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# Qualitative and quantitative estimation of phytosterols in *Achyranthes aspera* and *Cissus quadrangularis* by HPLC

**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 naturally occurring phytosterols of *Achyranthes aspera* and *Cissus quadrangularis*. The separation of filtered plant extracts as well as a mixture of authentic standard phytosterols samples of Campesterol, Stigmasterol, β-Sitosterol, Stigmastanol was done within 25 min. Running conditions included: injection volume 10µl; mobile phase: acetonitrile: water (50:50) containing 3% water, flow rate 1 ml/min; and detection at 205nm. Identifications of specific phytosterols are made by comparing their retention times with those of the standards. Three phytosterols in *Achyranthes aspera* namely Campesterol, Stigmasterol, β-Sitosterol and two phytosterols namely Campesterol, Stigmasterol in *Cissus quadrangularis* were detected by HPLC.

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

## 1. Introduction
Plants are the most exclusive sources of drugs for majority of world population and plant product constitute about 25% of prescribed medicines. The medicinal properties of plants are associated with their chemical constituents. These produce specific physiological action in the human body. A wide range of organic compounds, responsible for the biological activity of herb, are synthesized in plants that are traditionally classified as primary and secondary metabolites. Medicinal plants are rich in secondary plant products, their compounds are termed as 'Medicinal' or 'Officinal'. These secondary metabolites exert in general a profound physiological effect on the mammalian system and are thus known as 'the active principle of the plant'. These active principles are used for curing ailments and therefore, these are natural drugs.

Phytosterols are economically useful class of secondary metabolites. These are a group of naturally occurring steroid alcohols found in plants. Sterols are known to be the starting materials for a number of plant steroids, which are used in pharmaceuticals and industries. They act as a structural component in the cell membrane. They have application in medicine, cosmetics and as food additives. Researches have shown that phytosterols are beneficial in treating various diseases and are essential nutrients that ensure optimal functioning of the body's defense mechanisms. The phytosterols have been found effective in treating high cholesterol as the plant sterols compete for absorption sites with cholesterol, they thus reduce the amount of cholesterol absorbed. The phytosterols have been found effective in treating high cholesterol and also contributes anti-inflammatory effect.

*Achyranthes aspera* Linn. (Amaranthaceae) and *Cissus quadrangularis* Linn. (Vitaceae) are important plants possessing a wide biological activity. These plants have been used in traditional Indian medicine for thousands of years to treat various disorders. 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 phytosterols 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. The dried samples were separately extracted by following the procedure of Kaul and Staba (1968). Plant samples were hydrolyzed with 30% (v/v) hydrochloric acid (2 gm/20ml) for 4 hours on water bath. The hydrolyzed test samples were washed separately with distilled water till the filtrate attained pH 7.0. Test samples so obtained were dried at 60 $^0$C for eight hours and soxhlet extracted in benzene (200 ml) for twenty four hours (Nag *et al.,* 1979) separately. Benzene extracts of various test samples were dried separately in vacuo and taken up in chloroform for further analysis.

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

Each of the crude extract was applied separately on silica gel 'G' coated and activated thin (0.2-0.3 mm) glass plates along with the standard reference samples of sterols (β-sitosterol, Campesterol, Stigmasterol, Spinasterol, Ergosterol, Fucosterol and Stigmastanol). The plates were developed in an organic solvent mixture of Benzene and Ethyl acetate (85:15 v/v), air dried, sprayed with 50% sulphuric acid and subsequently heated at 100 $^0$C for 10 minutes. Some other solvent systems; hexane: acetone (80:20, v/v); acetone: benzene (1:2); hexane: ethyl acetate (3:1) were also tried but Benzene: Ethyl acetate (85:15) solvent mixture gave excellent results, in the present investigation.

### 2.3 Sample preparation for HPLC analysis

Five grams of each plant sample powder was extracted in soxhlet apparatus with 150 mL of methanol 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.

### 2.4 HPLC analysis

The concentrate was then dissolved in 10mL of HPLC-grade methanol: chloroform (1:1). 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 10µl; mobile phase: acetonitrile: water (50:50) containing 3% water, running time 25 min., flow rate 1 ml/min; and detection at 205nm. The separation of filtered plant extracts as well as a mixture of authentic standard samples of Campesterol, Stigmasterol, β-Sitosterol, Stigmastanol was done. The separation of standard phytosterols showed 4 fine peaks in chromatogram and Three phytosterols in *Achyranthes* and two phytosterols in *Cissus* were detected by HPLC. The peak area of standards and samples was calculated to determinate concentration.

## 3. Result and Discussion

Three spots coinciding with those of the authentic samples of β-sitosterol (Rf 0.60), stigmasterol (Rf 0.64) and campesterol (Rf 0.76) were marked in *A. aspera* whereas two spots coinciding with those of standard samples of stigmasterol (Rf 0.64) and campesterol (Rf 0.76) were observed in *C. quadrangularis*

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), Campesterol (Rt= 11.60 min.), Stigmasterol (Rt= 12.00 min.), β-Sitosterol (Rt=12.90 min.), Stigmastanol (Rt= 14.20 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), Campesterol (Rt= 11.10 min.), Stigmasterol (Rt= 12.30 min.), β-Sitosterol (Rt=13.10 min.) in chromatogram of *Achyranthes aspera* and 2 peaks {(Figure3) Campesterol (Rt= 11.60 min.), Stigmasterol (Rt= 11.90 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.

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| | |
|---|---|
| Acquired by | : Admin |
| Sample Name | : phytosterols standards |
| Sample ID | : phytosterols standards |
| Tray # | : 2 |
| Vail # | : 1 |
| Injection Volume | : 10 µL |
| Conc. | : 0.05 µmole $ml^{-1}$ |
| Data File Name | : phytosterols standards data new.lcd |
| Method File Name | : phytosterols standards Meth.lcm |
| Report File Name | : Default.lcr |
| Data Acquired | :1 2/12/2016 10:35 PM |
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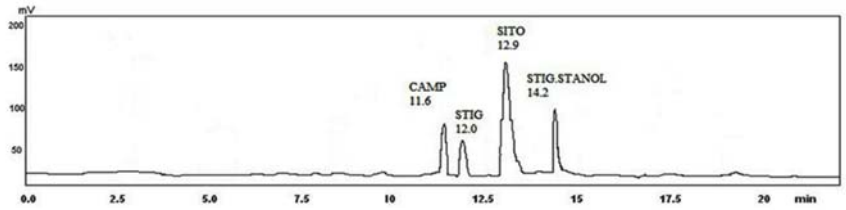


| Peak | Name | Retention Time (min) |
|---|---|---|
| 1. | Campesterol (CAMP) | 11.60 |
| 2. | Stigmasterol (STIG) | 12.00 |
| 3. | β- Sitosterol (SITO) | 12.90 |
| 4. | Stigmastanol (STIG.STANOL) | 14.20 |

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| | |
|---|---|
| Acquired by | : Admin |
| Sample Name | : Achy. phytosterols |
| Sample ID | :Achy. phytosterols |
| Tray # | : 2 |
| Vail # | : 1 |
| Injection Volume | : 10 µL |
| Conc. | : |
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| Method File Name | :Achy. phytosterols Meth.lcm |
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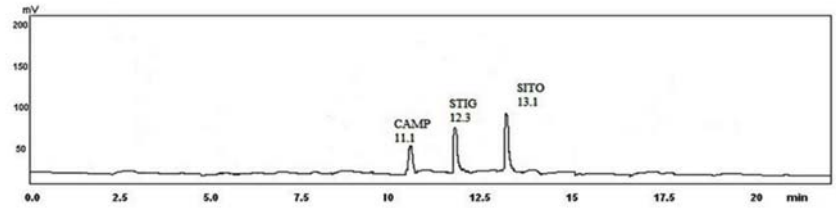


| Peak | Name | Retention Time (min) | Area($mm^2$) | Conc.(µg $g^{-1}$) |
|---|---|---|---|---|
| 1. | Campesterol (CAMP) | 11.10 | 6.11 | 7.03 ±0.23 |
| 2. | Stigmasterol (STIG) | 12.30 | 8.43 | 8.29 ±0.15 |
| 3. | β- Sitosterol (SITO) | 13.10 | 10.02 | 10.12 ±0.09 |

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| | |
|---|---|
| Acquired by | : Admin |
| Sample Name | : Cissus phytosterols |
| Sample ID | :Cissus phytosterols |
| Tray # | : 2 |
| Vail # | : 1 |
| Injection Volume | : 10 µL |
| Conc. | : |
| Data File Name | : Cissus phytosterols data new.lcd |
| Method File Name | : Cissus phytosterols Meth.lcm |
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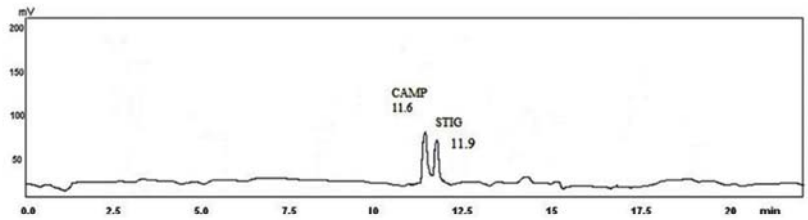


| Peak | Name | Retention Time (min) | Area(mm²) | Conc.(µg g⁻¹) |
|---|---|---|---|---|
| 1. | Campesterol (CAMP) | 11.60 | 7.02 | 8.72 ± 0.02 |
| 2. | Stigmasterol (STIG) | 11.90 | 6.01 | 5.99 ± 0.16 |

## 4. Conclusion

The results above showed, therefore, that *Achyranthes aspera* and *Cissus quadrangularis* are rich in the presence of the important biologically active phytosterols Campesterol, Stigmasterol, β-Sitosterol. The described HPLC procedure could be useful for the qualitative and quantitative analysis of phytosterols in plant materials. Phytosterols have beneficial effects against a wide variety of human ailments. Phytosterols help reduce serum cholesterol by enhancing excretion of cholesterol, interfering with cholesterol synthesis, and competing for cholesterol acceptor sites in the intestinal walls. Some animal studies have also shown that phytosterols inhibit or slower down tumor development. They may also normalizes blood sugar and insulin levels in Type-II diabetics. Therefore determination of phytosterols is very important related to the quality of medicinal plants. The described HPLC procedure could be useful for the qualitative and quantitative analysis of phytosterols in plant materials. It can also be used in the quality control of phytopreparations containing phytosterols.

**5. Conflict of interest:** None

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