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"Phytochemical Screening of Prosopis Cineraria (L.) Stem Bark and Leaves."

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Abstract : Prosopis cineraria is locally known as Khejri one of the most common tree of the Indian desert belonging to family Fabaceae. The plant is known as "Golden tree" or "Wonder tree" of the desert. Phytochemical screening is an important step which leads to the isolation of new and novel compounds. Stem bark and leaves of Prosopis cineraria were selected for photochemical 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. Benzene, Chloroform, Ethanol, methanol and Water revealed that Alcohol & Water to be the best solvent in extracting metabolites from Prosopis cineraria. The qualitative chemical analysis of extracts were found positive for alkaloids, proteins, carbohydrates, flavonoids, saponins and tannins in alcohol and aqueous solvent extracts. These studies provide referential information for correct identification and standardization of this plant material.

Keywords: Alkaloids, Flavonoids, Phytochemical, Solvent Extracts, Saponins, Tannins.

I. INTRODUCTION

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials **(1).**

In the present work we have the done the phytochemical screening of the stem of Prosopis cineraria plant.

Prosopis cineraria (L.) Druce (family: Fabaceae) commonly known as "Khejari" in Rajasthan. It is the State tree of Rajasthan, India **(2).** Khejari is the golden tree of Indian deserts, plays a vital role in preserving the ecosystem of arid and semi-arid areas. Since all the parts of the tree are useful, it is called kalpatru. It is also known as the 'wonder tree' and the 'king of desert' **(3-4)** It is commonly found in dry and arid regions of north-western India, southern India, Afghanistan, Pakistan, Arabia and Iran **(5).** The Prosopis cineraria plays an important role in the socio-economic development of the farmers. The wood of the prosopis is the main part of the tree that have economic importance it is used for fuel, firewood and charcoal. Dry pods of the prosopis is known as sangri and it is the main part of some rajasthani dishes and also have a broader range of pharmaceutical application like in pain, High cholesterol level, Diabetes, Anaemia, Kidney & Liver disorders. Cooked pods of Khejri are used as a functional food in Rajasthan, for the amelioration of numerous illnesses **(6-7).** 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 **(8).**

The leaf of the tree is known as loom and they have high nutrient content like Carbohydrate, Protein, Fat, Minerals and Vitamins **(9).** "Loong". Leaf paste of P. cineraria is applied on boils and blisters, including mouth ulcers in livestock **(10).** The smoke of the leaves is considered good for eye troubles **(11).** Leaf extracts of P.cineraria have shows antibacterial, anti-hyperglycemic, anti-hyperlipidemic and antioxidative activities **(12).**

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. The bark of the tree has abortifacient and laxative

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properties and is also used as a remedy for rheumatism in the central provinces.

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 **(13).** It is used as anti-hyperlipidemic, 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 **(14).**

II. HABIT & DISTRIBUTION

Tree of dry condition, found in sandy plains and grows abundantly on the dry, arid and exposed habitat like wasteland, cultivated lands, road sides and surrounding plains of hills. The most common occurrence of the prosodies is the dry places of the world it most commonly found at western Rajasthan, Delhi, Punjab and Gujarat state ofthe India.

Vernacular Names

Bengali (shami);

Gujarati (khijado,sumri,semru,sami,kamra);

Hindi (janti,banni,jand,sangri,shami,chaunkra,khejiri);

Sanskrit (jhind,jhand)

Trade name (jand,kandi,khejri);

Urdu (jandi,thand,kan)

III. MATERIALS AND METHOD

The brief description of the glass ware, instruments, reagents and chemicals which were used in the study are given below.

A. Glass Ware

Conical flask, Funnel, Glass rod, Pipettes, Measuring cylinder, Reagent bottle, Test tube, Beaker, Slide, Brush, Dropper, Crucible, Capillary tube, Iodine flask.

B. Instrument

Water bath, Electronic weighing machine, Rotatory flask shaker, Hot air oven, HPTLC, Fluorescence microscope (Binocular microscope), Soxhlet extraction unit, Desiccato,r Test sieves, Mixer Grinder, Spatula, Heating mantle, Needle, Microtome machine.

C. Reagents

Fehling's solution A & B, Dragendorff's reagent, Mayer's reagent, Alpha nephthol solution, Wagner's reagent, Anthrone's reagent, Folin Denis reagent, Million's reagent, Hager's reagent, Aqueous basic lead acetate solution, Ammonia solution, Phosphoric acid.

D. Chemicals

10% Sodium hydroxide, Chloroform, Concentrate sulphuric acid, Ethanol, Distilled water, 50% Sulphuric acid, 50% Nitric acid. Sod. Tungustate, Iodine water, 1.5% Hydrochloric acid, 2M Hydrochloric acid, Potassium iodine, 3% Copper sulphate, Concentrate nitric acid, 0.2% Ninhydrin solution, Acetone, Sodium bicarbonate, Glycerin, 5% ferric chloride, 1N NaOH in methanol, 1N NaOH in water, Glycial acetic acid, 1N Hydrochloric acid, 50% HCl, 50% ammonia solution, 50% KOH.

E. Sample Collection and Powder Preparation

The plant Prosopis Cineraria is widely found throughout the India. For our work plant shami was collected from university field at chitrakoot, Satna, M.P., India.The plant was identified by Dr. Manoj Tripathi, Botanist. The stem was cleaned, dried and grounded to fine powder by using electric mill grinder. The powder was sieved with 30# mess size (No.). Finally the powder was stored in air tight container to prevent moisture and was used for further analyse. In the present study I have done the following tests.

- Determination of moisture content (Loss on drying, LOD at 105oC)
- Determination of ethanol soluble extractive (ESE)
- Determination of water soluble extractive (WSE)
- Determination of chloroform soluble extractive
- Determination of benzene soluble extractive
- Determination of methanol soluble extractive
- Determination of total ash
- Fluorescence Study
- Preliminary Phytochemical analysis
- Quantitative determination
- HPTLC fingerprint profile

F. Physicochemical Parameters

The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica. The total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. The Loss on drying test is designed to measure the amount of water & volatile matters in a sample when the sample is dried under specified conditions. The extractive value of the powdered material was analyzed successively with the different known solvents i.e. Benzene, Chloroform, Ethanol, Methanol and Water.

G. Preparation of Sample Extracts

For analysis of phytochemicals, macerated the 2g air dried powder with 100 ml alcohol and distilled water separately in a closed iodine flask for 24 hours, shaking frequently during first 6 hours and allowed to stand for 18 hours. Then the solution was filtered by using whatman filter paper No.1.Both the extracts (alcoholic & aqueous) were used for the analysis of different bioactive constituents.

H. Preliminary Phytochemicals Screening

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like Alkaloids, Carbohydrates, Proteins, Resins, Saponins, Starch, Flavonoids, Steroids, Coumarins, Tannins, and Phenolic compounds was analysed by following methods.

a). Test for Alkaloids

- Mayer's test: Added few drops of Mayer's reagents to 1 ml of the acidic, aqueous extract of the powder.
- Dragendroff's test: Dissolved few mg of alcoholic or aqueous extract of powder in 5 ml of distilled water, added 2 M HCl until an acid reaction occurs, then added 1 ml of Dragendorff's reagent.
- Hager's test: To 1 ml of alcoholic extract of powder and added few drops of Hagers reagent.

b). Test for Carbohydrate

- Anthrone's test: To 2 ml of anthrone's test solution, added 0.5 ml of aqueous extract of powder.
- Fehling's test: To 2 ml of aqueous extract of powder, added 1 ml of mix. of equal parts of Fehling's solution A and Fehling's solution B and boiled the content of the test tube for few minutes.
- Molish's test: To 2 ml of aqueous extract of the powder, added 2- drops of freshly prepared 20% alcoholic solutions of naphthol and Poured 2 ml of conc. H_2SO_4 so as to form a layer below the mixture.

c). Test for Proteins

- Biuret test: To 1 ml of hot aq. extract of sugarcane powder, added 5 – 8 drops of 10% w/v NaOH solution followed by 1 or 2 drops of 3% w/v $CuSO_4$ solutions.
- *Xantho protein test:* A little residue was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it.
- Ninhydrin test: The Ninhydrin reagent is 0.1% w/v solution of Ninhydrin in n-butanol. A little of this reagent was added to the test extract.

d). Test for Resins

Dissolved 1 ml of extract in 1 ml of acetone and poured the solution into 5 ml distil water.

e). Test for saponins

- Foam test: To 5 ml of aq. extract of Sugarcane powder, added few drops of sodium bicarbonate. Shaked vigorously and left it for few minutes.

f). Test for Starch

Dissolved 0.015g of iodine and 0.075g of KI in 5 ml of distilled water and added 2 -3 drops of an aq. extract of Sugarcane powder.

g). Test for Flavonoids: Shinoda's Test

To 0.5 ml of alcoholic extract of sugarcane powder, added 5-10 drops of concentrate HCl followed by small 0.5g of 'Mg' metal.

- *Alkaline reagent test:*To the test solution added sodium hydroxide solution.

h). Test for Steroids

- Salkowski's reaction: Added 1 ml of concentrate H_2SO_4 to 2 ml of chloroform extract of the Sugarcane powder carefully, from the side of test tube.

i). Test for Coumarins:

Plant extracts (1ml) were treated with alcoholic sodium hydroxide.

- *Test for tannins: Ferric Chloride test***:** To 1 – 2 ml of extract of Sugarcane powder, added few drops of 5% $FeCl_3$ solutions.

j). Test for phenolic compounds

The extract was taken in water and warmed; to this added 2 ml of ferric chloride solution and observed.

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k). Fluorescence characters of the plant powder

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study. The treatment of powdered sample with different chemical reagents reveals the presence of different chemical constituents with fluorescence character in UV light. Therefore, the results obtained from the present fluorescent studies will also help to check any impurities present in plant powder.

IV. QUANTITATIVE ANALYSIS

Carbohydrates, Proteins, Tannins, Alkaloids and Flavonoids was carried out using for standard methods .

1. Estimation of total Carbohydrate by Anthrone method **(20)**
2. Protein estimation by Lowry's Method **(21-22)**
3. Estimation of Tannin by Folin-Denis method **(20)**
4. Estimation of Alkaloids by Dragendorff's Reagent method **(23)**
5. Determination of Flavonoid by Bohm and Kocipai – Abyazan (1994) **(24)**

V. RESULTS & DISCUSSION

The observations are tabulated in the given tables below:-

Sr.No.	Parameters	% w/w for leaves	% w/w for bark
1	Total Ash value	9.0975	7.695
2	Loss on drying	3.893	4.0856

Table1: Physicochemical Parameters of Powdered of Stem Bark & Leaves of Prosopis Cineraria(L.).

Sr.No.	Solvent extract	Yield %w/w for leaves	Yield %w/w for bark
1	Benzene	4.965	3.885
2	Chloroform	5.09	3.25
3	Ethanol	12.985	4.87
4	Methanol	21.65	5.495
5	Water	25.375	11.69

Table 2: Extractive Values of Stem Bark & Leaves of Prosopis Cineraria(L.)

S. No.	Powder + Reagents	Observation in day light	Observation at 366 nm
1.	Powder as it	Light green colour	Green colour
2.	Powder + 1N HCl	Light green colour	Green colour
3.	Powder + Concentrate H_2SO_4	Dark brown	Black colour
4.	Powder + Glacial acetic acid	Green colour	Red colour
5.	Powder + Iodine water	Green colour	Black colour
6.	Powder + 50% KOH	Greenish colour	Muddy colour
7.	Powder + 1N NaOH in H_2O	Yellowish colour	Black colour
8.	Powder + 1N NaOH MeOH	Yellow colour	Orange colour
9.	Powder + 50% HNO_3	Brown colour	Black colour
10.	Powder + 50% H_2SO_4	Yellow colour	Dark green colour

Table 3: Fluorescence Analysis of Leaves of Prosopis Cineraria(L.)

S. No.	Powder + Reagents	Observation in day light	Observation at 366 nm
1.	Powder as it	Muddy colour	Bluish colour
2.	Powder + 1N HCl	Muddy colour	Black colour
3.	Powder + Concentrate H_2SO_4	Dark brown colour	Black colour
4.	Powder + Glacial acetic acid	Muddy colour	Green colour
5.	Powder + Iodine water	Muddy colour	Green colour
6.	Powder + 50% KOH	Muddy colour	Green colour
7.	Powder + 1N NaOH in H_2O	Muddy colour	Green colour
8.	Powder + 1N NaOH MeOH	Muddy colour	Green colour
9.	Powder + 50% HNO_3	Brown colour	Black colour
10.	Powder + 50% H_2SO_4	Black colour	Dark green colour

Table4: Fluorescence Study of Steam Bark.

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S. No.	Name of experiments	Observation	Result
1.	Alkaloids		
	a. Mayer's test	Pale yellow colour appear	Present
	b. Wagner's test	Yellow colour appear	Present
	c. Dragendorff's test	Light yellow colour appear	Present
2.	Carbohydrate		
	a. Anthrone's test	Green colour appear	Absent
	b. Fehling's test	Brick-red colour appear	Present
	c. Molish's test	Blue-white ring is appear	Present
3.	Proteins		
	a.Ninhydrin test	purple colour appear	Present
	b.Xanthoproteic test	Yellow colour appear	Present
4.	Resins	Turbidity are seen	Present
5.	Saponin test		
	Foam test	Honey comb-like structure are formed	Present
6.	Starch test	Greenish colour appear	Absent
7.	Tannin a. Feeric chloride test b. Lead acetate test c. Potassium dichromate test	 Dark green colour appear Yellow ppt appear Dark yellow colour appear	 Present Present Present
8.	Phenolic compound	Yellow brown colour appear	Absent
9.	Flavonoid test		
	a. Shinoda's test	White foam appear	Absent
	b. Alkaline reagent test	Yellow colour appear	Present
10.	Steroid test		
	Salkowski's test	Green colour appear	Absent
11.	Coumarins test	Dark yellow colour appear	Present

Table 5: Preliminary Phytochemical Screening of Aqueous Extract of Leaves

S. No.	Name of experiments	Observation	Result
1.	Alkaloids		
	d. Mayer's test	White colour appear	Present
	e. Wagner's test	Yellow colour appear	Present
	f. Dragendorff's test	Dark yellow colour appear	Present
2.	Carbohydrate		
	d. Anthrone's test	Green colour appear	Present
	e. Fehling's test	Blue colour appear	Absent
	f. Molish's test	Red violet ring is appear	Present
3.	Proteins		
	a.Ninhydrin test	purple colour appear	Present
	b.Xanthoproteic test	White colour appear	Absent
4.	Resins	Turbidity are seen	Present
5.	Saponin test		
	Foam test	Honey comb-like structure are formed	Present
6.	Starch test	Yellow colour appear	Absent
7.	Tannin d. Feeric chloride test e. Lead acetate test f. Potassium dichromate test	 Yellow colour appear White ppt appear Dark yellow colour appear	 Absent Present Present
8.	Phenolic compound	Yellow colour appear	Absent
9.	Flavonoid test		
	a. Shinoda's test	Magenta colour appear	Present
	b. Alkaline reagent test	Yellow colour appear	Present
10.	Steroid test		
	Salkowski's test	Yellow colour appear	Absent
11.	Coumarins test	Dark yellow colour appear	Present

Table 6: Preliminary Phytochemical Screening of Aq. Extract of Stem Bark

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S. No.	Name of experiments	Observation	Result
1.	Alkaloids		
	g. Mayer's test	Green colour appear	Absent
	h. Wagner's test	Pale yellow colour appear	Present
	i. Dragendorff's test	Orange colour appear	Present
2.	Carbohydrate		
	g. Anthrone's test	Green colour appear	Present
	h. Fehling's test	Brick-red colour appear	Present
	i. Molish's test	Blue-red ring is appear	Present
3.	Proteins		
	a.Ninhydrin test	Bluish colour appear	Present
	b.Xanthoproteic test	Yellow colour appear	Present
4.	Resins	Turbidity are seen	Present
5.	Saponin test		
	Foam test	Honey comb-like structure are not formed	Absent
6.	Starch test	Greenish colour appear	Absent
7.	Tannin c. Feeric chloride test d. Lead acetate test e. Potassium dichromate test	 Dark green colour appear Green ppt appear Greenish yellow colour appear	 Present Present Present
8.	Phenolic compound	Yellow colour appear	Absent
9.	Flavonoid test		
	a. Shinoda's test	Magenta colour appear	Present
	b. Alkaline reagent test	Yellow colour appear	Present
10.	Steroid test: Salkowski's test	Yellow colour appear	Absent

Table 7: Preliminary Phytochemical Screening of Ethanolic Extract of Leaves

S. No.	Name of experiments	Observation	Result
1.	Alkaloids		
	j. Mayer's test	Pale yellow colour appear	Present
	k. Wagner's test	Yellow colour appear	Present
	l. Dragendorff's test	pale yellow colour appear	Present
2.	Carbohydrate		
	j. Anthrone's test	Green colour appear	Present
	k. Fehling's test	Blue colour appear	Absent
	l. Molish's test	Red violet ring is appear	Present
3.	Proteins		
	a.Ninhydrin test	purple colour appear	Present
	b.Xanthoproteic test	Light yellow colour appear	Present
4.	Resins	Turbidity are seen	Present
5.	Saponin test Foam test	Honey comb-like structure are not formed	Absent
6.	Starch test	Yellow colour appear	Absent
7.	Tannin f. Feeric chloride test g. Lead acetate test h. Potassium dichromate test	 Green colour appear Yellow ppt appear Dark yellow colour appear	 Present Present Present
8.	Phenolic compound	Yellow colour appear	Absent
9.	Flavonoid test		
	c. Shinoda's test	Pink colour appear	Present
	d. Alkaline reagent test	Yellow colour appear	Present
10.	Steroid test		
	Salkowski's test	Yellow colour appear	Absent

Table 8: Preliminary Phytochemical Screening of Ethanolic Extract of Stem Bark

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Sr.No.	Name of constituents	mg/ml or %
1	Tannin	0.28715 mg/ml
2	Total Carbohydrates a. For 0.5 ml b. For 1 ml	 a. 1.5389 mg/ml b. 1.465 mg/ml
3	Protein a. For 0.1 ml b. For 0.2 ml	 a. 1.3691 mg/ml b. 3.220 mg/ml
4	Alkaloid	43.3793 mg/ml
5	Flavonoid	6.1652 %

Table 9: Quantitative Analysis of Stem Bark of Prosopis Cineraria (L.).

Sr.No.	Name of constituents	mg/ml or %
1	Tannin	1.35437 mg/ml
2	Total Carbohydrates c. For 0.5 ml d. For 1 ml	 c. 1.1278 mg/ml d. 1.0265 mg/ml
3	Protein c. For 0.1 ml d. For 0.2 ml	 c. 2.064 mg/ml d. 4.077 mg/ml
4	Alkaloid	43.3793 mg/ml
5	Flavonoid	21.0386 %

Table10: Quantitative Analysis of Leaves of Prosopis Cineraria (L.).

VI. DISCUSSION

Table-1 reveals the moisture contents at 105 ^{0}C and the total ash value and it was 3.893% & 9.0975% for leaves and 4.0856% & 7.695% for bark respectively. Table-2 reveals the benzene, chloroform, ethanol, methanol & water soluble extractive value and it was 4.965%, 5.09%, 12.985%, 21.65%, 25.375% for leaves and 3.885%, 3.25%, 4.87%, 5.495%, 11.69% for stem bark respectively. The fluorescence characteristics of powderd sample with different reagent were observed under day light and UV light (366nm). It is a tool for the determination of constituents present in the plant that gives an idea on its chemical nature.

Preliminary photochemical screening of aqueous extract of stem was performed for screening and identification of bioactive chemical constituents present in them and their findings revealed the presence of various phytochemicals such as carbohydrates, proteins, resin, saponin, flavonoids, tannin but starch and steroids are absent. In case of ethanolic extract, presence of alkaloids, carbohydrate, protein, resin, tannin was seen but saponin, starch and steroids were absent.

The quantitative determination of the bioactive constituents in the present study shows the presene of tannin in leaf 1.3544mg/ml and in stem bark is 0.2872mg/ml, total carbohydrates in leaf and stem bark, for 0.5 ml is 1.1278mg/ml, 1.5389mg/ml, & for 1 ml is 1.0265mg/ml, 1.465mg/ml respectively, alkaloid in leaf is 75.381mg/ml and in bark is 43.379 mg/ml & flavonoid content in stem bark and in leaf was, 6.1652%, 21.0386% respectively.

VII. CONCLUSION

Ayurvedic herbal medicines ensure physical and mental health without side effects containing the natural ingredients. The Ayurvedic herbal medicines help bring arogya to human body and mind ("arogya" means free from disease) while allopathic drugs/medicines have more side effects due to the presence of various toxic chemicals.

From the study it is quite clear that the plant Prosopis cineraria produce several compounds including alkaloids, tannins, saponins, flavonoids, proteins, coumarins and resins . The constituents of leaves and stem bark of Prosopis have several medicinal properties and can be utilized for the treatment of various diseases. So, it can be concluded that this plant can be used for treating various ailments without any side effects.

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