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Pharmacognostic, Physicochemical and Phytochemical Investigation of *Prosopis spicigera* [L.] Leaves

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Abstract

Objective: The study was intended to investigate requisite detail about pharmacognostic characteristic and phytochemical profile of *Prosopis spicigera* (L.) leaves. **Methods:** Pharmacognostic investigation of the anatomical section of the dried leaves as well as powder microscopy was carried out to determine its morphological, anatomical and phytochemical diagnostic features. The qualitative microscopy, quantitative microscopy and other WHO suggested parameters of standardization of dried leaves and powder were carried out as per standard procedures. **Results:** Pharmacognostic assessment of *Prosopis spicigera* leaves shows lamina and midrib region. Lamina exhibits upper and lower epidermis and shows multicellular covering trichomes, mesophyll comprises of palisade and spongy parenchyma. Midrib exhibits are shaped vascular bundle enclosed by pericyclic fibres and collenchyma. Vascular bundle consists of xylem and phloem. Preliminary phytochemical examination demonstrated the presence of Proteins, carbohydrates, tannins, saponins, alkaloids, phenolics, steroids and flavonoids as phytoconstituents. The previously stated phytochemicals were further confirmed by Thin-layer chromatography (TLC). **Conclusions:** It can be presumed that the pharmacognostic profile of the *Prosopis spicigera* (L.) leaves might be supportive in setting some diagnostic indices for the identification and the preparation of the monograph of the plant.

Keywords
Procurement, pharmacognostic study, phytochemical Screening, *P. spicigera,* Shami.

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INTRODUCTION

Genus of Prosopis is widespread in 44 species that has medicinal benefits on their pharmacological activity. One of the medicinal plants from this genus is *Prosopis spicigera L*., taxonomically known as *Prosopis cineraria L* family Fabaceae. In Indian states for example Rajasthan it is known as Khejri, Janti,

Jand, and Sangri, Maharashtra it is known as Shami, in Punjab is known as Jand, in Sindh it is known as Kandi, in Tamilnadu it is known as Vanni, in Karnataka it is known as Banni, and in Gujarat it is known as Sami and Sumri. In Sanskrit it is known as Shami, Sankhphala, Keshahantri, Sivaphala, Mangalya, and Papanasini, In the United Arab Emirates it is called as Ghaf [1]. Shami is a rare medicinal tree and can grow in very harsh climatic conditions and in poor soil. It is the State tree of Rajasthan, India [2]. *P. spicigera* are small to medium size tree evergreen and thorny. It is also known as "wonder tree" and "king of desert" as all the parts of tree are useful.

Yellow green flowers are borne on 5 to 23 cm long spike like racemes. On maturity they become attractively bright yellow and usually 0.6 cm broad. After pollination flowers produce specialized fruits in clusters. Fruits of Shami are pods containing up to 25 dull brown seeds, 0.3 to 0.8 cm long. Pods are light green-yellow in colour. They grow up to 8 to 19 cm. These are locally called as Sangar or Sangri in Rajasthan. The dried pods are locally called as Kho–Kha. These are eaten by the poor people. Dried pods form rich animal feed and are liked by all animals. Humans eat Shami fruits in boiled form when these are young and soft. These are also used as famine food and are reported to be known by even the pre-historic men. Many communities in central India worship the Shami during Dasara. They soak the leaves of the Shami in water and on the day before Deepavali, bath with this water. It is the State tree of Rajasthan, India **[13]**. *P. cineraria* are small to medium size tree evergreen and thorny. It is also known as "wonder tree" and "king of desert" as all the parts of tree are useful.

Review of the literature reveals the presence of some imperative phytoconstituents such as Vitamins, phenolics, and carotenoids are three major groups of natural antioxidants possessing defensive property present in fruits and vegetables [3]. extracts possess alkaloids, glycosides, saponins and flavonoids [4]. The chemical constituents isolated from leaves include spicigerine from the whole plant; and steroids, namely, campesterol, stigma sterol, sitosterol, cholesterol, alcohols, namely, octacosanol and triacontan-1-ol, and alkane [5]. Flowers contain patuletin glycoside patulitrin, sitosterol, spicigerine, Flavone derivatives Prosogerin A and Prosogerin B. Leaves contain steroids like campesterol, cholestrol, sitosterol and stigmasterol, actacosanol, hentriacontane, methyl docosanoate, Diisopropyl-10, 11-dihydroxyicosane1, 20-dioate, Tricosan-1-ol, and 7,24-Tirucalladien-3-one along with a piperidine alkaloid spicigerine Seeds contain Prosogerin C, Prosogerin D, Prosogerin E, Gallic acid, patuletin, patulitrin, luteolin and rutin.[6].

The importance of the medicinal value of this tree has been highlighted in our ancient literature [7] such as *p. spicigera* has a potential new antimicrobial activity [8]. A thorn of *P. spicigera* has been detected in vivo in two cases of chronic skin granulomas [9]. Stem bark shows anti inflammatory properties [1]. The primary compounds that are thought to provide the protection afforded by fruit and vegetables are the antioxidants. The bark of the tree has abortifacient and laxative properties and is also used as a remedy for rheumatism in the central provinces. The leaves are of high nutritive value and locally called ''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 been reported to show antibacterial,[12]anti-hyperglycemic, anti-hyperlipidemic and anti-oxidative activities [13]. Khejri or Shami was reported to have antimicrobial activity against S. aureus and E. coli [14]. Pods are brown to chocolate in colour on ripening, each containing several seeds embedded in sweet dry yellow pulp. *P. cineraria* pods are locally called ''sangar'' or ''sangri''. The pod is considered astringent in Punjab. Sangri pods are known to prevent protein and mineral deficiency [7]. **[14]**. Leaves and pods are extensively used as fodder for cattle, camels and goats. *P. species* have also been extensively used in indigenous system of medicine as folk remedy for various ailments like leprosy, dysentery, bronchitis, asthma, leucoderma, pile, muscular tremors and wandering of the mind [15, 16]. It is also known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties. It is also known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties. Leaf infusion is used on open sores on the skin [17-20]. In view of the various medicinal constituents and uses credited to *P. spicigera* endeavor is made to examine anatomical and other physicochemical parameters required for quality control of the crude drug material. Thus, this pharmacognostic research was undertaken with an aim to assess various parameters like macroscopic, microscopic, physicochemical and phytochemical properties of *P. spicigera L*.

MATERIALS AND METHODS

Procurement of plant materials:

The leaves of *P. spicigera* were collected from Nanded of Marathwada region in Maharashtra during January, 2012, was authenticated by Dr. Vishal R. Marathe, Department of Botany, Science College, Nanded.

The Leaves of *P. spicigera* were collected from Nanded of Marathwada region in Maharashtra during January, 2012, cleaned and dried at room temperature in shade, away from direct sunlight.

Identification of the plant was done by Dr. Vishal R. Marathe, Department of Botany, Science College, Nanded, authenticated plant by comparing morphological features and a sample voucher specimen of plant is deposited for future reference.

Drying and size reduction of plant:

The leaves of P. spicigera were separated from other parts, cleaned to evacuate the adhered foreign material and were washed under tap water, air dried, homogenized to powder.

Procurement of chemicals:

All reagents and chemicals used for testing were analytical grade obtained from Ranbaxy Fine Chemicals Ltd., New Delhi and Loba Chemie, Mumbai, India.

Pharmacognostic studies:

Organoleptic evaluation

Diverse sensory parameters of the plant material (Colour, Odor, Taste, Size and Shape) were examined by organoleptic evaluation.

Qualitative microscopy.

In this study, transverse sections of leaves were examined under the microscope (10X and 40X). Staining reagents Phloroglucinol- Hydrochloric) were employed according to standard techniques. The different distinguishing characters were observed with or without staining and images were recorded [21, 22]

Physiochemical analysis

The physicochemical constant for example percentage of total Ash value, acid-insoluble ash value, Foreign organic matter, water-soluble extractive value, alcohol soluble extractive value, pet-ether soluble extractive value, methanol soluble extractive value and ethyl acetate soluble extractive value were determined. Moisture content determination was performed according to the WHO guidelines [23-25].

Preliminary Phytochemical Screening

The leaves of p. spicegera were procured, dried in the shade and subsequently powdered in a homogenizer. The powdered leaves were used for extraction. Powder drug was passed through 120 mesh to remove the fine powder. Coarse powder material (500g) was employed for successive extraction with petroleum ether, ethyl acetate, methanol and ethanol in Soxhlet apparatus. Crude extract obtained was vacuum dried to get solvent-free dry extract. All fractions were concentrated under reduced pressure using a rotary evaporator and dried in vacuum and subjected to phytochemical screening for the detection of various class of phytochemicals [26-29].

Chromatographic evaluation [30, 31]

On the basis of preliminary phytochemical tests, all extracts were subjected to thin layer chromatography (TLC). Preperative silica gel plates were used for development of chromatograph. Different solvent systems used were Acetic acid: Chloroform (1:9), Ethyl acetate: benzene (9:11), Benzene: Methanol: Acetic Acid (45:8:4), Benzene: Chloroform (1:1). After development, initially spots were visualized in the Ultraviolet (UV) chamber (254, 365 nm) the present study freshly prepared vanillin-sulphuric acid and anise aldehyde solution was used as visualizing agent to detect the bands on the TLC plates. The distance travelled by phytochemicals was noted by calculating its retention factor (Rf) value. After visualized using visualizing agents, the spots were observed in different colours. The Rf values were measured and the chromatogram was photographed and described in the tabulated form.

RESULTS AND DISCUSSION

Pharmacognostic characteristics:

Macroscopy:

The leaflets are compound, bipinnate, stipulate, stipules modified into spines, alternate, petiolate. Leaflets are ovate, apex mucronate, base unequal, margin entire and reticulate venation. Size of leaf is 1-1.5 cm long and 0.4-0.6 cm broad. [Figure 1]

Microscopy:

Microscopical study shows on leaves of *P. spicigera*, transverse section of leaf consists of lamina and midrib region. Lamina exhibits upper and lower epidermis and shows multicellular covering trichomes, mesophyll comprises of palisade and spongy parenchyma. Midrib exhibits are shaped vascular bundle enclosed by pericyclic fibres and collenchyma. Vascular bundle consists of xylem and phloem [Figure2].

Powder microscopy:

The upper epidermal view contains palisade cells and simple trichomes, prisms of Ca-oxalate present in veins, lower epidermal view shows lower palisade cells and stomata namely anisocytic and paracytic are seen. [Figure 3, 4, 5, 6]

Physico-chemical analysis:

In the physicochemical analysis, endeavor is made to evaluate various parameters of *P. spicigera* leaves such as ash value and extractive values which are mentioned in [Table 1 & 2]. Foreign organic matter of powdered leaves indicates that there is no contamination by any unnecessary matters. Ash value of leaves predicts that *P. spicigera* leaves comprise of a massive amount of calcium oxalate crystals. Extractive values display that leaves contains non-polar as well as polar constituents. Generally, extractive values are indications of constituents present in the drug and are useful in the determination of adulterated drugs and exhausted drug [Table 2].

Preliminary Phytochemical Screening:

The preliminary phytochemical screening revealed the presence of proteins, carbohydrates and flavonoids in the petroleum ether extract. Proteins, carbohydrates, tannins, saponins, alkaloids, phenolics, steroids and flavonoids present in ethyl acetate extract. Proteins, carbohydrates, tannins, saponins, alkaloids and fixed oil present in methanolic extract Proteins, carbohydrates, tannins, alkaloids, flavonoids and fixed oil presents in ethanolic extract which could make the plant useful for treating different ailments as having a potential of providing useful drugs for human use[Table 3].

Thin layer chromatography (TLC):

TLC profile of different extracts of *P. spicigera* leaves revealed the presence different compounds (Table 4) having R_f values of 0.88, 0.91, 0.13, 0.33, 0.44, 0.63, 0.76 0.92, 0.86, 0.93, 0.15, 0.88 and 0.96, 0.98 after derivatization with visualizing agents. It was also observed in the UV chamber at wavelength 254 nm and 365 nm which is shown in Figure 7.

Table 1. Physicochemical parameters of *P. spicigera* leaves extract

Sr. No.	Parameters	Values (%w/w)
1	Total ash	5.6
2	Acid-insoluble ash	2.14
3	Water soluble ash	68.5
4	Moisture soluble ash	26.6
5	Foreign organic matter	1.4

Table 2. Extractive Values of *P. spicigera* leaves extract

Sr. No.	Parameters	Values (%w/w)
1	Water soluble	15.8
2	Alcohol soluble	9.2
3	Pet.ether soluble	3.85
4	Methanol soluble	3.84
5	Ethyl acetate soluble	12.19

Table 3. Qualitative phytochemical analysis of *P. spicigera* leaves

Sr. No.	Constituents	Pet. ether extract	Ethyl acetate extract	Methanolic extract	Ethanolic extract
1.	Protiens**ns**	+	+	+	+
2.	Carbohydrates	+	+	+	+
3.	Tannins	-	+	+	+
4.	Saponins	-	+	+	-
5.	Alkaloids	-	+	+	+
6.	Phenols	-	+	-	-
7.	Steroids	-	+	-	-
8.	Flavonoids	+	+	-	+
9.	Fixed oils	-	-	+	+

+: indicates presence of constituents,
-: indicates absence of constituents

Table 4. Chromatographic analysis of *P. spicigera* leaves extracts

Extracts	Mobile phase	R_f values
Pet.ether	Acetic acid:Chloroform (1:9)	0.88, 0.91
Ethyl acetate	Ethyl acetate:benzene (9:11)	0.13, 0.33, 0.44. 0.63, 0.76, 0.92
Methanol	Benzene:Methanol: Acetic Acid(45:8:4)	0.86, 0.93
ethanol	Benzene:Chloroform (1:1)	0.15, 0.88, 0.96, 0.98

Figure 1. Morphology of *P. spicigera* leaves

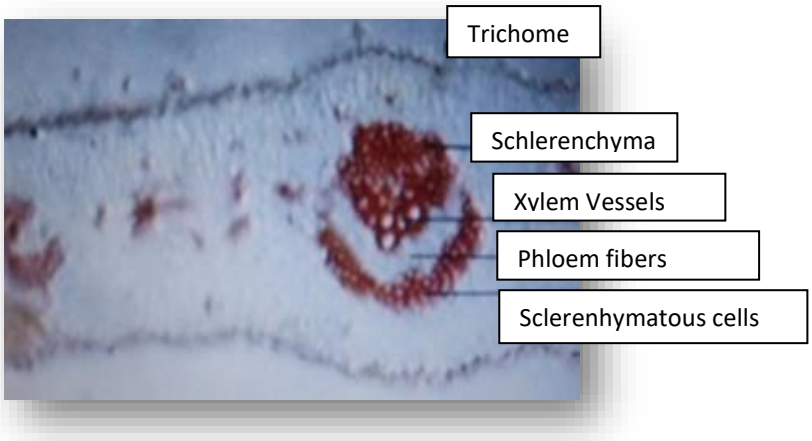

Figure 2. Transverse section of *P. spicigera* leaves

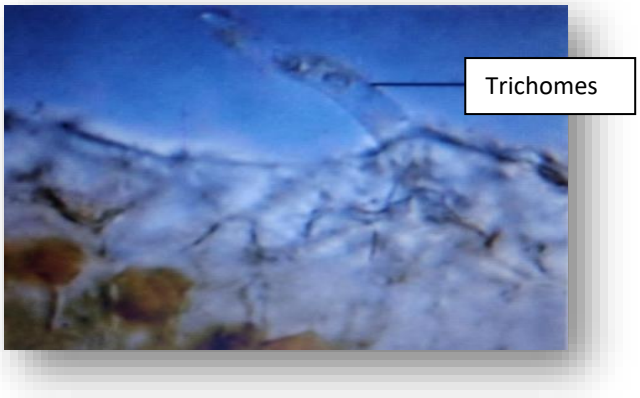

Figure 3. Surface view of Upper epidermis showing Trichomes of *P. spicigera* leaves

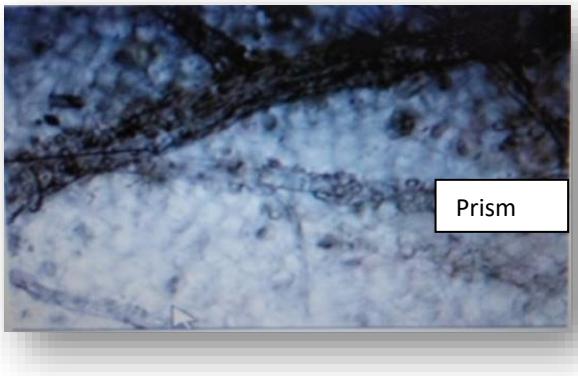

Figure 4. Prism of Ca-oxalate in vein of *P. spicigera* leaves

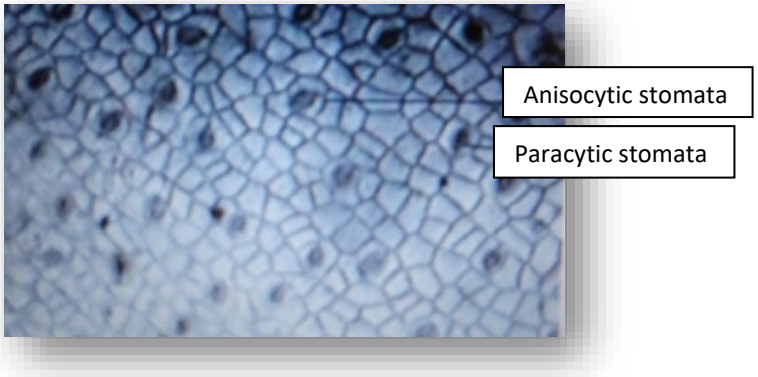

Figure 5. Anisocytic and Paracytic stomata of *P. spicigera* leaves

Figure 6. **Palisade cells of *P. spicigera* leaves**

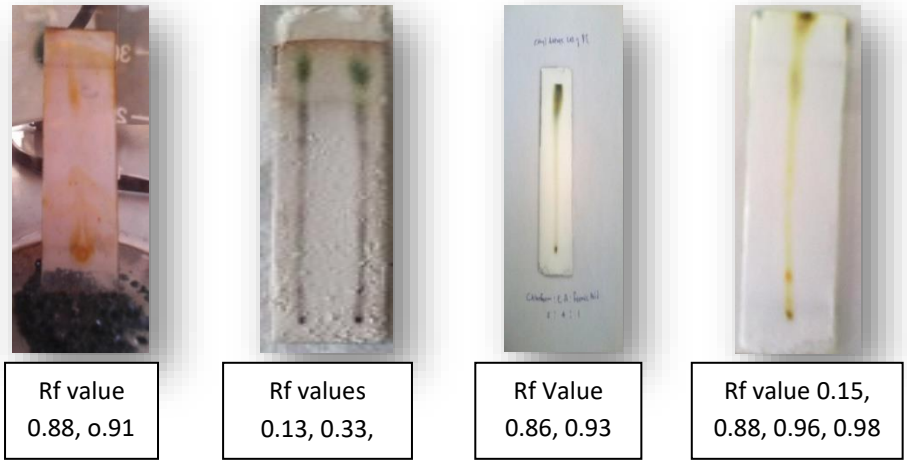

Figure 7. Thin Layer chromatography of *P. spicigera* leaf extract

CONCLUSION

With a specific end goal to standardize an herbal medication with various parameters like macroscopic, microscopic, TLC, Phytochemical examination can be concluded that it can serve as a valuable resource of pharmacognostic and phytochemical information. The present work was embraced with a view point to set down bench marks which could be valuable in recognizing the authenticity of this medicinal herb. Microscopical studies have demonstrated the presence of characters on lamina and midrib region. Lamina exhibits upper and lower epidermis and shows multicellular covering trichomes, mesophyll comprises of palisade and spongy parenchyma. Midrib exhibits are shaped vascular bundle enclosed by pericyclic fibres and collenchyma. Vascular bundle consists of xylem and phloem. Phytochemical screening showed the presence of Proteins, carbohydrates, tannins, saponins, alkaloids, phenolics, steroids and flavonoids as phytoconstituents. The TLC analysis of leaves can provide standard fingerprints and it can be used as a reference for the standardization and quality control of the drug. Physicochemical parameters established importance in detecting adulteration and mishandling of the crude drug. This pharmacognostical studies will provide helpful inputs for standardizing crude drugs and can also be useful in detecting and differentiate closely related species. In future, isolation and identification of individual phytochemicals, *in-vivo* studies are essential for better understanding of their mechanism of action.

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