REVIEW

Ethnomedicinal, phytochemical and antibacterial activities of medicinal flora of Pakistan used against *Pseudomonas aeruginosa*-A Review

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Abstract: Medicinal plants have been used from ancient time against different infectious diseases caused by microorganisms across the globe. The present review represents different medicinal plants of Pakistan used traditionally for the treatment of variety of ailments caused by *Pseudomonas aeruginosa*, their *in-vitro* activities against *P. aeruginosa* and phytochemistry. These plants were extracted with different solvents that showed good *in-vitro* activities against *P. aeruginosa*, due to the presence of active phytoconstituents including alkaloids, terpenoids etc. Among all the solvents used for extraction process, alcoholic extracts were mostly preferred in Pakistan. However, non-alcoholic solvents like ethyl acetate and chloroform also showed good anti-*P. aeruginosa* activities. Statistically, increase in concentration (mg/ml) of ethyl acetate and chloroform extracts significantly increase ($p=0.000$ and $p= 0.046$) inhibitory activity against *P. aeruginosa.* This review provides scientific validation of the traditional knowledge in using medicinal plants for the treatment of different diseases caused by this bacterium. Reported Pakistani medicinal plants contain variety of phytochemical compounds that could be very useful in the production of new drugs with fewer side effects on living system compared to some allopathic drugs. This review also provides baseline information for future research studies on the phytochemistry of unexplored plants. Further research studies should be carried out on non-alcoholic extracts that could be helpful in the extraction new compounds, which could lead to the development of some novel drugs in the pharmaceutical industries of Pakistan.

Keywords: Medicinal plants, traditional medicines, phytochemistry, *in vitro* activities, *Pseudomonas aeruginosa.*

INTRODUCTION

Medicinal plants have been traditionally used for the treatment of different ailments from the ancient times. These medicinal plants become an important source of medicines after various observations and experimentations (Marwat *et al*., 2008). Different methods like biological screening, isolation as well as clinical trials for a variety of plants have been developed in last decades, in order to reveal the secrets of ancient herbal remedies (Bibi *et al.,* 2011). Thus medicinal plants are considered to be a source for drugs because of its traditional practice, which facilitates the discovery of new drugs (Budovsky and Fraifeld, 2012). Approximately, 61% of new drugs developed between 1981 and 2002 are from natural products (Cragg and Newman, 2005). Globally 422,000 flowering plants found and more than 50,000 are used for medicinal purposes while 33% contribution in drug production worldwide (Kadir *et al.,* 2015). According to World Health Organization more than 80% of individuals from developing countries rely on the medicinal plants for their primary health care (Bashir *et al.,* 2012). In early 1950s, 84% of Pakistani population was dependent on local medicines (Hocking, 1958) but now such practices are limited to remote rural areas due to modernization (Ibrar *et al.* 2007; Mahmood *et al*., 2011).

Drugs derived from medicinal plants have curative potential and have a significant role in the treatment of diseases caused by microorganisms (Kubmarawa *et al.,* 2007). Different bacteria are responsible for causing serious infectious diseases in humans. These bacteria include gram positive and gram negative like *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* etc. *Pseudomonas aeruginosa* is gram-negative type of bacterium and can cause severe nosocomial infections like pneumonia, urinary and respiratory infections because of its multiple environmental habitats and ability to survive in harsh conditions (Bibi *et al.,* 2011; Levin *et al.,* 1999). It is

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scientifically proved that the antibiotics are an important weapon in fighting bacterial infections. However in last few decades antibiotics showed less activity due to the emergence of drug resistant bacteria (Mateen, 2011). The emergence of drug resistant *P. aeruginosa* causing infections in humans is a growing problem all over the world. According to Sader *et al.* (1993), *P. aeruginosa* showed resistivity to commercially available antibiotics like penicillins, imipenem and monobactams. Due to the challenge of growing incidences of drug-resistant pathogens, medicinal plants containing active compounds (phenolic compounds, resins, acidic compounds, tannin, sterol, fats and lipids) have the ability to overcome such resistivity with fewer side effects compared to antibiotics (Kaufman *et al.,* 1999; Ali *et al*., 2012). Furthermore, the antibiotics are costly and are non affordable for the individuals belonging to developing areas (Walsh and Amyes, 2004). This might be the reason that people in developing countries prefer traditional plant medicines for ailments (Dubey *et al*., 2004).

Microbial resistance to antibiotics is a global health related issue and hence, actions are required to overcome this problem. The aim of this review is to compile the available fragmented literature on Pakistani medicinal plants used against *P. aeruginosa* and suggest measures on newer and safer herbal drugs for the diseases caused by this bacterium. This review will provide ethnomedicinal knowledge and active phytochemical of those Pakistani medicinal plants having antibacterial potential. Thus, this review will enable the pharmacists to carry forward the knowledge towards the identification of effective antimicrobial drugs against gastrointestinal, respiratory, skin and urinary diseases caused by *P. aeruginosa*.

MATERIALS AND METHODS

Literature selection

Research papers on the antibacterial activity of medicinal flora of Pakistan were searched on three online bibliographic databases, Google Scholar, ISI Web of Knowledge and Science Direct Navigator, as well as libraries sources. A huge published and unpublished literature was found, in which medicinal plants of Pakistan were chemically extracted by different solvents like methanol, ethanol, ethyl acetate, n-hexane and chloroform for *in-vitro* screening. Approximately, 124 species were found, whose different parts were extracted and used *in-vitro* against *P. aeruginosa.* We only focused those plant species, whose extract concentrations were mentioned quantitatively i.e. milligrams of the extracts dissolved in milliliters of the respective solvents (methanol, ethanol, ethyl acetate, n-hexane, chloroform and aqueous) or in dimethyl sulfoxide (DMSO) solvent. Thus, as a whole 36 medicinal plants were selected for the present study. This review is not only limited to antibacterial activity against *P. aeruginosa*, but it also contains ethno botanical and phytochemical aspects of the selected medicinal plants. The ethno botanical data was selected from Pakistani literature while the phytochemical data was mostly from foreign literature.

Data organization and statistical analysis

This review consists of two tables and three main figs. table 1 was consisted data on ethnobatanical uses and phytochemical constituents of the selected medicinal plants of Pakistan. table 2 contains data on the antibacterial activity of selected plant species against *P. aeruginosa* in Pakistan. Data on the concentrations of plant extracts and zone of inhibition of *P. aeruginosa* were uniformed through conversion of other units into mg/ml and mm, respectively. fig. 1 shows total number of medicinal plants traditionally used against the *P. aeroginosa* in Pakistan in comparison to the selected species for review. This fig. justifies the selection criteria of species for this review article. For instance, data in the fig. reveals that methanolic extraction has been used for the majority of medicinal plants against *P. aeruginosa.* However, only those plant species were included in this review, whose concentrations were mentioned (fig. 1). fig. 2 shows graphical representations of the data; where inhibition zones of *P. aeruginosa* and extracts concentrations were Bivariately correlated with Pearson correlation coefficients for two tailed significances. fig. 3 presents structures of three most active phytoconstituents isolated from the selected plant species. These structures were drawn with the help of Chemdraw software.

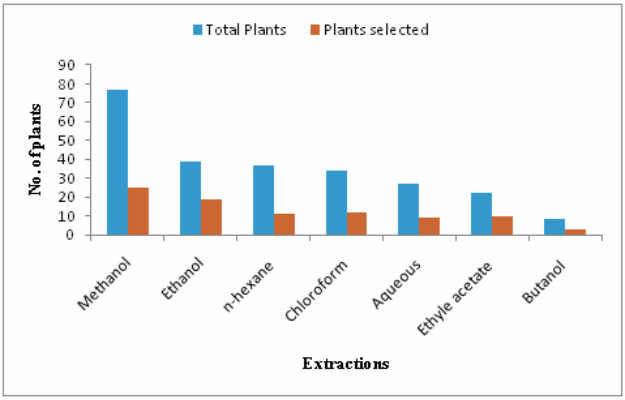

Fig. 1: Number of medicinal flora of Pakistan extracted with different solvents.

Medicinal plants extraction

Extraction is the process of separating active metabolites of medicinal plants by using solvents through standard procedures. General techniques for medicinal plant extractions in Pakistan include Maceration, Infusion, Percolation, Decoction and Soxhlet. Of these, Soxhlet and Maceration techniques are mostly used (Irshad *et al.,* 2011; Hussain *et al.,* 2010). Soxhlet is the hot continuous extraction technique while in Maceration, powdered plant drug is mixed with the solvent in a container for a defined period with frequent agitation until the soluble matter

dissolved (Ncube *et al.,* 2008). Most important solvents used throughout the Pakistan for medicinal plant extractions are discussed below.

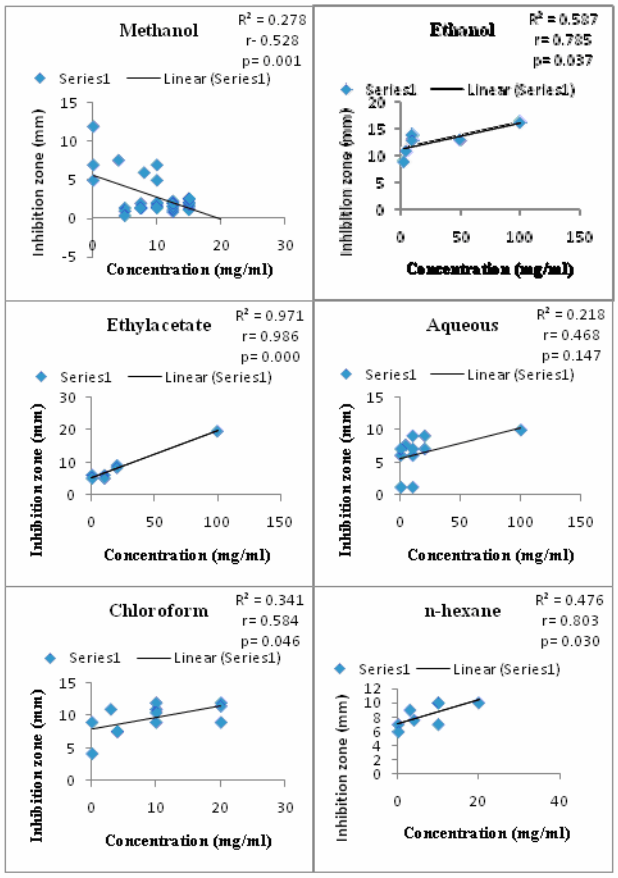

Fig. 2: Relationship between concentrations of plants extracts dissolved in DMSO and their respective solvents and inhibition zone of *P. aeruginosa*.

DISCUSSION

Methanolic and ethanolic extractions

Methanol and ethanol are organic compounds belonging to alcoholic groups. These colorless simple liquids are used all over the world for the extractions of medicinal plants (Aliero and Afolayan, 2006). Likewise other countries of the world, these solvents are also preferred in Pakistan for medicinal plants extraction. Out of 124 medicinal plants, 75 were extracted with methanol and 40 with ethanol for *in-vitro* screening against *P. aeruginosa* (fig. 1). Their increased preference is reflecting their higher antibacterial activities might be due to presence of active phytochemicals extracted by these solvents. However, the antibacterial activity of methanolic and ethanolic extracts on dissolving in the DMSO solvent, are not that satisfactory. For instance the methanolic extract of a medicinal plant at 10mg/ml in methanol showed 24.33mm inhibition against *P. aeruginosa*. On the other side, similar concentration of another medicinal plant showed only 2mm inhibition against the same bacterium when dissolved in DMSO solvent (table 2). We also statistically proved that a significant decrease (R^2=0.28; p<0.001) occur in zone of inhibition against *P. aeruginosa* by increasing the concentration of methanolic extract in DMSO solvent. Furthermore, we statistically proved a significant increase in the inhibition zone of the studied bacterium by increasing the extract concentration (mg/ml) of ethanol at R^2=0.59; p=0.037 in their respective solvent (fig. 2).

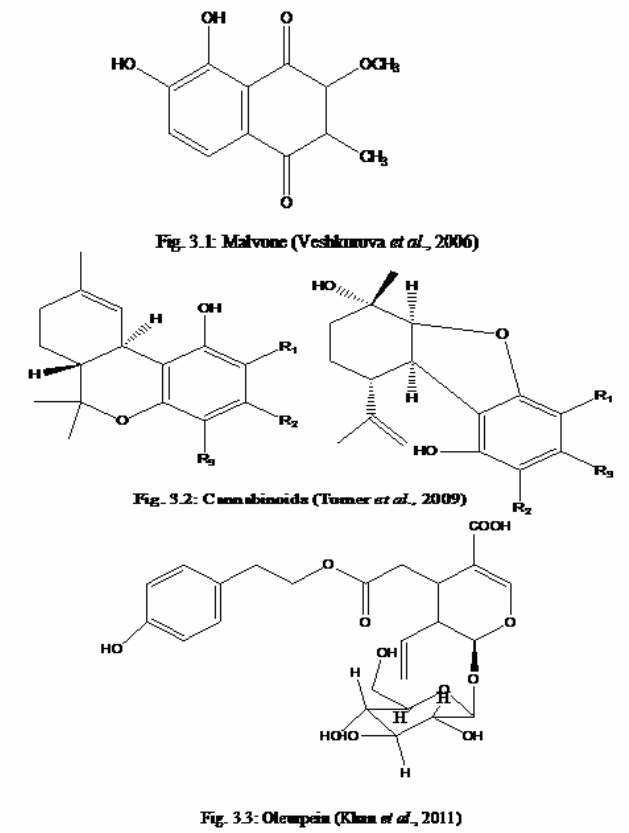

Fig. 3: Structure of chemical compounds isolated from medicinal plants.

Chloroform and n-hexane extractions

Chloroform and n-hexane both being organic solvents are used for the extraction of medicinal plants all over the world (Mateen *et al*., 2011). Chloroform is a colorless and sweet smelling compound also known as trihalomethane, while n-hexane is carbon and hydrogen containing compound. Medicinal flora of Pakistan showed good antibacterial activity against *P. aeruginosa* when extracted with chloroform and n-hexane in both DMSO and in their respective solvents (table 2). Out of 124 plants, only 37 and 38 Pakistani medicinal plants were extracted with chloroform and n-hexane, respectively. fig. 2 contains Pearson correlation between zone of inhibition of *P. aeruginosa* and concentration of plants extracted with chloroform and n-hexane. It is obvious from the graph that increase in the concentration (mg/ml) of chloroform and n-hexane, significantly increase the zone of inhibition against *P. aeruginosa* at R^2=0.34; p=0.046 and R^2 =0.48; p=0.030, respectively (fig. 2).

Table 1: Reported Ethnobotany and phytochemistry of medicinal plants of Pakistan in literature.

Plant name/ Family name	Area	Part used	Ethnobotany	Mode of preparation	Known Phytoconstituents	Citations
Althaea officinalis L./ Malvaceae	Rawalpind, Muzaffar abad	Flowers, leaves, roots, fruits and seeds.	Demulcent, used for burns, snake bite, asthma, bronchitis pneumonia, cough, cold. Seeds are useful in kidney and bladder problems.	Leaves, flowers and roots are applied in burns and soften the skin. Plant boiled in wine or milk, relieve diseases of the chest, coughs, bronchitis, whooping-cough.	n-hexacos-2-enyl-1,5-olide (altheahexacosanyl lactone), 2β-hydroxycalamene (altheacalamene), 5,6-dihydroxycoumarin-5-dodecanoate-6β-D-glucopyranoside (altheacoumarin glucoside), lauric acid, β-sitosterol, lanosterol, dihydrokaempferol 4'-O-glucoside, Tiliroside and Hypolaetin 8-0-gentiobioside	Hussain *et al.,* 2008; Shahzeb *et al.,* 2013; J. Gudej and Bleganowska, 1990; Rani *et al.,* 2010.
Calandula arvensis L./Asteraceae	Cherat, Mardan, Malakand, Kohat	Leaves	Disinfectant, antispasmodic, diuretic, wounds healing.	The decoction of the flower heads has been used for treating burns. Their crushed leaves are topically applied on wounds.	Sesquiterpene Glycosides, triterpene saponin arvensoside A, B, C, Calandulasoids, 28-O-β-D-glucopyranoside-3-β-O-(O-β-D-galactopyranosyl (1→3)-β-D-glucopyranoside.3-β-O-(O-β-D-galactopyranosyl (1→3)-β-D-glucopyranoside.	Babadjamiay *et al.,* 1987; Ahmad *et al.,*1993; Arora *et al.,*2013.
Calotropis procera (Aiton) Dryand/ Apocynaceae	Kohat	Stem, leaf, latex	Dermatitis, antiseptic, wounds, expectorant, anthelmentic, cholera, asthma, earache, gastrointestinal diseases.	Cut into portions to secrete the milky juice, which is used for curing dermatitis. Stem latex is used in earache and asthma. Infusion of leaves used for stomach problems. Poultice on dog bitten wounds and ringworm	alkaloids, steroids, triterpenoids,	Adnan *et al.,* 2014; Murad *et al.,* 2013; Haq *et al.,*2011; Hassan *et al.,*2006.
Cannabis sativa L./ Cannabaceae	Cherat, Mardan, Malakand, Kohat .	Leaves, Flowering	Narcotic, Sedative, intoxication, diuretic, laxative, throat soreness, astrointestinal, antiseptic, aches, urinogenital diseases.	Flowering tops are given in the case of stomachache. Leaves mixed with 50 to 60 grams of sugar and one glass of water reduce general body inflammation, intoxication and to improve the appetite of an addictive person. Boiled leaves and seeds in water used dermally for painfull condition.	Anhydrocannabisativine Cannabisativine cannabinoids N-(p-hydroxy-β-phenylethyl)-p-hdroxy-(trans)-cinnamide	Hayat *et al.,* 2008; Imtiaz *et al.,* 2013; Turner *et al.,*1980 ElSohly and Slade, 2005; Adnan *et al.,*2013.
Cichorium Intybus L./ Compositae (Asteraceae)	Sawabi, Gawadar.	Whole plant	Vomiting, Diarrhea, acne.	Whole plant is used for diarrhea and bilious attacks. A decoction from the seeds is used in obstructed menstruation and for checking bilious vomiting.	[lup-12,20(29)-dien-3β-ol-3β-L-arabinofuranosyl-2'-hexadecanoate] [lup-12,20(29)-dien-3β-olyl hexadecanoate][4β-(pent-2-enylolactone)-hexatriacontane]	Kumari *et al.,* 2012; Hussain *et al.,*2008; Malik *et al.,*2011.
Convolvulus arvensis L./ *Convolvulaceae*		Whole plant	Anthelminthic, control dandruff, Emollient & stomach Disorders, Constipation	Grind fresh plant into a paste. Add sugar and water to the paste. Filter the mixture and use the filtrate to treat constipation. Plant paste is also applied to hairs to treat dandruff.	NA	Hayat *et al.,* 2008; Imtiaz *et al.,*2013.

Plant name/ Family name	Area	Part used	Ethnobotany	Mode of preparation	Known Phytoconstituents	Citations
Cordia dichotoma G. Forst. (=*Cordia latifolia* Roxb.)/ Boraginaceae	Abottabad	Flower,Fruit	Cough and flu	Decoction is used	NA	Walter *et al.*, 2011
Delonix regia (Hook.) Raf./ Leguminosae		Stem, bark, leaves	Abdominal pains, bronchitis and pneumonia.	Root decoction is used for abdominal pains. The leaf extract was used as anti-inflammatory. Bark is used for bronchitis and pneumonia.	lupeol, epilupeol, b -sitosterol, stigmasterol, aromatic compound p -methoxybenzaldehyde	Jahan *et al.*,2010; Sung and Fowden, 1969.
Dodonaea viscose Jacq./ Sapindaceae	Cherat, Mardan, Malakand, Kohat	Leaves, stem, root	Rheumatism and burns	Grind the leaves and add small amount of water to make fine paste for dermal use.	Tannins, Saponins, Flavanoids and Terpenoids	Prakash *et al.*, 2012, Adnan *et al.*, 2014
Elaeagnus angustifolia L./ Eleagnaceae	Bannu	Whole plant	Cough, cold, dyspepsia, constipation, asthma.	Fruit and leaf latex is used for treating patients of dyspepsia, constipation and cough. Fruit is dried and powdered for treating asthma. Its fruit is used for cough and cold as a ingredient of 'Joshanda'.	Phenolic compounds, rutin, gallic acid, hydroxy cinnamic acids, hydroxybenzoic acids and chlorogenic acid	Sabir *et al.*,2007; Ahmad *et al.*,2006;
Ephedra gerardiana Wall. Ex Stapf./ Ephedraceae	Baluchistan	Whole plant	Asthma.	It is a source of fuel wood, soil binder and ephedrine extracted from it is used as a nasal drops. It is mixed with tobacco for preparation of quality of Niswar. It is also used for treatment of asthma.	Ephedrine.	Afzal *et al.*,2009; Adnan *et al.*,2014
Eucalyptus cammaldulensis Dehnh./ Myrtaceae	Cherat, Mardan, Malakand, Kohat.	Leaves	Common cold, nose infections.	NA	Ellagitannins, flavonoids, phloroglucinol derivatives and galloyl esters.	Sultana *et al.*,2006, Singab *et al.*,2011
Ficus carica L./ Moraceae	Cherat, Mardan, Malakand, Kohat.	Leaves	Wound healing, respiratory, gastro intestinal, urinary, demulcent, laxative, constipation, flatulence, measles and dysentery.	Ash of leaves is sprayed on the wounds dermally. Decoction is used for intestinal problems. Fresh fruit used in anemia, respiratory diseases and constipation. Latex obtained from the stem and leaves for checking bleeding.	Steroids, triterpenoids, cumarines, flavanoids, glycoside, carbhydrates, phenolic compounds, resins, acidic compounds, tannin, sterol, fats and lipids.	Ali *et al* 2012; Adnan *et al.*,2014; Hussain *et al.*,2008; Jeong *et al.*,2009.
Geranium wallichanum D. Don./Genraniaceae	Abbottabad	Rhizome Leaves	Dysentry, mouth ulceration and leucorrhoea	The roots are used in mouth ulceration. An efficient astringent which is given for chronic dysentery, diarrhea and leucorrhoea.	β-sitosterol , β-sitosterol-glactoside , stigmasterol, 2,4,6-trihydroxyethylbenzoate	Ali *et al.*,2011; Ismail *et al.*,2012
Glycyrrhiza glabra L./ Papilionaceae	Peshawer	Leaves	Respiratory problems, coughing	The whole plant is boiled in water and the decoction is used for cough and chest problems.	Glycyrrhizin	Varsha and Agrawal, 2013; Tareen *et al.*, 2010.

Plant name/ Family name	Area	Part used	Ethnobotany	Mode of preparation	Known Phytoconstituents	Citations
Hyssopus officinalis L./ Lamiaceae	Azad Kashmir	Leaves	Cough reliever, other respiratory and stomach problems	Decoction is used as an expectorant and stomach tonic and upper respiratory tract infections, coughs in children, etc.	a-Glucosidase inhibitors. quercetin 7-O-β-D-apiofuranosyl-(1→2)-β-D-xylopyranoside and quercetin 7-O-β-D-apiofuranosyl-(1→2)-β-D-xylopyranoside3'-O-β-D-glucopyranoside	Wang and Yang, 2010; Walter *et al.*, 2011.
Justicia adhatoda L./ Acanthaceae	Margalla Hills	Whole plant	Rheumatism, Stomachache, ear ailments, antiseptic, asthma, cough, bronchitis, antispasmodic and expectorant.	Grind the leaves and mix it with honey, paste is used for swelling. The roots are useful in bronchitis and other chronic coughs. Leaves are very effective in the treatment of asthma and rheumatism.	Alkaloids	Corrêa and Alcântara, 2011; Adnan *et al.*, 2013; Haq *et al.*, 2011.
Malva sylvestris L./ Malvaceae	Rawal Dam (Islamabad), Dir, Punjab.	Leaves, roots and flowers	Soothe mucous membrane inflammations, skin disorders, chronic bronchitis, jaundice and urinary disorders.	The whole plant is boiled in water and the decoction is used in jaundice.	8-hydroxyflavonoid glucuronides ,malvone A (2-methyl-3-methoxy-5,6-dihydroxy-1,4-naphthoquinone), Terpanoids	Walter *et al.*, 2011; Jan *et al.*, 2009; Billeter *et al.*,1991. Veshkurova *et al.*, 2006.
Mentha longifolia L./ Labiatae	Kohat	Leaves	Carminative, diarrhea, dysentery and stomachache.	Leaf extract used against vomiting and dysentery. Leaf in dry powdered form used in asthma and as condiment.	Longifone, (longiside-A and -B) flavanone-glycoside (longitin). tricetin 7-O-methylether 3'-O-glucoside 5'-O-rhamnoside. tricetin 3'-O-glucoside 5'-O-rhamnoside. tricetin 3'-O-rhamnosyl- 1→4 – rhamnoside.	Sharaf *et al.*, 1999; Ali *et al.*,2012; Haq *et al.*,2011; Ali *et al.*,2011.
Morus alba L./ Moraceae	Punjab	Whole Plant	Liver problems, tonsils, purgative, anthelmentic, sore throat, dyspepsia and constipation treatment.	Fruits are laxative and relieve constipation, refrigerant in fever and used as a remedy for sore throat, dyspepsia. Bark is dried, crushed in to powder, mixed with milk and gur, after boiling filter through a cloth, used as purgative and anthelmentic.	NA	Imtiaz *et al.*, 2013; Jan *et al.*, 2008.
Morus nigra L./ Moraceae	Punjab	Fruit, leaves, Root	Sore throat, cough, stomach problem, carminative and blood purifier.	NA	NA	Imtiaz *et al.*, 2013
Olea europaea L./Oleaceae	Cherat, Mardan, Malakand, Kohat	Leaves, fruits	diuretic, emollient, laxative, febrifuge, skin cleanser, urinary infections, gallstones, asthma and diarrhea	Decoctions of the dried fruit and of dried leaf are taken orally for diarrhea and to treat respiratory and urinary tract infections.	Oleuropein, Hydroxytyrosol, Leteoline-7-glucoside	Benavente *et al.*, 2000; Khan *et al.*, 2007

Plant name/ Family name	Area	Part used	Ethnobotany	Mode of preparation	Known Phytoconstituents	Citations
Onosma bracteata Wall. (=Onosma bracteatum Wall.)/ Boraginaceae	Naran valley (Himalaya), Northern Areas, Baluchistan	Whole plant	Bronchitis, asthma, urinary complaints, stomach irritation.	Decoction of leaves and dried flowers is used to cure fever, heart palpitation, and irritation of stomach, anti-dandruff. Dried leaves and stems are used to treat bronchitis and asthma and urinary complaints.	Tannins, Glycosides, resins, alkaloids	Khan *et al.*,2013; Shinwari and Gilani 2003; Arya and Gupta 2011; Ahmad *et al.*, 2009
Paeonia emodi Royle./ Paeoniaceae	Swat	Whole plant	Diarrhea, vomiting, cholera, emetic, purgative and eye diseases.	The dried flowers are used against diarrhea. The seeds are purgative and emetic.	Paeonins A and B, new monoterpene galactosides	Raiz *et al.*, 2003.
Rydingia limbata (Ben th.) Scheen & V.A.Albert (=*Otostegia limbata* Benth.) / Lamiaceae	Abottabad Cherat, Mardan, Malakand, Kohat	Aerial parts Leaves	Antiulcer, antispasmodic, antidepressant, Opthalmia and gums diseases.	Fresh leaf extract is taken orally twice a day for 3-4 days for mouth gums and throat ache.	limbatolide A, limbatolide B and limbatolide C. oleanic acid and b-sitosterol	Abbasi *et al.*, 2010; Ahmad *et al.*, 2005.
Salvia cabulica Benth./ Lamiaceae		Leaves	Flu, lung diseases, stomach and fever	The leaves are soaked in water and the decoction is used for stomach ache, fever and flu liver and disorder.	Monoterpenes,diterpenes, flavones, flavone glycosides, anthocyanins and proanthocyanins	Rashid *et al.*, 2009
Solanum virginianum L. (=*Solanum xanthocarpum* Schrad. & H. Wendl./ Solanaceae	Lahore	Root	Respiratory disorder, stomach disorder, throat sore and gums.	Paste of roots applied on gums, in snake bite and scorpion bite.	Carpesterol, steroidal glycosides, alkaloids, sterols, saponine, flavonoids and Glycosides.	Singh *et al.*, 2010; Malik *et al.*, 2011.
Trigonella foenum graecum L./Leguminosae	Karachi	Seeds, stem Leaves	Anticancer, antiseptic, wound healing, chronic cough, heart disease, diarrhea.	200 g seeds are ground and the resulting powder is used orally for washing urethra.	Rich in proteins, minerals, vitamin C	Hussain *et al.*, 2008; Abassi *et al.*, 2010
Viscum album L./ Santalaceae	Azad Jammu and Kashmir	Leaves	Anti-inflammatory, Emetic, Purgative , Anti – Diabetic, Hernia	NA	4'-O-[β-D-Apiosyl(1→2)]-β-D-glucosyl]-5-hydroxyl-7-O-sinapylflavanone , 3-(4-acetoxy-3,5-dimethoxy)-phenyl-2E-propenyl-β-D-glucopyranoside , 3-(4-hydroxy-3,5-dimethoxy)-phenyl-2E-propenyl-β-D-glucopyranoside , 5,7-dimethoxy-4'-O-β-D-glucopyranoside flavanone , 4',5-dimethoxy-7-hydroxy flavanone	Biswas *et al.*, 2011
Withania somnifera L. Dunal/ Solanaceae	Kohat	Fruits, seeds	Carminative, diuretic, opthalmia, asthma, fever, constipation, Rheumatic.	Put 2-3 seeds or fruits into the water and then eat as a carminative, painful swellings diuretic and ulcer. Fruit is applied to wounds, used in asthma.	Withaferin, Withanolides, Steroidal lactone.	Adnan *et al.*, 2014; Hussain *et al.*, 2010; Ahmad *et al.*, 2008; Sundaram *et al.*,2011.
Ziziphus jujube Mill. (=*Ziziphus vulgaris* Lam.)/ Rahmanaceae	Mianwali	Fruits	Colds and coughs	Both fresh and dried fruits are used for the treatment of cold and cough.	Zizyphusine, Alkaloids, Coclaurine, Nuciferine	Walter *et al.*, 2011.Han and Park., 1987

Table 2: Antibacterial activity of Pakistani medicinal plants used against *P. aeruginosa in vitro* at different concentrations

Ziziphus jujube Mill. (=Ziziphus vulgaris Lam.)/ Rahmanaceae	Mianwali	Fruits	Colds and coughs	Both fresh and dried fruits are used for the treatment of cold and cough.	Zizyphusine, Alkaloids, Coclaurine, Nuciferine	Walter *et al.*, 2011.Han and Park., 1987
Plant name	Area	Part used	Extract	Concentration	Inhibition zone of P. aeruginosa	Citations
A. officinalis	Muzaffarabad	Root, Leaves, Flower	Methanol	15 (D)	2.1	Walter *et al.*, 2011.
				12.5 (D)	2.1	
				10 (D)	2.1	
				7.5 (D)	2	
				5 (D)	1.4	
C. arvensis	Cherat, Mardan, Malakand, Kohat.	Leaves	Ethanol	10 (C)	13	Subhan *et al.*, 2013
C. procera	Kohat	Stem	n-Hexane Methanol Aqueous Chloroform Butanol	4 (D) 4 (D) 4 (D) 4 (D) 4 (D)	7.6 7.6 7.6 7.6 7.6	Bibi, 2013
C. Sativa	Cherat, Mardan, Malakand, Kohat.	Leaves	Ethanol	10 (C)	14	Subhan *et al.*, 2013
C. Intybus	Mardan	Roots	Methanol	20 (D)	13.5	Sattar, 2010
			n-Hexane	20 (D)	8.5	
			Chloroform	20 (D)	11.5	
			Ethyl acetate	20 (D)	8.3	
			Aqueous	20 (D)	11.4	
C. Noeanum	Sawabi,Gawadar.		Methanol	20 (D)	7	Ahmad, 2010
			n-Hexane	20 (D)	8	
			Chloroform	20 (D)	9	
			Ethyl acetate	20 (D)	6	
			Aqueous	20 (D)	7	
C. arvensis	Peshawar	Leaves	Methanol	10 (D)	7	Raza, 2010
			n-Hexane	10 (D)	10	
			Chloroform	10 (D)	11	
			Ethyl acetate	10 (D)	9.9	
			Butanol	10 (D)	4	
			Aqueous	10 (D)	7	
C. latifolia	Abottabad	Flower, Fruit	Methanol	15 (D)	1.9	Walter *et al.*, 2011.
				12.5 (D)	1.6	
				10 (D)	1.7	

Plant name	Area	Part used	Extract	Concentration	Inhibition zone of P. aeruginosa	Citations
				7.5 (D)	1.4	
D. regia	Karachi	Flower	Ethanol	100 (C)	15	Khursheed *et al*., 2012.
D. viscosa	Cherat, Mardan, Malakand, Kohat.	Leaves	Ethanol	10 (C)	13	Subhan *et al*., 2013
E. angustifolia	Bannu	Whole Plant	Methanol	5 (D)	0	Khan *et al*., 2013.
			n-Hexane	5 (D)	18	
			Ethyl acetate	5 (D)	25	
E. gerardiana	Baluchistan	Whole Plant	Methanol	15 (D)	1.2	Walter *et al*., 2011.
E. cammaldulensis	Cherat, Mardan, Malakand, Kohat.	Leaves	Ethanol	10 (C)	15	Subhan *et al*., 2013
F. carica	Cherat, Mardan, Malakand, Kohat.	Leaves	Ethanol	10 (C)	20	Subhan *et al*., 2013
G. wallichanum	Abbottabad	Rhizome	Methanol	1 (D)	14.33	Ismail *et al*., 2012.
G. glabra	Peshawar	Root	Methanol	15 (D)	2.6	Walter *et al*., 2011.
				12.5 (D)	1.6	
				10 (D)	1.4	
				7.5 (D)	1.4	
				5 (D)	1	
H. strigosum	Malakand	Whole Plant	Ethyl acetate	10 (D)	9	Hussain *et al*., 2010.
			Chloroform	10 (D)	12	
			Aqueous	10 (D)	9	
			n-Hexane	10 (D)	10	
H. officinalis	Azad Kashmir	Leaves	Methanol	15 (D)	2.1	Walter *et al*., 2011.
J. adhatoda	Margalla Hills	Leaves	Methanol	15 (D)	2.6	
				12.5 (D)	2	
				10 (D)	1.5	
				7.5 (D)	1.4	
				5 (D)	0.4	
M. sylvestris	Rawal Dam	Leaves, Roots, Flowers	Methanol	15 (D)	1.6	Walter *et al*., 2011.
				12.5 (D)	1.2	
M. longifolia	Cherat, Mardan, Malakand, Kohat .	Leaves	Ethanol	10 (C)	12	Subhan *et al*., 2013
M. alba	Kohat	Fruits	n-Hexane	3 (D)	10	Maqsood, 2012
			Ethyl acetate	3 (D)	10	
			Chloroform	3 (D)	16	
M. nigra	Kohat	Leaves	Ethanol	3 (D)	10	Maqsood, 2012
			n-Hexane	3 (D)	10	
			Ethyl acetate	3 (D)	10	
			Chloroform	3 (D)	16	
		Fruits	Ethanol	3 (D)	9	

Plant name	**Area**	**Part used**	**Extract**	**Concentration**	**Inhibition zone of P. aeruginosa**	**Citations**
			n-Hexane	3 (D)	9	
			Ethyl acetate	3 (D)	10	
			Chloroform	3 (D)	11	
D. viscose	Kohat	Arial Parts	Ethanol	3.2 (D)	12.4	Khurram *et al*., 2009.
O. europaea	Cherat, Mardan, Malakand, Kohat.	Leaves	Ethanol	10 (C)	14	Subhan *et al*., 2013
O. bracteata	Kashmir	Leaves	Methanol	15 (D)	2.6	Walter *et al*., 2011.
				12.5 (D)	2.3	
				10 (D)	2	
				7.5 (D)	1.4	
P. emodi	Swat	Roots	Methanol	5 (C)	14	Ullah *et al*., 2011.
R. limbata	Cherat, Mardan, Malakand, Kohat.	Leaves	Ethanol	10 (C)	13	Subhan *et al*., 2013
	Abottabad	Aerial Parts	Ethanol	8 (C)	6	Anwar *et al*., 2009
			Methanol	8 (C)	6	
S. cabulica	Mach and Ziarat	Aerial Parts	Methane	1 (D)	9	Rashid *et al*., 2009.
			Aqueous	1 (D)	15	
S. xanthocarpum	Karachi	Leaves, Stem, Flower, Root	Ethanol	5 (C)	11.02	Ghani *et al*., 2010.
				50 (C)	13.12	
				100 (C)	16.32	
T. foenum graecum	Karachi	Seeds	Ethanol	100 (C)	7	Khursheed *et al*., 2012.
V. foetens	Abottabad	Aerial Parts	Chloroform	10 (C)	0	Awan *et al*., 2013.
			Ethanol	10 (C)	22.5	
			Methanol	10 (C)	24.33	
V. vervosum	Abottabad	Aerial Parts	Petroleum Ether	10 (C)	9.33	Awan *et al*., 2013.
			Chloroform	10 (C)	10.5	
			Ethanol	10 (C)	15.33	
			Methanol	10 (C)	10.33	
V. album	Azad Jammu And Kashmir	Leaves, Twigs	Ethyl acetate	100 (C)	19.66	Hussain *et al*., 2011.
			Chloroform	100 (C)	10	
			Ethanol	100 (C)	13.83	
			Methanol	100 (C)	19.83	
			Aqueous	100 (C)	9.9	
W. somnifera	Cherat, Mardan, Malakand, Kohat (Kpk)	Leaves	Ethanol	10 (C)	16	Subhan *et al*., 2013
Z. jujube	Mianwali	Fruits	Methanol	15 (D)	1.2	Walter *et al*., 2011.
				12.5 (D)	0.9	

Ethylacetate and aqueous extractions

Ethyl acetate is a colorless sweet smelling organic compound while water is a colorless tasteless universal solvent. Like methanol, ethanol and chloroform, ethyl acetate and aqueous solvents are also used for the extractions of medicinal plants around the world. In Pakistan, out of 124 medicinal plants, only 20 were extracted with ethyl acetate and 27 with aqueous. Furthermore, medicinal plants extracted with these solvents showed good antibacterial activity against *P. aeruginosa.* Statistically, significant increase (R^2=0.97; p =000) was observed in the inhibition of *P. aeruginosa* due to increasing concentration of ethyl acetate. However, in case of aqueous extraction this increase was not significant (fig. 2).

Ethnobotany, antibacterial activity and phytochemistry of medicinal flora Pakistan

Pakistan has a good climate and is blessed with diverse flora. Since long, medicinal flora of Pakistan is traditionally used for the treatment of different infections caused by microorganisms (Haq *et al.,* 2011). Traditionally used medicinal plants have recently fascinated the interest of the pharmaceutical and scientific communities. This has triggered the isolation and identification of secondary metabolites form plants and their use as antibacterial agents against pathogens (Ali *et al.,* 2009; Haq *et al.,* 2011). Literature showed that traditionally used Pakistani medicinal plants contain variety of phytoconstituents that showed excellent antibacterial activity against *P. aeruginosa* (Bibi *et al*., 2011).

Justicia adhatoda L. Acanthaceae

Justicia adhatoda is an evergreen shrub distributed in Panama, Indonesia, Malaya, India and Pakistan. Ethnobotanically, whole plant is used for the treatment of different diseases caused by *P. aeroginosa* like gastrointestinal, ear ailments, asthma, cough and bronchitis (Adnan *et al.,* 2014). Moreover, it is widely used in the Ayurvedic and Unani system of medicine (Ali and Qaiser, 2010). Mostly, roots are useful in bronchitis and other chronic coughs while leaves are very effective in the treatment of asthma (table 1). Walter *et al*. (2011) studied the inhibitory effect of methanolic extract of some Pakistan medicinal plants at different concentrations dissolved in DMSO solvent and concluded that *J. adhatoda* exhibited maximum inhibition zone (2.6mm) against *P. aeruginosa* at 15mg/ml (table 2). Their good antibacterial activity might be due to the presence of active compounds from alkaloids (Corrêa and Alcântara, 2011).

Malva sylvestris L. Malvaceae

M. sylvestris is usually a biennial herb distributed in West Europe, North Africa and Asia (Ali and Qaiser, 2010). Traditionally it is used for the treatment of skin disorders, throat infections, chronic bronchitis, jaundice and urinary disorders caused by *P. aeruginosa*. Whole plant is boiled in water and the decoction is used for jaundice treatment (Walter *et al*., 2011; Ahmad, 2007). Its roots, leaves and flowers are preferred for various ethnomedicinal recipes. Methanolic extracts of these parts when dissolved in DMSO solvent inhibit the growth of *P. aeruginosa* (1.6 mm) at 15mg/ml *in vitro* (Walter *et al*., 2011). Similarly, according to a study, the alcoholic extracts of *M. sylvestris* were active against *P. aeruginosa* (Alkofahi *et al*., 1996). Their activity against the bacterium might be due to the presence of active phytoconstituents like terpanoids 8-hydroxyflavonoid glucuronides and malvone A (2-methyl-3-methoxy-5, 6-dihydroxy-1,4-naphthoquinone) (fig. 3.1).

Cannabis sativa L. Cannabinaceae

C. sativa is an annual herbaceous plant distributed in Russia, China, India, Pakistan, and Iran (Ali and Qaiser, 2010). It is traditionally considered a valuable medicinal plant. Mostly, its leaves and flowers are used for the treatment of narcotics, intoxication, diuretic, throat soreness, gastrointestinal aches and urinogenital diseases. Its leaves are mixed with 50-60grams of sugar and one glass of water is used to reduce general body inflammation, intoxication and to improve the appetite of an addictive person (Adnan *et al*., 2014). The ethanolic extract of *C. sativa* leaves inhibits the growth of *P. aeruginosa* (14mm) at 10mg/ml in their respective solvent (Subhan *et al.,* 2013). Similarly, a study revealed that the growth of this bacterium can be inhibited by the alcoholic extract of *cannabis sativa,* as it is resistant to drugs like benzyl penicillin, ampicillin and cloxacillin (Ali *et al.,* 2012). Thus, excellent antibacterial activity of this plant might be due to active compounds like anhydro cannabisativine, cannabisativine, cannabinoids (fig. 3.2)

Delonix regia (Hook.) Raf. Leguminosae

D. regia is large deciduous tree. It is traditionally a good medicinal plant used against the diseases caused by *P. aeruginosa*. Its stem, root, bark and leaves are used for the abdominal pains, bronchitis and pneumonia. In case of abdominal pains, the root decoctions are preferred and leaf extracts are used as anti-inflammatory (Jahan *et al*., 2010). According to Khursheed *et al*. (2012), the ethanolic extract of *D. regia* at 100mg/ml in their respective solvent showed good inhibitory activity (15 mm) against the *P. aeruginosa* (table 2). Various secondary metabolites of this plant might be responsible for this activity. A study also concluded that different extracts of *D. regia* showed good activity against *P. aeruginosa* (ATCC 27853). Moreover, the study linked the antibacterial activity with the phytoconstituents of the plant like lupeol, epilupeol, β-sitosterol, stigmasterol, aromatic compound p-methoxybenzaldehyde (Jahan *et al.,* 2010).

Olea europaea L. Oleaceae

Traditionally, leaves and fruits of *Olea europaea* are beneficial in the treatments of diuretic, urinary, bronchial,

asthma and diarrhea. For this purpose, decoctions of the dried fruit and leaf are taken orally in order to treat diarrhea, respiratory and urinary tract infections (Khan *et al.,* 2007). The ethanolic extract of leaves (10mg/ml) also showed good activity (13mm) against *P. aeruginosa in-vitro* when dissolved in its respective solvent i.e. ethanol (table 2). A study confirmed that different extracts of *O. europaea* inhibit the growth of *P. aeruginosa* (Pereira *et al*., 2007). The *O. europaea* contain some active phytoconstituents like Oleuropein (fig.3) Hydroxy tyrosol and Leteoline-7-glucoside (Khan *et al*., 2007), which might be responsible for excellent anti-*P. aeruginosa* activities.

Withania somnifera L. Dunal Solanaceae

W. somnifera is an annual herb, which is widely used for different ailments traditionally. As an example, decoction of 2–3 fruits mixed in water is used as carminative, relief painful swellings and ulcer. The fruits are also applied to wounds, used in asthma and biliousness. The seeds are used for the treatment of diuretic (Adnan *et al.,* 2014; Hussain *et al.,* 2010). Its crude extract at 10mg/ml concentration showed 16mm inhibition (table 2). Literature review showed that it contains some steroidal lactone such as Withaferin and Withanolides, which may be responsible for its biological activities (table 1).

Viscum album L. Santalaceae

Leaves of *V. album* are used traditionally as purgative and treating diabetes, hernia and inflammatory diseases in Pakistan (Hussain *et al.,* 2011). Different extracts show different inhibition zone at same concentration but methanol presents more inhibition (Hussain *et al.,* 2011). Different flavones compounds have been isolated from this plant (Biswas *et al.,* 2011).

Solanum xanthocarpum Burm. Solanaceae

Roots of *S. xanthocarpum* are used traditionally in snake bite, scorpion bite and respiratory diseases. Root paste is applied on gums and sore throat. Extract of different aerial parts show good inhibition against *P. aeruginosa* with increasing concentrations (Ghani *et al.,* 2010). Phytoconstituents like steroidal glycosides, alkaloids, sterols, saponin, flavonoids and glycosides are the reported active phytochemicals present in different parts of this plant (Singh *et al.,* 2010).

Cichorium intybus L Asteraceae

Ethno medicinally, the whole plant is used against diarrhea. A decoction from the seeds is used in obstructed menstruation and for checking bilious vomiting (Hussain *et al.,* 2008; Malik *et al.,* 2011). Methanol and aqueous extract of the plant have strong zone of inhibition and contain different phytochemicals which may be responsible for its different biological activities (tables 1, 2).

Other plants

Other plants also show good inhibitory activities against *P. aeruginosa*. Awan *et al*. (2013) reported that different extract of *Viburnum vervosum* shows different inhibitions at same concentration although it is not reported for its traditional uses in medicine. Ethanol and methanol extract of *Viburnum foetens* have 22.4 mm and 24.33 mm zone of inhibition against *P. aeruginosa* at 10mg/ml. Similarly, methanol extract of *Paeonia emodi* have 14 mm zone of inhibition at 5 mg/ml and have scientific validation for its traditional uses (Ullah *et al.,* 2011). Fresh leaf extract of *Rydingia limbata* is taken orally twice a day for 3-4 days for mouth gums and throat ache, antidepressant and opthalmia diseases. Its ethanolic and methanolic extracts have 6 mm inhibition at 8 mg/ml (Anwar *et al.,* 2009) while Subhan *et al.,* 2013 reported 13 mm inhibition zone of ethanolic extract at 10 mg/ml. Limbatolide A, B and C, oleanic acid and b-sitosterol are isolated compounds from *R. limbata*, which may be responsible for its biological activities. Methanol extract of *Onosma bracteata* show good inhibition with increasing concentration and give scientific proof for its traditional uses (Walter *et al.,* 2011). Decoctions of the *Olea europaea* dried fruit and dried leaf are taken orally for diarrhea and to treat respiratory and urinary tract infections. Its extract shows 14 mm inhibition at 10 mg/ml against *P. aeruginosa* (Subhan *et al.,* 2013). *Morus nigra* is used traditionally to treat sore throat, cough, stomach problem and act as blood purifier (Imtiaz *et al.,* 2013). Different extract have good inhibition at low concentration (Maqsood, 2012).

CONCLUSION

It is concluded from the above discussion that local people of Pakistan mostly belong to remote areas are traditionally using large number of medicinal plants for different types of diseases caused by *P. aeruginosa*. These medicinal plants have been proved for their strong antibacterial activities *in-vitro*. Different types of chemical compounds like alkaloids, flavonoids and terpenoids have been extracted from these medicinal plants that are responsible for their anti-*P. aeruginosa* potential. In Pakistan, different solvent like methanolic, ethanolic, aqueous and chloroform are used for the extraction of medicinal plants for biological screening. Most researchers preferred methanolic and ethanolic extracts, however the present review shown that non-alcoholic extraction like chloroform, n-hexane and ethyl acetate have also shown good inhibition against *P. aeruginosa*. These non-alcoholic extracts should be explored in future studies for extraction of medicinal plants couple with phytochemistry of still unexplored medicinal flora. This could be helpful in the isolation of new compounds and would lead to the development and introduction of some new novel drugs for pharmaceutical industries with fewer side effects on the living system as

compared to the present allopathic drugs. Toxicological studies are also recommended in order to check the toxic effects of these plants.

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