *Emblca officinalis*. *P. harmala* showed highest inhibition against *K. pneumoni*. These findings however disagree with Takhi et al. (2011) who reported no antibacterial activity on *E. coli*, *S. aureus* and *P. aeruginosa* with alkaloidic extracts of *P. harmala*.

*T. terrestris* showed highest inhibition against *S. aureus* and these findings support the results of Hussain et al. (2009). Al-Bayati et al. (2008) and Jain et al. (2010) also reported similar results. *F. cretica* and *P. harmala* showed maximum % inhibition of *P. aeruginosa*, while *T. terrestris*, *C. tinctoria* and *R. communis* showed maximum % inhibition of *S. aureus*. The present results clearly showed that the antibacterial activity varied with the plant species and the extracts used and it agrees with Mahesh and Satish (2008). The t-test done for comparing the antibacterial activity of methanolic and n-hexane plant extracts against various microbes revealed that there was no significant difference between the two solvents used in the present study. The n-hexane extract of *F. cretica* and *P. harmala* were more inhibitory than methanolic extract. Walter et al. (2011) reported similar results. Methanolic

extract of *T. terrestris* was also more inhibitory than the n-hexane extract. This is supported by Devi et al. (2009) and Amadi et al. (2010) who found similar results. Devi et al. (2009) reviewed that methanol extraction yielded higher antimicrobial activity than n-hexane. The variation of antibacterial activity among different crude extracts of this investigation might be due to distribution of varied antimicrobial substance. Similarly, Owoseni et al. (2010) reviewed that different extracts of plants show different antimicrobial activities on an organism.

Comparing the microorganism's inhibition, the methanolic extract of *F. cretica*, *P. harmala* and *R. communis* showed highest % inhibition of *S. aureus* and this agrees with Arote et al. (2009) who found that methanolic extract of *Pongamia pinnata* was effective against *S. aureus*. The n-hexane extract of *F. cretica* and *P. harmala* showed maximum % inhibition of *P. aeruginosa*, while *T. terrestris*, *C. tinctoria* and *R. communis* were highly inhibitory against *S. aureus*. The factors responsible for this high susceptibility of the bacteria to the extracts are not exactly known, but may be attributed to the presence of secondary plant metabolites or which is soluble in solvents tested and to the structural differences in the cell envelope compositions of the Gram negative and the Gram positive bacteria (Devi, 2009). The present study showed that Gram negative bacteria were more sensitive to the tested methanolic and n-hexane extracts as compare to the Gram positive bacteria. The highest sensitivity of *S. aureus* may be due to its cell wall structure and outer membrane (Shan et al., 2007). This, however, disagree with the findings of Shan et al. (2007) who found that *S. aureus* was the most sensitive. *S. aureus* and *E. coli* were resistant in the present study; similar results were also reported by Shan et al. (2007).

### Phytochemical screening

The bio-activity of natural products is due to phytochemicals

having therapeutic, prophylactic, nutritional and anti-bacterial properties. Phytochemical screening of plant materials is thus vital in the knowledge of their therapeutic properties. They have been found to inadvertently confer anti-microbial protections to humans due to compounds synthesized in the secondary metabolism as well as being immunomodulatory (Al-Bayati and Al-Mola, 2008). Saponins have antibiotic and antimicrobial activity (Mandal et al., 2005) and this activity of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell. The methanolic extract of *F. cretica*, *P. harmala*, *C. tinctoria* revealed the presence of saponins in *T. terrestris* and *R. communis*. This agrees with Raja and Venkataraman (2011) who also reported that *T. terrestris* and *Tribulus alatus* showed the presence of saponins.

Alkaloids have analgesics, antimicrobial and antioxidant activity (Igbinosa et al., 2009; Govindappa et al., 2011). Alkaloids were present in all the plants. Raja and Venkataraman (2011) reported similar findings. Alkaloids interfere with cell division, hence the presence of alkaloids in the studied plants could account for the inhibition of bacterial species in this study. Mughal et al. (2010) and Siddiqui et al. (2009) reported similar results in Euphorbiaceae members. Tannins had antibacterial activities and they decrease the bacterial proliferation by blocking key enzymes at microbial metabolism (Mungole et al., 2010). The methanolic extract of *C. tinctoria* and *R. communis* contained tannins. Singh et al. (2010) reported similar results. Ogunwenmo et al. (2007) reviewed that water-soluble tannins are toxic to bacteria. Flavonoids have antimicrobial activity and are probably due to their ability to complex with extracellular and soluble proteins and bacteria cell wall (Njoku and Obi, 2007). Flavonoids were found in the methanolic extract of *F. cretica*. This agrees with Khalik et al. (2000) who also reported flavonoids and triterpenoids saponins in *F. cretica*. Flavonoids were found in the methanolic extract of *P. harmala*. Arshad et al. (2008) reported similar findings. Flavonoids were found in the methanolic extract of *T. terrestris* and it agrees with Rajkala et al. (2011) who reported flavonoids in the fruits of *T. terrestris*. Flavonoids were also present in *C. tinctoria* and *R. communis*. Jain et al. (2010); Jain and Nafis (2011) also reported similar findings. Tannins, saponins, alkaloids, phenols, flavonoids, oil, ricinoleic acid, ricin and monoterpenoides were found in the leaf extract of *R. communis* (Kensa and Yasmin, 2011).

Furthermore, glycosides were present in the methanolic extract of *F. cretica*, *P. harmala*. Terpenoids were found in the methanolic extract of *P. harmala*. These results agree with Siddiqui et al. (2009) who reported flavonoids in *Euphorbia hirta*, steroids and cardiac glycosides in *Jatropha gossypifolia*, tannins in *Phyllanthus niruri*. Steroids were present in the methanolic extract of *P. harmala*, *T. terrestris* and *C. tinctoria*. Amino acids were present in the methanolic extract of *F. cretica* and *P.*

*harmala*. Reducing sugars were present in the methanolic extract of *F. cretica*, *P. harmala* and *T. terrestris*. This agrees with the findings of Hussain et al. (2011) who reported the reducing sugar in stem, leaves and fruits of *T. terrestris*. Phenolics were found in the methanolic extract of *P. harmala*. Phenolics have anti-inflammatory, antioxidant, and antimutagenic and anticarcinogenic activities. As they are widespread in plant-based foods, humans consume phenolic acids on a daily basis (Stalikas 2007). In the present study chemical group tests showed that the methanolic extract of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* revealed the presence of OH, carboxylic and phenolic groups. The methanolic and n-hexane extract of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis* showed the absence of aldehyde or and ketone group.

Phytochemical screening of the n-hexane extract of all the plants showed the absence of saponins, alkaloids, tannins, phlobatannins and anthraquinones in the present study. Flavonoids were found in the n-hexane extract of *F. cretica*, *P. harmala*, *T. terrestris*, *C. tinctoria* and *R. communis*. Glycosides were present in the n-hexane extract of *F. cretica* and *T. terrestris*, while terpenoids were found in the n-hexane extract of *F. cretica*, *T. terrestris*, *C. tinctoria* and *R. communis*. Steroids were present in the n-hexane extract of *F. cretica*, *P. harmala*. Amino acids were found in the n-hexane extract of *P. harmala*, *T. terrestris*, *C. tinctoria*. Rafique and Chaudhry, (1999) stated that phytoconstituents are known to vary with ecological factors as well as with the seasonal variations.

In the present study chemical group tests revealed that the n-hexane extract of *F. cretica*, *T. terrestris*, *C. tinctoria* and *R. communis* contained OH, phenolic, carboxylic group. In the phytochemical screening, oils were found in stems and fruits of *F. cretica*, in the roots, leaves and fruits of *T. terrestris*, in roots, stems and fruits and in stems and leaves of *R. communis*. Mucilage was also present in the stems and fruits and of *F. cretica*, in the roots, leaves and fruits of *T. terrestris*. Meanwhile, it was present only in leaves of *C. tinctoria*. It was also present in the stems and leaves of *R. communis* in the present study. It is observed and not surprising that there are differences in the antibacterial effects of plant species in the present study. It is quite possible that some of the plants that showed ineffectiveness in this study could be due to less constituents, just not in sufficient concentrations so as to be effective or the active chemical constituents were not soluble in methanol or hexane (Parekh and Chanda, 2007). An interesting finding in the present study is that as far as solvent is concerned, the n-hexane extracts exhibited the maximum antibacterial activity as compared to methanol. This suggests that n-hexane is a best solvent to extract active principles from the plant material than the other solvent used.

The variations of the present results with the previous findings are not unexpected as phytoconstituents are known to vary with ecological factors (Upadhyay et al., 2010). Sometimes the composition of the chemicals change with the orientation of the plant parts (Sayal et al., 1999). The environmental factors such as rain fall or drought enhances the quality of active compounds or diminishes it (Upadhyay et al. 2010). Other factors like soil nature, temperature, (Yang et al., 2011), and length of the sunny period also counts very much (Upadhyay et al., 2010).

The present study shows that the plants possess antibacterial activity which in the extracted form can be utilized successfully to treat infectious diseases and protect the host from microbial infections. In this regard, plants used in the traditional system of medicine offer a great reservoir for the discovery of not only antimicrobial drugs but some other drugs as well.

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## REFERENCES

- Afzal S, Afzal N, Awan MR, Khan TS, Gilani A, Khanum R, Tariq S (2009). Ethno-botanical studies from Northern Pakistan. *J. Ayub. Med. Coll. Abbottabad* 21(1):52-57.
- Akhtar N, Begum S (2009). Ethnopharmacological important plants of Jalala, District Mardan, Pakistan. *Pak. J. Plant Sci.* 15(2):95-100
- Al-Bayati FA, Al-Mola HF (2008). Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. *J. Zhejiang Univ. Sci. B* 9(2):154-159.
- Ali SI, Qaiser M (2008). Flora of Pakistan. Department of Botany, University Karachi, Pakistan.
- Amadi JES, Salami O, Eze CS (2010). Antifungal properties and phytochemical analysis of the leaves and the root bark of *Parinari curatellifolia* Planch Ex Benth (Chrysobalanaceae). *Cont. J. Pharm. Sci.* 4:1-5.
- Arote SR, Dahikar SB, Yeole PG (2009). Phytochemical screening and antibacterial properties of leaves of *Pongamia pinnata* Linn. (Fabaceae) from India. *Afr. J. Biotechnol.* 8 (22):6393-6396.
- Arshad N, Neubauer C, Hasnain S, Hess M (2008). *Peganum harmala* can minimize *Escherichia coli* infection in poultry, but long term feeding may induce side effects. *Poult. Sci.* 87:240-249.
- Attaur R (1983). International Series of Monographs in Chemistry.
- Barkatullah IM, Hussain F (2009). Ethnobotanical studies of Charkotli Hills, District Bathkella Malakand, Pakistan. *Frontier Biol.* 4(4):539-548.
- Bukhari N, Choi JH, Jeon CW, Park HW, Kim WH, Khan MA, Leet SH (2008). Phytochemical studies of the alkaloids from *Peganum harmala*. *Appl. Chem.* 12 (1):101-104
- Carballo LJ, Hernandez-inda LZ, Perzer P, Gravalos MD (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *Biol. Med. Cent.* 2:1-10.
- Carron R, Maran A, Montero JM, Fernandezlago, Derminguez A (1987). *Plantes. Med. Phytother.* 21:195.
- Chakrabarti MH, Ahmad R (2008). Transesterification studies on castor oil as a first step towards its use in biodiesel production. *Pak. J. Bot.* 40:1153-1157.
- Chaudhry NMA, Tariq P (2006). Anti-microbial activity of *Cinnamomum cassia* against diverse microbial flora with its nutritional and medicinal impacts. *Pak. J. Bot.* 38(1):169-174
- Chopra RN, Nayar SL, Chopra IC (1956). Glossary of Indian Medicinal Plants, CSIR, New Delhi, India, p. 100.
- Cox GW (1967). Laboratory Manual of General Ecology. Wm. C. Brown Co. Pub- Iowa.
- Delazar A, Talischi B, Nazemiyeh H, Rezazadeh H, Nahar L, Sarker SD (2006). Chrozophorin: a new acylated flavone glucoside from *Chrozophora tinctoria* (Euphorbiaceae) *Rev. Bras. Farmacogn.* 16(3):286-290
- Devi K, Thirumaran G, Arumugam R, Anantharaman P (2009). Antibacterial activity of selected medicinal plants from Parangipettai coastal regions; Southeast coast of India. *World Appl. Sci. J.* 7(9):1212-1215.
- Govindappa M, Sadananda TS, Channabasava R, Jeevitha MK, Pooja KS, Raghavendra VB (2011). Regular article antimicrobial, antioxidant activity and phytochemical screening of *Tecoma stans* (L.) Juss. ex Kunth. *J. Phytol.* 3(3):68-76.
- Harborne JB (1973). Phytochemical methods, Chapman and Hall Ltd., London, pp. 49-188.
- Hayet E, Maha M, Mata M, Mighri Z, Laurent G, Mahjoub A (2010). Biological activities of *Peganum harmala* leaves. *Afr. J. Biotechnol.* 9(48):8199-8205.
- Hussain AA, Mohammed AA, Ibrahim HH, Abbas AH (2009). Study the biological activities of *Terrestris terrestris* extracts. *World Acad. Sci. Eng. Technol.* 57:433-435.
- Hussain I, Khan FA, Khan H, Shafiq ur R, Badrullah H (2011). Antimicrobial, phytochemical and fluorescence studies on *Tribulus terrestris*. *J. Pharm. Res.* 4(5):1556-1558.
- Igbinosa OO, Igbinosa O, Aiyegoro OA (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr. J. Pharm. Pharmacol.* 3(2):58-62.
- Jain P, Bansal D, Bhasin PA (2010). Antimicrobial activity and phytochemical screening of five wild plants against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. *J. Pharm. Res.* 3(6): 1260-1262.
- Jain P, Nafis G (2011). Antifungal activity and phytochemical analysis of aqueous extracts of *Ricinus communis* and *Punica granatum*. *J. Pharm. Res.* 4(1):128-129.
- Javale P, Sabnis S (2010). Antimicrobial properties and phytochemical analysis of *Embliba officinalis*. *Asian J. Exp. Biol. Sci.* pp. 91-95.
- Kensa VM, Yasmin S (2011). Phytochemical screening and antibacterial activity on *Ricinus communis* L. *Plant Sci. Feed* 1(9):167-173.
- Khalik SMA, Miyase T, El-Ashaal HA, Melek FR (2000). Triterpenoid saponins from *Fagonia cretica*. *Phytochemistry* 4(8):853-859.
- Khan FA, Hussain I, Farooq S, Ahmad M, Arif M, Inayat Ur R (2011). Phytochemical screening of some Pakistanian medicinal plants. *Middle-East J. Sci. Res.* 8(3):575-578.
- Khan FM (2009). Ethno-veterinary medicinal usage of flora of greater Cholistan Desert (Pakistan). *Pak. Vet. J.* 29(2):75-80
- Kianbakht S, Jahaniyani F (2003). Evaluation of antibacterial activity of *Tribulus terrestris* L. growing in Iran. *Iran. J. Pharm. Therap.* 2(1):22-24.
- Mahesh B, Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agric. Sci.* 4(S):839-843.
- Mahesh B, Satish S (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agri. Sci.* 4(S):839-843.
- Mandal P, Babu SPS, Mandal NC (2005). Antimicrobial activity of saponins from *Acacia auriculiformis*. *Fitoterapia* 76(5):462-465.
- Mariam TG, Murthy PN, Ranganathan P, Hymete A, Daka K (1993). Antimicrobial screening of *Rumex abyssiricus* and *Rumex nervosus*. *East. Pharm.* 36(33):131-133.
- Marwat SK, Khan MA, Ahmad M, Zafar M, Rehman FU (2008). Ethnophytomedicines for treatment of various diseases in D. I. Khan District. *Sarhad J. Agric.* 24(2):305-315.
- Mathew KM (1991). The useful plants of India. Publ. inf. Directorate Counc. Sci. Ind. Res. pp. 1-10.
- Mohanty A, Das C, Dash S, Sahoo DC (2010). Physico-chemical and antimicrobial study of polyherbal formulation. *Glob. Pharm.* 4:1-3.



- Mughal T, Mamona A, Saddique Z, Qureshi S, Mehboob S (2010). Phytochemical and pharmacognostical evaluation of Euphorbiaceae species from Lahore region, Pakistan. *J. Appl. Pharm.*, 3(2): 79-85.
- Mungole AJ, Awati R, Chaturvedi A, Zanwar P (2010). Preliminary phytochemical screening of *Ipomoea obscura* (L.) a hepatoprotective medicinal plant. *Int. J. Pharm. Technol. Res.* 2(4):2307-2312.
- Nasir E, Ali SI (1973-1995). *Flora of West Pakistan*. NARC Islamabad 4:2-55
- Ngoci SN, Mwendia CM, Mwaniki CG (2011). Phytochemical and cytotoxicity testing of *ndigofera lupatana* Baker F. *J. Anim. Plant Sci.* 11(1):1364-1373.
- Njoku VO, Obi C (2007). Phytochemical constituents of some selected medicinal plants. *J. Pure Appl. Chem.* 3(11):228-233.
- Ogunwenmo KO, Idowu OA, Innocent C, Esan EB, Oyelana OA (2007). Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. *Afr. J. Biotechnol.* 6(20):2400-2405.
- Owoseni AA, Ayanbamiji TA, Yejide O, Ewegbenro IB (2010). Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridelia ferruginea*. *Afr. J. Biotechnol.* 9(7):1031-1036.
- Perveen A, Qaiser M (2005). Pollen flora of Pakistan-XLVII. Euphorbiaceae. *Pak. J. Bot.* 37(4):785-796.
- Qureshi R, Waheed A, Arshad M, Umbreen T (2009). Medicobotanical inventory of tehsil Chakwal, Pakistan. *Pak. J. Bot.* 41(2):529-538.
- Rafique M, Chaudry FM (1999). Seasonal variation in the composition of essential oil of *Eucalyptus camaldulensis* from Pakistan. *Pak. J. Sci. Ind. Res.* 42(8):282-285.
- Raja M, Venkataraman R (2011). Pharmacognostical studies on *Tribulus terrestris* and *Tribulus alatus*. *Pharm. Sin.* 2(4):136-139.
- Rajkala R, Thakar AB, Shukla VJ, Harisha CR (2011). A preliminary physio-chemical assay of Gokshura-Punarnava Basti - a pilot study. *Int. J. Res. Ayurveda Pharm.* 2(2):343-349.
- Saeed S, Tariq P (2007). Antibacterial activity of Oregano (*Origanum vulgare* Linn) against gram positive bacteria. *Pak. J. Pharm. Sci.* 22(4):421-424.
- Semerdjieva I (2011). Studies on leaf anatomy of *Tribulus terrestris* L. (Zygophyllaceae) in populations from the Thracian floristic region. *Biotech. Biotechnol.* 25(2):2373-2378.
- Shah SRU, Hassan G, Rehman A, Imtiaz I (2006). Ethnobotanical studies of the Flora of district Musakhel and Barkhan in Balochistan, Pakistan. *Pak. J. Weed Sci. Res.* 12(3):199-211.
- Shan B, Cai YZ, Brooks JD, Corke H (2007). The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *Int. J. Food Microbiol.* 17:112-119.
- Siddiqui S, Verma A, Rather AA, Jabeen AA, Meghvansi MK (2009). Preliminary phytochemical analysis of some important medicinal and aromatic plants. *Advan. Biol. Res.* 3(5-6):188-195.
- Singh B, Chopra A, Ishar MPS, Sharma A, Raj T (2010). Pharmacognostic and antifungal investigations of *Elaeocarpus ganitrus* (*Rudrakasha*). *Indian J. Pharm. Sci.*, 72( 2): 261-265.
- Sofowora A (1993). *Medicinal Plants and Traditional Medicines in Africa*. Chichester John Wiley & Sons New York, pp 97- 145.
- Spooner DF, Sykes GJ (1972). *Methods in microbiology*. Academic Press, London. P. 69.
- Stalikas CD (2007). Review extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci.*, 30: 3268 – 3295.
- Stepanovic, S, Antic, N, Dakic I, Svabic-Vlahovic. M (2003). *In vitro* antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. *Microb. Res.* 158(4):353-357.
- Takhi D, Quinten M, Yousfi M (2011). Study of antimicrobial activity of secondary metabolites extracted from spontaneous plants from the area of Laghouat, Algeria. *Advances Environ. Biol.* 5(2):469-476.
- Tauseef S, Bano S, Khan A, Kamal K, Sohail M, Amyun A, Naz A, Siddiqi H, Lubna A, Tahir SF, Faizi S, Ahmad A (2007). Studies on antimicrobial activity of extracts and fractions of *Tagetes erecta*. *Proc. 6<sup>th</sup> Int. Conf. Pak. Soc. Microbiol.* pp. 3-9.
- Trease GE, Evans WC (1989). *Pharmacognosy*. 13<sup>th</sup> ed., english language book, society, Baillere Tindall, Oxford Univ. Press. P. 546.
- Upadhyay B, Dhaker AK, Singh KP, Kumar A (2010). Phytochemical analysis and influence of edaphic factors on lawsone content of *Lawsonia inermis* L. *J. Phytol.* 2(6):47-54.
- Verma SC, Rani R, Pant P, Padhi MM, Jain CL, Babu R (2011). Quantitative determination of ferulic Acid in *Ricinus communis* Linn. leaves and its geographical variation using HPTLC C Fingerprint. *Der Chem. Sin.* 2(5):127-135.
- Walter A, Samuel W, Peter A, Joseph O (2011). Antibacterial activity of *Moringa oleifera* and *Moringa stenopetala* methanol and n-hexane seed extracts on bacteria implicated in water borne diseases. *Afr. J. Microbiol. Res.* 5(2):153-157.
- Wazir SM, Dasti AA, Shah J (2004). Common medicinal plants of Chapursan Valley, Gojal II, Gilgit, Pakistan. *J. Res. Sci., BZU, Multan*, 15:41-47.
- Wazir SM, Saima S, Dasti AA, Subhan M (2007). Ethnobotanical importance of salt range species of district Karak, Pakistan. *Pak. J. Plant Sci.* 13(1):29-31.