

PHYTOCHEMICAL ANALYSIS OF DIFFERENT PARTS OF *PROSOPIS JULIFLORA*

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ABSTRACT

Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Different parts of *Prosopis juliflora*, such as leaves, pods, flowers, stem and roots were selected for phytochemical screening to identify the different classes of secondary metabolites. Solvent extract of the plant material with the help of different solvents, hexane, chloroform, acetone, ethanol and water revealed ethanol and water to be the best solvent in extracting metabolites from *P.juliflora*. Quantitative 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, root and stem. Phytochemical analysis of the extracts revealed presence of tannins, phenolics, flavonoids, alkaloids, terpenes and steroids in most parts of *P. juliflora*.

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

INTRODUCTION

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 (Akerlele, 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 of 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.

The medicinal value of these plants can be observed from the chemical agents they possess which may alter certain physiologic actions in the human body. The most important of these bioactive constituents of plants are terpenes, alkaloids, flavonoids and phenolic compounds (Gurib-Fakim, 2006). Terpenes are used as insecticides and their pharmacological properties include antibacterial, antifungal, antihelmintic, antimalarial and molluscicidal (Gurib-Fakim, 2006), similarly phenolic compounds have a wide range of pharmaceutical activities such as anti-inflammatory, analgesic, antitumor, anti-HIV, anti-infective, vasodilatory, immunostimulant and antiulcerogenic (Potmeisel and Pinedo, 1995; Beisert and Schwarz, 2002). Recently, flavonoids have attracted interest due to the discovery of their pharmacological activities (Pietta, 2000; Brahmachari and Gorai, 2006). Alkaloids are pharmaceutically significant and are used as analgesic, antimalarial, antiarrhythmic, antispasmodic, in the treatment of coughs and pain, in the treatment of gout, and as pupil dilatin (Buss and Waigh, 1995).

Hence, knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of economic materials, such as tannins, oils, gums and precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies.

Prosopis juliflora, a member of family Leguminosae, is found in arid and semi-arid regions of India. It has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat and in 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). 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). In view of therapeutic importance of this plant, the present research work aims

at screening different parts of *P.juliflora* for the presence of important phytochemicals, so that full pharmacological potential of the plant could be exploited.

MATERIALS AND METHODS

Plant material

Plant material (leaf, pod, flower,) of *P. juliflora* were collected from their natural habitat in the Shekhawati regions of Rajasthan, India. The plant was identified with the help of "Flora of Rajasthan" (Shetty and Singh, 1993). Plant parts were washed thoroughly under tap water and air dried. Plant material was air dried under shade for two weeks and oven dried for 24 hrs at 40°C. The dried plant material was grounded to form fine powder and filtered through sieve of 345 micron pore size. The ground plant materials were stored in a refrigerator at 4°C.

Extract preparation

Plant sample was extracted with the help of various organic solvents (hexane, chloroform, acetone and ethanol) in the order of their increasing polarity and finally with distilled water.

500 grams of dry powder was taken in beaker and hexane was added to it so that the plant material gets totally immersed in the solvent. This whole setup was kept for 48 hours with frequent shaking. It was first filtered with a muslin cloth, then with whatman filter paper (No.1) finally centrifuged at 5000 rpm for 5 mins. Whole process was repeated 3 times and supernatant were collected and pooled together. Plant material left after filtration was air dried to evaporate the hexane completely and then was immersed with chloroform. It was extracted 3 times with chloroform. The above process was applied with acetone, ethanol and water and there supernatant were collected. Extracts were concentrated to 1/20th of their initial volume with the help of rotary evaporator (Buchi Rotavapor R-200/205) at 40 °C under different pressure conditions for the solvent. All the extract were kept in air tight bottle at 4°C.

Phytochemical screening

Extracts were subjected to phytochemical screening as described by method of Harborne, 1984

Test for saponins: Boiled 300 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 Phlobatanins: Deposition of red precipitate when extract was boiled with 1% aqueous hydrochloric acid indicates presence of phlobatanins

Test for phenolic compounds: Formation of intense green, purple, blue or black colours with 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: 200mg plant material was taken in 10 ml chloroform and then filtered. In 2ml filtrate, 2ml acetic anhydride and small amount of H₂SO₄ was added, appearance of blue green ring indicates presence of steroids

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

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

Test for cardiac glycoside: Keller-Kiliani test: 5ml of extract was treated with 1ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates the presence of deoxysugar characteristic of cardenolides.

RESULTS

The yield of extracts obtained by extracting 50 g of plant material by various solvents is shown in Table 1. Among different solvents used, ethanol was found to be the best solvent generating highest yield 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. Hexane 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.

leaf and pod were found to contain highest concentration of plant metabolite (15.72 g and 15.30 g respectively), followed by flower (13.68 g), root (10.48 g) and least in stem (7.95 g).

Table 1: Plant metabolites (in gram) extracted by various solvents from 50gm of dry plant material

Plant part	Solvents				
	Hexane	Chloroform	Acetone	Ethanol	Water
Leaf	1.31	2.85	0.96	5.63	4.97
Stem	0.82	1.51	0.68	3.08	2.48
Pod	1.05	2.23	0.72	5.55	5.84
Flower	0.95	2.62	0.77	5.04	4.30
Root	0.74	0.79	0.56	3.04	2.35

Table 2: Phytochemicals present in the extracts, obtained from various parts of *P.juliflora*

Phytochemicals	Plant Parts				
	Leaf	Pod	Flower	Stem	Root
Tannin	+	+	-	-	++
Phlobatannin	-	-	-	-	-
Phenolics	+++	+++	+++	+	++
Flavonoid	+++	++	+++	+	++
Cardiac glycoside	-	-	-	-	-
Alkaloid	+++	+++	++	-	+
Terpenes	+++	++	+	+	++
Steroids	+++	++	+	+	+
Saponin	-	-	-	-	+

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

The phytochemical screening of leaves, pod, flower, stem and root showed presence of various phytochemicals, however their concentration varied in different parts of the plants (Table 2). Phytochemical analysis revealed the presence of alkaloids, terpenoids, steroids, flavonoids, phenolics and tannins in leaf and pod extracts. The flower extract showed the presence of alkaloids, terpenoids, steroids, phenolics and flavonoids. Stem showed least number of compounds with phenolics, flavonoids, terpenes and steroids in low concentrations, while roots extracts showed the presence of tannins, phenolics, flavonoids, alkaloids, saponin, terpenes and steroids. Phlobatannin and cardiac glycoside were absent in all the tested parts of the plant, whereas saponin was found only in roots.

DISCUSSION

A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effect of herbal plants has been attributed by phytochemicals, which are the non-nutrient compounds (Gurib-Fakim, 2006). Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The phytochemical screening of the plant parts of *P.juliflora* showed that the leaves, pod, flower,

stem and root contain most of the secondary metabolites analyzed. They were shown to possess alkaloids, phenolic compounds, flavonoids glycosides, steroids, tannins and triterpenoids. Leaf extract was found to be the richest source of secondary metabolites, followed by pod and flower, as evident by the presence of high concentrations of most of the tested metabolites. These results are in accordance with high yield of leaf, pod and flower extracts with different solvents, whereas small amount of extract was obtained from stem and root. Among different solvents chosen ethanol was found to be the best solvent generating highest yield, this was because both the polar and nonpolar compounds get extracted in ethanol.

Phytochemical study on *P.juliflora* has been earlier reported (Rastogi and Mehrotra, 1993) and several compounds has been identified from different parts of the plant, however in current screening step, certain important metabolites are shown to be present, not only in reported part, but also in other plant parts, such as tannin reported in root (Kapoor, 1990) is shown in leaves and pod, alkaloids reported in leaves (Ahmad *et al.*, 1989) are also evident in flower and pod. Hence these plant parts could also provide a good source for isolation of important metabolites. Phenolic compounds and flavonoids are present in most part of the plant, as mentioned in earlier reports (Kapoor, 1990; Khan *et al.*, 2003).

The present screening study proved useful tool for the comparative studies of the amount of bioactive principles present in different parts of the plant. These data can help us to choose appropriate plant part for extraction of medically and therapeutically important phytochemically with greater quantity.

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