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Phytochemical And Chromatographic Analysis Of *Prosopis cineraria* In Different Polar Solvents.

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ABSTRACT

Prosopis Cineraria is an important tree which is easily available in India. It has also medicinal potent value for cure the medical problems. In this study, we analyze the different kind of phytochemical (carbohydrates, amino acids, flavonoids, alkaloids, saponins, tannins *etc.*) in *Prosopis Cineraria* leaves and stem and made extract with different polar solvents methanol, ethanol, acetone and chloroform, respectively. Furthermore, we used to study five solvent systems for TLC analysis of leaves and stem samples and conclude the R_f values of different phytochemicals in our study.

Keywords: *Prosopis Cineraria,* phytochemical tests, chromatography, medicinal plant, solvent

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INTRODUCTION

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 *Prosopis cineraria*. Qualitative analysis of the total metabolite presents 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 *Prosopis cineraria* (Khandelwal *et al*., 2016).

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 (Sharma *et al*., 2012). 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 (Kumar *et al*., 2011). Medicinal plants are the important source for the new chemical substances with potential therapeutic effects (Velmurugan *et al*., 2012). A promising multipurpose tree species commonly found is *Prosopis cineraria. 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 (Purohit and Ram, 2012). *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 (Gupta *et al*.,2014). 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' (Sachdeva *et al*., 2014). It is also known as "Golden tree" or "Wonder tree" of the desert (Pareek *et al*., 2015). 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 (Garg and Mittal, 2013). It is used as antihyperlipidemic, antioxidative, anthelmintic, antibacterial, antifungal, antiviral, anticancer, in treatment of dysentery, bronchitis, asthma, leukoderma, 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 (Sharma and Singla, 2013).

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 (Khandelwal *et al*., 2015). *Prosopis 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 (Rani *et al*., 2013).

MATERIALS AND METHODS

Collection of samples

The plant sample (leaves and stem) of *Prosopis Cineraria* is collected from Bada-Gawn Jhansi Uttar Pradesh during month of august 2021 and authenticated and verified from Regional Ayurveda Research Institute (RARI) Gwalior Road, Jhansi (U.P.). *Prosopis Cineraria* leaves and stems was washed with tap water to remove soil and dust and washed with distilled water. then cut into small pieces. Put the sample in clean and dry place on blotting paper. Dried in shade of sun light for 15 to 20 days. after drying transferred into fine powder by using electric grinder and ready to use for further analysis.

Extraction method by using Soxhlet apparatus

The plant material (leaves and stem of *Prosopis Cineraria*) was dried in shade for 15 to 20 days then grinded to uniform powder then 25 gram was placed in Soxhlet apparatus and then run for 15 hours, the dried material was extracted in different organic solvents such as methanol, ethanol, acetone, chloroform and stored in refrigerator at 4°C. Extraction was performed with 300ml (AR grade) solvents for 15 hours, at a 60°C temperature and after completion of Soxhlet extraction extract was filtered with Whatman filter paper No. 41 (110mm) and then through cotton wool and stored in a refrigerator at 4°C until use.

Preliminary phytochemical screening of *Prosopis Cineraria* leaves and stem

Phytochemical screening for primary metabolites (carbohydrates, starch, proteins, amino acid, oils and fat) and secondary metabolites (anthraquinones, quinines, glycosides, cardiac glycosides, phenol, tannins, flavonoids, phytosterols, saponins, and steroids) was done (Shaikh and Patil., 2020).

Test for carbohydrates

Benedict Test: To 1ml sample, few drop of benedict reagent was added, mind heated for 5-10min to red green or yellow.

Test for Proteins and amino acids

Biuret test: To 1ml sample, few drop of 10% NaOH sol was added and then heat. Few drops of 0.7% copper sulphate solution were add violet or blue colour.

Test for Saponins

Forth test; To 1ml sample, few drops of diluted with 2ml distilled water was added, shaken gently for 2 min and observed to layer of indicates the presence of saponin.

Test for Tannins

10% ferric chloride test; To 1ml sample, few drops of 10% sol was added, blue, green colour appeared.

Test for Triterpenes

Salkowski test: 1ml sample was treated with 1 ml chloroform was added, filtered few drops H_2SO_4 shaken allowed to stand and lower layer red steroid yellow layer.

Steroid test

Liebermann-Burchard's test: 50gm extract is dissolved in 2mL acetic anhydride then 1-2 drops of conc. H_2SO_4 (along the side of test tube) and observe an array of colour change.

Test for Alkaloids

Wagner test: To 1ml sample, few drop of Wagner reagent was added, formation red brown colour.

Test for Flavonoids

Alkaline test: To 1ml sample, few drop of sodium hydroxide add to yellow colour.

Test for Phenol

5% Ferric chloride test: To 1ml sample, few drops of 5% ferric chloride was added to blue, green, or violet appearance.

Test for Glycosides

Bontrager test: To 1ml sample, few drop of dil. H_2So4 was added boiled for 5 minutes filtered after cooling 1ml of benzene or chloroform was added to pink to red colour.

Test for Lipids

Saponification test: Mix extract and few drops of 0.5N alcoholic KOH then a drop of phenolphthalein (Heated for 2hr.). Soap formation or partial neutralization of alkali was observed.

Thin Layer Chromatography (TLC)

Leaves and stem of *Prosopis Cineraria* samples (Soxhlet) were used for TLC analysis. There were five solvent systems with different ratio to analyze the Rf values of such samples. TLC was performed on a silica gel TLC plates grade G-250 to determine the number of compounds present in the plant crude extract. A total of sample was spotted at 1 cm from the bottom of silica gel plates using capillary tubes. Development of the chromatogram was done in closed tanks, in which the atmosphere has been saturated with eluent vapour by wetting a filter paper lining. The chromatogram was visualized under UV light.

Table 1: Mobile phase ratio for TLC chamber

Mobile phase or solvent system	Solvents	Ratio
I	Ethyl acetate: Methanol: Water	5:3:2
II	Chloroform: Methanol	6:4
III	Ethyl acetate: Toluene: Formic acid	6:2:2
IV	Hexane: Acetone: Ethanol	2:5:3
V	Benzene: Ethanol: Acetone	2:3:5

R_f value = Distance travelled by solute (sample)/Distance travelled by solvent.

RESULTS AND DISCUSSION

Phytochemical screenings showed the moderate percentage presence of various secondary metabolites (alkaloids, flavonoids, triterpenes, saponins, phenols, glycosides). Due to the presence of these chemical compound plants showed different type medicinal activities like antimicrobial, antitumor, antioxidant etc. and these compounds have therapeutic properties against microorganisms. In our study, table-1 and table-2 shows phytochemical study i.e., carbohydrates, protein, saponins, tannins, triterpenes, steroids, alkaloids, flavonoids, phenol, glycosides and lipids. Out of all tests, wangers test showed positive result in all different solvents methanol, ethanol, acetone and chloroform. The moisture contents at 105°C and the total ash value and it was 3.893% & 9.0975% for leaves and 4.0856% & 7.695% for bark respectively. 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 powdered 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. The quantitative determination of the bioactive constituents in the present study shows the presence of tannin in leaf 1.3544mg/ml and in stem bark is 0.2872mg/ml, total carbohydrates in leaf and stem bark, for 0.5ml is 1.1278mg/ml, 1.5389mg/ml, and for 1ml is 1.0265mg/ml, 1.465mg/ml respectively, alkaloid in leaf is 75.381mg/ml and in bark is 43.379 mg/ml and flavonoid content in stem bark and in leaf was, 6.1652%, 21.0386% respectively (Pathak and Kumar., 2017).

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Table 1: *Prosopis cineraria* Leaves phytochemical tests

Phytochemical compounds	Tests	Methanol	Ethanol	Acetone	Chloroform
Carbohydrates	Benedicts test	+	-	-	-
Protein	Biurets test	+	-	+	+
Saponin	Froths test	-	-	-	-
Tannins	Ferric chloride test	-	-	-	-
Triterpenes	Salkowski test	+	-	-	+
Steroids	Liebermann-Burchard's test	-	-	-	+
Alkaloids	Wagner's test	+	+	+	+
Flavonoids	Alkaline chloride test	+	-	+	+
Phenol	Ferric chlorides test	+	-	+	+
Glycosides	Modified Bontrager's test	+	-	+	+
Lipids	Saponification test	+	+	-	-

The phytochemical analysis is very much important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for targeted isolation of compounds and to perform more precise investigations. Extraction of a phytochemical from the plant material is mainly dependent on the type of solvent used. Similarly, the test applied for phytochemical analysis determines the presence or absence of a phytochemical in the sample (Shaikh and Patil., 2020).

Table 2: *Prosopis cineraria* Stem phytochemical tests

Phytochemical compounds	Tests	Methanol	Ethanol	Acetone	Chloroform
Carbohydrates	Benedicts test	-	-	-	-
Protein	Biurets test	+	-	+	+
Saponin	Froths test	-	-	-	-
Tannins	Ferric chloride test	-	-	-	-
Triterpenes	Salkowski test	+	-	-	-
Steroids	Liebermann-Burchard's test	-	-	-	+
Alkaloids	Wagner's test	+	+	+	+
Flavonoids	Alkaline chloride test	-	-	-	+
Phenol	Ferric chlorides test	-	-	-	-
Glycosides	Modified Bontrager's test	-	-	+	+
Lipids	Saponification test	+	-	-	-

Phytochemical analysis of *Prosopis cineraria, Curcuma amada* and *Citrullus colocynthis* plant extracts showed the presence of various constituents which are known to exhibit medicinal as well as physiological activities (Sofowara, 1993). *Prosopis cineraria* gives positive result for all constituents whereas *Curcuma amada* showed negative result for saponins and positive for all other on the other hand *Citrullus colocynthis* gave positive result for carbohydrates, alkaloids, flavonoids terpenoids etc (Soni *et al*., 2017).

Table 3: *Prosopis cineraria* Leaves TLC plates

Solvents system	R_f values	Compounds	Color
Ethyl acetate: Methanol: Water	0.55	Chlorophyll b	Green
Chloroform: Methanol	0.82 0.92	Xanthophyll Carotene	Green Yellow
Ethyl acetate: Toluene: Formic acid	0.88 0.95	Carotene Xanthophyll	Green Yellow green
Hexane: Acetone: Ethanol	0.69 0.83	Carotene Xanthophyll	Green Yellow green
Benzene: Ethanol: Acetone	0.63 0.78	Carotene Xanthophyll	Green Yellow green

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Table 4: *Prosopis cineraria* Stem TLC plates

Solvents system	R_f values	Compounds	Color
Ethyl acetate: Methanol: Water	0.58 0.72 0.95	Xanthophyll Pheophytin b Carotene	Yellow Green gray Orange
Chloroform: Methanol	0.63	Xanthophyll	Green yellow
Ethyl acetate: Toluene: Formic acid	0.45 0.96 0.98	Carotene β-Carotene β-Carotene	Orange Green Green yellow
Hexane: Acetone: Ethanol	0.61	Xanthophyll a	Yellow green
Benzene: Ethanol: Acetone	0.62	Xanthophyll a	Yellow green

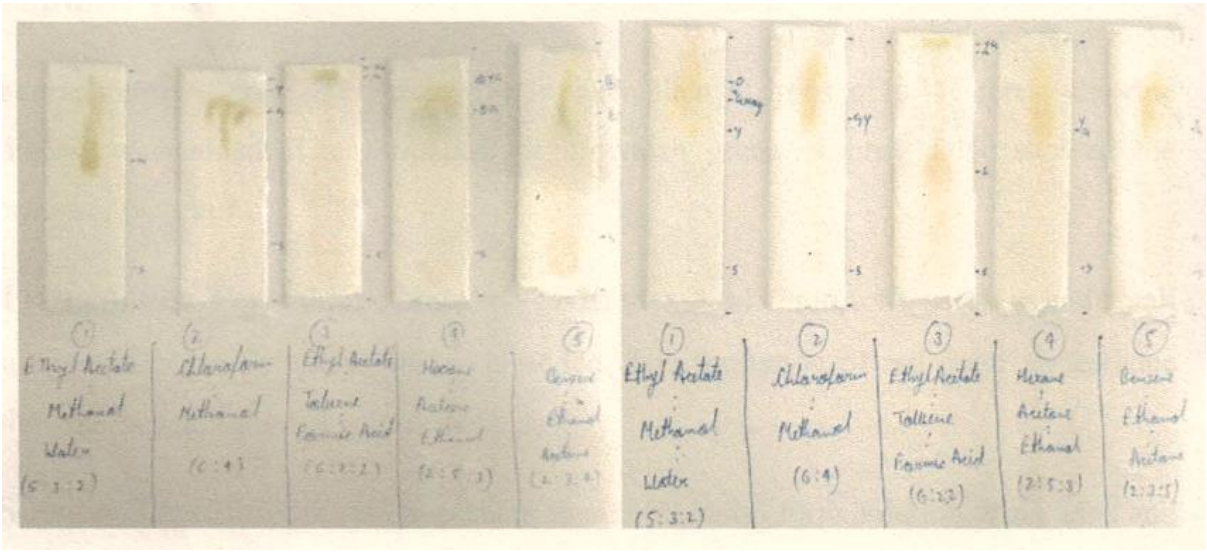

Figure 1: Thin layer chromatography of Leaves and Stem of *Prosopis Cineraria*

Thin layer chromatography analysis of *Prosopis Cineraria* was done with different solvent system Ethyl acetate: Methanol: Water (5:3:2), Chloroform: Methanol (6:4), Ethyl acetate: Toluene: Formic acid (6:2:2), Hexane: Acetone: Ethanol (2:5:3) and Benzene: Ethanol: Acetone (2:3:5) and analyzed R_f values of leaves and stem sample. Table-3, table-4 and figure-1 showed the R_f values with their respective compounds (Chlorophyll, carotenes, xanthophyll, pheophytin etc.) in form of green, yellow and orange, respectively. The phytoconstituents *i.e.,* alkaloids and flavonoids are active principles of plants.

These active principles provide defensive mechanism of the plants against different pathogens (Hafiza, 2000). The terpenoids have significant pharmacological activities, such as anti-viral, anti-bacterial, anti-malarial, anti-inflammatory, inhibition of cholesterol synthesis and anti-cancer activities (Mahato and Sen,1997). Saponins which are used to stop bleeding and in treating wounds and ulcers as it helps in red blood cell coagulation (Okwu and Josiah, 2006). Further studies will need to isolate and characterize the bioactive chemical entities of these plants.

CONCLUSION

Prosopis cineraria leaves and stem can be considered as an alternate protein source for protein-energy-malnutrition among the economically weaker people. The plant Prosopis 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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