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Original Article

ISOLATION AND IDENTIFICATION OF STEROIDS FROM DIFFERENT PARTS OF *PROSOPIS JULIFLORA*

KHANDELWAL PREETI[1*], SHARMA R. A.[2], AGARWAL MALA[3]

[1,2]Dept. of Botany, University of Rajasthan, Jaipur, Rajasthan, India, [3]Dept. of Botany, B. B. D. Government P. G. College, Chimanpura (Shahpura), Jaipur, Rajasthan, India
Email: preeti45khandelwal@gmail.com

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ABSTRACT

Objective: The present study was carried out to investigate the steroid content present in the leaves, stem, pods and callus of *Prosopis juliflora*.

Methods: 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 evidence (TLC, IR and GC-MS).

Results: The study concluded that a single type of steroid Diosgenin was found in the selected plant species.

Conclusion: Diosgenin is an important steroidal metabolite used as a starting material for the synthesis of steroidal drugs, as it exhibits estrogenic activity.

Keywords: *Prosopis juliflora*, GC-MS, Steroids, Diosgenin, Thin Layer Chromatography

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INTRODUCTION

Prosopis juliflora is one of the most economically and ecologically important tree species in arid and semi-arid zones of the world [1]. It is an important component of desert Ecosystem of India as biomass producer and as the Leguminous tree, it enriches the desert soil, fixes atmospheric nitrogen and provides a green coverage [2]. All parts of *P. Juliflora* have a wide range of uses and also provide many types of secondary plant metabolites such as Steroids and many more [3]. Steroids are triterpenoids made up of isoprene units containing 30 carbon atoms, which are pharmaceutically important for human life [4]. Steroids are derivatives of cyclopentanoperhydrophenanthrene and includes steroidal sapogenins, steroidal glycosides and cardiac glycosides [5]. Steroidal sapogenins are of immense value so they could provide eminent sources for steroidal drugs [6].

MATERIALS AND METHODS

Each of the dried plant parts of *Prosopis juliflora* viz. Leaves, Stem, Pods and Callus 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 a water bath. Each hydrolysate was filtered and the filtrate extracted thrice with ethyl acetate. The ethyl acetate fractions of each sample were 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 (250mμ thick) were dried at room temperature, thereafter kept at 1000C for 30 min to activate. The freshly prepared activated plates were used for qualitative as well as quantitative analysis [7].

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 [8] and anisaldehyde reagent (composed of 0.5 ml of anisaldehyde, 1 ml of conc. sulphuric acid and 50 ml of acetic acid) separately and heated to 1000C until the characteristics colors developed. The fluorescence response, as well as permanent black zones, was recorded. The time required for the initial appearance of a color reaction, the initial color in daylight and after heating for 10 min and the colour in UV light (360 nm) were recorded. A combination of other solvent systems such as benzene and ethyl acetate (85:15); [9] and acetone and benzene (1:2); [10]. Were also used but a solvent system of chloroform, hexane and acetone (23:5:2) was comparatively better than another solvent system. Three replicates were run and Rf 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 20 mg 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 [11] and examined for mp, mmp and infra–red spectral studies.

RESULTS

In the present investigation, Diosgenin has been characterized and quantified in *Prosopis juliflora.* While assessing relative levels of the isolated steroids was found in maximum concentration in Pods of *P. juliflora* (3 mg/gdw) followed by Leaves (2.3±0.08 mg/gdw) followed by Callus (1.8±0.01 mg/gdw) and lowest concentration was found in the stem (1±0.01 mg/gdw). Total Steroid content in *Prosopis juliflora is* given in table 1 TLC Chromatogram of isolated phytosteroids from different parts of *Prosopis juliflora* is given in fig. 1. The IR fig. of Diosgenin is given in fig. 2.

Table 1: Total steroid content in *prosopis juliflora* (in mg/gdw)

Plant part	Steroids
Leaf	2.3±0.08
Stem	1±0.01
Pod	3±0.11
Callus	1.8±0.01

Values are the mean±SEM (n = 3 replicates in each group). *P<0.05; **P<0.001 compared with the control; P<0. 001.

Fig. 1: Tlc chromatogram of isolated phytosteroids from different parts of *Prosopis juliflora* l, Abbreviations–Dio-Diosgenin, PJC-*Prosopis juliflora* callus PJL-*Prosopis juliflora* Leaves, PJP-*Prosopis ju; liflora* Pods, PJS-*Prosopis juliflora* Stem

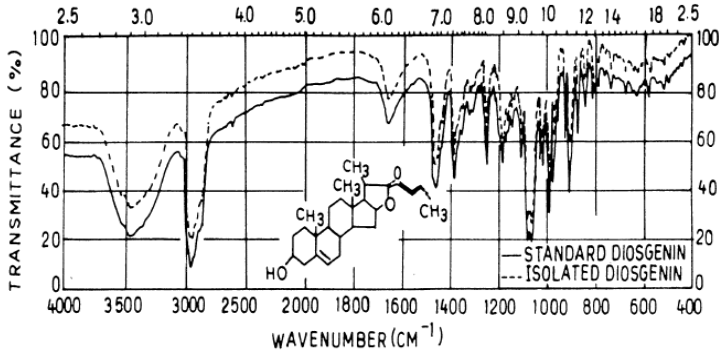

Fig. 2: Infra-red spectra of isolated and standard diosgenin

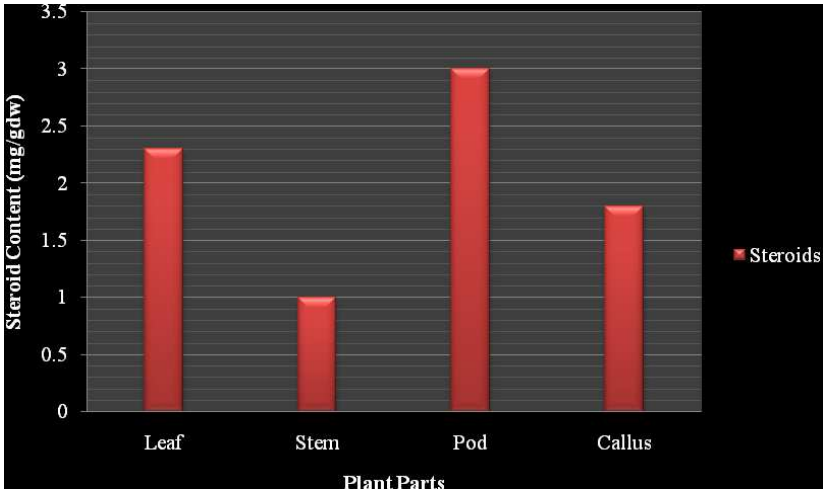

Fig. 3: Graphical representation of total steroids present in different parts of plant *prosopis juliflora* L.

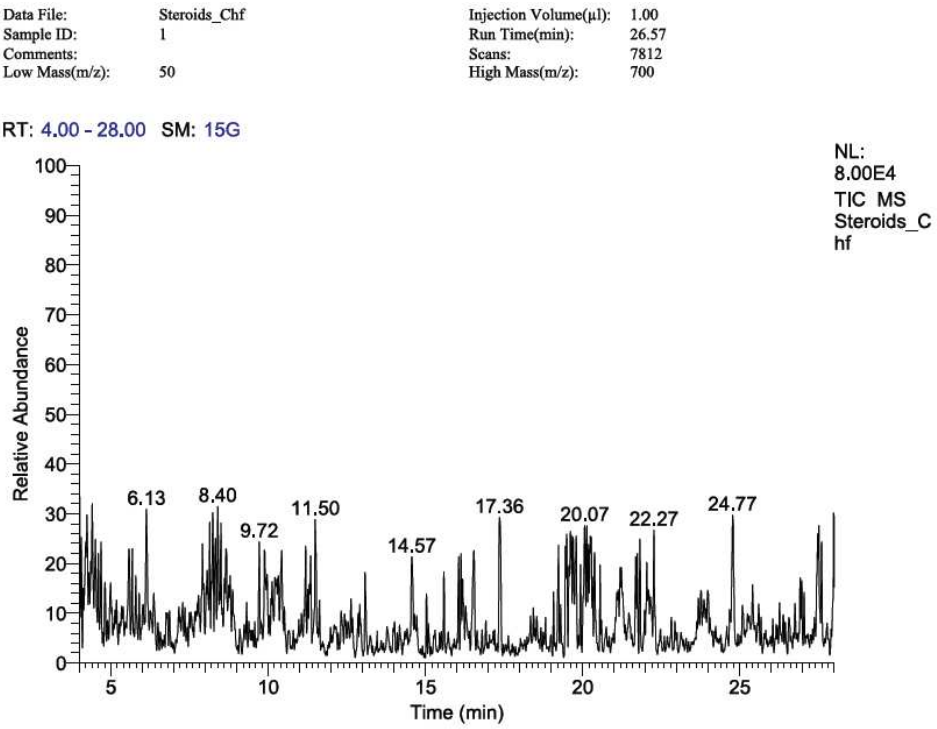

Fig. 4: Gc-Ms result of steroids

Table 2

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 dithiopropanonate	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-dicaboxylic 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.

DISCUSSION

The discovery of diosgenin in *Prosopis juliflora* has made it the most researched and studied plant. The presence of steroids in *Prosopis juliflora* has also confirmed by Velmurugan *et al.*, 2010 [12]. Our result also shows the presence of diosgenin in pods, leaves and stem of experimental plants. Diosgenin is principally obtained from Dioscoria roots (4 to 6% DW) for conversion to commercially useful drugs. 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 [13].

CONCLUSION

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. 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. [14-16].

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CONFLICT OF INTERESTS

Declare none

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