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Phytochemical Analyses of Various Parts of *Prosopis cineraria*

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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 cineraria*, such as leaves, pods, flowers, stem and seeds were selected for phytochemical screening to identify the different classes of metabolites. Solvent extract of the plant material with the help of different solvents in the increasing order of polarity was taken. Petroleum ether, benzene, chloroform, acetone, ethanol and water revealed that ethanol and water to be the best solvent in extracting metabolites from *P. cineraria*. 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 pods, leaves, flowers, seeds and stem. Phytochemical analysis of the extracts revealed presence of carbohydrates, proteins, tannins, flavonoids, alkaloids, terpenes and steroids in most of the parts of *P. cineraria*.

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

1. Introduction

Plants and plant products have been used as medicine from the ancient time. It is estimated by the World Health Organization that approximately 75-80% of the world's population uses plant medicines either partly or entirely as medicine [1]. Recently there has been a shift in universal trend from synthetic to herbal medicine, which we can say 'Return to Nature'. Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments. Nature has bestowed our country with an enormous wealth of medicinal plants, therefore India has often been referred to as the Medicinal Garden of the world (Sharma et al., 2008) [2]. Medicinal plants are the important source for the new chemical substances with potential therapeutic effects [3]. A promising multipurpose tree species commonly found is *Prosopis cineraria* (L.) Druce. *Prosopis cineraria* is known as a boon tree of the Thar desert by its multiple uses and its medicinal values. It is widely used for traditional therapeutic purposes [4].

Prosopis cineraria belongs to family Leguminosae, grows in dry and arid regions of Arabia and in India mainly Rajasthan, Haryana, Punjab, Gujarat, Western Uttar Pradesh and drier parts of Deccan and extends as far as South India. It is also known as Khejri, Jand, Janti and Sangri in Rajasthan, Jand in Punjab, Kandi in Sindh, Banni in Karnataka, Vanni or Jambu in Tamil Nadu, Sami and Sumri in Gujarat [5].

The tree holds an important place in the rural economy in the northwest region of Indian subcontinent. Since all parts of the tree are useful, it is called 'Kalptaru' [6]. It is also known as "Golden tree" or "Wonder tree" of the desert [7]. Various phytoconstituents like tannins (gallic acid), steroids (stigmasterol, campesterol, sitosteroletc), Flavone derivatives (Prosogerin A, B, C, D and E), alkaloids (spicigerine, prosophylline) etc has been isolated from the plant [8]. It is used as antihyperlipidemic, antioxidative, anthelmintic, antibacterial, antifungal, antiviral, anticancer, in treatment of dysentry, bronchitis, asthma, leucoderma, piles, leprosy, muscular tremors and wandering of the mind. It has analgesic and antipyretic activities. It is also used as a remedy for rheumatism. Applied on boils and blisters, mouth ulcers in livestock and on open sores on the skin, good for eye, prevent miscarriage, anti-diabetic agent, help in preventing

protein calorie malnutrition and iron calcium deficiency in blood [9].

It is an important component of desert Ecosystem of India as biomass producer and as Leguminous tree it enriches desert soil, fixes atmospheric nitrogen and provides a green coverage. It contributes to ecological stability of the region and providing extensive support to human beings, livestock and the nutrient deficient soils. Pods of this plant locally called "Sangri" are considered as dry fruit of desert and are one of the main ingredients of quintessential Rajasthani dish - The Panchkuta [10]. *P. cineraria* pods provide protein, iron, vitamins A and C and other micro minerals Unripe pods are also nutritious and are used to prepare curries and pickles [11].

2. Materials and Methods

2.1. Sample Collection

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

2.2. 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].

2.3. 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, each of the test samples 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].

3. 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.

3.1. 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 [17].

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 [17].

3.2. 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 [17].

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 [17].

3.3. 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 [17].

3.4. 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 [17].

3.5. 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 [17].

3.6. Test for Steroids

200 mg plant material was taken in 10 ml chloroform and then filtered. In 2ml filtrate, 2ml acetic anhydride and small amount of H_2SO_4 was added, appearance of blue green ring indicates presence of steroids [18].

3.7. Test for Alkaloids

200 mg plant extract is dissolved in 10 ml methanol and then filtered. In 1ml filtrate 6 drops of Dragondroff's reagent is added. Appearance of orange precipitate indicates presence of alkaloids [18].

3.8. 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 [18].

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

Phytochemicals	Plant Parts				
	Leaf	Pod	Flower	Stem	seed
Carbohydrates	-	+++	+	-	+
Proteins	+	++	-	-	+
Tannin	+	+	-	-	+
Flavonoid	+	++	+++	-	+ +
Cardiac glycoside	-	+	-	-	-
Alkaloid	++	++	++	-	+
Terpenes	+	+	-	+	++
Steroids	+++	_	+	-	++

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

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

Phytochemicals	Plant Parts				
	Leaf	Pod	Flower	Stem	seed
Carbohydrates	-	+++	+	_	+
Proteins	+	++	-	-	+
Tannin	+	+	-	-	+
Flavonoid	+	+	++	+	++
Cardiac glycoside	-	+	-	-	-
Alkaloid	++	++	++	-	++
Terpenes	+	+	-	+	+
Steroids	+++	-	+	-	+

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

4. Result and Discussion

The presence of metabolites obtained by extracting 50 g of plant material by various solvents is shown in both the Tables. Among different solvents used, ethanol was found to be the best solvent while pods and leaves 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 to lesser amount of compounds in the plant, which could be dissolved in these solvents.

5. Conclusion

P. cineraria pods and seeds can be considered as an alternate protein source for protein-energy-malnutrition among the economically weaker people. The plant *P. cineraria* produce 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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