Phytochemical evaluation and quantification of beta-sitosterol in geographical variation of *Withania coagulans* Dunal by HPTLC analysis


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Abstract

The recent global resurgence of interest in herbal medicines, has led to an increase in demand for herbal drugs and consequently a decline in their quality, particularly due to a lack of adequate evidence proof data for assessing the quality of drug. The dried seeds of *Withania coagulans* Dunal of Solanaceae family, play a major role in indigenous system of medicine for the treatment of ulcers, dyspepsia, rheumatism, dropsy, etc. Organoleptic parameters are not much reliable in establishing the standards of herbal drugs for which an attempt was made through analytical analysis, providing a more concrete picture regarding the qualitative and quantitative aspects which were widely accepted in the quality assessment of herbal drugs such as TLC and HPTLC studies. In the present study, β-sitosterol has been quantified in methanol and ethyl acetate extracts of *Withania coagulans* Dunal from different states or regions, showing the variation of β-sitosterol content in the drug due to geographical variation. TLC carried with mobile phase Toluene: Ethyl acetate: Glacial Acetic acid (6:1.5:0.5 (v/v)) on Precoated aluminium silica gel plates (Merck) and densitometric determinations was done at 254 nm. Calibration curve was prepared and the amount of β-sitosterol estimated in the extracts by comparing the respective peak areas with that of the standard. A faster, reliable and sensitive HPTLC method has been developed and validated for the analysis of β-sitosterol in seeds of *Withania coagulans*. Other parameters studied such as phytochemical screening, morphology, heavy metals, aflatoxin contamination and fluorescence behaviour to lay down the standard for the genuine drug.

Key words: Phytochemical screening, Safety evaluation, HPTLC studies, β-sitosterol, Quantification

Introduction

The traditional medicines are increasingly solicited through the traditional practitioners and herbalists in the treatment of infectious diseases. Medicinal plants play a vital role for the development of new drugs. The bioactive extract should be standardized on the basis of active compounds (Mathur and Agrawal, 2011). Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. Seeds of *Withania coagulans* Dunal used in Unani System, known as Tukm-e-Hayath which is a small genus of shrubs, popularly known as Indian cheese maker. It is common in Iran, Afghanistan and East India, and also used in folk medicine. It is known differently in various languages such as English - Vegetable rennet; Persian - Arusaka, Paneer-bad; Urdu - Paneerband, Tukme-Hayath; Telugu - Panneru-gadda. It is fairly common in dry hot and stony places up to 1700 m, found in North-west India, Shimla, Punjab, Gujarath, Garhwal.

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Chemical constituents: Dihydrostigmasterol and β-sitosterol, Withanolides, Esterases, Fatty oil, Essential oil, Triacantane, and Amino acids.

Materials and Methods

Collection of material

Withania coagulans Dunal seeds were procured from the local market of different states or regions i.e., Andhra Pradesh, Bhopal, Delhi, Aligarh and Mumbai and are authenticated with the help of a Botanist, Dr. V.C. Gupta at Central Research Institute of Unani Medicine before carrying out the study.

All solvents were of HPLC grade. β-sitosterol (98% purity) was procured from Sigma-Aldrich, Bangalore for reference standard.

The present investigation includes parameters such as morphology, physicochemical analysis, TLC and HPTLC fingerprint, phytochemical screening, fluorescence study, safety evaluation, quantification of active principle etc. Physicochemical parameters were determined according to the methods described in ‘The Unani Pharmacopoeia of India’ (Anonymous, 2009). Fluorescence analysis was carried out as per the method described by Trease and Evans (1972) and GBC-908 AA model Atomic Absorption Spectrophotometer (AAS) was used to determine the concentration of heavy metals. Microbial load and aflatoxins contamination were analyzed as per the methods described in WHO guidelines (Anonymous, 1998). Phytochemical screening was carried out in methanol and ethyl acetate extracts of drug as per the methods described by Trease and Evans (1972) to know the nature of phytoconstituents present in the drug.

Preparation of the sample drug extract

Five grams of powdered drug each of Withania coagulans from different states or regions were macerated in 100 ml of methanol and ethyl acetate separately, in a stoppered 250ml conical flask and was kept for 2 hours while shaking at regular intervals. Later the contents were filtered through Whatmann No. 41 paper and evaporate the solution to 20 ml. The solution, thus, obtained was used as sample for the determination of components.

Preparation of the standard solution of β-sitosterol

Stock solution (100μg/ml) of β-sitosterol was prepared by dissolving with methanol in 10ml standard volumetric flask.
and different aliquots were prepared. For standard drug, methanol is used and for sample drug, methanol and ethyl acetate is used to check in which solvent standard concentration is more.

**Chromatography**

HPTLC was performed on 20 cm × 10 cm Precoated Aluminium Sheets of Silica Gel 60 F254 (Merck). Samples solution of about 10μl were applied as 6 mm width bands using automatic TLC applicator system of the DESAGA Sarstedt Gruppe (Germany). A linear ascending development with Toluene: Ethyl acetate: Glacial Acetic acid (6:1.5: 0.5) (v/v) as mobile phase was carried out in a twin trough glass chamber previously saturated with mobile phase vapour for 20 min. at room temperature (25 ± 2°C). The development of solvent distance was 85 mm. After development, plates were air-dried. Scanning was performed using densitometer of DESAGA Sarstedt Gruppe (Germany) at 254nm and 366nm wavelength and operated by ProQuant 1.06 version software. The source of radiation was a deuterium lamp emitting a continuous UV spectrum in the range 190-400 nm. The slit dimensions were 4 mm × 6 mm.

**Development of HPTLC technique**

After the development, TLC plate was then removed dried completely and detected with under UV Cabinet system for detection of spots. Further, it is scanned with the Densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 254nm, and 366nm as shown in the figures. A corresponding densitogram was then obtained in which peaks are appeared for the corresponding spots being detected in the densitometer while scanning and the peaks area under the curve corresponds to the concentration of the component in the sample for the concentration that applied on the TLC plate.

**Results and Discussion**

**Organoleptic characters**

The crude drug consists of the seeds of Withania coagulans Dunal. of Solanaceae family, having yellow colour.

**Morphology of Withania coagulans Dunal**

**Macroscopic:** Seeds: Dark brown, ear shaped, glabrous, pulp brown, having yellow, berry, globose, 1.5-1 cm long, 0.7-1 cm width, seeds oval to rounded, yellowish brown, 41-59 in number, 0.1-0.3cm long, 0.2-0.3cm wide, dotted (Figure 1).

**Microscopic:** The T. S. section of seeds shows a single layer of epidermis, followed by a layer of highly flattened thin walled sub epidermal cells. Under the sub-epidermis, there is a layer of highly lignified palisade like cells having narrow lumen. The epidermis of the seed coat inner comprise of 1-2 layer of thin-walled parenchymatous cells which are at places are collapsed showing hyaline-like structure. The endosperm is represented by cells showing strong cellulosic thickening filled with aluerone grains without any globoide. The cotyledon shows thin walled radially elongated cells enclosing a wide zone of round to oval to polyhedral parenchymatous cells.

**Quantitative estimation of β-sitosterol by HPTLC analysis**

TLC of the β-sitosterol along with methanol and ethyl acetate extracts of Withania coagulans developed with the mobile phase solvent system and Rf values for the β-sitosterol is 0.45±0.02 and the corresponding spot at the same Rf value was obtained in the other drug extracts at 254nm and under UV 366nm of chromatogram is shown in the Figure 4 and an overlay of densitometric scan of Withania coagulans Dunal, collected from different regions and β-sitosterol in fluorescence 254nm was shown in Figure 2.

The Rf value 0.45±0.02 of β-sitosterol is in correspondence with all the spots at the same Rf value in other drug extracts and were tabulated with respect to position, area percentage and the β-sitosterol concentration corresponds to each peak in the solution applied on the TLC plate are shown in the Table 7 in drugs extracts of different states or region. Further, in Table 8, amount of β-sitosterol in g% with respect to drug is mentioned. A graph illustrated for the amount of β-sitosterol in g% with respect to Withania coagulans Dunal, collected from different states or regions has been depicted in the Figure 3.
Figure 3: A graph showing the amount of β-sitosterol in g% with respect to *Withania coagulans* Dunal collected from different states or regions

TLC plate of Tukhme-Hayath of different extracts at U.V. 254nm

TLC plate of Tukhme-Hayath of different extracts at U.V. 366nm

Figure 4: TLC chromatogram of methanol and ethyl acetate seed extracts of *Withania coagulans* Dunal collected from different regions and β-sitosterol at UV 254nm and 366nm

**Physicochemical studies**

Physicochemical data of *Withania coagulans* such as total ash, acid insoluble ash, water soluble ash, alcohol soluble matter, water soluble matter, pH of 1% and 10% aqueous solution, loss of weight on drying at 105°C and volatile oil are summarized in Table 1.
Table 1: The physicochemical parameters data expressed here as mean values of the three readings calculated

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Results found</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total ash</td>
<td>3.95 - 4.803 g%</td>
</tr>
<tr>
<td></td>
<td>Acid insoluble ash</td>
<td>0.04 - 0.06 g%</td>
</tr>
<tr>
<td></td>
<td>Water soluble ash</td>
<td>1.58 - 1.93 g%</td>
</tr>
<tr>
<td>2.</td>
<td>Alc. sol. matter</td>
<td>10.6 - 11.12 g%</td>
</tr>
<tr>
<td></td>
<td>water sol. matter</td>
<td>25.52 - 26.58 g%</td>
</tr>
<tr>
<td>3.</td>
<td>pH values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. 1% Aqueous solution</td>
<td>4.36 - 4.43</td>
</tr>
<tr>
<td></td>
<td>b. 10% Aqueous solution</td>
<td>4.39 - 4.41</td>
</tr>
<tr>
<td>4.</td>
<td>Loss of weight on drying at 105°C</td>
<td>9.17 g%</td>
</tr>
<tr>
<td>6.</td>
<td>Moisture content</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(by Karlfsicher Titrator Method)</td>
<td>0.3301%</td>
</tr>
<tr>
<td>7.</td>
<td>Volatile oil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Phytochemical screening of *Withania coagulans* Dunal was carried out in different solvent such as ethanol, methanol, chloroform, aqueous, ethyl acetate, petroleum ether extracts to know the nature of compounds present. Qualitative tests for phytoconstituents were carried out for alkaloids, carbohydrates, resin, glycosides, phenols, saponins, proteins, starch, steroids, tannins and flavonoids which are tabulated in Table 2.

Table 2: Phytochemical screening of the nature of compounds present in different solvent extracts of *Withania coagulans* Dunal

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytoconstituents</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Aqueous</th>
<th>Ethyl acetate</th>
<th>Pet ether</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1. Dragendorff’s reagent</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2. Mayer’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1. Benedict’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2. Molisch’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Resinified volatile oils</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Phenols: 1. FeCl₃ test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Proteins: 1. Millon’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Phytosterols(Steroids)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>1. Salkowski reaction test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Tannins: 1. Ferric chloride test:</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Flavonoids: 1. Shinoda test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Fluorescence analysis of powdered drug reaction with chemicals ordinary light and UV light were observed and reported in Table 3. Fluorescence analysis of powdered drug extracts in different solvents were observed and reported in Table 4. Heavy metals and aflatoxins contamination along with their permissible limits were given in Tables 5 and Table 6, respectively. β-sitosterol detection in correspondence with all the spots at the same Rf value in other drug extracts were tabulated with respect to position, area percentage and the β-sitosterol concentration corresponds to each peak in the solution applied on the TLC plate are shown in the Table 7 in drugs extracts of different states or region. Further, in Table 8 amount of β-sitosterol in g% with respect to drug is mentioned.

### Table 3: Fluorescence analysis of powdered drug

<table>
<thead>
<tr>
<th>S.No</th>
<th>Reagents</th>
<th>UV light</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Short 254nm</td>
<td>Long 366nm</td>
<td>Visible light</td>
</tr>
<tr>
<td>1.</td>
<td>Powder as such</td>
<td>Brown</td>
<td>Black</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Powder treated with 1N NaOH in methanol</td>
<td>Black</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Powder treated with 1N NaOH in water</td>
<td>Black</td>
<td>Dark brown</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Powder treated with 1N HCl</td>
<td>Black</td>
<td>Light brown</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Powder treated with 50% HNO₃ aqueous</td>
<td>Black</td>
<td>Blackish green</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Powder treated with 50% H₂SO₄ aqueous</td>
<td>Black</td>
<td>Black</td>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Powder treated with glacial acetic acid</td>
<td>Black</td>
<td>Pale yellow</td>
<td>Dark brown</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Fluorescence analysis of powdered drug extracts in different solvents

<table>
<thead>
<tr>
<th>S.No</th>
<th>Extraction solvent</th>
<th>UV light</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Short 254nm</td>
<td>Long 366nm</td>
<td>Visible light</td>
</tr>
<tr>
<td>1.</td>
<td>Acetone extract</td>
<td>Black</td>
<td>Pale yellow</td>
<td>Yellow</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Alcoholic extract</td>
<td>Greenish black</td>
<td>Light green</td>
<td>Light brown</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform extract</td>
<td>Black</td>
<td>Pale yellow</td>
<td>Brown</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Petroleum ether extract</td>
<td>Black</td>
<td>Pale yellow</td>
<td>Yellow</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Methanol extract</td>
<td>Greenish black</td>
<td>Pale yellow</td>
<td>Dark brown</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Ethyl acetate extract</td>
<td>Greenish black</td>
<td>Light blue</td>
<td>Yellow</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Aqueous extract</td>
<td>Black</td>
<td>Pale yellow</td>
<td>Dark brown</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Heavy metal analysis

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter analyzed</th>
<th>Results</th>
<th>Permissible limits as per WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Arsenic</td>
<td>Nil</td>
<td>Not more than 3.0 ppm</td>
</tr>
<tr>
<td>2</td>
<td>Cadmium</td>
<td>Nil</td>
<td>Not more than 0.3 ppm</td>
</tr>
<tr>
<td>3</td>
<td>Lead</td>
<td>Nil</td>
<td>Not more than 10.0 ppm</td>
</tr>
<tr>
<td>4</td>
<td>Mercury</td>
<td>Nil</td>
<td>Not more than 1.0 ppm</td>
</tr>
</tbody>
</table>

### Table 6: Aflatoxin contamination

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter analyzed</th>
<th>Results</th>
<th>Permissible limits as per WHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B1</td>
<td>Nil</td>
<td>Not more than 0.50 ppm</td>
</tr>
<tr>
<td>2</td>
<td>B2</td>
<td>Nil</td>
<td>Not more than 0.10 ppm</td>
</tr>
<tr>
<td>3</td>
<td>G1</td>
<td>Nil</td>
<td>Not more than 0.50 ppm</td>
</tr>
<tr>
<td>4</td>
<td>G2</td>
<td>Nil</td>
<td>Not more than 0.10 ppm</td>
</tr>
</tbody>
</table>
Table 7: Concentration of β-sitosterol in μg present in different solvent extracts of *Withania coagulans* Dunal collected from different states or regions

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Y-Pos</th>
<th>Area (%)</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH meoh A.P.</td>
<td>40.8 mm</td>
<td>156.608</td>
<td>22.0 μg</td>
</tr>
<tr>
<td>TH meoh Bhopal</td>
<td>40.3 mm</td>
<td>105.676</td>
<td>14.2 μg</td>
</tr>
<tr>
<td>TH meoh Delhi</td>
<td>40.0 mm</td>
<td>99.625</td>
<td>13.2 μg</td>
</tr>
<tr>
<td>TH meoh Aligarh</td>
<td>40.4 mm</td>
<td>155.310</td>
<td>21.4 μg</td>
</tr>
<tr>
<td>TH meoh Mumbai</td>
<td>40.3 mm</td>
<td>169.451</td>
<td>27.0 μg</td>
</tr>
<tr>
<td>TH EA A.P.</td>
<td>42.5 mm</td>
<td>346.539</td>
<td>74.2 μg</td>
</tr>
<tr>
<td>TH EA Bhopal</td>
<td>40.1 mm</td>
<td>236.844</td>
<td>42.1 μg</td>
</tr>
<tr>
<td>TH EA Delhi</td>
<td>40.1 mm</td>
<td>230.391</td>
<td>41.8 μg</td>
</tr>
<tr>
<td>TH EA Aligarh</td>
<td>42.1 mm</td>
<td>228.992</td>
<td>43.6 μg</td>
</tr>
<tr>
<td>TH EA Mumbai</td>
<td>42.9 mm</td>
<td>291.142</td>
<td>58.7 μg</td>
</tr>
</tbody>
</table>

Table 8: Amount of β-sitosterol in g%(w.r.t drug) present in different solvent extracts of *Withania coagulans* Dunal collected from different states or regions

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Concentration</th>
<th>Amount of β-sitosterol in gm%(w.r.t drug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH meoh A.P.</td>
<td>22.0 μg</td>
<td>0.880 %</td>
</tr>
<tr>
<td>TH meoh Bhopal</td>
<td>14.2 μg</td>
<td>0.568 %</td>
</tr>
<tr>
<td>TH meoh Delhi</td>
<td>13.2 μg</td>
<td>0.528 %</td>
</tr>
<tr>
<td>TH meoh Aligarh</td>
<td>21.4 μg</td>
<td>0.856 %</td>
</tr>
<tr>
<td>TH meoh Mumbai</td>
<td>27.0 μg</td>
<td>1.080 %</td>
</tr>
<tr>
<td>TH EA A.P.</td>
<td>74.2 μg</td>
<td>2.968 %</td>
</tr>
<tr>
<td>TH EA Bhopal</td>
<td>42.1 μg</td>
<td>1.648 %</td>
</tr>
<tr>
<td>TH EA Delhi</td>
<td>41.8 μg</td>
<td>1.672 %</td>
</tr>
<tr>
<td>TH EA Aligarh</td>
<td>43.6 μg</td>
<td>1.744 %</td>
</tr>
<tr>
<td>TH EA Mumbai</td>
<td>58.7 μg</td>
<td>2.348 %</td>
</tr>
</tbody>
</table>

**Conclusion**

The drug under study was subjected for physicochemical analysis, which is very much supportive in establishing the standard along with the other parameters such as macroscopic, microscopic, fluorescence behavior as reported in the present investigation including heavy metals, aflatoxins contamination found nil, allowing in the permissible limits of WHO. HPTLC studies were thoroughly studied in methanol and ethyl acetate extract and β-sitosterol successfully quantified and observed the variation of content with respect to the drug. Amount of β-sitosterol content found maximum in ethylacetate extract of A.P. with 2.96 g%. On the basis of these data, the drug was broughtup in determining and ascertaining its quality and standardization of drug. Thus, the study is likely to help in the quality assurance of drug with HPTLC technique which is cost effective, less time consuming and fast analysis to lead formulation with the more therapeutic efficacy due to chemical constituents present in it.

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Graphical abstract

- A faster, reliable and sensitive HPTLC method has been developed and validated for the analysis of β-sitosterol in seeds of *Withania coagulans* Dunal.
- Standardization and quantification of β-sitosterol.
- Used as a reference standard in quality control of *Withania coagulans* in marketed samples. Active marker species content can be compared within the market products.

References


