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PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF SOME UNEXPLORED MEDICINAL PLANTS OF CHOLISTAN DESERT

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Abstract

Ethnobotanical survey plays a vital role for the discovery of novel crude drugs from reported medicinal plants. In folklore traditions the plant extracts or decoctions have been used to treat different diseases representing a basis of chemical entities but without knowledge of their nature. The present study was planned to conduct biochemical profiling of five indigenous medicinal plants of Cholistan desert of Pakistan to be used as potential chief constituents in the drug discovery practice. Qualitative phytochemical analysis of these plants confirmed the presence of various important secondary metabolites viz isoflavones, sesquiterpene lactones, phenolics, flavonoids and non-alkaloids. Methanolic extracts of *Suaeda fruticosa* and *Solanum surattense* showed significant antibacterial activities with mean halo diameters of 19.5 ± 0.3 mm and 14.8 ± 0.5 mm respectively, and inhibited the growth of *Escherichia coli*. Biochemical profiling of selected plants is reported for the first time. The methanolic extracts of *Fagonia indica* and *S. fruticosa* were found to have maximum numbers of phytochemicals. The occurrence of various bioactive compounds confirmed the studied plants against various diseases as local people of Cholistan desert still have a solid belief on herbal remedies for their elementary healthcare issues. Isolation of individual active constituents from each plant reported here may proceed to find some novel natural drugs.

Key words: Medicinal plants; Cholistan desert; Biochemical profiling; Antibacterial activity; Secondary metabolites.

Introduction

Medicinal plants have been used as a source of medicine in all cultures since time immemorial (Malik *et al.,* 2005). The natural flora is a very useful resource for health improvement and to cure many diseases across various human communities. A variety of plant species are offered which are still in use in many parts of the world viz. Asia (Duraipandiyan *et al.,* 2006), South America (Bolzani *et al.,* 2012) and Africa (Khalid *et al.,* 2012) for remedies against several diseases. Even though World Health Organization reported that the major health care system for the 60% population of the world is represented by the traditional medicines (WHO-AFRO, 2010). However, a great number of plant species with potential biological activities are still unexplored.

Pakistan is very rich in botanical wealth and has a variety of aromatic and medicinal plants because of its exclusive geographical distribution due to varied climatic and edaphic factors such as soil conditions and multiple ecological regions. Majority of the plants growing in Cholistan desert possess therapeutic properties and native people utilize those to treat different diseases (Shafi *et al.,* 2001). However, such plants are being used without the knowledge of active ingredients. The treasure of medicinal plants is being reduced with the passage of time and sound efforts are needed to conserve it (Saqib *et al.,* 2014). The current study was aimed to find the effects of plants' extracts from five candidate species from Cholistan desert, the Eastern side of province of Punjab, Pakistan. The main objective of the study was to investigate biochemical profiling to provide clues of active secondary compounds in these plants species. This will open new opportunities towards isolation of potent novel bioactive compounds from unexplored medicinal plants.

Materials and Methods

Chemicals: All chemicals were of analytical grade or HPLC grade and purchased from Sigma Chemical Co., Missouri USA, unless otherwise stated.

Study areas: Cholistan desert is a part of Great Indian Desert and is situated in southern Punjab of Pakistan, between 27° 42' and 29° 45' north and 69° 52' and 73° 05' east (Akhter & Arshad, 2006). The desert surrounds thirty kilometers from Bahawalpur city of Punjab, Pakistan and covers an area of 16,000 km^2 (Chouhan *et al.,* 2002). The climate of Cholistan desert is considered as low and infrequent rainfall. The most conspicuous feature of the Cholistan desert is drought with dry and wet years that occur in clusters (Akhter & Arshad, 2006). The vegetation of Cholistan desert comprises of xerophytic species due to adaptation of extreme seasonal temperature, a wide variety of edaphic conditions and moisture fluctuations.

Collection and identification of medicinal plants: Five medicinal plants used by local societies and herbalists of Cholistan desert for treatment of different diseases were collected (Table 1) and brought to Herbarium, University of Agriculture, Faisalabad, Pakistan. For each medicinal plant, the botanical name, vernacular name, plant family, traditional medicinal uses, part(s) used and method of preparation are also given in Table 1. For preservation, the plants were shade dried, pressed and 1% $HgCl_2$ solution was used to poison and finally mounted on herbarium sheets. Herbarium specimens were identified by Dr. Mansoor Hameed (Plant Taxonomist), Department of Botany, University of Agriculture, Faisalabad, Pakistan. After verifying plants identification by matching with the Flora of Pakistan (Ali & Nasir, 1989-1991; Ali & Qaiser, 1993-2011) voucher specimens were submitted in the herbarium.

Preparation of plant extracts: A representative amount (1 g) of whole plant samples collected was chopped into small pieces, shade dried, powdered and extracted at room temperature for 48 h with methanol (Piaza *et al*., 2010). The extracts were filtered through a Whatmann filter paper No. 42 (125 mm) and 5 g of Na_2HSO_4 was added to each solution and left for 10 h to absorb water. The extracts were filtered and concentrated on a rotary evaporator.

LC/MS analyses: Plant extracts (1 mg/mL) were filtered from 0.2 µm filter and 10 µL of each solution were injected for LC-MS analysis. All samples were subjected to LC-MS analyses using a Hewlett-Packard series 1100 LC-MS system having a reversed-phase C18 column (Phenomenex Luna, 4.6 mm by 100 mm; pore size, 5 µm) using a solvent gradient with a range of 5% to 100% CH_3CN for 23 min, a flow rate of 0.7 mL/min, and UV detection. The mass data of low-resolution was obtained in the positive mode; the following instrumental parameters were used: nitrogen gas temperature 350°C, drying gas flow rate 11.0 l/min, capillary voltage was 4000 V, ESI voltage, 6.0 kV; capillary temperature, 200°C; auxiliary and sheath gas pressure, 5 units and 70 lb/in^2, respectively.

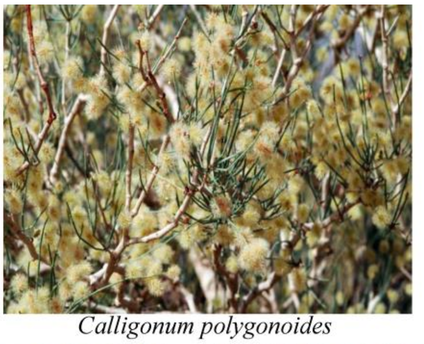
Antibacterial activity: Gram-negative, *E. coli* was used in this study as a reference strain of human pathogen. The agar disc diffusion method was used to determine antibacterial activity of the plant extracts according to (Qaralleh *et al*., 2010) with some adaptation. Concisely, inoculum containing 10 CFU/mL was spread on Mueller-Hinton agar plates for bacteria. The sterile filter papers of 6 mm diameter having crude extracts of 10 mg/mL and 1 mg/mL, and Dimethyl sulfoxide (DMSO) as negative control were placed on the surface of inoculated agar plates with sterile forceps. Sensi-Discs of Ciprofloxacin were used as reference antibiotic. The plates were incubated at 37 °C for 24 h. The samples were tested in duplicates and the inhibition zones were measured as millimeter diameters.

Results

Biochemical profiling of selected medicinal plants: In the present work we explored the usage of LC-MS analysis of the methanolic extracts of five selected medicinal plants (Fig. 1) for the separation and identification of bioactive compounds rapidly. Library search was conducted to find out active ingredients from each extract. The most potent compounds were compared with those reported in literature. LC-MS analysis revealed that the active constituents from plants had strong activities against a broad range of diseases (Table 2). Maximum numbers of active compounds were detected in *Fagonia indica* and *Suaeda fruticosa*. The search was made for each of the compound detected in the medicinal plants for its potential use as a medicine or drug as found in literature.

Antibacterial study of medicinal plants: Plants extracts were tested for their antibacterial activities against *E. coli* DH5α by disc diffusion assays. Inhibition of *E. coli* growth was observed with most of the plant extracts tested. The negative control, DMSO, did not affect *E. coli* growth. The methanol extracts of *Suaeda fruticosa* and *Solanum surattense* showed substantial inhibition. The mean halo diameters were 14.8 ± 0.5 mm for *Solanum surattense*, 10.5 ± 0.9 mm for *Calligonum polygonoides*, 12.3 ± 0.7 mm for *Fagonia indica*, 19.5 ± 0.3 mm for *Suaeda fruticosa* and 9.3 ± 1.3 mm for *Heliotropium strigosum* against a mean halo diameter of 42.5 ± 0.1 mm for the Ciprofloxacin as positive control and 6 ± 0 mm for DMSO as negative control. The results were found to be highly significantly different from the controls in each case (p<0.01). The results demonstrate that these extracts have strong antibacterial activities towards *E. coli*.


Solanum surattense


Calligonum polygonoides


Fagonia indica


Suaeda fruticosa


Heliotropium strigosum

Fig 1. Medicinal plants.

Table 1. Indigenous medicinal plants used in the study with their traditional medicinal uses.

Plant species/ voucher number	Vernacular name	Family	Habit [a]	Parts used	Preparations	Traditional therapeutic uses	References
Calligonum polygonoides (822-38-2014)	Phok	Polygonaceae	Wd, Sh, An	Fruit, Stem, Leaves, Flower	Decoction, Juice, Infusion, Powder	Abortifacient, Antibacterial, Emollient, Epilepsy, Diuretic, Dysentery	(Ahmed *et al.*, 2014)
Fagonia indica (822-50-2014)	Dramaaho	Zygophyllaceae	Wd, Sh, An	Whole plant	Decoction, Juice, Infusion, Powder, Paste	skin eruption, Cold, Cooling effect, Cancer, Hypertension, Pneumonia, Sedative, Analgesic Antimicrobial	(Sharma *et al.*, 2009)
Heliotropium strigosum (822-15-2014)	Kharsan/ Gorakh Pam	Boraginaceae	Wd, Hr, An	Leaves	Paste	Wounds	(Qureshi & Bhatti, 2008)
Solanum surattense (822-45-2014)	Kundiari, Momoli	Solanaceae	Wd, Hr, An	Whole plant	Pills	Chest pain, vomiting, burning feet, cough, asthma, expectorant, stomachache, diuretic, gonorrhea, urinary, gastro-intestinal diseases	(Mahmood *et al.*, 2012)
Suaeda fruticosa (822-23-2014)	boi booti/ Khaari	Amaranthaceae	Wd, Sh, An	Stem, Leaves	Decoction, Juice	Antibacterial, conjunctivitis, Blood purifier, Cancer, Skin diseases, Snake bites	(Ahmed *et al.*, 2014)

[a] Wd, Wild; Hr, Herb; An, Annulus; Sh, Shrub

Table 2. List of compounds present in each medicinal plant with their medicinal use.

Medicinal plant species / Compounds with reported activities	Classification of the bioactive compounds	*Solanum surattense*	*Calligonum polygonoides*	*Fagonia indica*	*Suaeda fruticosa*	*Heliotropium strigosum*
Myricetin Antioxidant (Larson, 1988)	Flavonoids		+	+	+	+
Quercetin Against Interstitial Cystitis (Katske *et al.*, 2001)	Flavonoids		+	+	+	+
Enhydrin Antibacterial Activity (Choi *et al.*, 2010)	Sesquiterpene lactones	+	+	+	+	
Dihydro-Parthenolide Anti-inflammatory activity (Feltenstein *et al.*, 2004)	Sesquiterpene lactones	+	+	+	+	+
Genistein Against genetic diseases (Wegrzyn *et al.*, 2010)	Isoflavones		ND		+	+
Genistin Anticancer Activity (Hamdy *et al.*, 2011)	Isoflavones		ND		+	
4-Hydroxybenzoic acid Vasodilative Compounds (Seki *et al.*, 2010)	Phenolics	+	ND	+		
Protocatechuic acid Cancer Chemo-preventive Activity (Tanaka *et al.*, 2011)	Phenolics			+		
Maytansin Anticancer Activity (Bell-McGuinn *et al.*, 2011)	Non-alkaloids	+	+	+	+	+
Indole-3-acetic acid Treatment of Acne (Huh *et al.*, 2012)	Auxins	+	+	+	+	+
Indole-3-carboxylic acid Antiinflammatory activity (Rapolu *et al.*, 2011)	Auxins		+	+	+	+
Phenylacetic acid Anticancer Activity (Neish, 1971)	Auxins	+	+	+	+	+
L-Tryptophan Antidepressent (Thomson *et al.*, 1982)	Free amino acid	+	+	+	+	+

ND: Not determined

Discussion

The phytochemical classes of compounds such as alkaloids, flavonoids, saponins, tannins and terpenoids possess strong physiological antibacterial, antifungal and antioxidant activities. The hot desert plants such as *Leptadenia pyrotechnica* (Forssk.) Decne have been reported for having all major groups of phytochemicals viz., alkaloids, flavonoids, saponins and tannins (Munazir *et al*., 2015). Screening of phytochemicals from five selected medicinal plants of Cholistan desert in the current study confirmed the presence of various important secondary metabolites which are discussed below:

Two flavonoids i.e., Quercetin and Myricetin were detected in four medicinal plants. Quercetin is responsible to inhibit cytotoxicity of *In vitro* low-density lipoprotein and oxidation (De Whaley *et al.,* 1990) and can decrease coronary heart disease or cancer risks (Yoshida *et al.,* 1990). Myricetin is one of the most active antioxidant and responsible for free radical scavenging activity in foods and also have significant activity of vitamin C sparing (Larson, 1988). Plant flavonoids are responsible for a wide range of biochemical and pharmacological properties including anti-inflammation, anti-thrombotic action, anti-oxidation, anti-platelet and anti-allergic effects (Cooks & Samman, 1996). The mechanism of action of flavonoids is through chelating or scavenging process (Kessler *et al.,* 2003) and also show anti-microbial activity through inhibiting those microbes which are resistant to antibiotics (Linuma *et al.,* 1994b).

Two different sesquiterpene lactones were found in the tested plant species. Enhydrin was found in four whereas dihydro-parthenolide was detected in all the five species. The plants having such compound have effects to cure migraine, pain and inflammation (Jain & Kulkarni, 1999). The other sesquiterpene lactone, enhydrin also showed similar activity and significantly blocks hyperalgesic response and attenuates edema response significantly (Feltenstein *et al.,* 2004). Sesquiterpene lactones which belong to lactones class are considered to be effective against inflammation (Hall *et al.,* 1980) and the mechanism of action of these lactones has been found to inhibit both cyclooxygenases (COX-2) and pro-inflammatory cytokines in macrophages (Hwang *et al.,* 1996).

Two isoflavones i.e. genistein and genistin were found in the plant species. Genistein was found in *Suaeda fruticosa* and *Heliotropium strigosum*, and genistin was found in *Suaeda fruticosa* only. Genistein belongs to the group of isoflavones, heterocyclic polyphenols which occur naturally in plants and it is also called 4′,5,7-trihydroxyisoflavone (Dixon & Ferreira, 2002). Genistein occurs naturally as a glycoside called genistin rather than as an aglycone (Nielsen & Williamson, 2007). A number of biological functions of genistein have been reported to date including phyto-oestrogenic, antioxidant and tyrosine kinase inhibitor activities (Szkudelska & Nogowski, 2007; Wegrzyn *et al.,* 2010) reported that genistein could also be used as a drug for as yet untreatable genetic diseases. Genistin was supplemented with selenium to provide antioxidant defense with high potential chemopreventive activity against tumors more than selenium alone (Hamdy *et al.,* 2011). Isoflavones belong to phytoestrogen class and are naturally occurring plant chemicals. For a range of hormone dependent conditions including cancer, cardiovascular disease, menopausal symptoms and osteoporosis the isoflavones are heralded as offering potential alternative therapies (Setchell & Cassidy, 1999).

In this study, 4-Hydroxybenzoic acid and protocatechuic acid were the two phenolics observed in some plant species. Protocatechuic acid (3,4-dihydroxybenzoic acid) is a simple phenolic which is widely distributed in nature and found in almost all plants like many other simple phenolic acids therefore, it is a common constituent of human diet (Liu, 2004). Tanaka *et al.* (2011) indicated that protocatechuic acid could be protective against epithelial malignancy development in different tissues and the cardiovascular diseases.

Maytansin is the only non-alkaloid found in all the five studied medicinal plant species. Maytansinoid toxins result in the disruption of microtubule activity and cell division and ultimately cell death through their binding to tubulin and inhibition of tubulin polymerization and microtubule assembly (Bell-McGuinn *et al.,* 2011).

The antibacterial agent Enhydrin (Choi *et al.,* 2010) is present in tested medicinal plants with higher antibacterial activity, but the optimal effectiveness of medicinal plants may not be due to only one main active compound. The combined action of different constituents originally in the plant could be the cause of optimal effectiveness against a particular disease (Chang *et al.,* 2001). A number of plants from Cholistan desert have been frequently used by the local inhabitants but the medicinal importance of these plants is still not determined. Many species including *Suaeda fruticosa* have been used for their vermicidal and anthelmintic properties against intestinal worms and for having antibacterial activities against *Klebsiella*, *Staphylococcus* and *E. coli* (Hameed *et al.,* 2011). Similarly, substantial antibacterial activity was also found in the methanolic root extracts of a desert plant *Leptadenia pyrotechnica* against *Staphylococcus epidermidis* (Munazir *et al*., 2012). In antibacterial studies, the observed *In vitro* minor interactions might not only result in significant synergism *In vivo* but they also made a difference to the *In vivo* durations for an effective drug level (Berenbaum, 1987). Herbal remedies have been used to treat infectious diseases throughout human history (Ozturk & Ercisli, 2006) but reliable and standardized antibacterial methods are needed to study potential antibacterial properties of plant-derived phytochemicals. In the past few years, a number of studies have been conducted in different countries to prove such efficacy (Tabuti *et al.,* 2010; Zakaria *et al.,* 2010) and antibacterial activity from medicinal plants (Khanna and Kannabiran, 2008). The presence of different phytochemicals in plants is responsible for antibacterial activity.

In conclusion, Pakistan has rich plant biodiversity with tradition of use of medicinal plants for healthcare issues, but without the knowledge of bioactive ingredients. The biochemical profiling of five selected medicinal plants is reported for the first time and listed active constituents these plants exhibit. The methanolic extracts of *S. fruticosa* and *S. surattense* revealed promising antibacterial activities and inhibited the growth of *E. coli* at par with the standard antibiotic (i.e. Ciprofloxacin). Outcome of current study will augment the use of traditional medicine with scientific background. These plants had several compounds that could be responsible for their bioactivity. Such plants may be investigated further for isolation of bioactive compounds for their therapeutic use.

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