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Investigation of Phytochemical Profile and Antibacterial Potential of *Prosopis glandulosa*

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Abstract: The present study describes the phytochemical screening and antibacterial study of chloroform, hexane, acetone and ethanol extract of *Prosopis glandulosa* leaves, flower, stem and seeds. The phytochemicals screening revealed that the solvent extracts contained terpenoids, carbohydrates, glycosides, saponins, alkaloids, flavonoids, tannins, phenols, coumarins and proteins. *In vitro* antibacterial studies on the solvent leaves and flower extracts were carried out on two medically important bacterial strains (*Escherichia coli* and *Staphylococcus aureus*) using the agar disc diffusion method. The bacterial strains were exposed to the following four different concentrations of extracts: 01µg/ml, 05 µg/ml, and 10 µg/ml solvent. The results of our antibacterial assay revealed that the extract showed good inhibitory activity against all the tested pathogens.

Keywords: *Prosopis Glandulosa*, Phytochemical, Antibacterial, Extraction

1. INTRODUCTION

Plants are considered as one of the important sources to deal with the maintenance in the bodily functions of life. They produce some chemical substances and turn into complicated articles which are imperative for human strength. Moreover, curative plants are used as traditional medicines by natives all around the world (Rahman *et al.,* 2018). Such plants have actually opened new vistas and avenues for the progress of various healing agents (Prejeena *et al.,* 2016). The parts of plants which contain the crude protein and carbohydrates make it useful for the production of the livestock food (Bashir *et al.,* 2016). So, it is clear that the cure of the any sort of disease through plants have actually started in the earlier human progress. After few surveys, it was observed that the plants are one of the most important sources for the vital medicines (Aberoumand, 2012). A particular activity of a particular plant is indebted to the presence of various secondary metabolites. Such secondary metabolites are reported to be observed for many pharmaceutical activities. Many techniques are being engaged to extract the maximal bioactive components from the plant. A technique which is time taking named as Maceration is equally effective procedure in order to get maximal secondary metabolites (Shah *et al.,* 2014).Plants are rich source of phytochemicals like amino acids, tannins, terpenes, alkaloids, fatty acids, saponins, sterols, flavonoids, and Glycosides (Samejo *et al.,* 2013).Those phytochemicals not only protect the plants but the new research reveals that these photochemical also protect animals and humans against many diseases (Muthee *et al.,* 2016).The diseases include cancer, diabetes and various cardiovascular diseases (Tiwari *et al.,* 2016).Tannins consume some anti-microbial effects and flavonoids partake antibacterial, anti-fungal, anti-inflammatory, bactericidal and antimicrobial actions. Terpenes and Steroids are identified to have bactericidal and anti-microbial properties against countless pathogens (Samejo *et al.,* 2011).

In the south west of United States and Mexico, a medium sized flowering tree is found which is commonly called Honey Mesquite (Rahman *et al.,* 2011). Mesquite actually belongs to a Leguminosae and family and Mimosaceae sub family and it is a gene of trees which has got about 44 species. These trees are found in arid as well as sub arid areas (Michel-López *et al.,* 2014). If we talk about *Glandulosa* (Mimosaceae), it is a medium sized shrub which can be called a small tree because it is thorny and branching. It is usually found in southern parts of India. People commonly call it Vanni or SeemaiParambai in Tamil. The leaves and barks of this shrub are used for making medicines to cure various diseases and issues like leprosy, asthma, dysentery, bronchitis, piles, leukoderma, tremors of the muscles, tumors, and various eye diseases (Kumar *et al.,* 2011). Some of its species like *Glandulosa* is found in vegetarian mosaic in Baja California, Mexico. The ecosystem where it is found is hyper arid and is called mezquittales (Abdelmoteleb *et al.,* 2017). In past, people of southwestern North America clearly depended upon Mesquite as one of the most important resources. They

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used it to make their food, fuels, fibers, cosmetics, medicine, dye, and many other things. Different parts of the plant were used for different practical as well as aesthetical purposes. The ecological importance and value of genus Prosopis is very high because they are resistant to heat, drought, and salinity, and that is why they have been studied in all over the world. This plant has made colonies of forests in north entre of the country (Abdelmoteleb *et al.,* 2017).

It is inevitable from a bibliographical study that the significant results are originated on *P. Glandulosa* flowers, leaves, seeds and stem phytochemical screening and its antibacterial activity. For this persistence, the purpose of this study was to explore the investigation of phytochemical screening and antibacterial study.

2. MATERIALS AND METHODS

Collection and identification of plant materials: *Prosopis glandulosa* flower, seeds, stem and leaves (800 g each) were collected from District Jamshoro (longitude: N 25.430400 and latitude: E 68.280900), Sindh, Pakistan in August 2017 and identified by a Taxonomist, Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. A voucher specimen of the species was deposited in the herbarium of the same Institute under the acquisition numbers 2671317.

Processing of plant material: The *Prosopis glandulosa* flowers seeds, stem and leaves were washed three times with sterilized water in order to remove dust and contaminated particles, dried in shade for 15 days, all the parts were ground individually using electric mixer and powdered was placed in different vessels before analysis.

Preparation of different extracts with various solvents: Taken 10gpowder of each part of *P.glandulosa* (e.g. stem, flowers, seeds and leaves) and separately Sonicated with four different solvents namely; Chloroform, hexane, acetone and ethanol the extracts were filtered through filter paper (Whatman no. 8). After it all the filtrates were analyzed for phytochemicals screening tests and determined their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

The phytochemical study of different crude extracts: Preliminary phytochemical examinations of all the extracts were assayed using standard procedures for the presence of active principles as described by terpenoid, Carbohydrates, glycosides, saponins, alkaloids, flavonoids, tannins, phenols, coumarins and proteins(Samejo *et al.,* 2013).

Screening Procedure

Coumarins test: 2 ml of each substance are taken for the citation in test tube. Furthermore, 3ml of 10% was added sodium hydroxide (NaOH), until yellow coloration was observed.

Alkaloids test (Wagner's reagent): A small amount of extract was mixed with 2ml of Wagner's reagent (2 g of KI and 1.27 g of I_2 in 100 ml of H_2O), until a precipitate or reddish coloration was appeared.

Cardiac glycosides test (Keller Kellan's test): Took 2 ml of glacial acetic acid (CH_3COOH) in a test tube and added 5 ml of each extract into it and added 1 ml of ferric chloride solution in the same test vial. This was warmed and cooled carefully and the entire solution was transferred into another test tube containing 2 ml of concentrated sulfuric acid. A brown ring on the borderline specifies the existence of deoxy sugar attribute of cardenolides. A violet ring may become visible below the ring while in the acetic acid layer, may be a formation of green ring.

Flavonoids test or alkaline reagent test: Few drops of 20% NaOH (sodium hydroxide) solution were added to 2 ml of acid extracts. The (hydrochloric acid), yellow color showed the existence of flavonoids.

Ferric chloridefor Phenols: A fraction of the extracts was added in 5% aqueous $FeCl_3$ (ferric chloride) and observed for the formation of black or intense blue color.

Molisch's Test for Carbohydrates: Took 2 ml of extract put into test tube and added few drops (2, 3) of Molisch's reagent and 2 to 3 drops of H_2SO_4 under walls of test tube slowly. The appearance of ring immediately confirmed the presence of carbohydrates.

Foam test for Saponins: Took 5 ml of water in a test tube and added 5ml of extract into a same tube the mixture was stirred vigorously; the formation of persistent frothing confirms the saponins.

Tannins test: 2ml of extract was mixed with 2 mlH_2O of 5% $FeCl_3$ (ferric chloride) solution, the appearance of green or blue color confirms the presence of tannins.

Terpenoidtest:2 ml of each extract was mixed with 1 ml of $CHCl_3$ (chloroform)followed by a few drops of concentrated H_2SO_4 (sulphuricacid). The rapid formation of red brown precipitates confirmed the existence of terpenoid.

Proteins test: 1 ml of $H_2SO_4$was treated with 1 ml of extract. White precipitate showed the presence of proteins.

Determination of antibacterial activities

The antibacterial activity of leaves, flowers, seeds and stems extracts of *Prosopis glandulosa*was determined by the disc diffusion method on Muller

Hinton Medium (MHA). The modified disc diffusion method was favored to check the antibacterial activity of all four parts (Rahman *et al.,* 2017).With the help of the American Type Culture Collection (ATCC), antibacterial activity was planned against two different microbes; *Escherichia coli* and *Staphylococcus aureus.* Muller Hinton Agar (MHA) medium was utilized for the growth of microorganism species(Rawat *et al.,* 2013).

Three successive concentrations of 10, 5 and 1µg/ml were prepared in 100% DMSO (dimethyl sulfoxide) to check out the antibacterial activity of leaves, flowers, seeds and stem extracts. Hence, DMSO was employed as the negative control (Bouchekrit *et al.,* 2016). The bacterial suspensions were expanding on the solid Petri dishes (size 90 mm) with the used of sterile cotton swab moistened with the bacterial suspensions and adjusted to 10^6 CFU/ml.

Subsequently, a soaked Whatman No. 1 filter paper (6 mm diameter) with 20 µl of different concentrations (Diluted in 100% DMSO) was positioned on the surface of the microbial Petri plates (Petri dishes) and placed in an incubator at 37°C for 24hours. After incubation stage, the antibacterial activity of stem, flowers. Seeds and leaves extracts were recorded against each microbial species by measuring the area of inhibition diameter in mm (millimeter) around the discs (Pavithra *et al.,* 2009)and calculated MIC values. All assays were performed in triplicate (Panhwar and Memon, 2011).

3. RESULTS AND DISCUSSION

The analysis of phytochemicals

The phytochemical screening of plant extracts has given the idea of the presence of phytochemicals. Results obtained for qualitative screening of phytochemicals in leaves, flowers, seeds and stem of *P.glandulosa* raveled that the total ten phytochemicals such as carbohydrates, glycosides, saponins, tannins, alkaloids, phenols, flavonoids, proteins, terpenoids and coumarins.

Table.1. Phytochemical screening results of *P. glandulosa* leaves, seed, stem, flowers

Phytochemicals	Leaves				Seed				Stem				Flowers			
	CHL	HEX	ACE	ETH	CHL	HEX	ACE	ETH	CHL	HEX	ACE	ETH	CHL	HEX	ACE	ETH
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Coumarins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+Present; -Absent

Chloroform (CHL),hexane (HEX), acetone (AC) and ethanol (ETH)extracts were used for the phytochemical screening of each part (leaves, flowers, seeds and stem) of *Prosopis glandulosa*. In four types of extracts leaves, flowers, seeds and stem gave positive results for tannins, saponins, proteins, carbohydrates, flavonoids, phenols, terpenoids and alkaloids in *P. glandulosa*. Similarly, cardiac glycosides and coumarins gave negative results in four types of extracts Chloroform (CHL), Hexane (HEX), Acetone (AC) and Ethanol (ETH) in all parts (leaves, flowers, seeds and stems) of *P. glandulosa.* The existence of above phytochemicals may demonstrate therapeutic activities of *Prosopis glandulosa*. Prior studies on plants showed that flavonoids, alkaloids, saponins, terpenoid, steroids and tannins have anti-inflammatory properties (Orhan *et al.,* 2007). The flavonoids, alkaloids, tannins and glycosides have hypoglycemic actions (Muthee *et al.,* 2016). Saponins possess anti-diabetic and hypercholesterolemia actions (Rupasinghe *et al.,* 2003). Tannins used for treatment of inflamed or ulcerated tissues. Alkaloids commonly used as an antimalarial, cytotoxic, anticancer us, and antibacterial agents (Wirasathien *et al.,* 2006). Similarly, saponins possess the antibiotic, fungicidal and insecticidal properties (Sparg *et al.,* 2004). Flavonoids hold anti-inflammatory, antineoplastic, antibacterial, antiviral, anti-thrombotic antiallergic, vasodilatorand antioxidant properties (Ahmed *et al.,* 2016).Terpenoids used in wound curing, skin strengthen and increase in concentration of antioxidants in injuries (Krishnaiah *et al.,* 2009). The Terpenoids and steroids have been reported to possess

analgesic properties (Malairajan *et al.*, 2006). Steroids and Saponins are also recognized to have central nervous system (CNS) actions (Argal and Pathak, 2006). Terpenes and tannins, particularly condensed tannins, have been revealed to have strong anthelmintic activity (Hostettmann *et al.*, 2000; Muthee *et al.*, 2016). Polyphenolic compounds alike tannins that have been stated to have anthelmintic properties (Max *et al.*, 2007). Similarly, the anthelmintic effects of flavonoids have been reported too (Trease and Evans, 2002; Lahlou, 2002). Additional studies have specified that flavonoids and alkaloids also possess anthelmintic properties (Jitendra *et al.*, 2011; Rubini *et al.*, 2012). Phenolic compounds showed therapeutic properties alike anti-atherosclerosis, anti-apoptosis, anti-inflammation, cardiovascular protection, anti-carcinogen, anti-aging and for the evolution of endothelial purpose (Yadav *et al.*, 2011).

Determination of antibacterial activities

The results of the antibacterial action of stem, flowers, seeds and leaves extracts of *Prosopis glandulosa* against two bacteria *E. coli* and *S. aureus* are tabulated in **(Table 2).** The four types of extracts such as the chloroform (CHL), hexane (HEX), acetone (AC) and ethanol (ETH), extracts were made for antibacterial activity of each part (stem, flowers, seeds and leaves) of the *Prosopis glandulosa*. From these four type of extracts ethanol extracts of leaves and flower shows the zone of diameter against *E-coli* (12, 7, and 4) and (10, 7 and 5) respectively, Hence against *S. aureus* ethanol extracts of leaves and flowers show the zone of diameter (10, 8 and 5) and (8, 7 and 5) respectively, likewise chloroform extracts of leaves and flower shows the zone of diameter against *E-coli* (11, 7 and 2) and (12, 8 and 5) respectively, Hence *S. aureus* chloroform extracts of leaves and flower show the zone of diameter (7, 6 and 3) and (7, 6 and 3) respectively, similarly acetoneextracts of leaves and flower shows the zone of diameter against *E-coli* (10, 6 and 4) and (11, 5 and 5) respectively, Hence *S.aureus* chloroform extracts of leaves and flower show the zone of diameter (9, 8 and 5) and (7, 5 and 3) respectively, Hence Hexane extracts of leaves and flower shows the zone of diameter against *E-coli* (10, 5 and 2) and (7, 5 and 2) respectively, Hence *S. aureus* chloroform extracts of leaves and flower show the zone of diameter (9, 7and 3) and(8, 5 and 2) respectively. While Ethanol, Chloroform, Hexane and Acetone extracts of seeds and stem of *P. glandulosa* were examine to be ineffective against *E-coli* and*S. aureus*.

Table. 2. Inhibition zones (mm in diameters) for antibacterial activities of Ethanol, chloroform, Acetoneand Chloroformextracts of leaves, flower, seeds and stem.

Solvents	Zone of inhibition in mm ± SD							
Con. (µg/ml)	*Escherichia coli* (MIC 01 µg/ml)				*Staphylococcus aureus* (MIC 01 µg/ml)			
Chloroform	Leaves	Seed	Stem	Flowers	Leaves	Seed	Stem	Flowers
10	11±0.02	-	-	12±0.3	7±0.02	-	-	7±0.03
05	7±0.01	-	-	8±0.02	6±0.01	-	-	6±0.02
01	2±0.00	-	-	5±0.02	3±0.02	-	-	2±0.01
Hexane								
10	10±0.02	-	-	7±0.02	9±0.02	-	-	8±0.04
05	5±0.02	-	-	5±0.01	7±0.01	-	-	5±0.02
01	2±0.01	-	-	2±0.01	3±0.01	-	-	2±0.00
Acetone								
10	10±0.3	-	-	11±0.05	9±0.2	-	-	7±0.03
05	6±0.02	-	-	5±0.01	8±0.01	-	-	5±0.03
01	4±0.01	-	-	5±0.02	5±0.01	-	-	3±0.01
Ethanol								
10	12±0.3	-	-	10±0.03	10±0.04	-	-	8±0.03
05	7±0.04	-	-	7±0.03	8±0.02	-	-	7±0.04
01	4±0.01	-	-	5±0.02	5±0.02	-	-	5±0.01

(Table 2) illustrate that Ethanol, Chloroform, Acetone and Hexane extracts of leaves and flowers of *Prosopis glandulosa* showed reactivity against *E. coli* and *S. aureus* at the concentration of 10, 5 and 1µg/ml, respectively. Hence, all above extracts of stem and seeds of *Prosopis glandulosa* showed no activity against *E. coli* and *S. aureus* at the concentration of 10, 5 and 1µg/ml, respectively. Hence, control DMSO also showed no any antibacterial activity against *E. coli* and *S. aureus*.

The minimum concentration at which it inhibits the growth of microorganisms (bacteria) usually known as the minimal inhibitory concentration (MIC) in vitro (Hossain *et al.*, 2012; Sokovic *et al.*, 2007). Different concentrations of stem, flowers and leaves such as 10, 5 and 1 µg/ml were prepared against two strains of bacteria. It is also shown in Table 2 that all extracts of flowers and leaves of *Prosopis glandulosa* observed to be effective in action against *E. coli*and *S. aureus* having a MIC of 1µg/ml, while all extracts of stem and

seeds of *Prosopis glandulosa* were observed ineffective against *E. coli* and *S. aureus* and hence no any MIC value were observed against two strains of bacteria.

Previous studies on of phytochemicals showed that aqueous (inorganic solvent) extracts of stem, seeds, flowers and leaves of *Prosopis glandulosa* specie revealed that proteins, terpenoid, saponins, phenols, carbohydrates, tannins, and alkaloids are present in all the parts of plant; tannins, phenols and flavonoids are present in flowers and leaves; However, glycosides and Coumarins were absent in all parts of plant. Comparing the achieved data from *Prosopis glandulosa* (Table 1) with those previously reported on *Prosopis glandulosa* shows that ethanol, chloroform, acetone and hexane extracts of stem, flowers, seeds and leaves of *Prosopis glandulosa* gave a much better results for a particular class of secondary metabolites such as proteins, saponins, tannins, alkaloids, phenols, flavonoids, terpenoid and carbohydrates.

4. CONCLUSION

The results of the phytochemical tests revealed that different extracts of *Prosopis glandulosa* stem, flowers, seeds and leaves gave a positive test for a particular class of secondary metabolites such as, saponins, tannins, alkaloids, phenols, proteins, flavonoids, terpenoid and carbohydrates. By using these phytochemicals, you can cure diseases without side effects. The present study also revealed that chloroform (CHL),hexane (HEX) acetone (AC) and ethanol (ETH) extracts from different parts of *Prosopis glandulosa* plants were found to be highly active in action against *E. coli* and *S. aureus*by a MIC of 1µg/ml. Therefore, this work justifies the use of *Prosopis glandulosa* in ethno medicine and in the future this plant may be valuable for the last potent antibacterial agent.

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