PHYTOCHEMICAL ANALYSIS OF THE LEAF EXTRACT OF *PROSOPIS SPICIGERA* L. AN IMPORTANT MEDICINAL PLANT USED TO TREAT HUMAN SPLEEN DISORDERS.

V. KAYALVIZHI and **V.C. SARALABAI***

Department of Botany, Government Arts College (Autonomous), Coimbatore - 641 018, Tamil Nadu, India.

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 ime 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

Prosopis 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 Arabia[1]. 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 ailments[1,2] 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 skin[3-6]. 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 won 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: Macrophytes, 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 ethno medicinally 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 leavess 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^{o} C, Interface temperature set as 250^{o} C, source kept at 220^{o}C. The oven temperature programmed as available, 75^{o} C for 2 mins, 150^{o}C @ 10^{o}C/min, up to 250^{o} C @ 10^{o}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 220ºC 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

Extract Name	Chemical Constituents	Result
Methanol	Alkaloids	++
	Flavonoids	++++
	Glycosides	++
	Tannins	+
	Saponins	++
	Steroids	+
	Terpenoids	+++

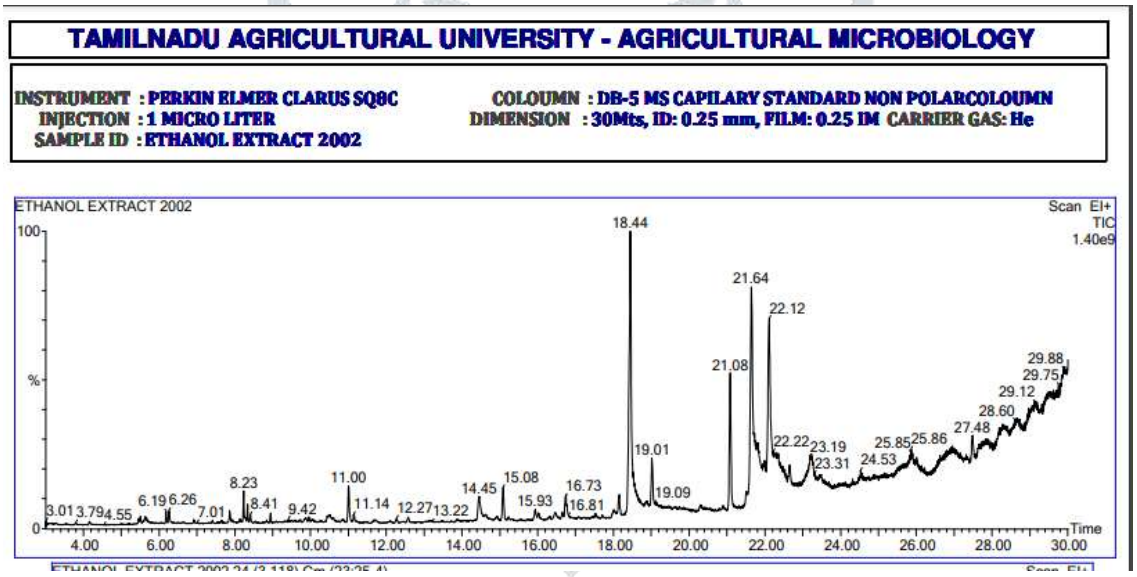

Figure 1: GC-MS studies of *Prosopis spicigera* Leaves

#	RT	Scan	Height	Area	Area %	Norm %
1	3.118	24	24,026,474	4,202,741.5	0.554	4.07

Pk #	RT	Hit	Compound Name	Match	R.Match	Prob.	CAS	Library
1	3.118	1	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	577	586	10.4	56599-45-2	mainlib
		2	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	566	602	7.1	16714-85-5	mainlib
		3	3-Chloropropionic acid, 3-pentadecyl ester	553	611	4.6		mainlib
		4	l-Gala-l-ido-octose	552	650	4.4		mainlib
		5	N-(5-Hydroxy-2-oxo-5-phenyl-1-aza-bicyclo[4.2.0]oct-3-yl)carbamic acid, benzyl ester	550	566	4.1		mainlib
		6	6,9,12-Octadecatrienoic acid, phenylmethyl ester, (Z,Z,Z)-	544	681	3.2	77509-03-6	mainlib
		7	Preg-4-en-3-one, 17à-hydroxy-17á-cyano-	543	600	3.1		mainlib
		8	2-Oxazolamine, 4,5-dihydro-5-(phenoxymethyl)-N-[(phenylamino)carbonyl]-	543	583	3.1	132786-21-1	mainlib
		9	Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)-	537	568	2.4	2111-90-2	mainlib
		10	Oleic Acid	532	593	1.9	112-80-1	replib

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

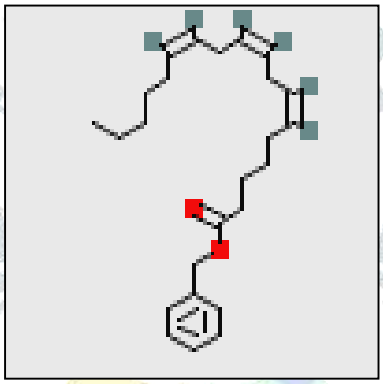

Figure 3: Structure of ($C_{25}H_{36}O_2$) 6, 9, 12-Octadecatrienoic acid, phenylmethyl ester, (Z, Z, Z)-Benzyl (6Z, 9Z, 12Z)-6, 9, 12-octadecatrienoate # (6Z, 9Z,12Z)-6, 9, 12-Octadecatrienoic acid benzyl ester77509-03-6

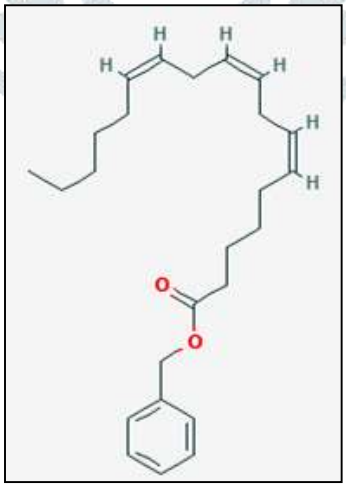

Figure 4: Structure of (2alpha, 16xi, 19S) -19, 20-Dihydroxycuran-17-oic acid methyl ester

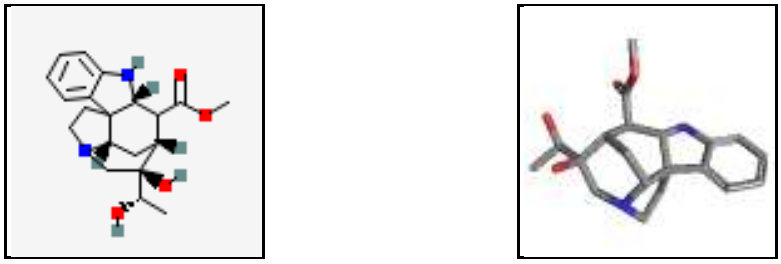

Figure 5: Structure of (2alpha, 16xi, 19S)-19, 20-Dihydroxycuran-17-oic acid methyl ester 2111-90-2

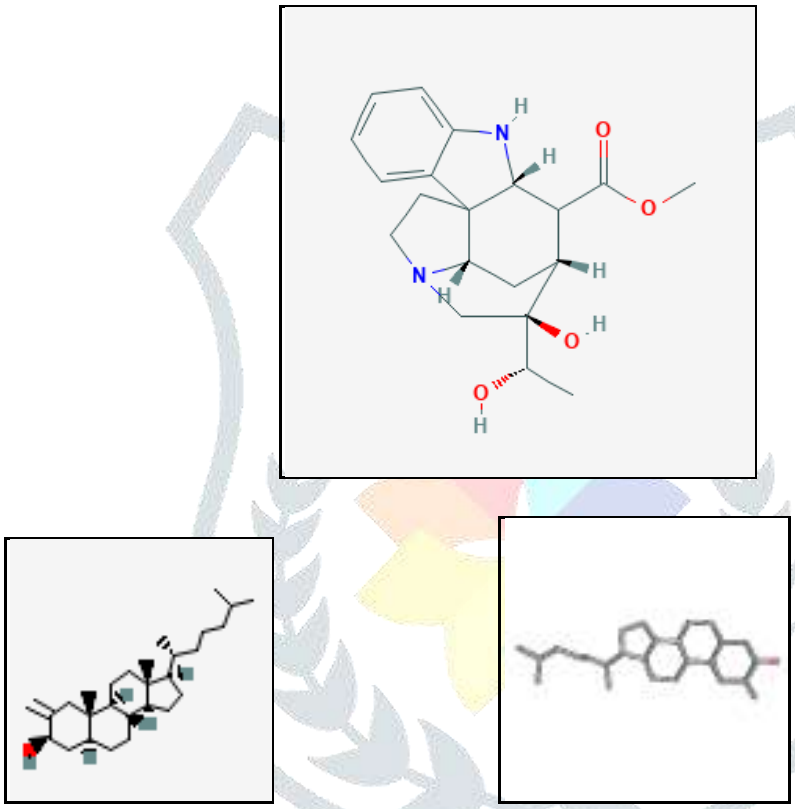

Figure 6: Structure of 5alpha-Cholestan-3beta-ol, 2-methylene

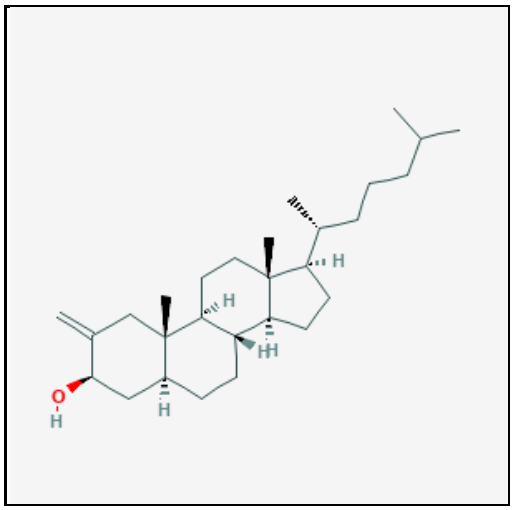

Figure 7: Structure of Tridecanedial

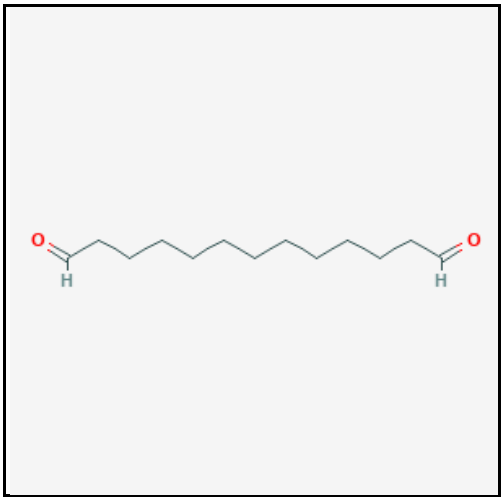

Figure 8: Structure of 6-Methylhept-5-en-2-ol

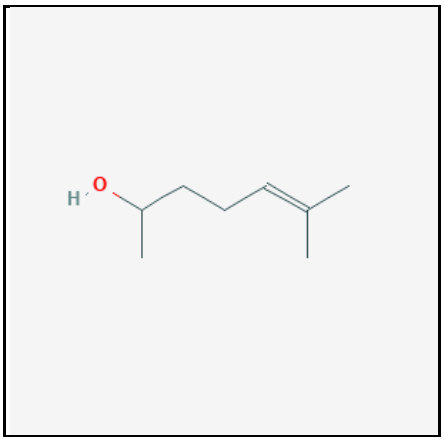

Figure 9: Structure of Sulcatol 6-Methylhept-5-en-2-ol 6-METHYL-5-HEPTEN-2-OL 1569-60-4 5-Hepten-2-ol, 6-methyl

Figure 10: Structure of N-Acetyl-N-depropionylaspidoalbine 2122-26-1

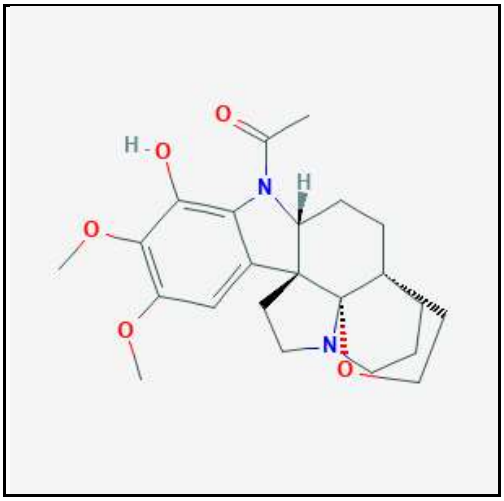

Figure 11: Structure of5alpha-Cholestan-3beta-ol, 2-methylene

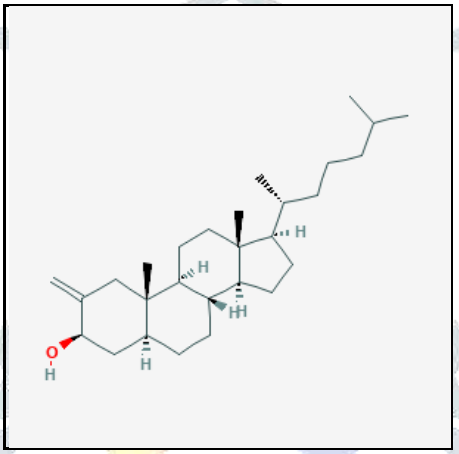

Figure 12: Structure 5.alpha.-Cholestan-3.beta.-ol, 2-methylene- 2-Methylenecholestan-3-ol # Cholestan-3-ol, 2-methylene-, (3.beta., 5.alpha.)-2-Methylene-5alpha-cholestan-3beta- ol 22599-96-8

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

The authors are thankful to the Botanical Survey of India (BSI) Southern Circle, Coimbatore 641 001 for identification of the plant and to the Department of Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore – 641 004, Tamil Nadu, for the GC-MS studies of Leaf sample of *Prosopis spicigera*.

References

[1].Kirtikar, K.R. and Basu, B.D. Indian Medicinal Plants, L.M. Basu, Allahabad, 1984. 910.

[2].Duke JA. Handbook of Energy Crops, 1983,https://www.hort.purdue.edu/newcrop/duke_energy/dukeindex.html

[3]. Chopra, RN, Chopra, I.C. Handa, K.L. and Kapur , L.D. In:Chopra's Indigenous Drugs of India. U.N. Dhur and Sons Pvt.Ltd., Calcutta, 1958, 52.

[4]. Chopra, RN, Nayar, S.L. and Chopra, I.C. Glossary of Indian Medicinal Plants. Council for Scientific and Industrial Research, New Delhi, 1956, 204.

[5]. Nadkarani, A.K. In: Indian Materia Medica. Popular Book Depart. Bombay, 1954. 1011.

[6.] Usmanghani, K., Saquib, Q.N.Jewers, K. Nagler, M.J., Zirvi, K.A., Amir, F.and Cottee, F.H. Pahlavi Med.J., 1974, 5, 1.

[7]. Jewers, K, Nagler, M.J., Zirvi, K.A. Amir, F. and Cottee, F.H. Pahlavi Med. 1974, 5, 1.

[8].Jewers, K, Nagler, M.J., Zirvi, K.A. Amir, F.Phytochem.1976, 15, 238.

[9.]. Janakai, A., Kaleena, P. K., Elumalai, D., Hemalatha, P., Babu, M., Velu, K., Sudha Ravi.(2017). Phytochemical Screening, Antioxidant and Antibacterial Activities of *Millingtonia hortensis* (L).*International Journal of Current Pharmaceutical Research*. 9: 162-167.

[10]. Hooker and Dalton, (1954). The Flora of British India, L. Reeva and Co, England, 4:376.