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PHYTOCHEMICAL ANALYSES OF *PROSOPIS JULIFLORA* SWARTZ DC.

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Abstract

The air dried leaves of *Prosopis juliflora* Swartz DC., commonly known as Vilayati Kikar was analyzed for chemical composition. The results revealed the quantitative analysis of following classes of natural constituents: flavonoids, alkaloids, saponins, phenols and tannins in the range of (16 ± 0.39), (3.6 ± 0.06), (2.2 ± 0.23), (0.66 ± 0.11) and (0.33 ± 0.07) % respectively. The crude fiber and ash content were also analyzed and found as (17.5 ± 0.14) and (9.5 ± 0.08) % respectively whereas, pectic substances were calculated as (4.9 ± 0.18) %. The proximate analysis of the leaves for this robust plant widely distributed in Pakistan has been carried out for the first time and has revealed high concentration of flavonoids.

Introduction

The genus *Prosopis* belongs to the family *Leguminosae* (Mimosaceae). About 45 species of this genus are known. Some of these are commonly found in Pakistan. The genus *Prosopis* has many uses in folklore medicine (Saidman & Vilardi, 1987; Nadeem, 1992). Flavonoids are water soluble phytochemical showing the antioxidant, anticancer and anti-inflammatory activities. These prevent cells from oxidative damage and carcinogenesis. Flavonoids are also used to cure some heart related diseases (Hussain *et al.*, 2011a). Flavonoids occur virtually in all parts of the plant, the root, heart wood, sap wood, bark, leaf, fruit and flower (Harborne, 1975). Alkaloids and their derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal activities (Harisaranraj *et al.*, 2009). Alkaloid rich fractions of *P. juliflora* are antifungal and antibacterial (Aqeel *et al.*, 1988 & 1989). Saponins are widely distributed in nature, occurring primarily in the plant kingdom. These are generally known as nonvolatile surface active compounds having sweet or bitter, foaming, emulsifying, pharmacological, medicinal and haemolytic properties, as well as antimicrobial, insecticidal, and molluscicidal activities (Vincken *et al.*, 2007). Phenols and phenolic compounds prevent the platelets from clumping and have the ability to block specific enzymes that cause inflammation; these are antioxidant, immune enhancer and hormone modulators and are also used for curing skin infections and other wounds (Hussain *et al.*, 2011a). Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes. Tannins present in the cells of plants are inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens (Harisaranraj, 2009; Dash, 2008). The crude fiber content is commonly used as a measure of the nutritive value of poultry and livestock feeds. It is analyzed in various foods and food products to assess adulteration, quality and quantity (Stary, 1998). Pectin or pectic substances are complex polysaccharides universally present in the cell walls of plants, especially in the spaces between the cell walls where these act as binders to hold adjacent cell walls together. These are reported to have anti-hypercholesterolemic property and helps in excreting heavy metal and lowering fat absorption properties (Leung & Foster, 1996).

Proximate analyses provide an initial quantification of the different secondary metabolites in the plants (Hussain *et al.*, 2011b; Sew *et al.*, 2010; Gulfraz *et al.*, 2011). On these bases detailed analyses can be performed on the screened plants in search of new phytomedicines. In recent past, the group has also extracted fresh leaves of *P. juliflora* with methanol, followed by extensive solvent-solvent extraction and fractionation. All fractions were subjected to larvicidal and repellent activities against *Aedes agypti* L., larvae and pupae and found that benzene soluble, benzene insoluble and chloroform fractions are more active (Ibrahim *et al.*, 2011).

Materials and Methods

Plant material: The fresh leaves, collected during Sep-Oct 2011, dried in air (20 days) were manually separated from the stem of Vilayati Kikar (*Prosopis juliflora* Swartz DC.). The plant was identified by Dr. Sher Wali Khan and a voucher specimen (No. 84795) is deposited in the Herbarium, Department of Botany, University of Karachi. After drying, leaves were grinded by electrical grinder and analyzed by adopting gravimetric as well as spectrophotometric methods.

Determination of flavonoids: 20g of sample was extracted with 150 ml of 80% aqueous methanol at room temperature. The extract was filtered using Whatman filter paper no. 42. The filtrate was transferred into a dish, evaporated to dryness and weighed (Okwu & Ukanwa, 2007).

Determination of alkaloids: 3g of the sample was taken in a beaker and 200 ml of 20% ethanolic acetic acid was added. The beaker was covered and left for 4 hours. The extract was filtered and the volume was reduced to one quarter of the original, using water bath. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation get completed. The precipitates were left for settling, filtered, dried and weighed (Poornima & Ravishankar, 2009).

Determination of saponins: 25g of sample was taken in 250ml of 20% aqueous ethanol and heated over water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200ml of 20% ethanol. The combined extracts were concentrated to 40ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and extracted thrice with 20 ml of diethyl ether each. The ether layer was discarded whereas the aqueous part was extracted thrice with 60ml of *n*-butanol. The combined *n*-butanol extract was washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was evaporated over water bath, dried in the oven and weighed (Aliyu, 2008).

Determination of phenols: Fat free sample was prepared by extracting 5 g of plant material twice with 100 ml *n*-hexane for 4 hours. 50 ml diethyl ether was added twice to the sample and heated for 15 min each. Combined ether extract was cooled and filtered into a separating funnel. About 50 ml of the 10% NaOH solution was added twice and shook well. The organic layer was separated and washed thrice with 25 ml deionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and extracted twice with 50 ml dichloromethane (DCM). Consequently, the organic layer was collected, dried and weighed (Hussain *et al.*, 2011a).

Determination of tannins: 60mg of plant sample was shaken constantly for 60 s with 3ml of methanol in a test tube and filtered. The tube was quickly rinsed with an additional 3 ml of methanol and the contents were poured at once into the filtering funnel. The filtrate volume was made up to 50 ml with distilled water and analyzed within an hour. 3ml of 0.1 M $FeC1_3$ in 0.1 N HC1 was added to the extract, followed immediately by timed addition of 3ml of 0.008 M $K_3[Fe(CN)_6]$. The absorbance was measured after 10 min in 1cm cell at 720 nm on spectrophotometer using water as blank. A reagent blank sample was also analyzed. Results were expressed as tannic acid equivalents, using standard calibration curves prepared immediately before this analysis (Price & Butler, 1977).

Determination of crude fiber: 2g of sample was boiled with 200 ml of 0.255 N sulfuric acid for 30 min with anti-bumping chips. The material was cooled and filtered through muslin. The residue was washed with boiling water till it was no longer acidic. The residue was boiled with 200 ml of 0.313 N of sodium hydroxide solution for 30 min and again filtered through muslin cloth. The residue was washed successively with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol respectively. The residue was transferred to weighed crucible, dried in oven for 2 hours at 130 ± 2°C weighed and was ignited for 30 min at 600 ± 15°C, Cooled in a desiccator and reweighed. Loss on ignition was calculated as crude fiber (Patil & Gaikwad, 2011).

Ash content: 1 g sample was taken in previously ignited and weighed crucible. The material was incinerated at 450°C until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weighing (Anon., 1973).

Determination of pectic substances: 5g of sample was boiled with 30 ml of 0.01 N HCl for 30 min and filtered under suction. The residue was washed with hot water and filtrate was collected. Same process was repeated with 25 ml of 0.05 N HCl for 20 min and 25 ml of 0.3 N HCl for 10 min. The combined filtrate was collected, cooled and made up to 1000 ml. 100 ml of aliquot was pipetted into a beaker and 50 ml of distilled water was added. The solution was neutralized with 1 N NaOH using phenolphthalein as indicator. 5 ml of 1 N NaOH was added in excess with constant stirring and left to stand overnight. 10ml of 1 N acetic acid was added, kept on standing for 5 min, followed by the addition of 3.3 ml of 1 N anhydrous calcium chloride solution with constant stirring. The solution was left to stand for 1 hour, boiled for 1 to 2 min and filtered through Whatman filter paper No. 1. The precipitates were washed with almost boiling water until the filtrate was free from chloride. The residue was dried overnight at 100 °C in oven, cooled in a desiccator and weighed (Ranganna, 1979).

Results and Discussions

A number of compounds have already been isolated from *P. juliflora.* Some of these compounds are listed in Table 1 with their bioactivities. Up till now it is found that most of these botanicals are alkaloids. As shown, other chemical constituents, which included saponins, flavonoids, tannins and phenols etc., are not reported yet. The present study not only investigated their presence but also quantifies these important botanicals from leaves of *P. juliflora* for the first time.

The air dried leaves of *Prosopis juliflora* Swartz DC., were analyzed for its chemical composition and to the best of our knowledge proximate analyses of the leaves of this plant has not been carried out. The results revealed the presence of following important classes of natural constituents; flavonoids, alkaloids, saponins, phenols and tannins. The crude fiber and ash content were also analyzed and quantified whereas; pectic substances were calculated as (4.9 ± 0.18) % (Table 2).

Table 1. Promising bioactive botanicals from *P. juliflora* Swartz DC.

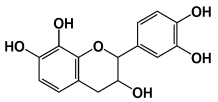
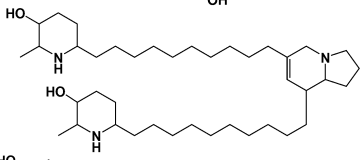
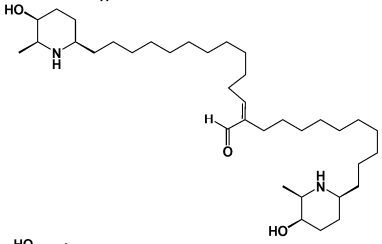
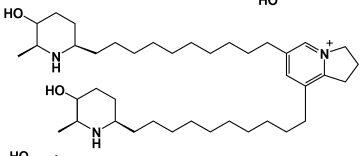
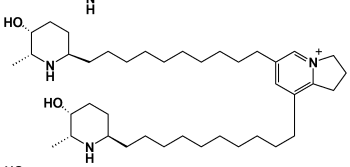
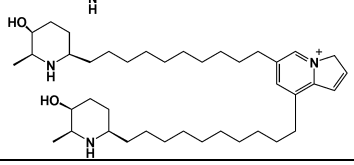
Name of compounds	Structures	Class of compound (source)	Pharmacological activity	References
(-)-Mesquitol		Flavonoid (Bark)	Antioxidant	Peter *et al.*, 2009
Juliflorine		Alkaloid (Leaves)	Antialzheimer agent	Choudhary *et al.*, 2005
Seco-juliprosopinal		Alkaloid (Leaves)	Plant growth inhibition	Hiroshi *et al*., 2004
Juliprosine		Alkaloid (Leaves)	Antifungal agent	Ahmad *et al.*, 1989
Isojuliprosine		Alkaloid (Leaves)	Antifungal agent	Ahmad *et al.*, 1989
Juliprosinene		Alkaloid (Leaves)	Antibacterial agent	Ahmad *et al*., 1989

Table 2. Quantitative phytochemical analyses of *Prosopis juliflora* Swartz DC.

Phytochemical	Concentration in %
Flavonoids	16 ± 0.39
Alkaloids	3.6 ± 0.06
Saponins	2.2 ± 0.23
Phenols	0.66 ± 0.11
Tannins	0.33 ± 0.07
Crude fiber	17.5 ± 0.14
Ash Content	9.5 ± 0.08
Pectic Substances	4.9 ± 0.18

Very high flavonoids content (16%) of *Propsopis juliflora* makes it a potential candidate bearing antioxidant and anticancer properties. Tannins and Phenols although found in low concentrations, (0.33 and 0.66% respectively) can synergize the antioxidant and anticancer potential of flavonoids. Phenols are reported to prevent the platelets from clumping and have the ability to block specific enzymes that cause inflammation. These also act as immune enhancers, anti-clotting and hormone modulators. Tannins in the plant cell inhibit hydrolytic enzymes like proteolytic macerating enzymes used by plant pathogens. The presence of alkaloids indicates the pharmacological importance of plant because alkaloids and their derivatives are used as basic medicinal agents due to their analgesic, antispasmodic and bactericidal activities. Table 1 presented the diversified bioactivity of alkaloids isolated from *P. juliflora*. The plant is an enriched source of alkaloids (3.6%). Saponins in the plant are responsible for antimicrobial, molluscidal and insecticidal activities and current analysis showed that leaves contain 2.2% saponins. High fiber content indicates the nutritive value of the plants. Younger leaves are palatable, although their intake is low. In no-choice droughts, cattle are found grazing foliage of this plant (Pasiecznik *et al*., 2001). Leaves of *P. juliflora* can also be used as low cost, highly nutritive and digestible forage after processing with molasses or other feeds (Heuze *et al.*, 2011). Ash content roughly represents minerals and 9.5% of ash content adds to the nutritive value of plant as fodder. Pectic substances are complex polysaccharides, present in the plant cell wall and act as binder. Pharmacologically pectins help in heavy metal excretion and act as anti-hypercholestrolemic.

Conclusion

The plant is a rich source of natural constituents, especially for flavonoids, alkaloids and saponins. The pharmacological and biological activities of these classes signify the importance of this plant as a potential candidate for deriving phytomedicines. In continuation of present work, further analyses and investigation has already been started using extraction and chromatography for isolation and purification of compounds, targeting flavonoids and alkaloids.

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