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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSES OF VARIOUS PARTS OF *PROSOPIS JULIFLORA*

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ABSTRACT

Objective: Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Different parts of *Prosopis juliflora (P.juliflora)*, such as leaves, pods, flowers, stem and seeds were selected for phytochemical screening to identify the different classes of metabolites. **Methods:** Solvent extract of the plant material with the help of different solvents taken were in the increasing order of polarity - Petroleum ether, benzene, chloroform, acetone, ethanol and water. **Results**: It is revealed that ethanol and water to be the best solvent in extracting metabolites from *P.juliflora*. Qualitative analysis of the total metabolite present in different parts of the plant, showed leaf and pod to be the richest source of plant metabolite, followed by flower, seeds and stem. **Conclusion**: Phytochemical analysis of the extracts revealed the presence of carbohydrate, proteins, tannins, phenolics, flavonoids, alkaloids, terpenes and steroids in most of the parts of *P. juliflora*.

Keywords: Phytochemical, *Prosopis juliflora*, Tannins, Flavonoids, Alkaloids, Terpenes, Steroids

INTRODUCTION

Despite the progress in conventional chemistry and pharmacology in the production of effective drugs, plants might provide a useful source of new medicines and may be used to replace existing drugs [1]. There are many natural products help to improve and care human health as crude drugs and herbal medicines [2]. Traditional medicine in general is turned out to be very useful in the discovery of natural products such as pharmaceutical drugs [3].

Plants have always been a source of natural product for the treatment of various diseases (Newman and Cragg, 2005). About 70–80% of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the World Health Organisation (Akerele, 1993). Plant-based medicines have an advantage over synthetic drugs in having low human toxicity and in addition, chemical diversity of secondary plant metabolites that results from plant evolution is equal or superior to that found in synthetic combinatorial chemical libraries. Despite having a wide historical background there are only a handful of plants that have been exhaustively studied for their potential value as a source of drugs [4].

Prosopis juliflora (*P.juliflora*) is a multipurpose leguminous tree, commonly known as Mesquite. It is native to Central and South America arid and semi-arid zones [5]. It was introduced into India in 1857. Now it occurs throughout the arid and semi-arid tropics of the Indian sub-continent due to its aggressive colonizing ability and adaptability [6]. In northern India, *Prosopis juliflora* is a pioneer species that rapidly colonizes denuded, abandoned ravines (Mwangi and Swallow, 2005). In the drylands of India, *P. juliflora* is considered one of the most valuable tree species (Pasiecznik et al. 2001). It serves as one of the main sources of fuel for the rural and urban poor in the country. It provides more than 90% of the fuelwood in some Indian villages (Sharma, 1981) because *P. juliflora* wood has excellent burning qualities [7].

The most important of these bioactive constituents of plants are terpenes, alkaloids, flavonoids and phenolic compounds (Gurib-Fakim, 2006). Alkaloids are pharmaceutically significant and are used as analgesic, antimalarial, antiarrythmic, antispasmodic, in the treatment of coughs and pain, in the treatment of gout, and as pupil dilatin (Buss and Waigh, 1995) [8]. 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) [9]. It has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat and in the healing of wounds (Hartwell, 1971). Several alkaloids have been isolated from leaf extracts having pharmacological properties (Ahmad et al., 1988,1989; Aqeel et al., 1989) [10]. Apart from alkaloids, other important compounds isolated from *P.juliflora* include flavone glycoside Patulitrin, Prosogerin D, Procyanidin, ellagic acid, tannin and polystyrenes (Rastogi and Mehrotra, 1993) [11].

MATERIALS AND METHODS

Sample collection

Green and dry plant parts leaf, stem, flowers, pod, seed samples of the exotic plant were collected from Chimanpura, Jaipur - Rajasthan. Samples were collected in plastic bags and then transported to the lab and stored at room temperature for later use [12].

Samples treatment and processing

The freshly cut plant parts were separated into component parts (stem and leaves) in the laboratory using a hand saw. All green and dried leaves, stem, pods were separated, then dried by spreading them out in the chemical hood at room temperature. After drying, the leaves were grinded by blender [13].

Extraction

50 grams of the powder of each plant part was weighed separately through electric balance, extracted successively with petroleum ether, benzene, chloroform, acetone, ethanol and water in order of increasing polarity using a soxhlet extractor for 72 h. Dry powder was taken in beaker and solvent was added to it so that the plant material got totally immersed in the solvent [14]. Soxhlet extraction apparatus which was protected from moisture absorption by a calcium chloride filled drying tube. Either one pure solvent or a mixture of solvents was used for each extraction. In most cases, the extraction period was 72 hours, but in few cases, the extraction period was 24 hours [15]. Powder was extracted with each solvent for four times using fresh solvents to exhaustively extract the constituents. At the completion of the extraction, the extract was filtered through Whatman No. 1 filter paper using a Bucher funnel and was transferred into a tared flask and the solvent was evaporated. Later, following the established protocols (Peach and Treacy, 1955) each of the test sample was processed further to used to evaluate the presence of carbohydrates, proteins, lipids, saponins, tannin, flavonoid, alkaloids etc. Before doing so each test sample was reconstituted in the respective solvents and divided into aliquots to perform the qualitative tests [16].

PHYTOCHEMICAL SCREENING

Phytochemical screening was performed using standard phytochemical procedures and the extracts were tested for carbohydrates, proteins, flavonoids, saponins, tannins, alkaloids, triterpenes and sterols.

Test for Carbohydrates

Fehling's test :To 2 ml of the aliquots, equal amount of freshly prepared Fehling's solution (prepared by mixing solutions A: 7:0 gm $CuSO_4$. $7H_2O$ in 100 ml distilled water and B: 24.0 g KOH and 34.6 gm Sodium Potassium tartarate in 100 ml distilled water) was added and the mixture was boiled in a water bath. The formation of rusty brown or red precipitate indicated the presence of carbohydrates.

Benedict's test: To 2 ml of the aliquots, a few drops of Benedict's solution (prepared by mixing 17.3 gm of sodium citrate, 10.0g of Na_2O_3 in 75 ml of distilled water, which was filtered and to this 17.3 gm of $CuSO_4.7H_2O$ dissolved in 20 ml of distilled water was added and the volume was raised to 100 ml with distilled water) was added, followed by boiling the mixture in a water bath. The sequential changes in the colour (green-blue-orange) indicated the presence of reducing sugars.

Test for Amino acid/ Protein

Ninhydrin test: To 2 ml of aliquots 2-3 drops of 1% ninhydrin reagent (in acetone) was added along with a few drops of pyridine and heated in boiling water bath for 10 minutes. Appearance of blue color shows the presence of amino acids.

Biuret test: To 2 ml of aliquots 2ml of 20% NaOH solution was added and mixed thoroughly. To this mixture 1 ml of 0.5% copper sulphate ($CuSO_4.5H_2O$) solution was slowly added. Formation of violet color confirms the presence of protein.

Millon's reagent test: To 2 ml of aliquots 2ml of millon's reagent was mixed. Formation of brick red precipitate indicates the presence of protein.

Test for saponins

Boiled 30 mg of extract with 5 ml water for two minutes. Mixture was cooled and mixed vigorously and left it for three minutes. The formation of frothing indicates the presence of saponins.

Test for tannins: To an aliquot of the extract added sodium chloride to make to 2% strength. Filtered and mixed with 1% gelatin solution. Precipitation indicates the presence of tannins.

Test for phenolic compounds: Formation of intense green, purple, blue or black colours with the addition of 1% ferric chloride solution to the extract.

Test for Triterpenes: 300 mg of extract mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution is then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicates the presence of triterpenes.

Test for steroids: 200 mg plant material was taken in 10 ml chloroform and then filtered. In 2ml filterate, 2ml acetic anhydride and small amount of H_2SO_4 was added, appearance of blue green ring indicates presence of steroids.

Test for alkaloids: 200 mg plant extract is dissolved in 10ml methanol and then filtered. In 1ml filtrate 6 drops of Dragondroff's reagent is added. Appearance of orange precipitate indicates presence of alkaloids.

Test for flavonoids: 5ml of dilute ammonia solution was added to the filtrate followed by concentrated sulphuric acid. A yellow colour observed indicates the presence of flavonoids [17].

RESULTS

The presence of metabolites obtained by extracting 50 g of plant material by various solvents is shown in Tables. Among different solvents used, ethanol was found to be the best solvent presenting highest number of metabolites from all parts of the plant. The high efficiency of ethanol can be attributed to its intermediate polarity leading to the extraction of both polar and non polar compounds (Harborne, 1984). Ethanol was followed by water and chloroform. Pet ether and acetone were found to be the least effective solvents in extracting phytochemicals, which could be due lesser amount of compounds in the plant, which could be dissolved in these solvents.

Table 1: Phytochemicals present in various parts of *P. juliflora* in ethanol extract.

Phytochemicals	Plant Parts				
	Leaf	Pod	Flower	Stem	Seed
Carbohydrates	+	++	+	-	+
Proteins	+	+	-	-	+
Tannin	+	+	-	-	+
Phenolics	+++	+++	+++	+	+
Flavonoid	+++	++	+++	+	++
Cardiac glycoside	-	-	-	-	-
Alkaloid	++	+++	++	-	+
Terpenes	+++	++	-	+	++
Steroids	+++	++	+	-	+
Saponin	-	-	-	-	+

+; low concentration, ++; moderate concentration, +++; high concentration, - ; absent

Table 2: Phytochemicals present in various parts of *P. juliflora* in aqueous extract.

Phytochemicals	Plant Parts				
	Leaf	Pod	Flower	Stem	Seed
Carbohydrates	+	+++	+	-	+
Proteins	+	++	+	+	+
Tannin	+	+	-	-	-
Phenolics	+++	+++	+++	+	++
Flavonoid	+++	++	+	+	++
Cardiac glycoside	-	-	-	-	-
Alkaloid	++	+++	++	-	+
Terpenes	+++	++	-	+	++
Steroids	+++	++	+	-	+
Saponin	-	-	-	-	+

+; low concentration, ++; moderate concentration, +++; high concentration, - ; absent

CONCLUSION

P. juliflora plant parts contain nutritional compounds pods and seeds can be considered as an alternate protein source to protein-energy-malnutrition (PEM) among the economically weaker people. The plant *P. juliflora* produces several compounds including alkaloid, tannin, phenolics, steroids, terpenes, flavonoid, proteins, sugars, and fatty acids. Some of these compounds may exhibit therapeutic activities such as antibacterial activity.

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