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# Phytochemical Composition, Antioxidant and Antimicrobial Activities of *Prosopis juliflora* (Sw.) Dc. Grown in State of Azad Jammu and Kashmir, Pakistan

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**Abstract.** Plants and their bioactive components have a long history of use in both traditional and modern medicine. Pharmaceutical medications and phytotherapy is based on substances that are produced from plants. According to the World Health Organization (WHO), 80% of the population still relies on these herbal medicines to cure their illnesses since they are simple to obtain, affordable and have less adverse effects than allopathic medications. Many *Prosopis* species have anthelmintic, antioxidant, antipyretic, antiulcer, cytotoxicity, antigiardial, amoebicidal and antipustule activity. *Prosopis juliflora* is wild plant native to Pakistan commonly known as jangli kikar has been used as a folk remedy for colds, dysentery, flu, inflammation, measles, sore throat, diarrhea and wound healing used in this study. The current research concludes the phytochemicals detection and biological prospective of different solvent extracts *i.e*. *n*-hexane, dichloromethane (DCM), ethyl acetate, 1-butanol and aqueous fractions of leaves, pods and bark of *P. juliflora*. Standard qualitative phytochemical screening exhibits that the *P. juliflora* contain alkaloids, glycosides, phenols, tannins, flavonoids, proteins, diterpenes, cumarins, steroids, terpenoids, anthraquinones, triterpenoids, quinones, carbohydrates and free amino acids. Quantitative analysis of total phenolic contents (TPC), total flavonoids contents (TFC) and antioxidant potency were determined by Folin-Ciocalteu method, aluminum chloride calorimetric method, DPPH and ABTS analysis respectively. Antibacterial activity was also carried out against six pathogenic gram positive and gram negative bacteria *i.e*. *Escherichia coli, Bacillus subtilus, Staphylococcus aureus, Bacillus subtilus*, *Klebsiella pneumoneae* and *Pseudomonas aeuroginosa* by disc diffusion method. These standard analyses elaborates that all parts of *P. juliflora* exhibits prominent antibacterial and antioxidant potential, proves the extraordinary medicinal significance of this wild and easily available plant of Pakistan.

**Keywords:** *Prosopis juliflora*, phytochemical screening, total phenolic contents, total flavonoid contents, antioxidant potential, antimicrobial activity

## Introduction

For thousands of years, plants have played an important role in sustaining human health and improving the quality of human life. Since the ancient time, people have collected plant for their necessary requirements, *i.e*. edible nuts, gums, fibres for shelter, medicines and clothing (Hapsari *et al*., 2017). Collecting high value things such as mushrooms and medicinal plants (black cohosh, ginseng) also resume in developed countries for economic reasons (Jones *et al*., 2002). Out of total 422,000 plant species worldwide it can be estimated that more than 70,000 plant species used for medicinal purposes (Bramwell, 2003). It is estimated by world health organization (WHO), Asian and African countries 80% people depend on the traditional herbal medicine for their health care and plant extracts are used as traditional herbal medicine (Cordell *et al*., 2001). Also, it is estimated by WHO that 25% of modern drugs have been derived from plants even in United States and minimum 7000 pharmaceutical compounds are derived from plants which are mostly used in modern pharmacopoeia. Many medicine used extensively derived from plants include Taxol, morphine, aspirin, reserpine and quinine (Dias *et al*., 2012). Herbalism is a type of traditional medicine or folk medicine that involves the use of plants and plant extracts. Herbal medicine is predicated on the idea that plants contain natural chemicals that can promote health and ease illness. Alkaloids, flavonoids, tannins and phenolic chemicals are the most important physiologically active plant constituents. Antioxidants derived from plant materials inhibit the action of free radicals, protecting the body from a variety of ailments. The antioxidant actions of medicinal plants may be attributable to the presence of phenolic compounds, which contain hydroxyl groups

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and confer the potential to donate hydrogen (Lakshmibai *et al*., 2015). *Prosopis juliflora* belongs to the Mimosaceae (Fabaceae) family, that grows extensively in the different areas of Pakistan *i.e*. Sindh and Punjab (Wazir *et al*., 2008). There are different names of *P. juliflora* in different languages in various regions of the world e.g. Honey mesquite (England), Algarobo (Latin America), Vilayti kikar (Marathi), Jangli kikar (Punjabi), Vilayti khejra (Haryana) and Mesquitebaum (German). The stem height of *P. juliflora* generally varies 1 to 3 m and the tree height varies from 4-12 m. The thickness of *P. juliflora* bark is 3-4 cm, dark gray with black shade. Branches contain thorns and leave in the form of cluster. Leaves are bipinnate and each pinna contains 12-25 pairs of dark green leaflets. The leaflets are normally 0.5-2.4 cm long, 0.15-0.52 cm wide. The inflorescence of *P. juliflora* is axillary, 80-100 mm long and mature flower bears yellow color (Tewari *et al*., 2000).

*P. juliflora* extracts have strong insecticidal potential against diseases like whitefly eggs and nymphs and mosquito vectors of malaria, dengue, chikungunya and filariasis (Rahul *et al*., 2015; Yadav *et al*., 2014; Bansal *et al*., 2012; Cavalcante *et al*., 2006). *P. juliflora* pod shows remarkable anti-bacterial activity against common pathogens like *E. coli* and *P. aeruginosa* (Tajbakhsh *et al*., 2015). The alkaloid rich fraction of leaves shows effective response against the fungi Cryptococcus (Valli *et al*., 2014). All major parts of *P. juliflora* mostly explored for ethno-medicinal uses. By boiling the wood chips of *P. juliflora*, a decoction is produced which is mostly employed in toning of the skin (Damasceno *et al*., 2017). Bark extract is used as a disinfectant and its gum can be used for treatment of eye problems. *P. juliflora* can also be used to prepare anti-AIDS and its anti-carcinogenic drugs (Dave and Bhandari 2013; Tiwari *et al*., 2013). The leaf, bark and flower extracts of *P. juliflora* possess antiplasmodial potential (Ravikumar *et al*., 2012). Juliflorine is an alkaloid, which is specific present in *P. juliflora* mostly used in suppressing acety-lcholinesterase and butyryl cholinesterase enzymes in a concentration dependent fashion and has potential against for Alzheimer's disease (Patocka, 2008; Choudhary *et al*., 2005). Syrup prepared from grounded pods of *P. juliflora* has various pharmacological values. It is very effective for children showing weight deficiency or retardation in their early growth. This syrup is also believed to increase lactation and to make other syrups, particularly for expectorants. Tea made from *P. juliflora* is found to be very effective for digestive disturbances and skin lesions (Qureshi *et al*., 2014). Experimental data shows that leaf extract of *P. juliflora* has found to be very effective against fungi *Pyricularia grisea* which causes disease rice blast (Kamalakannan *et al*., 2001). Its leaves can also control late leaf spot and rust diseases in groundnut (Kishore and Pande, 2005). The methanolic extracts of their leaves has found to be effective against tomato bacterial leaf spot disease as erythromycin and penicillin (Ahmed *et al*., 2007). Its alkaloid-enriched extracts mostly used against *Xanthomonas pathovars* as synthetic antibiotics (Raghavendra *et al*., 2010). Pellets obtain from its leaves incorporated in soil completely inhibits the colonies of *Fusarium* spp. *Macrophomina phaseolina* and *Rhizoctonia solani* (Ikram and Dawar, 2016). It also reduces the post-harvest anthracnose incidence in banana and mango (Deressa *et al*., 2015; Bazie *et al*., 2014). *P. juliflora* was chosen for present research because of its enormous ethnobotanical importance and year-round availability in Pakistan. The majority of the previous literature research has relied on the leaves of this plant outside the Pakistan but our aim of work to explore bark, pods and leaves of *P. juliflora*, which grows in Pakistan's Azad Jammu and Kashmir region.

## Material and Methods

**Sample preparation.** A healthy and disease free leaves, pods and bark of *P. juliflora* were collected from Mirpur Azad Jammu and Kashmir, Pakistan. These parts were then properly washed first with tap water and then again with ultra-distilled water. The plant pieces were dried for 7-9 days without sunshine under shade to avoid photochemical reactions. After carefully weighing the plant material, an electrical grinding machine is used to crush it. Finally, plant materials (leaves, pods and bark) were obtained in the fine powdered form. (Orhan *et al*., 2007a).

**Crude extract preparation.** Dry powdered bark, pods and leaves weighing 250 g of each were soaked in 1.0 dm3 of methanol in glass jars for seven days. Sample mixtures were shaken regularly to assure the proper mixing and removal of unwanted gases. Buckner's funnel along with filter paper used to filter the samples. Residue on filter media was again soaked in methanol and filtration procedure was repeated 2-3 times over and over by soaking the residue in methanol to obtain the maximum methanolic extracts of plant parts. After repeated filtration, the methanolic extracts were subjected

in Rotary Evapourator by maintaining temperature at 45 °C for concentration. Rotary evapourator was continuously used until only sticky and gummy was remained at the bottom of the round bottom flask. Crude extracts of *P. juliflora* were then stored at 4 °C in fridge to prevent from fungal attack. Later, the crude extracts were weighed by using digital weighing balance (Orhan *et al*., 2007b).

**Fractionation of crude extract by different solvents.** Crude extract was further subjected to fractionation by using solvents from nonpolar to more polar *i.e*. n-hexane, DCM, ethyl acetate and 1-butanol. The fractions obtained were concentrated by vacuum evapouration in rotary evapourator. The scheme for preparing different solvent fractions of crude extract is shown in Fig. 1 (Orhan *et al*., 2007b).

**Phytochemical analysis.** Phytochemicals such as alkaloids, diterpenes, glycosides, steroids, triterpenoids, quinone, anthraquinone, coumarin, phalobatannins, tannins, flavonoids, fats, saponins, phenolic compounds, proteins, carbohydrates and amino acids were detected in plant extracts by using the standard methodologies. The stock solutions of plant extracts were prepared in respective solvent (1mg/1 mL) used for different qualitative analysis.

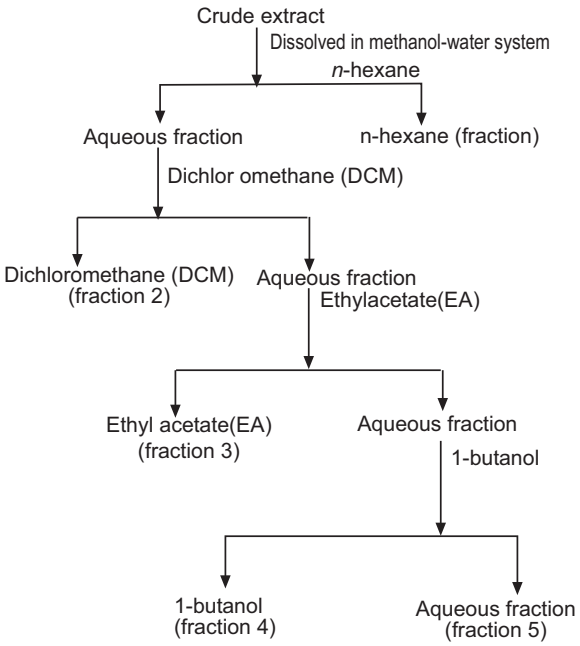



**Fig. 1.** Scheme of solvent fractions from crude extract.

**Test for alkaloids.** 5 mg of powdered plant extract was added to 10 mL of 5 % hydrochloric acid and filtered. Now this filtrate was then used for further analysis.

**Mayer's test.** Filtrate was then treated with Mayer's reagent (prepared by dissolving 0.78 g of $HgCl_2$ and 2.5 g of KI in 50 mL of $H_2O$). Formation of white precipitate suggested the presence of alkaloids in sample (Hadi and Bremner, 2001).

**Wagner's test.** Add 4-5 drops of Wagner's reagent (prepared by dissolving 1 g of I2 and 3 g of KI in 50 mL of $H_2O$) in the filtrates and the formation of red precipitates indicates the presence of alkaloids (Jha *et al*., 2012).

**Dragendorff's test.** The filtrate was mixed with Dragendorff's reagent (prepared by treating 0.38 g of $BiNO_3$ with 7.5 mL concentrated HCl. In separate beaker 3 g of KI was added in small quantity of $H_2O$ and stir it. Add both solutions give dark orange solution) in china dish) and the formation of red/brown precipitates indicates the presence of alkaloids (Raal *et al*., 2020).

**Hager's test.** Filtrate was mixed with 1.5 mL of Hager's reagent (prepared by dissolving 1.5 g of picric acid in 150 mL of $H_2O$). Formation of yellow coloured precipitates confirms the presence of alkaloids in the plant sample (Firdouse and Alam, 2011).

**Test for carbohydrates.** Plant extracts were added in distilled water and then filtered. The filtrates were then used for different analysis to detect carbohydrates.

**Molisch's test.** Add 2-4 drops of a-naphthol in filtrate and the formation of green-violet ring suggested the presence of carbohydrates in the plant extract (Elzagheid, 2018).

**Iodine test for starch.** 2 mL of filtrate was mixed with 3 mL of Lugol's solution ($I_2$/KI solution). Formation of black/blue colouration indicates the presence of starch (Guo *et al*., 2017).

**Benedict's test.** In this analysis, the filtrate was treated with 2.5 mL of Benedict's solution (prepared by treating 4.08 g of $Na_3C_6H_5O_7$, 2.5 g of $Na_2CO_3$, 8.65 g of $CuSO_4.5H_2O$ into the flask) and then boiled, formation of reddish brown precipitates shows the presence of carbohydrates (Elzagheid, 2018).

**Test for glycosides.** ***Keller-Kiliani test.*** In this test, few drops of acetic acid were mixed with plant extract followed by the addition of ferric chloride solution then add 2-3 drops of conc. $H_2SO_4$ very slowly and carefully.

Appearance of two layers lower with reddish brown and upper bluish green indicates the glycosides (Ashraf *et al*., 2022).

**Modified Borntrager's test.** In this test, 2 mL of plant extract react with 2 mL of $FeCl_3$ solution and 2 mL of dil. Hydrochloric acid in it. Heat it for five minutes and filtered it. Then add equal volume of benzene in it and shake it. Separate the organic layer and add ammonia solution in it. Ammonia layer turns pink or red suggested the vicinity of glycosides (Lalam, 2020).

**Test for phenols.** ***$FeCl_3$ test.*** 2 mL of plant extract mixed with 2-3 drops of 10 % $FeCl_3$ solution then appearance of bluish black or deep green colour shows the presence of phenols (Rahimah *et al*., 2019).

**Follin-ciocalteu test.** 2 mL freshly prepared sodium carbonate solution treated with 2.5 mL of plant fraction and add 3-4 of Follin-Ciocalteu chemical in it then formation of intense green color shows the presence of phenols (Lamuela-Raventós, 2018).

**Test for saponins.** ***Froth floatation test (Foam test).*** 2 mL of extract mixed with 5 mL of water then shake it well for twenty minutes. Appearance of 1.0 cm thick layer of froth shows the presence of saponins (Jain *et al*., 2020).

**Test for lipids.** ***Emulsion test.*** 2.5 mL of plant fraction was added in 3 mL of ethanol followed by the addition of 6 mL cold water in it. Formation of white cloudy emulsion displays the presence of lipids (Grunbaum *et al*., 2016).

**Test for tannins.** ***$FeCl_3$ test.*** Add 1.5 mL 1 % freshly prepared $FeCl_3$ solution in the 2.5 mL of plant fraction. Formation of brown-green colour displays the vicinity of tannins (Alinejhad *et al*., 2016).

**Tests for flavonoids.** ***Alkaline reagent test.*** 2.5 mL of plant fraction was first mixed with 3-5 drops of NaOH solution. As a result of this deep pale color produced which became disappeared by the addition of 3-4 drops of dilute HCl confirms the presence of flavonoids (Panchal and Parvez, 2019).

**Test for proteins.** ***Xanthoproteic test.*** 2.5 mL of plant extract mixed with 1.25 mL of Conc. $HNO_3$ then appearance of pale colour indicated the existence of proteins in sample (Sruthi and Indira, 2016).

**Test for diterpenes.** 1 mL water was treated with the 1 mL of plant fraction then add 3-5 drops of copper acetate solution in it. Appearance of green color shows the existence of diterpenes (Yadav *et al*., 2017).

**Test for coumarins.** 2.5 mL of plant fraction was treated with 3 mL of 12 % NaOH solution. Emergence of deep yellow colour indicates the presence of coumarin (Roghini and Vijayalakshmi, 2018).

**Test for steroids.** ***Salkowki's test.*** Plant extract was first treated with $CHCl_3$ and then filters. Add 2-5 drops of conc. $H_2SO_4$ carefully. The colour change from violet to blue/red indicates the presence of steroids (Panchal and Parvez, 2019).

**Test for phlobatannins.** Boil the plant extract with 1 % aqueous hydrochloric acid. Production of reddish precipitates suggested the vicinity of phlobatannins (Solihah *et al*., 2012).

**Test for terpenoids.** In this test, 3 mL of plant extract was mixed with 1.0 mL of $CHCl_3$ then add 1.5 mL conc. $H_2SO_4$. Formation of red ring indicates the proximity of terpenoids (Abdel-Rahman *et al*., 2019).

**Test for anthraquinones.** In this analysis, 2 mL of plant fraction mixed with 1 mL of benzene and filter the mixture, then add 4.5 mL of 12 % $NH_3$ solution in it. Emergence of violet color at lower phase displays the existence of anthraquinone (Sankhalkar and Vernekar, 2016).

**Test for triterpenes.** ***Salkowki's test.*** In this method, plant fraction was first treated with $CHCl_3$ and then filters. Add 2-5 drops of conc. $H_2SO_4$ carefully, the generation of greenish colour indicates the presence of steroids. (Sasikala and Sundaraganapathy, 2017).

**Test for quinones.** 1.5 mL of plant fraction was treated with 4-6 drops of alcoholic KOH. Formation of reddish to bluish colour was the indication of quinones (Maria *et al*., 2018).

**Test for free amino acids.** ***Ninhydrin test***. In this test, the plant extract was mixed with 3-4 drops of 0.2 % ninhydrin solution and heat it. Generation of dark purple colour shows the presence of amino acid (Guembe-García *et al*., 2021).

**Total phenolic contents (TPC).** Total phenolic contents (TPC) present in different solvent extracts of *Prosopis julifora* were calculated by the Folin-Ciocalteu standard method with slight modification (Lamuela-Raventós, 2018). The stock solutions of plant fractions were prepared in respective solvents (1 mg/1 mL). 1.5 mL

of plant fraction was treated with 3 mL of Folin-Ciocalteu solution (10%). Allow to stand this mixture for 12 min at 25 °C then adding 2.25 mL of $Na_2CO_3$ solution (7.5%) and then stand it 25 °C for 35 min at dark. The UV spectrophotometer was used to measure the absorbance of mixture by setting the wavelength at 765 nm. Similar steps were used to determine the absorbance of gallic acid at different concentrations to gain the calibration curve. TPC of all freshly prepared samples mixture were measured in mg/mL by using the calibration curve. The TPC was expressed in milligram of gallic acid equivalent per gram of dried extract.

**Total flavonoid contents (TFC).** Total flavonoid contents (TFC) of crude fractions were determined by aluminum chlouride colourimetric method (Shraim *et al*., 2021). The stock solutions of plant fractions were prepared in respective solvent (1 mg/1 mL). In brief, 1 mL of plant fraction was treated with 3 mL $H_2O$ and 2 mL 4.9 % $NaNO_3$ solution. After 5 min of incubation, 1 mL of 10 % $AlCl_3$ solution was added in the reacting mixture and stand it for 5 min, add 1 mL of NaOH and 2 mL doubly distilled $H_2O$. Allow standing the above mixture for 25 min at 25 °C and absorbance was determined at wavelength 510 nm. Similar steps were used to determine the absorbance of rutin at different concentration to gain the calibration curve. The TFC was expressed as milligram of rutin equivalent per gram of dry weight.

**Determination of antioxidant potential. *DPPH analysis.*** The antioxidant potency of the *P. juliflora* fractions were evaluated through DPPH test with some modification (Sirivibulkovit *et al*., 2018). Ascorbic acid was used as reference in this analysis. In this plant extracts were dissolved in their respective solvents (1mg/1mL) as stock solution. Four mM solution of DPPH was prepared in absolute methanol and different concentrations (20, 40, 60, 80 and 100 µL) were treated with 1.8 mL of stock DPPH solution. Now standing the reacting mixture for 25-30 min in the dark and the absorbance was determined at wavelength 517 nm. The percentage antioxidant potency was measured by this formula:

DPPH percentage antioxidant potential = $[(A_c - A_i) / A_c] 100$

$A_c$ = Absorbance value of stock DPPH solution

$A_i$ = Absorbance value of extract treated with DPPH stock solution

**ABTS analysis.** The antioxidant potency of *P. juliflora* fractions were determined by using the ABTS test with some modification for experimental purposes (Munteanu and Apetrei, 2021). Rutin was used as standard in this test. In this plant extracts were dissolved in their respective solvents (1mg/1mL) as stock solution. 2.45 mM solution potassium persulfate ($K_2S_2O_8$) was allowed to react with 7.0 mM ABTS solution at ideally 25 °C in totally dark condition for 18 h. When reaction completed, then the ABTS stock solution turns green in colour. The ABTS assay was started by mixing the 2 mL of ABTS solution with different concentrations of plant fractions (20, 40, 60, 80 and 100µL). The mixture was allowed reacts for 5 min at 25 °C and the absorbance was measured with help of UV spectrophotometer at wavelength 734 nm. The percentage antioxidant potency was determined by using following expression:

ABTS% antioxidant potential = $[(A_c - A_i) / A_c] 100$

$A_c$ = Absorbance value of stock ABTS Solution without plant extract

$A_i$ = Absorbance value of extract after treating with ABTS solution

**Antibacterial activity. *Sample preparation.*** Extracts of leaves, bark and pods of *P. juliflora* were dissolved in respective solvents as the solution has concentration 1mg/1mL. These concentrations of plant extract were directly applied against the pure pathogenic bacteria culture.

**Bacterial cultures.** The following pathogenic bacteria cultures were used to evaluate the antibacterial activity of *P. juliflora* extracts shown in Table 1. The source of all bacterial cultures was the "Biotechnology Department, Mirpur University of Science and Technology (MUST)".

**Growth or refreshing the bacteria.** L.B (Luria Bertani) Broth medium was used for refreshing the bacterial cultures. L.B Broth was prepared by mixing 0.6 g tryptone, 0.61 g sodium chloride and 1.6 g of yeast extract in 500 mL conical flask. Add 100 mL of distilled water in the flask, placed a magnetic stirrer in it. Stir the mixture on hot plate about 4-5 min at high temperature to get homogeneous mixture. After that put

**Table 1.** Bacteria used in antimicrobial activity

| Bacteria name | Type |
|---|---|
| *Klebsiella pneumoneae* | Gram-negative |
| *Pseudomonas aeuroginosa* | Gram-negative |
| *Escherichia coli* | Gram-negative |
| *Streptococcus aureus* | Gram-positive |
| *Bacillus subtilus* | Gram-positive |
| *Bacillus pumilus* | Gram-positive |

out, the magnetic stirrer from the flask and closed the mouth of flask by cotton wool and aluminum foil. On the other side took six Folcan tubes, clean them with distilled water, dry them and covered with paper tightly. Now, put flask containing L.B. Broth and six Folcan tubes in the autoclave for sterilization at 121 °C for 30 min. After sterilization, the six Folcan tubes and flask containing the L.B. Broth was opened in the Laminar flow which was washed with 70 % ethanol, air blower and U.V lamp was kept on. Put 10 mL of L.B Broth in each Folcan tube. Took a sterilized coil, dipped and round it on pure pathogenic pure culture and then transfer the bacteria from culture in the Folcan tube containing L.B Broth with the help of coil. Repeat this same process for all 6 bacteria. Now place these Folcan tubes in shaking incubator at 37 °C for 24 h. Change in colour/turbidity of L.B Broth in the Folcan tubes was the indication of bacterial growth (Belbekhouche *et al*., 2019).

**Media preparation.** L.B agar medium was used to grow bacteria on petri dish for antibacterial analysis. L.B agar was produced by treating 2 g tryptone, 1 g sodium chloride, 1 g yeast and 3 g agar in a flask and adds 200 mL distilled water in it. Mixed the mixture throughout with help of stirrer and then closed the mouth of flask with the help of cotton plug and aluminum foil. Now place the flask for sterilization in autoclave at 121 °C for 20 min (Mohammadi *et al*., 2019).

**Disc diffusion method.** L.B agar medium was poured in sterilized petri plates (15 mL/plate) and allow it stand for few minutes for solidification. When agar medium gets solidified in the plate then 10 µL of fresh bacterial culture was placed on it and spread the bacterial culture throughout the plate with help of sterilized spreader. After few minutes, sterilized discs of Whatman filter paper of 6 mm diameter was soaked in the plant extract solution and placed carefully on the prepared plate. Now plates were subjected to 24 h incubation period at 37 °C temperature. The standard antibiotic Ciprofloxacin was used as standard antibiotic. The antibacterial potential of plant extracts was evaluated by determining the diameter (mm) of zone of growth inhibition of bacteria (Vaishali and Geetha, 2018).

## Results and Discussion

**Phytochemical screening (qualitative).** The presented phytochemical analysis was performed on the n-hexane, DCM, ethyl acetate, 1-butanol and water fractions of leaves, bark and pods of *P. juliflora*. Through standard tests discussed in methodology section, the presence of alkaloids, diterpenes, carbohydrates, phlobatannins, glycosides, saponins, steroids, amino acids, triterpenoids, lipids, tannins, flavonoids, proteins, quinones, anthraquinones, coumarins, phenols and terpenoids were confirmed in all fractions. Phlobatannins was not present in any extract, while Saponin was not detected in pods extracts of *P. juliflora*. Through these standard phytochemical tests, it was confirmed that the *P. juliflora* occurs in Mirpur Azad Jammu and Kashmir, Pakistan rich source of bioactive compounds which assure the medicinal importance of this plant. The results are summarized in Table 2. In literature few phytochemicals of leaves ethanolic extract was analyzed.

**Total phenolic contents (TPC).** TPC present in different solvent extracts of *P. julifora* were determined by the Folin-ciocalteu standard analysis. The stock solutions of plant extracts were prepared in respective solvent (1mg/mL). Gallic acid was used in concentration ranging 0.005-0.025 mg/mL. The standard calibration curve ($R^2$ = 0.9878) was obtained by plotting its concentration against absorption value. TPC were measured by using this calibration curve shown in Fig. 2. Aqueous fraction of leaves and 1-butanol fractions of pods and bark shows maximum TPC (3.04, 3.14 and 4.3 mg/mL respectively) while n-hexane fractions of leaves, pods and bark shows minimum TPC (1.12, 0.61 and 0.96 mg/mL respectively). TPC results are summarized in Table 3.

**Total flavonoid contents (TFC).** TFC present in leaves, pods and bark of *P. julifora* were determined by the aluminum chloride colorimetric method.

Rutin was used in concentration ranging 0.005-0.025 mg/ mL to draw the standard calibration curve.The

**Table 2.** Phytochemical analysis of *P. Juliflora* (leaves, pods and bark)

| | | Leaves | | | | | Pods | | | | | Bark | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Solvent extracts | | | | | Solvent extracts | | | | | Solvent extracts | | | | |
| Phytochemical Constituents | Reagent/Test | *n*-Hexane | DCM | Ethyl acetate | 1-Butanol | Water | n-Hexane | DCM | Ethyl acetate | 1-Butanol | Water | n-Hexane | DCM | Ethyl acetate | 1-Butanol | Water |
| Alkaloid | Mayer's test | - | + | - | - | - | + | + | + | + | + | - | - | - | + | + |
| | Wagner's test | - | - | - | - | + | + | + | + | + | + | - | - | - | + | + |
| | Dragendorff's test | - | - | - | + | + | + | + | + | + | + | - | - | - | + | + |
| | Hager's test | - | + | - | + | + | + | + | + | + | + | - | - | - | + | + |
| Carbohydrate | Molish's test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | Iodine test for starch | + | + | - | + | - | + | + | + | + | + | + | + | + | + | + |
| | Benedict's test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | Killer-Kiliani test | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Glycoside | Borntrager's test | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Phenol | $FeCl_3$ test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | Follin-ciocalteu test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Saponin | Froth floatation test | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - |
| Lipids | Emulsion test | + | + | - | - | - | + | + | - | - | - | - | + | - | - | - |
| Tannins | $FeCl_3$ test | + | + | + | + | + | + | - | + | + | + | + | - | + | + | + |
| Flavonoids | Alkaline reagent test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Proteins | Xanthoproteic test | + | + | - | + | + | + | - | - | + | + | + | + | + | - | + |
| Diterpenes | Copper acetate test | - | + | + | + | + | + | - | - | + | + | - | - | + | + | + |
| Cumarines | 10 %NaOH | - | - | - | - | + | + | + | - | + | + | - | + | + | - | + |
| Steroids | Salkowki's test | - | - | - | - | + | + | + | - | - | + | + | - | - | + | - |
| Phlobatannins | 1 % HCl | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Terpenoids | $CHCl_3/H_2SO_4$ | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Anthraquinone | Benzene/$NH_3$ | - | + | - | + | + | + | + | + | - | + | + | + | + | + | - |
| Triterpenoids | Salkowki's test | - | + | + | + | + | - | - | + | + | - | + | + | + | - | - |
| Quinone | Alc. KOH | + | + | - | + | - | - | - | - | - | - | + | - | - | - | - |
| Amino acids | Ninhydrin test | + | + | - | + | - | + | - | - | + | + | + | - | - | - | - |
| | Present (+) | | | | | | | | Absent (-) | | | | | | | |

**Table 3.** Total phenolic contents in extracts of *Prosopis juliflora* leaves, pods and bark

| Plant extracts | Absorbance | TPC (mg/mL) | Absorbance | TPC (mg/mL) | Absorbance | TPC (mg/mL) |
|---|---|---|---|---|---|---|
| | Leaves | | Pods | | Bark | |
| *n*-Hexane | 0.55 | 1.12 | 0.33 | 0.61 | 0.48 | 0.96 |
| (DCM) | 0.68 | 1.41 | 0.75 | 1.58 | 1.21 | 2.61 |
| Ethyl acetate | 1.08 | 2.32 | 1.23 | 2.66 | 1.47 | 3.22 |
| 1-Butanol | 1.22 | 2.64 | 1.63 | 3.14 | 1.95 | 4.3 |
| Water | 1.40 | 3.04 | 1.01 | 2.15 | 1.26 | 2.73 |

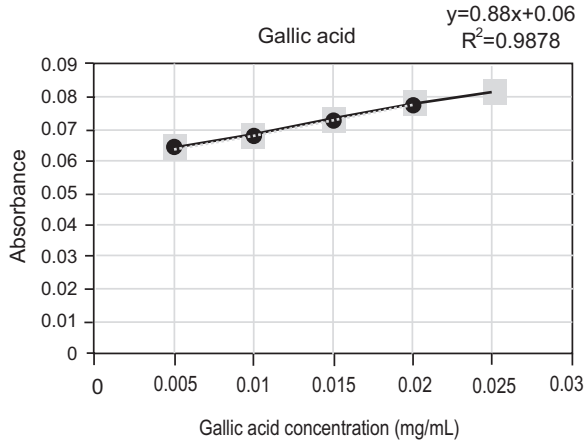



**Fig. 2.** Gallic acid calibration curve.

standard calibration curve ($R^2 = 0.9863$) was obtained by plotting its concentration against absorption value. The total flavonoids contents (TFC) were calculated by using rutin calibration curve shown in Fig. 3. The aqueous fraction of leaves and 1-butanol fractions of pods and bark shows maximum TFC (4.56, 3.89 and 5.21 mg/mL respectively) while n-hexane fractions of leaves, pods and bark shows minimum TFC (0.78, 0.38 and 0.51 mg/mL respectively). All the results of TFC are shown in Table 4.

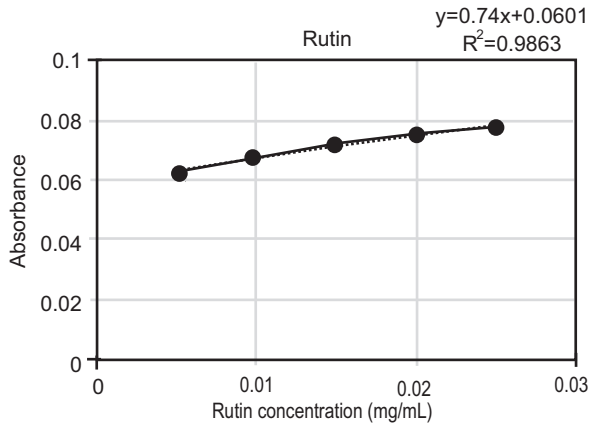
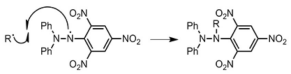



**Fig. 3.** Rutin calibration curve.

**Estimation of antioxidant potential. *DPPH Analysis*.** In this analysis, freshly prepared DPPH stock solution (in methanol) mostly exists in dark purple colour and shows its maximum absorption in spectrophotometer at 517 nm. The antioxidants present in the plant extracts will reacts with free radical of DPPH and its color fades ultimately it becomes colorless. Present work elaborates the antioxidant potential of leaves, Bark and pods of *Prosopis juliflora* with the help of DPPH radical scavenging assay. The aqueous fraction of leaves and 1-butanol fraction of pods and bark show maximum percentage antioxidant potential, while *n*-hexane fractions of leaves, pods and bark shows minimum percentage antioxidant. Ascorbic acid was used as standard in analysis. Results are summarized in Table 5. The following mechanism involved in DPPH action.



Purple colour (Radical form) Colourless (reduced form)

**ABTS analysis.** The mechanism of ABTS assay involves the formation and scavenging of ABTS free radical. The potassium persulfate being a good oxidizing agent reacts with ABTS and converted it into ABTS free radical form with solution appeared as dark green color. These ABTS free radical inhibited by the hydrogen given by the antioxidant present in the plant extract and the green colour of the solution fades ultimately solution becomes colourless. Rutin was used as standard in this analysis. Present work elaborates the antioxidant

**Table 4.** Total flavonoid contents in extracts of *P. juliflora* leaves, pods and bark

| Plant extracts | Absorbance | TFC (mg/mL) | Absorbance | TFC (mg/mL) | Absorbance | TFC (mg/mL) |
|---|---|---|---|---|---|---|
| | Leaves | | Pods | | Bark | |
| *n*-Hexane | 0.350 | 0.78 | 0.200 | 0.38 | 0.249 | 0.51 |
| (DCM) | 0.500 | 1.19 | 0.780 | 1.94 | 0.963 | 2.44 |
| Ethyl acetate | 0.820 | 2.05 | 0.920 | 2.32 | 1.783 | 4.66 |
| 1-Butanol | 1.380 | 3.57 | 1.500 | 3.89 | 1.989 | 5.21 |
| Water | 1.749 | 4.56 | 0.880 | 2.22 | 1.144 | 2.93 |

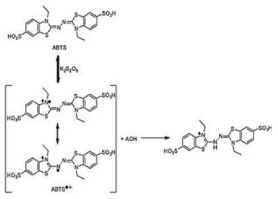
**Table 5.** DPPH antioxidant activity of *P. juliflora* leaves, pods and bark

| Solvents fractions | Conc. (µL) | Leaves | | Pods | | Bark | |
|---|---|---|---|---|---|---|---|
| | | DPPH inhibition | % inhibition | DPPH inhibition | % inhibition | DPPH inhibition | % inhibition |
| | 20 | 1.288 | 16.90 | 1.400 | 9.68 | 1.321 | 14.77 |
| *n*-Hexane | 40 | 1.257 | 18.90 | 1.371 | 11.55 | 1.274 | 17.81 |
| | 60 | 1.226 | 21.35 | 1.356 | 12.52 | 1.226 | 20.90 |
| | 80 | 1.198 | 22.71 | 1.325 | 14.52 | 1.207 | 22.13 |
| | 100 | 1.173 | 25.32 | 1.297 | 16.32 | 1.191 | 23.16 |
| Dichloromethane | 20 | 1.268 | 18.19 | 1.228 | 20.77 | 1.178 | 24.00 |
| (DCM) | 40 | 1.241 | 19.94 | 1.203 | 22.39 | 1.092 | 29.55 |
| | 60 | 1.219 | 20.90 | 1.183 | 23.68 | 1.023 | 34.00 |
| | 80 | 1.171 | 24.45 | 1.148 | 25.94 | 0.986 | 36.39 |
| | 100 | 1.154 | 25.55 | 1.086 | 29.93 | 0.945 | 39.03 |
| Ethyl acetate | 20 | 1.226 | 20.90 | 1.187 | 23.42 | 0.947 | 38.90 |
| | 40 | 1.202 | 22.45 | 1.128 | 27.22 | 0.901 | 41.87 |
| | 60 | 1.182 | 23.74 | 1.051 | 32.19 | 0.812 | 41.61 |
| | 80 | 1.144 | 26.19 | 1.033 | 33.35 | 0.717 | 53.74 |
| | 100 | 1.117 | 27.93 | 0.992 | 36.00 | 0.622 | 59.87 |
| 1-Butanol | 20 | 1.017 | 34.39 | 1.004 | 35.22 | 0.888 | 42.71 |
| | 40 | 0.940 | 39.35 | 0.921 | 40.58 | 0.799 | 48.45 |
| | 60 | 0.872 | 43.47 | 0.849 | 45.23 | 0.726 | 53.16 |
| | 80 | 0.765 | 50.64 | 0.717 | 53.74 | 0.674 | 56.52 |
| | 100 | 0.652 | 57.93 | 0.629 | 59.42 | 0.534 | 65.55 |
| Water | 20 | 1.000 | 35.48 | 1.201 | 22.51 | 1.108 | 28.52 |
| | 40 | 0.913 | 41.10 | 1.159 | 25.23 | 1.049 | 32.32 |
| | 60 | 0.818 | 47.23 | 1.081 | 30.26 | 0.995 | 35.81 |
| | 80 | 0.707 | 54.39 | 1.062 | 31.48 | 0.961 | 38.00 |
| | 100 | 0.587 | 62.13 | 1.022 | 34.06 | 0.910 | 41.29 |
| Ascorbic acid | 20 | 0.857 | 44.77 | 0.857 | 44.77 | 0.857 | 44.77 |
| | 40 | 0.699 | 54.90 | 0.699 | 54.90 | 0.699 | 54.90 |
| | 60 | 0.519 | 66.52 | 0.519 | 66.52 | 0.519 | 66.52 |
| | 80 | 0.341 | 78.00 | 0.341 | 78.00 | 0.341 | 78.00 |
| | 100 | 0.202 | 86.97 | 0.202 | 86.97 | 0.202 | 86.97 |

potential of leaves, Bark and pods of *Prosopis juliflora* with the help of ABTS assay. The water fraction of leaves and 1-butanol fractions of pods and bark shows maximum percentage antioxidant and n-hexane extracts of leaves, pods and bark shows minimum percentage antioxidant. The results of ABTS assay are summarized in Table 6. The following mechanism involved in ABTS action.

**Table 6.** ABTS antioxidant activity of *Prosopis juliflora* leaves, pods and bark

| Solvents fractions | Conc. (µL) | Leaves | | Pods | | Bark | |
|---|---|---|---|---|---|---|---|
| | | ABTS inhibition | Percentage inhibition | ABTS inhibition | Percentage inhibition | ABTS inhibition | Percentage inhibition |
| | 20 | 1.724 | 7.46 | 1.823 | 2.15 | 1.748 | 6.17 |
| | 40 | 1.695 | 9.02 | 1.798 | 3.49 | 1.721 | 7.62 |
| *n*-Hexane | 60 | 1.664 | 10.68 | 1.784 | 4.24 | 1.698 | 8.86 |
| | 80 | 1.631 | 12.45 | 1.721 | 7.62 | 1.677 | 9.98 |
| | 100 | 1.604 | 13.90 | 1.668 | 10.47 | 1.651 | 11.38 |
| Dichloromethane | 20 | 1.684 | 9.61 | 1.591 | 14.60 | 1.499 | 19.54 |
| (DCM) | 40 | 1.635 | 12.24 | 1.523 | 18.25 | 1.433 | 23.08 |
| | 60 | 1.561 | 16.21 | 1.505 | 19.22 | 1.346 | 27.75 |
| | 80 | 1.513 | 18.79 | 1.488 | 20.13 | 1.217 | 34.67 |
| | 100 | 1.460 | 21.63 | 1.412 | 24.21 | 1.004 | 46.11 |
| Ethyl acetate | 20 | 1.645 | 11.70 | 1.552 | 16.69 | 1.341 | 28.02 |
| | 40 | 1.578 | 15.30 | 1.472 | 20.99 | 1.181 | 36.61 |
| | 60 | 1.528 | 17.98 | 1.371 | 26.41 | 1.043 | 44.01 |
| | 80 | 1.450 | 22.17 | 1.259 | 32.42 | 0.918 | 50.72 |
| | 100 | 1.378 | 26.03 | 1.121 | 39.82 | 0.801 | 56.95 |
| 1-Butanol | 20 | 1.505 | 19.22 | 1.517 | 18.57 | 1.307 | 29.84 |
| | 40 | 1.478 | 20.66 | 1.411 | 24.26 | 1.148 | 38.38 |
| | 60 | 1.428 | 23.35 | 1.263 | 32.20 | 0.925 | 50.35 |
| | 80 | 1.334 | 28.39 | 1.074 | 42.35 | 0.790 | 57.59 |
| | 100 | 1.275 | 31.56 | 0.910 | 51.15 | 0.668 | 64.14 |
| Water | 20 | 1.421 | 23.72 | 1.567 | 15.88 | 1.492 | 19.91 |
| | 40 | 1.307 | 29.84 | 1.502 | 19.37 | 1.392 | 25.28 |
| | 60 | 1.228 | 34.08 | 1.486 | 20.24 | 1.323 | 28.98 |
| | 80 | 1.141 | 38.75 | 1.465 | 21.36 | 1.178 | 36.77 |
| | 100 | 1.007 | 45.95 | 1.341 | 28.02 | 0.961 | 48.42 |
| Rutin | 20 | 1.016 | 45.46 | 1.016 | 45.46 | 1.016 | 45.46 |
| | 40 | 0.896 | 51.90 | 0.896 | 51.90 | 0.896 | 51.90 |
| | 60 | 0.769 | 58.72 | 0.769 | 58.72 | 0.769 | 58.72 |
| | 80 | 0.658 | 64.68 | 0.658 | 64.68 | 0.658 | 64.68 |
| | 100 | 0.513 | 72.46 | 0.513 | 72.46 | 0.513 | 72.46 |



**Antibacterial activity.** Present study explains the potency of extracts obtained from three parts (leaves, pods and bark) of *P. juliflora* against different bacterial cultures. *Escherichia coli*, *Bacillus subtilus*, *Staphylococcus aureus*, *Bacillus subtilus*, *Klebsiella pneumoneae* and *Pseudomonas aeuroginosa* were used for the evaluation purposes.

In leaves extracts of *Prosopis juliflora*, the maximum antibacterial potential was shown by dichloromethane (DCM) fraction against *E. coli*, *B. pumilus, S. aureus* and *P. aeruginosa* with percentage inhibitions (91.67, 92.31, 75 and 113.6 % respectively), 1-butanol fraction against *E. coli, S. aureus*, *B. subtilus*, *P. aeruginosa* and *K. pneumoneae* with percentage inhibitions (75, 75, 71.4, 77.3 and 75 % respectively), ethyl acetate fraction of leaves with percentage inhibition 104.16 % and water fraction against *B. subtilus* with percentage inhibition 78.57 %.

In pods extracts of *Prosopis juliflora*, the maximum antibacterial potential was shown by dichloromethane (DCM) fraction against *S. aureus* with percentage

**Table 7.** Antibacterial analysis of *P. juliflora* leaves, pods and bark

| Plant extracts | Microbial cultures | Leaves | | Pods | | Bark | |
|---|---|---|---|---|---|---|---|
| | | Inhibition zone (mm) | Percentage inhibition | Inhibition zone (mm) | Percentage inhibition | Inhibition zone (mm) | Percentage inhibition |
| Control (Ciprofloxacin) | | 24 | 100 | 24 | 100 | 24 | 100 |
| *n*-Hexane | *Escherichia* | 14 | 58.33 | 10 | 41.67 | 0 | 0 |
| Ciprofloxacin) | *coli* | 22 | 91.67 | 12 | 50 | 0 | 0 |
| Dichloromethane | | 25 | 104.16 | 08 | 33.33 | 08 | 33.33 |
| Ethyl acetate | | 18 | 75 | 14 | 58.30 | 0 | 0 |
| 1-Butanol | | 13 | 54.17 | 08 | 33.33 | 08 | 33.33 |
| Water | | | | | | | |
| Control (Ciprofloxacin) | | 13 | 100 | 13 | 100 | 13 | 100 |
| *n*-Hexane | *Bacillus* | 07 | 53.85 | 07 | 53.85 | 06 | 46.15 |
| Dichloromethane | *pulmilus* | 12 | 92.31 | 06 | 46.15 | 08 | 61.54 |
| Ethyl acetate | | 08 | 61.54 | 08 | 61.54 | 09 | 69.23 |
| 1-Butanol | | 09 | 69.20 | 12 | 92.30 | 10 | 76.90 |
| Water | | 07 | 53.85 | 11 | 84.62 | 07 | 53.85 |
| Control (Ciprofloxacin) | | 12 | 100 | 12 | 100 | 12 | 100 |
| *n*-Hexane | *Staphylococcus* | 0 | 0 | 08 | 66.67 | 0 | 0 |
| Dichloromethane | *aureus* | 9 | 75 | 10 | 83.33 | 08 | 66.67 |
| Ethyl acetate | | 0 | 0 | 10 | 83.33 | 09 | 75 |
| 1-Butanol | | 9 | 75 | 11 | 91.70 | 11 | 91.70 |
| Water | | 0 | 0 | 07 | 58.33 | 0 | 0 |
| Control (Ciprofloxacin) | | 14 | 100 | 14 | 100 | 14 | 100 |
| *n*-Hexane | *Bacillus* | 07 | 50 | 0 | 0 | 0 | 0 |
| Dichloromethane | *subtilus* | 09 | 64.29 | 09 | 64.29 | 06 | 42.86 |
| Ethyl acetate | | 08 | 57.14 | 07 | 50 | 08 | 57.14 |
| 1-Butanol | | 10 | 71.40 | 10 | 71.40 | 06 | 42.90 |
| Water | | 11 | 78.57 | 0 | 0 | 0 | 0 |
| Control (Ciprofloxacin) | | 22 | 100 | 22 | 100 | 22 | 100 |
| *n*-Hexane | *Pseudomonas* | 12 | 54.55 | 10 | 45.45 | 0 | 0 |
| Dichloromethane | *aeruginosa* | 25 | 113.6 | 09 | 40.91 | 0 | 0 |
| Ethyl acetate | | 0 | 0 | 0 | 0 | 07 | 31.81 |
| 1-Butanol | | 17 | 77.30 | 14 | 63.60 | 08 | 36.40 |
| Water | | 07 | 31.82 | 0 | 0 | 0 | 0 |
| Control (Ciprofloxacin) | | 12 | 100 | 12 | 100 | 12 | 100 |
| *n*-Hexane | *Klebsiella* | 0 | 0 | 0 | 0 | 0 | 0 |
| Dichloromethane | *pneumoneae* | 0 | 0 | 07 | 58.33 | 0 | 0 |
| Ethyl acetate | | 08 | 66.66 | 10 | 83.33 | 08 | 66.66 |
| 1-Butanol | | 09 | 75 | 09 | 75 | 06 | 50 |
| Water | | 07 | 58.33 | 0 | 0 | 0 | 0 |

inhibition 83.33 %, ethyl acetate fraction against *S. aureus* and *K. pneumoneae* with percentage inhibitions (83.33 and 83.33 % respectively), 1-butanol fraction against *B. pumilus*, *S. aureus*, *B. subtilus*, *P. aeruginosa* and *K. pneumoneae* with percentage inhibitions (92.3, 91.7, 71.4 and 75 % respectively) and water fraction against *B. pumilus* with percentage inhibition 84.62 %.

In bark extracts of *Prosopis juliflora*, the maximum antibacterial activity was shown by ethyl acetate fraction against *S. aureus* with percentage inhibition 75 %, 1-butanol fraction against *B. pumilus* and *S. aureus* with percentage inhibitions (76.9 and 91.7 % respectively). This detailed antibacterial analysis on 5 solvent fractions of bark, pods and leaves of *P. juliflora* against 6 pathogenic cultures were not performed previous literature. Only ethanolic or methanolic fraction of leaves against few pathogens was reported. All the results of antimicrobial activities are shown in Table 7.

## Conclusion

The plants are rich source of natural constituents especially phenols, flavonoids, alkaloids and saponins. The pharmacological and biological activities of these classes signify the importance of plant as a potential candidate for deriving phytomedicines. Since antioxidants generated from plants lower the risk of chronic diseases including cancer and heart issues, several medication development companies place a heavy emphasis on plant research. In the present work phytochemical screening, antioxidant potential evaluation and antibacterial activity were performed on different solvent extracts of leaves, pods and bark of *P. julifora*. In phytochemical screening alkaloids, carbohydrates, glycosides, phenols, saponins, lipids, tannins, flavonoids, proteins, diterpenes, cumarins, steroids, terpenoids, anthraquinone, triterpenoids, quinones and amino acids were detected by using standard methods in different fractions of *P. juliflora*. Phlobatannins were not detected in any fraction and saponins were not present in pods of *P. juliflora*. Quantitative analysis of total phenolic and total flavonoid contents shows that the different extracts of *P. juliflora* are teemed with them which enhanced their antioxidant potential. The antioxidant potential was measured by DPPH and ABTS assays. The water extract of leaves and 1-butanol fractions of pods and bark shows maximum percentage antioxidant. Disc diffusion method was used to evaluate the antibacterial activity. Results concluded that the different extracts of *P. juliflora* display outstanding antibacterial activity bacteria. Our works have superiority on previous literature work because there is no research on *P. juliflora* of Mirpur, Azad Jammu and Kashmir, Pakistan, in the literature. The majority of earlier research relied solely on methanolic or ethanolic extracts taken from *P. juliflora* leaves. Very few phytochemicals were detected, antioxidant potential was assessed using traditional methods and only a small number of pathogens were subjected to antimicrobial activity studies in the literature. We choose the location Mirpur Azad Jammu and Kashmir, Pakistan for collection of plant because they are preferable to the work done there previously. We analyzed phytochemical screening of 18 different valuable phytochemicals. We also examined the therapeutic value of *P. juliflora's* bark and pods in addition to its leaves. Instead of using only one methanolic or ethanolic crude extract, we prepared five distinct solvent fractions and evaluated each fraction thoroughly. Finally we reached at conclusion from our study that the leaves, pods and bark of *P. juliflora* occurs at Mirpur Azad Jammu and Kashmir, Pakistan could be a rich source of natural phytochemicals, antioxidants and have great antibacterial potential that could have great importance as therapeutic agents.

## Acknowledgment

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**Conflict of Interest.** The authors declare that they have no conflict of interest.

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