PHYTOCHEMICAL ANALYSIS OF THE LEAF EXTRACT OF _PROSOPIS SPICIGERA_ L. AN IMPORTANT MEDICINAL PLANT USED TO TREAT HUMAN SPLEEN DISORDERS.

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Abstract: The objective of the present study was to report the phytochemical analysis of 70% ethanol extract of leaf of _Prosopis spicigera_. The phytochemical analysis of the ethanolic extracts of these plants parts was carried out to explore their phytoconstituents present in this plant. The leaf material was collected from the Maruthamalai Forests, Coimbatore, Tamil Nadu, India and extracts was prepared by using 70% ethanol. Leaves of _Prosopis spicigera_ in isolation of methyl docosanoate, diisopropyl-9, 10-dihydroxyicosane-1, 20-dioate, tricosan-1-ol and 7, 24-tirucalladien-3-one. While diisopropyl- 10, 11-dihydroxyicosane-1, 20-dioate is a hitherto unreported compound, methyl docosanoate, tricosan-1-ol and 7, 24-tirucalladien-3-one are being reported for the first time from _Prosopis spicigera_. These compounds have been characterized on the basis of spectral and other data.

Index Terms: _Prosopis spicigera_, ethanol extract, secondary metabolites, GC-MS

INTRODUCTION

_Prosocis spicigera_ L (family: Leguminosae, sub family: Mimosaceae) is prickly tree or shrub and commonly found in dry and arid regions of north-western India, southern India, Pakistan, Afghanistan, Iran and Arabia¹. Leaves and pods are extensively used as fodder for cattle, camels and goats. _Prosopis_ species have also been extensively used in indigenous system of medicine as folk remedy for various ailments¹⁻² like leprosy, dysentery, bronchitis, asthma, leucoderma, piles, muscular tremors and wandering of the mind. It is also known to possess anthelmintic, antibacterial, antifungal, antiviral, anticancer and several other pharmacological properties. Leaf paste of _P. spicigera_ is applied on boils and blisters, including mouth ulcers in livestock and leaf infusion on open sores on the skin³⁻⁶. The smoke of the leaves is considered good for eye troubles. Phytochemicals in the leaves of _P.
spicigera. and reported alkaloid namely spicigerine; steroids namely campesterol, cholesterol, sitosterol, stigmasterol; alcohols namely octacosanol and triacontan-1-ol; and alkane hentriacontane\(^7,8\).

The leaf extract of Prosopis spiciger is used in indigenous medicine for human spleen disorders. The Spleen is located in the left hypochondriac region of abdomen between the funds of the stomach and diaphragm. The spleen is an organ which not only effectively uses its own immune cells but also mobilizes the body’s immune cells for the immune surveillance and protecting other vital organs including heart, kidney and brain. The cells play which play an important role in spleen functions are: Macrophages, monocytes, natural killer cells and beta and T – cells. Enlargement of spleen may occur due to anaemia, infections, inflammations, cancer, metabolic activities such as to increase the number of platelets in the blood. The present study undertakes the reinvestigations on the chemical examination of its leaves and we isolated one new ketone along with three known compounds, reported for the first time, from the methanol extract of the plant leaves.

Materials and Methods

Herbarium

All the ethnomedicinally important plants were collected as per information given by the local population and numbers were given accordingly. Herbarium specimens were prepared according to the method described earlier [9]. The leaves of Prosopis spicigera were identified with the help of Flora of Presidency of Madras [10] and confirmed with the authentic herbarium of Government of India, Botanical Survey of India (Southern Circle) Coimbatore. All the prepared herbarium specimens were deposited in the Department of Botany, Government Arts College, Coimbatore – 641 018, Tamil Nadu, India.

Collection and storage of plant material

The fresh leaves were collected from the Maruthamalai Forests of Coimbatore, Tamil Nadu, India. It was ensure that the plant was healthy and uninfected, then cleaned it up. The particular amounts of leaves were dried under shadow at room temperature. The dried samples were powdered in a Wiley Mill (Scientific Equipment’s Works, New Delhi, India) to be 60 mesh in size. Care was taken to clean the Wiley Mill thoroughly after powdering sample and before starting to powder a new sample to avoid
mixing up of samples. The powder samples were stored in polythene containers at room temperature. The leaf samples were chemically screened to detect the presence of certain biologically active compound(s).

**Identification of certain biologically active compounds using preliminary qualitative test**

The plant species *Prosopis cineraria* (Leaves) were chemically screened to find out the presence or absence of certain bioactive compound(s). The method of extraction is common for all the aforesaid plants and respective test procedures are given below. For each experiment triplicates were maintained.

**Test for alkaloids**

Two ml aliquot of the extract was treated with the following reagents to test the presence or absence of alkaloids. Mayer’s reagent - white precipitate or turbidity will be observed.

**Test for steroids and sterols**

**Salkowski’s test**

The extract was dissolved in 1 or 2 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red revealing the presence of steroid and sterol compounds in the extract.

**Libermann-Burchard’ test**

The extracts were in 2 ml of chloroform and 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicates the presence of steroids.

**Test for Triterpenoids**

**Hirshorn test**

The extracts were dissolved in 2 ml of chloroform and heated for 10 min. after the addition of 2 ml of trichloro acetic acid. Appearance of yellowcolour to red indicates the presence of triterpenoids.
Libermann-Burchard’ test

The extracts were in 2 ml of chloroform and 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid were added. Appearance of red to violet colour indicates the presence of triterpenoids.

Test for Proteins and Amino acids

Biuret test

One ml of extract, 1 ml of 40 per cent sodium hydroxide solution and 2 drops of 1 per cent copper sulphate solution were added. The appearance of violet colour indicates the presence of proteins/amino acids.

Ninhydrin test

One ml of the extract, 2 drops of freshly prepared 0.2 per cent ninhydrin reagent was added and heated. The appearance of blue colour indicates the presence of proteins, peptides or amino acids.

Test for Carbohydrates

Benedict’s test

Five ml of Benedict’s solution was added to the extract and boiled in water bath. The appearance of red yellow or green precipitate indicates the presence of reducing sugars.

Tests for volatile oils

Two -ml aliquot of extract was evaporated on a porcelain crucible. If the residue has an aromatic smell, it indicates the presence of volatile oils.

GC-MS Analysis

Chromatographic Analysis by GCMS Prosopis cineraria Leaves. The Clarus SQ 8C Gas Chromatography - Mass Spectrometer from Perkin Elmer, were engaged for analysis. The instrument was set as follows, Injector port temperature set to 220° C, Interface temperature set as 250° C, source kept at 220°C. The oven temperature programmed as available, 75° C for 2 mins, 150°C @ 10°C/min, up to 250° C @ 10°C/min. Split ratio set as1:12 and the injector used was splitless mode. The DB-5 MS capillary
standard non-polar column was used whose dimensions were 0.25mm OD x 0.25μm ID x 30 meters length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 550 Da. The source was maintained at 220°C and 4.5e-6 mtorr vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has inbuilt libraries for searching and matching the spectrum. NIST MS Search 2.2v contain more than five lakh references.

**Identification of compounds**

Interpretation of mass spectrum of GC – MS was done using the database of National Institute Standard and Technology (NIST14). The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library.

**RESULTS AND DISCUSSION**

**Phytochemical Screening**

In the present study the results of phytochemical screening of the potential medicinal plant such as *Prosopis spicigera* are shown in Table.1.

All the five samples of the investigated five plant species were extracted with solvents of increasing polarity such as benzene, acetone, chloroform, methanol and qualitatively analyzed for certain bio-active compounds such as alkaloids, steroids, triterpenoids, protein, amino acids, carbohydrates, volatile oils and fatty acids. These biologically active constituents originally are secondary metabolites which are present in a significant amount in the ethanolic extract of *Prosopis spicigera* plant. These secondary metabolites comprises of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and different kinds of terpenoids as shown in the Table 1.
Table 1. Phytochemical Analysis of the ethanolic extract of *Prosopis spicigera* leaves

<table>
<thead>
<tr>
<th>Extract Name</th>
<th>Chemical Constituents</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Flavonoids</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>Glycosides</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Terpenoids</td>
<td>+++</td>
</tr>
</tbody>
</table>

Figure 1: GC-MS studies of *Prosopis spicigera* Leaves
Table 1: Summary of Identified Compounds in Prosopis spicigera leaves

<table>
<thead>
<tr>
<th>Pk #</th>
<th>RT</th>
<th>Scan</th>
<th>Height</th>
<th>Area</th>
<th>Area %</th>
<th>Norm %</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>3.118</td>
<td>24</td>
<td>24,026,474</td>
<td>4,202,741.5</td>
<td>0.364</td>
<td>4.07</td>
</tr>
</tbody>
</table>

Figure 2: Isolated other compounds of *Prosopis spicigera* Leaves

Figure 3: Structure of \( \text{(C}_{25}\text{H}_{36}\text{O}_{2}) \) 6, 9, 12-Octadecatrienoic acid, phenylmethyl ester, \((Z, Z, Z)\)-Benzyl \((6Z, 9Z, 12Z)-6, 9, 12-octadecatrienoate \# (6Z, 9Z,12Z)-6, 9, 12-Octadecatrienoic acid benzyl ester77509-03-6

Figure 4: Structure of \((2\alpha, 16\xi, 19S)\) -19, 20-Dihydroxycuran-17-oic acid methyl ester
Figure 5: Structure of (2α, 16ξ, 19S)-19, 20-Dihydroxycuran-17-oic acid methyl ester 2111-90-2

Figure 6: Structure of 5α-Cholestan-3β-ol, 2-methylene

Figure 7: Structure of Tridecanedial
Figure 8: Structure of 6-Methylhept-5-en-2-ol

Figure 9: Structure of Sulcatol 6-Methylhept-5-en-2-ol 6-METHYL-5-HEPTEN-2-OL 1569-60-4 5-Hepten-2-ol, 6-methyl

Figure 10: Structure of N-Acetyl-N-depropionylaspidalbine 2122-26-1
CONCLUSION

In view of the importance of plants as cure for diseases and as a source of medicinal lead compounds, there is a need for proper and systematic biological and phytochemical investigations of the plant. Plants are the biggest source of medicine in future. Nowadays scientists from divergent fields are investigating plants with a view to decipher antimicrobial usefulness and they found hundreds of phytochemicals which have inhibitory effects on all types of microorganism in *vitro*. In the present study chemical composition relevant to the disease for which they are used. The secondary metabolites *Prosopis spicigera* (Leaves) are reported for the first time in our laboratory. The optimal effectiveness of medicinal plants many not be due to one main bioactive constituent, but in fact to the combined action of different secondary metabolites originally present in the plant.
ACKNOWLEDGEMENTS

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References


