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HPTLC DETECTION OF B-SITOSTEROL IN *ANETHUM GRAVEOLENS* SEEDS: IMPLICATIONS FOR ITS POTENTIAL ON SKIN MODULATION VIA GUT-SKIN AXIS

MISHRA S[1*], NAYAK S[1], JOSHI K[1], PATEL S[2], MISHRA S[2]

1: Dept. of Dravyaguna, DY Patil Deemed to be University, School of Ayurveda. Nerul. Navi Mumbai-400706

2: K.G. Mittal Ayurveda College, Netaji Subhash Road, Vaidyaraj Ramgopal Sharma Chowk, Mumbai – 400 002, India

***Corresponding Author: Dr. Shivangi Mishra: E Mail: shivangi.mishra@dypatil.edu**

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ABSTRACT

The *Ayurvedic Pharmacopoeia of India (API)* catalogues over 100 herbs essential to traditional medicine, including *Anethum graveolens*. While its gastrointestinal benefits, volatile oils & phytochemical profile are well-documented, the specific role of its phytosterol content — particularly beta-sitosterol (β-sitosterol) — remains under-researched.

Our study aims to determine the presence of β-sitosterol in *A. graveolens* seeds using the CAMAG HPTLC method. Our analysis successfully identified the compound, yielding a characteristic purple band at an R_f value of 0.39, consistent with the β-sitosterol standard.

Building on this analytical validation, we further explored the therapeutic potential of β-sitosterol via the gut-skin axis. Through a comprehensive literature review, we propose that β-sitosterol's influence on gut microbiome modulation allows *A. graveolens* to exert systemic effects beyond relieving gastrointestinal disorders alone. Given its established anti-inflammatory and antioxidant properties, we suggest that *A. graveolens* holds significant potential for improving skin health via the gut-skin axis. This positions the herb as a candidate for treating skin disorders through targeted gut modulation.

Keywords: Anethum graveolens, beta-sitosterol, CAMAG HPTLC, gut-skin axis, microbiome

INTRODUCTION

Medicinal plants are a rich source of diverse and novel phytochemicals, which are the main contributors to their extensive therapeutic profile. *Anethum graveolens L.,* or commonly known as Dill or *shatpushpa,* is one such plant that comes from India's rich medicinal plant reserves. The word 'Anethum' from its Latin name originates from the Greek word 'aneeson'or 'aneeton', which means strong-smelling. The common name Dill comes from the Norse word dylla or dilla, which might mean to soothe **[1]**. *Anethum graveolens L.* (*A. graveolens*) is known for its diverse biological activities, and often, it is synonymously known as *Anethum sowa*. *Shatpushpa* has been mentioned across *Ayurveda* literature and even used in numerous classical formulations. It is an aromatic herb traditionally used primarily for its carminative, stomachic, aromatic, stimulant, diuretic, galactogogue and emmenagogue properties **[2]**. *Bhavaprakash Nighantu* describes the *dravya* to have *dipaniya* (improves digestive fire)*, jwara-hara* (relieves fever)*, vata-kapha hara* (alleviates *vata* & *kapha doshas*)*, vrana ropan* (wound healing)*, shula hara* (relieves pain) and *kshiro roga hara* (relieves disorders related to the ENT) **[3]**.

Studies on Dill have reported its proven pharmacological activities, viz, antioxidant, antimicrobial, anti-inflammatory, analgesic, antiparasitic, hepatoprotective, antidiabetic, diuretic, antiulcer, antidiarrheal, carminative, hypolipidemic, antispasmodic, and antifungal **[4]**. Repeated phytochemical screening of *A. graveolens* seed extracts have recorded the presence of polysaccharides, reducing sugars, proteins, lipids, tannins, catechic tannins, gallic tannins, flavonoids, leucoanthocyanins, saponisides, alkaloids, reducing compounds, carbohydrates (oses and holosides), mucilages, sterols, triterpenes and volatile essential oils **[5]**.

Phytochemicals, being structurally diverse, can be classified into several categories, including polyphenols, terpenoids, sulphur-containing compounds, nitrogen-containing compounds, and others. Of these compounds, volatile and non-volatile components, carvone, kaempferol, anethole, umbelliferone, quercetin and others have been isolated and extensively studied from extracts of different parts of the Dill plant. Beta-sitosterol (β-sitosterol), a naturally occurring phytosterol within the plant kingdom, has not been extensively explored in *A. graveolens*. These compounds can be absorbed into the body through the gastrointestinal tract and enter the hepatic and the circulatory system. In vivo studies have determined that β-sitosterol influences the gut microbiota, thus affecting disease outcome. Interestingly, current emerging

views on the gut-skin axis present us with a different perspective on the utility of Dill in not only gastrointestinal disorders but also skin diseases **[6, 7]**.

Thus, to further expand on the understanding and knowledge of the phytochemical profile of *A. graveolens*, we aim to study the presence of *β-sitosterol* through HPTLC. We will further explore its potential impact on the gut-skin axis modulation and its eventual role as a skin rejuvenator.

MATERIAL AND METHODS

Plant material

Plant material was sourced from Nashik, and authentication was performed independently by the Department of Botany, Xavier College.

HPTLC chemometric analysis

Sample preparation

Anethum graveolens: 600 mg of raw sample was weighed and dissolved in 6 mL of methanol (100mg/mL). This sample was first vortexed for 5 to 10 minutes, followed by sonication for 15 minutes. It was then centrifuged at 3000 rpm for 15 minutes. The clear solution was transferred into a vial for application.

Standard Preparation

β-sitosterol: 10 mg of the standard was weighed and dissolved in 10 ml of methanol (1mg/mL). This sample was first vortexed for 5 to 10 minutes, followed by sonication for 15 minutes. It was then centrifuged at 3000 rpm for 15 minutes. Further dilution was done in a ratio of 1: 10 with the diluent and vortexed for 2 minutes. 0.1 mg/mL of the clear solution was transferred into a vial for application.

Chromatographic conditions

- Stationary Phase Used: TLC Silica gel 60 F_{254} By Merck Product no. 1.05554.0007 plate
- Development Distance: 70 mm

1. Mobile Phase Used:
   - Toluene: Ethyl acetate (8:2v/v)
   - Saturation Time: 20 minutes.
2. Derivatisation reagent:

Anisaldehyde Sulphuric Acid reagent (ASR): 170ml of methanol was added to a 200ml glass bottle and was cooled down in a water-ice cube bath. To the ice-cold methanol, slowly and carefully 20ml of acetic acid and 10ml of sulphuric acid were added and mixed well. The mixture was allowed to cool to room temperature, then 1mL of anisaldehyde was added. The plates were sprayed with the reagent, followed by heating at 150 °C for 5 min on a CAMAG plate heater.

Further, we applied 5µl *Anethum graveolens* solution on track 1 and track 2 and doubled the concentration to 10µl for track 4 and track 5. Track 3 was used for application of the standard at a concentration of 5µl.

Additionally, to establish the potential benefits of beta-sitosterol and its influence

on the gut-skin axis, we will review the available literature on databases such as Google Scholar and PubMed.

RESULTS

HPTLC analysis was performed to detect the presence of the phytoconstituent β-sitosterol in *A. graveolens* seeds. Chromatographic separation was achieved using a mobile phase of toluene: ethyl acetate (8:2v/v) and silica gel TLC plates. Following development and derivatisation with ASR reagent visualization was done under white light to confirm the presence of purple bands distinct to β-sitosterol.

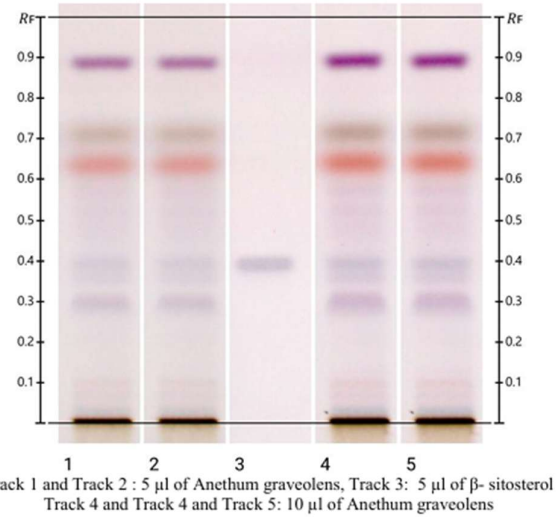

Figure 1: HPTLC chromatogram of *Anethum graveolens* and β-sitosterol standard after derivatisation with ASR (Visible under white light)

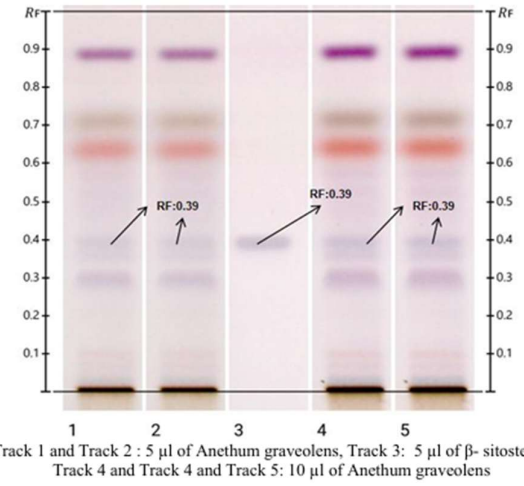

Figure 2: HPTLC chromatogram with R_f markers indicating β-sitosterol identification at *Rf* 0.39 in both standard and sample tracks

β-sitosterol standard (Track 3 in **Figures 1 and 2**) was used for verification and identification of the R_f values. A distinct characteristic purple coloured band at R_f of 0.39 was revealed in track 3. A corresponding band at the identical band of

R_f value 0.39 was visualised in tracks 1,2,4 and 5 **(Figures 1 and 2)**. The intensity of the purple bands in the sample tracks increased proportionally with the application volume (5µl vs. 10µl), further supporting the qualitative identification.

DISCUSSION

Plant sterols, or phytosterols, occur naturally as free alcohols and as fatty acid esters, which humans can only acquire through plant sources, as the body is unable to generate them. β-sitosterol is a phytosterol that, besides herbal plant sources, can be found in nuts, including natural oils, cereals, pulses, legumes, vegetables, fruits and soy products **[8]**. The bioactive compound's chemical formula is $C_{29}H_{50}O$ **(Figure 3) [9]**.

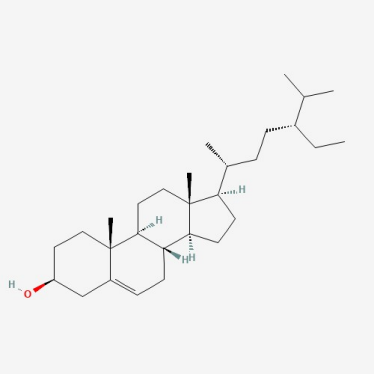

Figure 3: Chemical structure of β-sitosterol

Our study successfully identified β-sitosterol in *A. graveolens* seeds using CAMAG HPTLC, yielding a distinct R_f value of 0.39. A pharmacognostical study of flowers, fruits and leaves of dill by Ortan *et al* **[10]** had revealed flavonoids (quercetin, rutin), hydroxycinnamic acid derivatives (caffeic acid, chlorogenic acid), coumarins (scopoletin), sterols (beta-sitosterol/stigmasterol) and mucilage to be the main classes of active principles. But no analytical study has yet been directed to detect the presence of β-sitosterol specifically from *A. graveolens* seeds, despite being extensively studied in some of the other medicinal herbs.

Anisaldehyde Sulphuric Acid reagent (ASR), being a universal reagent, is often used for detecting and identifying a wide range of natural and synthetic compounds like phenols, steroids, essential oils, and others due to the resultant colour changes that occur upon heating the plates **[11]**. These are distinct in nature for different compounds, which was true in our study as well. Here, we observed the distinct purple band indicating the presence of β-sitosterol, which is a type of steroid.

Medicinal plants are often studied for specific phytochemicals, and β-sitosterol was also specifically isolated through analytical studies. Dubtsova *et al*, **[12]** studied the fractional composition of sterols in *Berberis vulgaris* fruit powder to find that the plant had the highest β-sitosterol concentration alongside campesterol and delta 5-avenasterol.

Gomathi *et al*, **[13]** isolated and characterised β-sitosterol from the combined ethanolic extracts of the rhizome of *Cyperus rotundus* and the stem of *Tinospora cordifolia.* Among the 54 chemical compounds detected, β-sitosterol held the highest concentration and its antioxidant as well as antidiabetic activity was reported.

Gahlaut *et al*, **[14]** successfully used the HPLC-QTOFMS (High-Performance Liquid Chromatography Coupled to Quadrupole Time-of-Flight Mass Spectrometry) technique to qualitatively and quantitatively analyse β-sitosterol in various crude extracts of *Saraca asoca.* The bark and leaves water extracts were found to have a higher concentration of β-sitosterol as compared to other parts.

Jain *et al*, **[15]** identified β-sitosterol in the crude methanol extracts of the leaves of *Atylosia barbata* Baker in amounts that attributed for its various biological activities. The quantification and the identification of compounds in the crude extract were done by TLC and using spectroscopic analysis.

Uttu *et al*, **[16]** Khan *et al*, **[17]** Nweze *et al*, **[18]** and Ved *et al,* **[19]** isolated β-sitosterol through chromatographic separation from extracts of *Strychnos innocua* (root bark), *Cassia sieberiana* leaves*, Punica granatum* stem*,* and *Operculina turpethum* roots, respectively. Besides these, some other plants from which β-sitosterol has been isolated recently are *Cassia fistula, Solanum nigrum, Dillenia indica, Celastrus paniculatus, Clerodendrum serratum,* and *Bauhinia vahlii* used extensively in *Ayurveda* formulations **[20-25]**.

As described earlier phytochemical profile of *A. graveolens* has been studied extensively but not specifically for β-sitosterol. Lote *et al,* **[26]** used a high-performance thin layer chromatography (HPTLC) method for the quantification of quercetin from the methanolic extract of *A. graveolens* seeds. Recent studies conducted on *A. graveolens* seeds specifically have been summarised in the table below, enlisting the molecules detected and the methods used **(Table 1)**. This further validates the specificity of our analytical study.

Table 1: Summary of the different phytochemicals detected in *A. graveolens* seeds and methods used in the studies

Molecule	Class	Method	Ref
α-Pinene, Camphene, Sabinene, β-Pinene, Myrcene, α-Phellandrene, α-Terpinene, Limonene, β-Phellandrene, Z-β-Ocimene, 1,8-Cineole, γ-Terpinene, Terpinolene, Fenchone, Linalool, trans-Pinene hydrate, Camphor, Terpinen-4-ol, Estragole, Carvone, Z-Anethole, ρ-Anisaldehyde, E-Anethole, α-Copaene, Z-Isoeugenol, Methyleugenol, Germacrene D, δ-Cadinene, E-Methylisoeugenol	Essential oils	gas chromatograph of the Thermo Electron type (Trace GC Ultra) coupled with a mass spectrometer	[27]
Medioresinol, Retusin, Kaempferide, 3-Hydroxyflavone, kaempferol, Apigenin 7-rhamnoside, Quercitine 7-glucuronide, Rhamnetin, Quercetin-3-O-galactoside, Quercetin 3-O-rhamnoside, Quercetin 3,3′-dimethyl ether, Apigenin, Apigenin-7-glucoside,	Flavonoid	HPLC/UV-ESI-MS	
Caffeic acid, Cinnamic acid, Syringic acid, Chlorogenic acid, trans-caftaric acid, 3-Feruloylquinic acid, Rosmarinic acid, caffeoyl-feruloyltartaric acid	Phenolic acid		
Scopoletin,Umbelliferone, Coumarin	Coumarin		
Pimelic acid	Fatty acid		
Carnosic acid	Diterpene		
Biotin, Riboflavin, δ-tocopherol, Folic acid	Vitamin		
Rosmanol, Methyl rosmarinate, Epicatechin gallate, 1-Caffeoyl-beta-D-glucose	Polyphenol		
Emmotin H	Sesquiterpenoid		
Homovanillic acid, Ascorbyl palmitate	Other		
Limonene, cis-Dihydro carvone, trans-Dihydro carvone, Carvone, Anethole, Myristicin, Dillapiole	Volatile Oil	GS/MS	[28]
Caprylic, Lauric acid, Myristic acid, Pentadycilic, Palmitoleic acid, Palmitic acid, Margaric acid, Linolenic acid, Linoleic acid, Oleic acid, cis-Vaccenic acid, Stearic acid, Eicosenoic acid, Arachidic acid, Erucic acid, Behenic acid, Lignoceric	Fatty acid	Gas chromatography mass spectrometry (GC/MS)	[29]
d-Limonene, 3-Hexanol, trans-Dihydrocarvone, Carvone, Apiol	Volatile oil		

The systemic importance of these phytochemicals extends beyond simple absorption. Recent evidence suggests that the therapeutic efficacy of sterols may be mediated through the gut-skin axis. Gut microbiota can influence metabolism and immunity; this could also have an impact on skin health. Gut microbiota transforms indigestible complex polysaccharides into essential nutrients, which include vitamins K and B associated with wound healing and repair. They also produce their own metabolites, such as short-chain fatty acids (SCFA), important signalling factors in the gut-skin axis responsible for alleviating skin inflammation **[30]**. Thus, phytochemicals like β-sitosterol could play a key role in maintaining skin health via the gut-skin axis. β-sitosterol has been known to have antioxidant, anti-inflammatory and immunomodulatory properties **[6]**. Recent studies have shown that it can also influence

the gut microbiome. *Ma et al,* **[31]** reported that β-sitosterol regulates the intestinal barrier function and reconstructs the gut microbiota structure by promoting the number of *Lactobacillaceae* and *Bifidobacteriaceae* bacteria. Recent in vivo studies have reported that, being a functional sterol, it has the potential to target functions in humans, beyond nutritional effects, promote health and well-being and/or reduce the risk of chronic diseases like PCOS, atherosclerosis, and renal fibrosis **[32-34]**.

Additionally, its antioxidant could further impact the skin health and integrity. Skin being constantly exposed to environmental factors and cellular respiration continuously produces reactive oxygen species (ROS). Some diseases like psoriasis, vitiligo and acne vulgaris show evidence of oxidative stress that creates an imbalance resulting in skin barrier dysfunction. In such conditions, *A. graveolens* could act as a valuable asset harnessing these properties **[35]**.

HPTLC is a rapid and easy method for qualitative detection of phytoconstituents, and when you are looking for confirmation of one in particular. While HPTLC provided robust qualitative confirmation, these findings establish a foundation for future quantitative analysis and validation using GC-MS and other advanced spectroscopic methods. Both skin and gut act as defence mechanisms of the body, and when it comes to skin disorders, a dysbiosis of both the skin and gut microbiota is also observed. The current study has reviewed the potential of *A. graveolens* phytochemical profile, which contains β-sitosterol can impact on the gut-skin axis. But further understanding is required to establish the therapeutic benefits of *A. graveolens* on the skin beyond its traditional applications.

CONCLUSION

In this study, we successfully demonstrated the presence of β-sitosterol in *A. graveolens* seeds using CAMAG HPTLC at the R_f value of 0.39 using a β-sitosterol standard. Further, we have theoretically laid the foundation for the use of *A. graveolens* seeds in skin disorders, not limiting it to gastrointestinal disorders alone. We propose that its potential impact on skin health is due to its anti-inflammatory, antioxidant and gut-skin axis modulation properties.

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