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HPLC analysis of saponins in *Achyranthes aspera* and *Cissus quadrangularis*

Tamanna Talreja, Mangesh Kumar, Asha Goswami, Ghanshyam Gahlot, Surendra Kumar Jinger and Tribhuwan Sharma

Abstract
Reversed phase high performance liquid chromatography (RP-HPLC) with UV detector was done for the analysis of four saponins of *Achyranthes aspera* and *Cissus quadrangularis*. The separation of filtered plant extracts as well as a mixture of authentic standard saponin samples of Gitogenin, Diosgenin, Hecogenin, Kryptogenin was done within 25 min. Running conditions included: injection volume 15µl; mobile phase: acetonitrile: water (40:60) running time 25 min., flow rate 1 ml/min; and detection at 203nm. Identifications of specific saponins are made by comparing their retention times with those of the standards. Three saponins in *Achyranthes aspera* namely Gitogenin, Diosgenin, Kryptogenin and two saponins namely Diosgenin, Kryptogenin in *Cissus quadrangularis* were detected by HPLC.

Keywords: *Achyranthes aspera*, *Cissus quadrangularis,* Saponins, retention time, HPLC

1. Introduction
Plants are excellent source for the discovery of new products of medicinal value for drug development. Plants are capable of synthesizing variety of low molecular weight organic compounds, called secondary metabolites usually with very unique and complex structures. Secondary metabolites are compounds biosynthetically derived from the primary metabolites and may be restricted to a particular taxonomic group. The role of secondary product has been rather ambiguous and earlier these were thought to be first waste materials. Now days, plant secondary metabolites are seen as tremendous source of pharmacological value for scientific and clinical research. Their biological activities have high therapeutic value, applicable in health care, drug development and synthesis of beneficial compounds.
Saponins are economically useful class of secondary metabolites. They are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, the foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have many health benefits. The pharmacological property of a steroidal saponin has been described including their hypocholesterolemia, anti-diabetic and antioxidant activities
Achyranthes aspera Linn. (Amaranthaceae) and *Cissus quadrangularis* Linn.(Vitaceae) are important plants possessing a wide biological activity. These plants revealed their potential to produce various valuable phytochemicals. The natural products obtained from plants provides a unique opportunity for the development of new drugs but due to their complex nature there is a need to isolate and purify the bioactive compounds from crude plant extract by advanced separation techniques and instrumentation. The compounds which are isolated from different natural plant sources by using various solvent systems and chromatographic techniques is very important. Practically most of them have to be purified by the combination of several chromatographic techniques.
HPLC is gaining increasing interest for the analysis of plant extracts. It has added a new dimension to the investigation of phytosterols in herbal plants. Although TLC is a powerful and simple technique used for this purpose, there are situations in which it can produce doubtful results. The separations by HPLC are far more rapid and accurate than classical methods and provide high resolution and sensitivity. Therefore, the aim of the present study was to do a simple and accurate HPLC analysis for the determination of saponins in *Achyranthes aspera* and *Cissus quadrangularis*.

2. Material and Method

2.1 Plant material and extract preparation

Cissus quadrangularis (stem) was collected from various parks of Bikaner where it is cultivated as ornamental plant whereas seed samples of *Achyranthes aspera* was purchased from the shop of herbal medicine and were identified by a well-known taxonomist of Bikaner. The fresh samples were dried separately and used for further analysis. Samples were hydrolyzed with 30% (v/v) hydrochloric acid (2 gm/20 ml) for 4 hours on a water bath. Hydrolyzed test samples were washed separately with distilled water and filtrate attained pH 7.0. Test samples obtained were dried at 60 °C for eight hours and soxhlet extracted in benzene (200ml) for twenty four hours separately. Benzene extract of each test sample was dried separately in vacuo and taken up in chloroform for analysis of its steroidal sapogenins.

2.2 Fractionation of crude extract by Thin Layer chromatography (TLC) for identification

Crude extracts with reference to sapogenins (diosgenin, gitogenin, hecogenin, kryptogenin, smilagenin, tigogenin and yamogenin) were dissolved in chloroform and applied separately on silica get 'G' coated and activated glass plates. These plates were developed in an organic solvent mixture of hexane and acetone (8:2, v/v). Developed glass plates were dried and visualized under UV light followed by spraying with 50% sulphuric acid and subsequent heating at 100°C for 10 minutes.

2.3 Sample preparation for HPLC analysis

Two grams of each plant sample powder was extracted in soxhlet apparatus with 150 mL of 70% ethanol for 7 hrs at 45°C. The extraction procedure was executed in triplicate for each plant sample. It was transferred into a flat bottom flask and concentrated with a rotary evaporator. The concentrate was then dissolved in 10mL of HPLC-grade methanol.

2.4 HPLC analysis

30 µl of these extracts were passed through. 45µm syringe filter and that filtrate was used for HPLC analysis. The HPLC system (Shimadzu lab chromo 2010 HT HPLC, UV detector) was used. The software package used for analyzing results was Shimadzu lab chromo HPLC control and auto-sampling. Chromatographic analysis was carried out using a c-18 column at 35 ^{0}C temperature. Prior to analysis, the column was equilibrated with the corresponding. Running conditions included: injection volume 15µl; mobile phase: acetonitrile: water (40:60) running time 25 min., flow rate 1 ml/min; and detection at 203nm. The separation of filtered methanolic plant extracts as well as a mixture of authentic standard samples of saponins was done. The separation of standard saponins showed 4 fine peaks in chromatogram and Three saponins in *Achyranthes* and two saponins in *Cissus* were detected by HPLC. The peak area of standards and samples was calculated to determinate concentration

3. Result and Discussion

Two spots (brown, Rf 0.43, Rf 0.22) coincided with that of the standard reference compound, diosgenin and kryptogenin were marked in *Cissus quadrangularis* whereas three spots coinciding with those of standard samples of gitogenin, diosgenin and kryptogenin (brown, Rf 0.56 Rf 0.43, Rf 0.22) were observed in *Achyranthes aspera*.

The method developed for HPLC provided a quick analysis of the plant extract. The conditions used led to a good separation of the peaks in standard solution which could be identified in the chromatogram (Figure1), gitogenin (Rt= 8.4 min.), diosgenin (Rt= 10.7 min.), hecogenin (Rt=12.2 min.), kryptogenin (Rt= 17.7 min.), Plant samples were identified by comparison with the chromatogram of the four reference compounds obtained under the same conditions and the respective UV spectra, obtained on line. The separation of flavonoids showed 3 fine and distinguished peaks {(Figure2), gitogenin (Rt= 7.9 min.),, diosgenin (Rt= 11.1 min.) and kryptogenin (Rt=17.1 min.) in chromatogram of *Achyranthes aspera* and 2 peaks {(Figure3) diosgenin (Rt= 10.5 min.) and kryptogenin (Rt= 17.6 min.) in *Cissus quadrangularis*. The data were processed by the Shimadzu lab chromo HPLC control and auto-sampling software (LC chrome software). The peak area of standards and samples was calculated to determinate concentration (Table1). The results of the quantitative analysis are the average of three samples and the data are summarized in Table 2 and Table 3

4. Conclusion

The results above showed, therefore, that *Achyranthes aspera* and *Cissus quadrangularis* are rich in the presence of the important biologically active gitogenin, diosgenin and kryptogenin. The described HPLC procedure could be useful for the qualitative and quantitative analysis of saponins in plant materials. Plants produce saponins to fight infections by parasites. When ingested by humans, saponins also seem to help our immune system and to protect against pathogens. The non-sugar part of saponins have also a direct antioxidant activity, which may results in other benefits such as reduced risk of cancer and heart diseases. Saponins have beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. Saponins bind with bile salt and cholesterol in the intestinal tract and cause a reduction of blood cholesterol by preventing its re-absorption. Therefore determination of saponins is very important related to the quality of medicinal plants. The described HPLC procedure could be useful for the qualitative and quantitative analysis of saponins in plant materials. It can also be used in the quality control of phytopreparations containing saponins.

5. Conflict of interest: None

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Acquired by	: Admin
Sample Name	: Saponin standards
Sample ID	: Saponin standards
Tray #	: 2
Vail #	: 1
Injection Volume	: 15 µL
Conc.	: 0.05 µmole ml^{-1}
Data File Name	: Saponin standards data new.lcd
Method File Name	: Saponin standards Meth.lcm
Report File Name	: Default.lcr
Data Acquired	: 19/12/2016 01:35 PM
Data Processed	: 19/12/2016 01:48 PM

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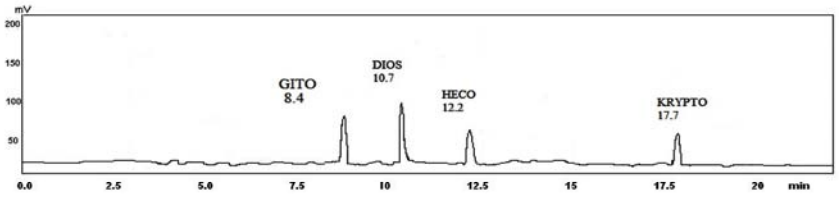

Peak	Name	Retention Time (min.)
1.	Gitogenin (GITO)	8.4
2.	Diosgenin (DIOS)	10.7
3.	Hecogenin (HECO)	12.2
4.	Kryptogenin (KRYPTO)	17.7

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Acquired by	: Admin
Sample Name	: Achy. saponin
Sample ID	: Achy. saponin
Tray #	: 2
Vail #	: 1
Injection Volume	: 15 µL
Conc.	:
Data File Name	: Achy. saponin data new.lcd
Method File Name	: Achy. saponin Meth.lcm
Report File Name	: Default.lcr
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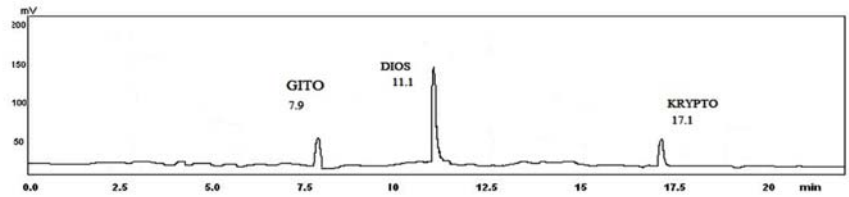

Peak	Name	Retention Time (min.)	Area(mm^2)	Conc.(µg g^{-1})
1.	Gitogenin (GITO)	7.9	5.06	4.11 ±0.58
2.	Diosgenin (DIOS)	11.1	7.38	6.32 ±0.20
3.	Kryptogenin (KRYPTO)	17.1	4.88	3.54 ±0.12

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Acquired by	: Admin
Sample Name	: Cissus Saponin
Sample ID	: Cissus Saponin
Tray #	: 2
Vail #	: 1
Injection Volume	: 15 µL
Conc.	:
Data File Name	: Cissus Saponin data new.lcd
Method File Name	: Cissus Saponin Meth.lcm
Report File Name	: Default.lcr
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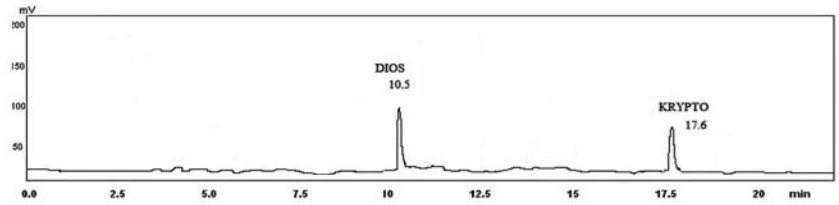

Peak	Name	Retention Time (min.)	Area(mm^2)	Conc.(µg g^{-1})
1.	Diosgenin (DIOS)	10.5	7.79	6.03 ± 0.11
2.	Kryptogenin (KRYPTO)	17.6	6.91	5.11 ± 0.02

6. Acknowledgement: Authors are thankful to UGC, New Delhi to provide the fund to pursue this research work.

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