

Isolation and identification of Steroids from Different Parts of *Prosopis Cineraria*

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Abstract

Prosopis cineraria are a tree of remarkable importance and a rich source of secondary metabolites. The present study was carried out to investigate the steroid content present in the leaves, stem and pods of *Prosopis cineraria*. The method of Tomita *et al.*, was used for isolation of steroids. The structure of the isolated compound was established on the basis of physical and chemical test and spectroscopic evidences (TLC, IR and GC- MS). The study concluded that a single type of steroid Diosgenin was found in all the parts of the selected plant species.

Keywords: *Prosopis cineraria*; Steroids; Diosgenin; Thin Layer Chromatography.

Introduction

Steroids are triterpenoids made up of isoprene units containing 30 carbon atoms, which are pharmaceutically important for human life [1]. Steroids have the potential to improve intrinsic seed yield significantly. These are capable of increasing plant tolerance/resistance to a wide range of biotic and abiotic stresses, such as drought, salinity, heat, cold, virus infection, and pathogen attack etc [2].

Material and Methods

Each of the dried plant parts of *Prosopis cineraria* viz. Leaves, Stem, Pods were powdered weighed and defatted separately in soxhlet apparatus in petroleum ether for 24 h on a water bath.

Each mixture was hydrolyzed with 15% ethanolic HCl (1g/5 mL: w/v) for 4 h by refluxing on water bath. Each hydrolysate was filtered and filtrate extracted thrice with ethyl acetate. The ethyl acetate fractions of each sample was pooled and washed to neutrality by repeated washings with distill water, dried in vacuo, reconstituted in chloroform, filtered, dried again and weighed.

Each test sample was replicated thrice.

Thin glass plates coated with silica gel (250µm thick) were dried at room temperature, thereafter kept at 100°C for 30 min to activate. The freshly prepared activated plates were used for qualitative as well as quantitative analysis[3].

Thin Layer Chromatography (TLC)

The crude steroidal sapogenin extract of each sample was examined on TLC, along with the reference steroidal sapogenin (diosgenin). The plates were developed in a solvent system of chloroform, hexane and acetone (23:5:2), air dried and sprayed with 50% sulphuric acid [4] and anisaldehyde reagent (composed of 0.5 mL of anisaldehyde, 1mL of conc. sulphuric acid and 50mL of acetic acid) separately and heated to 100°C until the characteristics colors developed.

The fluorescence response as well as permanent black zones was recorded. The times required for the initial appearance of a color reaction, the initial color in day light

and after heating for 10 minutes and the colour in UV light (360nm) were recorded. A combination of other solvent systems such as benzene and ethyl acetate (85:15); [5] and acetone and benzene (1:2);[6]. were also used but solvent system of chloroform, hexane and acetone (23:5:2) was comparatively better than other solvent system. Three replicates were run and R_f values were calculated.

Quantitative Analysis

Preparative Thin Layer Chromatography (PTLC)

PTLC was used to isolate diosgenin from crude steroidal sapogenin extract on silica gel G plates by using solvent mixtures of chloroform, hexane and acetone (23:5:2). The spots were marked on TLC by spraying with anisaldehyde reagent, to one of the columns on each plate and spots corresponding to the standard diosgenin were marked and scrapped separately from the unsprayed plates/column.

The PTLC was repeated until about 20mg of the substance was obtained. Co-TLC of

crystallized isolated substance along with reference marker (standard diosgenin) was carried out to test the purity of isolated compounds. Such chromatograms were also visualized by spraying a solution of antimony trichloride in conc. HCl. After PTLC the diosgenin was crystallized from methanol-acetone [7] and examined for mp, mmp and infra-red spectral studies.

Results & Discussions

In the present investigation Diosgenin has been characterized and quantified in *Prosopis cineraria*. While assessing relative levels of the isolated steroid was found in maximum concentration in leaves of *P.cineraria* (4 ± 0.15 mg/gdw) followed by pods (3.5 ± 0.08 mg/gdw) and lowest concentration in stem (1.5 ± 0.05 mg/gdw).

TLC Chromatogram of isolated phytosteroids from different parts of *Prosopis cineraria* L is given in Fig 1. The IR figure of Diosgenin is given in Fig-2. Total Steroid content in *Prosopis cineraria* L is given in Table-1

Table 1: Total Steroid content in *Prosopis cineraria* L. (in mg/gdw)

Plant part	Steroids
Leaf	4 ± 0.15
Stem	1.5 ± 0.05
Pod	3.5 ± 0.08



Fig 1: TLC Chromatogram of isolated phytosteroids from different parts of *Prosopis cineraria*

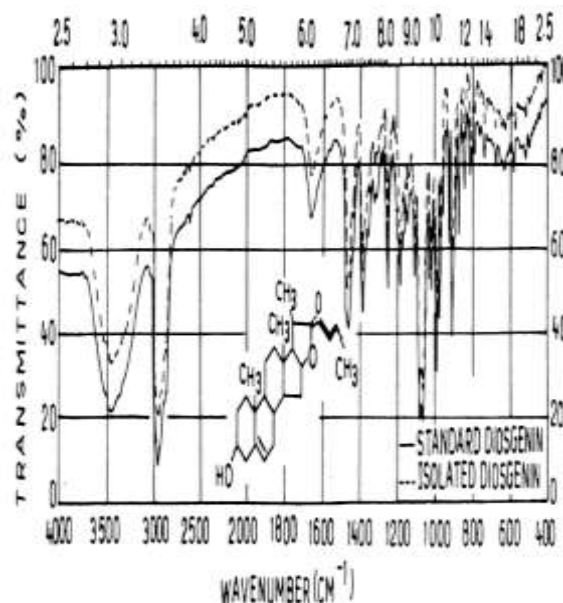


Fig 2: Infra-red Spectra of Isolated and Standard Diosgenin

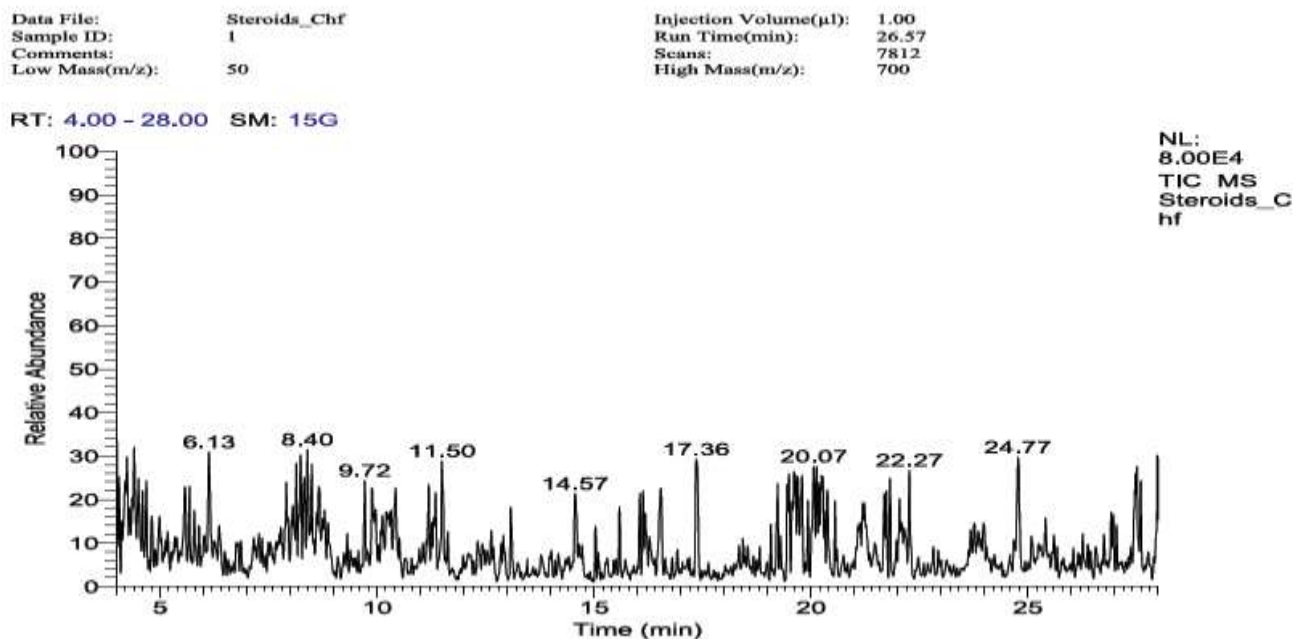


Fig 3: GC-MS result of Steroids

RT	Compound Name	Area	Area %	RSI	Cas#	Library
6.13	1-Methoxy-3-Methyl-5-Nitrobenzene	44609	3.29	631	NA	mainlib
8.40	1, 3 Benzodioxole, 4, 5 dimethoxy-6-[-2methylsulfinyl]-2-methylthio) ethenyl]	32960	2.43	711	75629-02-6	mainlib
9.72	2,3-Diphenyl-6,7-dimethylquinoxaline	25788	1.90	776	13362-56-6	mainlib
11.49	Prop-1-enyl dithiopropionate	40460	2.98	725	NA	mainlib
17.36	2-(14-Carboxytetradecyl)-2-ethyl-4,4-dimethyl-1,3-oxazolidine-N-oxyl	65019	4.79	761	53034-38-1	mainlib
20.07	1H-Imidazole-4,5-dicarboxylic acid, 1-benzyl-, dimethylester	39206	2.89	625	321970-25-6	mainlib
22.27	Methanone, (3,4-dimethoxyphenyl) (3,4,5-trimethoxy-2-methylphenyl)-	22973	1.69	566	56890-08-5	mainlib
24.77	Benzamide, N,N-dipropyl-	52607	3.88	789	14657-86-4	mainlib

Abbreviations: RT= Retention time, MF= Molecular formula, M Wt= Molecular weight.

Conclusions

Steroids are used commercially as biologically active compounds and generally high value-low volume products than the primary metabolites, which are used in drug manufacture by the pharmaceutical industries. Steroids are pharmaceutically important in the preparation of sex hormones, corticosteroids and contraceptives [8]. Diosgenin is an important steroidal metabolite used as a starting material for the synthesis of steroidal drugs, as it exhibits estrogenic activity. Diosgenin has indicated the effect of reducing the level of serum cholesterol. It is mainly used as the

initial material for partial synthesis of oral contraceptives, sex hormones and other steroids.[9,10,11].

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