INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.2.2022.12(37)

# PHYSICO-CHEMICAL EVALUATION OF PHYTOCONSTITUENTS IN *FOENICULUM VULGARE* SEED-A QBD APPROACH

**Indira. Bindu[1]*, Devendra Reddy[1], Chennu Surendra[1],**

**V. Devika[2], NS. Prakash and R. Sundaram[1]**

[1]Analytical Development Laboratory, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru – 562162, India.
[2]Department of Pharmacognosy, R&D Centre, Himalaya Wellness Company, Makali, Bengaluru – 562162, India.

**ABSTRACT**

**Introduction**: *Foeniculum vulgare* seed commonly known as fennel belongs to Apiaceae family. It is an important medicinal and aromatic plant with wide chemical constituents and various therapeutic effects. It is an integral part of many polyherbal formulations. It is a challenging task to ensure the quality of herb from batch to batch during the commercialization of herbal formulation. **Objective:** The study was aimed to find out the physicochemical variation in the seeds, seeds with 2 to 5% stalk, 95% stalk, immature, mature, mixed seeds of washed and unwashed sample with water. **Methods:** The physical and chemical parameters were performed for all the samples. The chemical parameters include total flavonoids, total polyphenols, HPLC profile of phenolic acids such as chlorogenic acid, 4-o-caffeoylquinic acid and flavonoids like rutin and quercetin 3-o-glucoronide and anethole, a volatile oil component were identified and quantified as marker principles. **Results and Discussion**: From the results, the seeds with 95% stalk has shown marginally lower extractive value compared to other two variants (only seeds and seed with 2 to 5% stalk). The same observation is found in the assay content of total flavonoids and total polyphenols. There is no significant difference in the chemical profile of analytical markers with respect to chlorogenic acid, 4-o-caffeoylquinic acid, rutin, quercein-3-o-glucoronide except anethole content. In immature seeds, the content of analytical markers such as total flavonoids, total polyphenols, chlorogenic acid, 4-o-caffeoylquinic acid, rutin, quercein-3-o-glucoronide and anethole is marginally higher than in the mature and mixed seeds. The analytical data indicates that during washing there is a loss of water-soluble phyto-constituents except anethole. As anethole is a volatile constituent and is insoluble in water and is intact even after washing. **Conclusion**: The seeds without stalk, immature seeds and unwashed seeds have higher content of chemical constituents compared to seeds with stalk, mature seeds, mixed and washed seeds.

**Keywords:** *Foeniculum vulgare*, Anethole, Phenolic acids and Flavonoids.

## 1. INTRODUCTION

*Foeniculum vulgare* Mill. is an annual, biennial or perennial aromatic herb belonging to the family Apiaceae. The seeds weigh between 6 and 7 mg, about 6 mm long and 2 mm width in the central portion. It is a highly aromatic herb and the seeds are used as flavouring agent in beverages, baked foods and herb mixtures[1]. It is used with purgative to alleviate the side effect. It is used as a food or drug to improve the milk supply of breast feeding mother[2]. Many studies revealed that pharmacological effects of *F. vulgare* are mainly due to the presence of essential oil and its individual phytoconstituents[3]. There is a continuous quest in medical science to discover new drug molecule to fight against emerging diseases. The chemical constituents from plant origin often act as a prime molecule for such discoveries[4]. A holistic approach of preparing and administrating the plant extracts has been successfully used traditionally. Recent years can be considered as revival of Ayurveda as herbal products has been reported to be safe and without any side effects[5].

The physicochemical character of the plant is varying in its composition depending upon the plant part used, maturity of plant collected and location where the plant is grown[6]. The characterization and estimation of phytochemical compounds are quite challenging due to the complex chemical composition in plants[7]. The present phytochemical studies were conducted on fennel seeds, seeds with stem stalk pieces and 95% stalk, immature seeds, mature seeds, mixed seeds (mature and immature), washed seeds and unwashed seeds[8-9]. The study aimed to identify the physio-chemical characters and quantification of analytical markers using high performance liquid chromatography[10-11]. The phenolic compounds were identified and quantified as chlorogenic acid and 4-o-caffeoylquinic acid and flavonoids as Rutin, and quercein-3-o-glucoronide[12].

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant material

The seeds of *F. vulgare* were collected from Patan, Unjha of Gujarat, GKVK of Bengaluru and some parts of South India. The seeds were identified and authenticated by Dr. Kannan, Botanist, R & D Centre, Himalaya Wellness Company, Bengaluru, Karnataka, India. The voucher specimen is deposited at the R&D archive centre of Himalaya Wellness Company.

### 2.2. Drying process

The samples collected contain seeds without stalk, seeds with 2 to 5% stalk, 95% stalk, immature, mature, mixed with mature and immature seeds, washed and unwashed seeds. In the present study, thirty-two samples were collected, and different variance were considered during the collection of samples. The variance includes place of collection, colour of seeds, maturity of the seeds and seeds with different proportions of stalks which vary from 2 to 5% and 95% stalk, washed and unwashed seeds.

### 2.3. Washing process

The seeds were washed under tap water (potable water) and dried under sunlight.

### 2.4. Powdering of the material

The dried samples of *F. vulgare* were ground using a rotary grinder, sieved through 25 mesh sieves and stored in airtight HDPE container at room temperature. The analytical variations can be minimized using the powdered sample which is used throughout the study for the physico-chemical and phytochemical evaluation.

### 2.5. Chemicals and Reagents

Analytical reagent grade or HPLC grade chemicals were used for the analysis. Trifluoro acetic acid was procured from Merck Life Science Pvt. Ltd, acetonitrile from Fischer Scientific, aluminium chloride from SD Fine Chemicals, ethyl acetate, acetic acid, butanol and formic acid from Rankem, methanol from Standard Reagents Pvt. Ltd. and ethanol from Honyon Intl, Inc. Purified water which is used for the preparation of mobile phase is from Milli-Q water purification system (Millipore, Pure Lab, Classic, ELGA). Filtration membranes (0.45 µm, cellulose acetate/cellulose nitrate mixed esters) were purchased from Millipore. The standard of Rutin hydrate, Anethole and Chlorogenic acid is procured from Sigma Aldrich.

### 2.6. Physico-chemical analysis

#### 2.6.1. Loss on drying

Weigh about 10 g of powdered sample and transferred in a tared evaporating dish and dried at 105$^0$C for 5 hours and weighed. The process was continued until constant weight[13].

#### 2.6.2. Total ash

Weigh about 2 g of powdered material and transferred in a previously weighed silica crucible and incinerated, gently at first, and gradually increased the temperature to 675$^0$C until free from carbon[13].

#### 2.6.3. Acid insoluble ash

The obtained ash was treated with 25 ml of 2M hydrochloric acid and boiled for 5 minutes. The insoluble matter was collected, washed with hot water and ignited at 600$^0$C in a silica crucible[13].

#### 2.6.4. Water soluble extractive value

Weigh about 5 g of powdered material and macerated with 100 mL of chloroform-water mixture in a closed flask for 24 hours, continuously shaken for 6 hours and allowed to stand for 18 hours. The solution was filtered, and 25 mL of the filtrate was dried completely in a tared flat-bottomed shallow dish at 105$^0$C and weighed which gives the percentage of water-soluble extractive value[14].

#### 2.6.5. Alcohol soluble extractive value

Weigh about 5 g of powdered material and macerated with 100 mL of absolute alcohol in a closed flask for 24 hours. It was continuously shaken for 6 hours and kept for 18 hours. The solution was filtered, 25 mL of the filtrate was transferred into a flat-bottomed flask and dried at 105$^0$C and weighed which gives the alcohol soluble extractive value[15].

**2.7. Total Flavonoids by UV**

Flavonoids are one of the most important pharmacologically active principles in *F. vulgare*. The plant extract react with aluminium chloride gives a yellow colour complex with flavonoids and can be measured using a spectrophotometer[16]. The calorimetric method was used for the estimation of total flavonoids using Shimadzu UV 1700 series. Rutin hydrate was used as a standard for the quantification of flavonoids. The samples were extracted with methanol and subjected to reaction with aluminium chloride reagent. The amount of the colour complex was measured at 410 nm against the prepared reagent blank.

**2.8 Total Polyphenols by UV**

Polyphenols are biologically active phyto-compounds with significant contribution in human health and the prevention of chronic diseases. They are aromatic compounds with hydroxyl groups attached to an aromatic hydrocarbon. The calorimetric method in which Folin-Ciocalteu reagent (FCR) was used which react with phenolic compounds present in plant material and yield the total amount of polyphenols in the sample. Pyrogallol standard was used as standard for the quantification of polyphenols. The samples were extracted with purified water and treated with Folin-Ciocalteu reagent and measured the absorbance at 760 nm.

**2.9. HPLC analysis**

A high-performance liquid chromatography (HPLC) with photo diode array detector (PDA) was used for the separation and identification of chlorogenic acid, 4-o-caffeoylquinic acid, rutin, quercein-3-o-glucoronide and anethole. The flavonoids identified and quantified was rutin and quercein-3-o-glucoronide using rutin as standard and polyphenols identified and quantified was chlorogenic acid and 4-o-caffeoylquinic acid using chlorogenic acid as standard. The HPLC used was Shimadzu Prominence I equipped with a photo diode array detector, SIL-20ACHT auto sampler, DGU-20A5 degasser, LC-20AD pump, CBM-20 A system controller, CTO-10 ASVP oven and LC solutions software. Many trials were conducted for the separation of the phyto-constituents. The best chromatographic condition was achieved using 0.1% v/v trifluoro acetic acid in water (mobile phase A) and acetonitrile (mobile phase B). The linear gradient elution was performed with the following ratio of mobile phase B 40%, 0 minutes, 90%, 10-15 minutes, 40%, 20-35 minutes at a flow rate of 1 mL/minute with a column oven temperature of 40°C. The approximate retention time for the elution of chlorogenic acid, 4-o-caffeoly quinic acid, Rutin, quercein-3-o-glucoronide and Anethole was about 8.3, 13.6, 14.3, 16.4 and 27.8 minutes respectively. The analytical markers identified and quantified include chlorogenic acid, 4-o-caffeoylquinic acid with respect to standard chlorogenic acid and cumulative value of chlorogenic acid and 4-o-caffeoylquinic acid is expressed as total phenolic acids. Rutin and quercein-3-o-glucoronide were calculated with respect to Rutin hydrate and amount of anethole was estimated by using anethole standard. The reference HPLC chromatogram with the retention time at the wavelength of 354 nm and 260 nm is shown in Fig.1a and Fig.1b.

**Test solution preparation**

2 g of powdered material was weighed and refluxed with methanol on water bath at $80^0$c for 30 minutes and the process was repeated for two more times. The dissolved extract was collected in to a 100 ml volumetric flask (Fig. 1a & Fig. 1b).

**2.10 TLC analysis**

Thin layer chromatography (TLC) fingerprinting technique is universally accepted and is a common tool for the identification of phyto-compounds in herbal products. Each plant has a specific set of phytoconstituents which can be identified by TLC using a stationary phase and a suitable solvent system (mobile phase). In the present study, TLC fingerprint was performed using a CAMAG TLC system with win CATS software including photo documentation unit, and TLC spotter. The USP general chapter < 203 > was referred for plate layout, sample application, conditioning of the plate, development, and visualization for the analysis.

The quality of the herbal materials can be evaluated by the TLC fingerprint by identifying the variation in the phytoconstituents in the different batches of the plant material. In this study the variation of the phytoconstituents were studied in the washed seeds, unwashed seeds, seeds mixed with stalk and seeds without stalk. The test solutions were prepared by dissolving 1 g of the material in methanol. The samples were spotted on precoated silica gel plate (Merck) using CAMAG Linomat V sample applicator. The mobile phase employed was Ethyl acetate: Acetic acid: Formic acid: Water in the ratio of 100:11:11:26. The plates were developed and visualized under UV 254 nm, 366 nm and at 366 nm after derivatization with aluminium chloride reagent. The TLC fingerprint confirms that bands intensity for chlorogenic acid, Rutin and quercein-3-o-glucoronide is less in

washed seeds compared to unwashed seeds which is shown in Fig.2. There is no difference observed in the TLC fingerprint of seeds mixed with different ratio of stalks and seeds without stalk which is shown in Fig.3.

## 3. RESULT AND DISCUSSION

*F. vulgare* seeds were collected from different locations and during the collection of materials, different variance were considered such as colour of seeds, maturity of seeds (immature, mature and mixed) and seeds admixed with different proportions of stalks such as 2 to 5 % and 95 % stalks, seeds after washing with potable water and seeds without washing with potable water which is shown in Table 1.

### Seeds, Seeds with 2 to 5% stalk and 95% Stalk

The seeds, seeds with 2 to 5% stalk and 95% stalk of immature, mature and mixed (immature and mature) samples of 32 batches were analysed for physico-chemical analysis and the results are reported in table 2 and 3. Three variances of seeds were green, green and brown and brown in colour. Based on the physico-chemical analysis results, there is no significant change between results of total ash and acid insoluble ash of seeds, seeds with 2 to 5% stalk and 95% stalk. The water extractive value of seeds, seeds with 2 to 5% stalk and 95% stalk were found to be 15.28 to 23.70%, 13.99 to 21.11% and 14.81% respectively. The alcohol extractive value of seeds, seeds with 2 to 5% stalk and 95% stalk is 7.84 to 13.91%, 5.45 to 11.28% and 3.5% respectively. From the results, the seeds with 95% stalk has shown marginally lower extractive value compared to other two variants. The same observation is also found in the assay content of total flavonoids and total polyphenols. The content of total flavonoids observed is between 0.12 to 0.41%, 0.11 to 0.30% and 0.30% in seeds, seeds with 2 to 5% stalks and 95% stalks respectively. The content of total polyphenol is 0.61to 0.94%, 0.58 to 0.76% and 0.59% in seeds, seeds with 2 to 5% stalks and 95% stalks. There is no significant difference in the chemical profiling of analytical markers with respect to chlorogenic acid, 4-o-caffeoylquinic acid, rutin, quercein-3-o-glucoronide except anethole content. The amount of anethole observed in seeds, seeds with 2 to 5% stalk and 95% stalk is 0.26 to 0.61%, 0.28 to 0.65 % and 0.14% respectively. The immature seeds with stalk has shown 0.11% of anethole content. There is no significant change in the HPTLC and HPLC pattern between seeds, seeds with 2 to 5% stalk and 95% Stalk.

### Immature, mixed, and matured seeds

One of the variance considered during the collection of sample is maturity of seeds. The analytical study conducted for immature, mixed and matured seeds and the colour of these seeds varies from green to brown. There is no significant change in ash value, acid insoluble ash value, water extractive value among the immature, mixed and matured seeds. The value range between 6.95 to 10.88% w/w for ash value, 0.17 to 1.49% for acid insoluble ash value and 15.28 to 23.70 % for water extractive value. The immature seeds (NPD/313/16ISD) has shown least alcohol extractive value (5.45% w/w) whereas in mixed and matured seeds it ranges from 7.84 to 12.61% w/w and 8.22 to 13.9% w/w respectively. In Immature seeds, the amount of analytical markers such as total flavonoids (0.4% w/w), total polyphenols (0.98% w/w), chlorogenic acid (0.1% w/w), 4-o-caffeoylquinic acid (0.0038% w/w), rutin (0.0126% w/w), quercein-3-o-glucoronide (0.25% w/w) and anethole (0.43% w/w) is marginally higher than in the mature and mixed seeds (table 2 and table 3). The chromatographic pattern of HPTLC and HPLC is similar in immature, mixed and matured seeds. The intense bands have observed in the immature seeds compared to mixed and matured seeds which has confirmed both by HPLC and HPTLC. The mixed green coloured unwashed certified seed NPD/823/15 collected from GKVK-Bengaluru has shown highest yield of quercetin-3-o-glucoronide (0.28%).

### Washed and unwashed seeds

The unwashed seeds are green to brown in colour. The green coloured seeds after washing with portable water became brown colour after drying. The physico-chemical results revealed that, there was a significant drop in water extractive value, alcohol extractive value and assay of active markers in washed samples compared to unwashed material. The water extractive values in the unwashed sample of NPD/325/16 and NPD/311/16 observed was 15.28% and 21.90% w/w respectively, where as in washed sample of same batch has observed 12.90% and 17.6% w/w respectively which is less compared to unwashed sample. The alcohol extractive values in the unwashed sample of NPD/325/16 and NPD/311/16 were 9.60% and 7.77% w/w respectively, where as in washed sample of same batch has observed the alcohol extractive value as 8.77% and 7.05% w/w respectively. There was a drastic reduction in the content of flavonoids from unwashed to washed samples of NPD/325/16 and NPD/311/16 from 0.32% to 0.19% w/w

and 0.17% to 0.09% w/w respectively. The content of polyphenols has reduced in unwashed sample compared to washed samples of NPD/325/16 and NPD/311/16 and the assay observed was 0.81% to 0.47% w/w and 0.68% to 0.61% w/w respectively. The amount of phyto-markers such as chlorogenic acid, 4-o-caffeoylquinic acid, rutin and quercetin -3-o-glucoronide were less in washed samples compared to unwashed sample but there is no significant change in the anethole content of washed and unwashed materials (table 2 and table 3). The same trend is observed in other three batches of washed and unwashed samples of NPD/307/16, NPD/308/16 and NPD/326 for water extractive value, alcohol extractive value, total flavonoids, total polyphenols, chlorogenic acid, 4-o-caffeoylquinicacid, rutin and quercetin-3-o-glucoronide (table 2 and table 3). The HPTLC fingerprint and HPLC chromatogram confirmed that there is a significant difference in the chromatographic pattern of washed and unwashed seeds.

**Table 1: Different Variance in the collection of *Foeniculum vulgare* seeds**

| No | Batch no | Grade | Variance 1 Purity | Variance 2 Percentage | Variance 3 Geography | Variance 4 Maturity | Variance 5 Colour | Variance 6 Washing |
|---|---|---|---|---|---|---|---|---|
| 1 | NPD/299/16 | Grade A | Seeds | 100 | Patan | Mixed | Green Colour Seeds | Un washed |
| 2 | NPD/300/16 | Grade B | Seeds | 98 | Patan | Mixed | Green Colour Seeds | Un washed |
| 3 | NPD/301/16 | Grade C | Seeds with Stalk | 96 | Patan | Mixed | Green & Brown colour Seeds | Un washed |
| 4 | NPD/302/16 | Farmer Grade | Seeds | 97 | Patan | Mixed | Green colour Seeds | Un washed |
| 5 | NPD/303/16 | Farmer Grade | Seeds | 97 | Patan | Mixed | Brown colour Seeds | Un washed |
| 6 | NPD/304/16 | Farmer Grade | Seeds | 97 | Patan | Mixed | Green & Brown Seeds | Un washed |
| 7 | NPD/305/UW/16 | Farmer Grade | Seeds with Stalk | 95 | Patan | Mixed | Green colour Seeds | Un washed |
| 8 | NPD/305/W/16 | Farmer Grade | Seeds with stalk | 95 | Patan | Mixed | Brown colour Seeds | Washed |
| 9 | NPD/306/16 | Farmer Grade | Seeds | 100 | Patan | Mixed | Green & Brown colour Seeds | Un washed |
| 10 | NPD/307/UW/16 | Not available | Seeds | 97 | Unknown | Matured | Brown colour Seeds | Un washed |
| 11 | NPD/307/W/16 | Innova 116 variety | Seeds | 97 | Unknown | Matured | Brown colour Seeds | Washed |
| 12 | NPD/308/W16 | Arya Farm Products | Seeds | 100 | Unknown | Matured | Brown colour Seeds | Washed |
| 13 | NPD/308/UW /16 | Arya Farm Products | Seeds | 100 | Unknown | Matured | Green colour Seeds | Un washed |
| 14 | NPD/309/UW/16 | Farmer Grade | Seeds | 97 | Patan | Mixed | Green colour Seeds | Un washed |
| 15 | NPD/309/W/16 | Farmer Grade | Seeds | 97 | Patan | Mixed | Brown colour Seeds | Washed |
| 16 | NPD/310/16 | Not available | Seeds with 95% Stalk Pieces | S90:sd10 | Patan | Mixed | Green & Brown colour | Un washed |
| 17 | NPD/311/UW /16 | Grade A | Seeds | 100 | Unjha | Matured | Green colour Seeds | Un washed |
| 18 | NPD/311/W/16 | Grade A | Seeds | 100 | Unjha | Matured | Brown colour Seeds | Washed |
| 19 | NPD/312/16 | Farmer Grade | Seeds with stalks | 97 | Patan | Mixed | Green & Brown colour | Un washed |
| 20 | NPD/313/IS/16 | Not available | Immature seeds with few stalks | 100 | Patan | Immature Seeds | Green & Brown colour | Un washed |
| 21 | NPD/313/IHS/16 | Not available | Immature Seeds with Stalk | Not available | Patan | Immature with hallow seeds | Green & Brown colour | Un washed |
| 22 | NPD/313/SD/16 | Not available | Immature Seeds with Hallow Stalk | Not available | Patan | Stalks & dust with immature seeds | Green & Brown colour | Un washed |
| 23 | NPD/821/15 | Not available | Seeds | 100 | Patan | Mixed | Green colour Seeds | Un washed |
| 24 | NPD/822/15 | Not available | Seeds | 100 | Patan | Mixed | Green & Brown colour | Un washed |
| 25 | NPD/823/15 | Not available | Seeds | 100 | GKVK Bengaluru | Mixed | Green colour Seeds | Un washed |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 26 | NPD/823b/15 | Not available | Seeds | 100 | GKVK Bengaluru | Mixed | Green to Brown colour Seeds | Un washed |
| 27 | NPD/325/UW/16 | Machine cleaned | Seeds | 100 | Unjha | Matured | Green colour Seeds | Un washed |
| 28 | NPD/325/W/16 | Machine cleaned | Seeds | 100 | Unjha | Matured | Brown colour Seeds | Washed |
| 29 | NPD/326/UW/16 | Not available | Seeds | 100 | Unjha | Matured | Brown colour Seeds | Unwashed |
| 30 | NPD/326/W/16 | Not available | Seeds | 100 | Unjha | Matured | Brown colour Seeds | Washed |
| 31 | NPD/327/16 | Farmer Grade | Seeds | 100 | Not available | Matured | Brown colour Seeds | unwashed |
| 32 | NPD/328/16 | Farmer Grade | Seeds | 100 | Not available | Mixed | Green & Brown colour Seeds | Unwashed |

W- Washed, UW-Unwashed

**Table 2: Physico-chemical analysis data of Fennel seeds**

| S.NO | B. No | Total ash (%w/w) | Acid insoluble ash (%w/w) | Water extractive value (%w/w) | Alcohol extractive value (%w/w) |
|---|---|---|---|---|---|
| 1 | NPD/299/16 | 7.24 | 0.24 | 16.51 | 8.18 |
| 2 | NPD/300/16 | 6.66 | 0.68 | 16.60 | 7.95 |
| 3 | NPD/301/16 | 9.00 | 0.43 | 16.21 | 8.68 |
| 4 | NPD/302/16 | 9.75 | 0.46 | 17.23 | 8.51 |
| 5 | NPD/303/16 | 7.41 | 1.19 | 16.84 | 7.72 |
| 6 | NPD/304/16 | 7.23 | 0.46 | 16.42 | 7.30 |
| 7 | NPD/305/16/W | 6.24 | 0.16 | 16.00 | 9.22 |
| 8 | NPD/305/UW/16 | 7.65 | 0.94 | 21.11 | 11.28 |
| 9 | NPD/306/16 | 7.82 | 1.22 | 23.70 | 12.61 |
| 10 | NPD/307/UW/16 | 7.64 | 0.95 | 21.22 | 8.53 |
| 11 | NPD/307/W/16 | 6.16 | 0.55 | 19.39 | 7.43 |
| 12 | NPD/308/W/16 | 6.91 | 0.72 | 17.09 | 11.25 |
| 13 | NPD/308/ UW /16 | 6.95 | 0.59 | 20.75 | 13.91 |
| 14 | NPD/309/UW /16 | 7.31 | 1.03 | 18.53 | 8.19 |
| 15 | NPD/309/W/16 | 6.86 | 0.82 | 17.64 | 7.16 |
| 16 | NPD/310/16 | 9.90 | 0.60 | 14.81 | 3.50 |
| 17 | NPD/311/ UW /16 | 8.35 | 1.49 | 21.90 | 9.77 |
| 18 | NPD/311/ W /16 | 7.81 | 0.57 | 17.61 | 7.05 |
| 19 | NPD/312/16 | 8.22 | 1.01 | 13.99 | 8.20 |
| 20 | NPD/313/IS/16 | 10.88 | 0.62 | 19.08 | 6.79 |
| 21 | NPD/313/HIS/16 | 12.16 | 4.45 | 18.00 | 3.99 |
| 22 | NPD/313/SD/16 | 7.94 | 0.23 | 15.25 | 5.45 |
| 23 | NPD/821/15 | 8.68 | 0.67 | 17.26 | 9.18 |
| 24 | NPD/822/15 | 8.71 | 0.36 | 16.94 | 10.62 |
| 25 | NPD/823/15 | 8.38 | 0.17 | 16.10 | 12.99 |
| 26 | NPD/823/B/15 | 8.62 | 0.35 | 18.78 | 12.16 |
| 27 | NPD/325/UW/16 | 7.12 | 0.76 | 15.28 | 11.12 |
| 28 | NPD/325/ W /16 | 6.00 | 0.47 | 12.90 | 8.77 |
| 29 | NPD/326/UW/16 | 7.51 | 0.79 | 16.73 | 9.60 |
| 30 | NPD/326/W/16 | 5.41 | 0.44 | 14.53 | 9.53 |
| 31 | NPD/327/16 | 8.52 | 0.92 | 16.92 | 8.22 |
| 32 | NPD/328/16 | 8.20 | 0.84 | 17.69 | 7.84 |

**Table 3: Quantification of Analytical markers by HPLC and UV in Fennel seeds**

| Sl. no | B.no | Total Flavonoids by UV % w/w | Total Polyphenols by UV % w/w | Chlorogenic acid % w/w | 4-o-caffeoyl quinic acid wrt to Chlorogenic acid % w/w | Rutin % w/w | quercetin-3-o-glucoronide wrt Rutin % w/w | Anethole w/w | Total phenolic acids wrt to chlorogenic acid by HPLC |
|---|---|---|---|---|---|---|---|---|---|
| 1 | NPD/299/16 | 0.18 | 0.60 | 0.11 | 0.0022 | 0.0056 | 0.18 | 0.51 | 0.20 |
| 2 | NPD/300/16 | 0.11 | 0.66 | 0.10 | 0.0033 | 0.0054 | 0.20 | 0.53 | 0.25 |
| 3 | NPD/301/16 | 0.14 | 0.67 | 0.06 | 0.0046 | 0.0048 | 0.16 | 0.32 | 0.17 |
| 4 | NPD/302/16 | 0.30 | 0.66 | 0.07 | 0.0032 | 0.0040 | 0.15 | 0.28 | 0.18 |
| 5 | NPD/303/16 | 0.28 | 0.76 | 0.06 | 0.0032 | 0.0048 | 0.15 | 0.49 | 0.15 |
| 6 | NPD/304/16 | 0.26 | 0.58 | 0.07 | 0.0029 | 0.0047 | 0.17 | 0.43 | 0.18 |
| 7 | NPD/305/ UW /16 | 0.25 | 0.63 | 0.07 | 0.0024 | 0.0042 | 0.15 | 0.56 | 0.16 |
| 8 | NPD/305/16/W | 0.13 | 0.44 | 0.00 | 0.0010 | 0.0000 | 0.01 | 0.58 | 0.01 |
| 9 | NPD/306/16 | 0.20 | 0.73 | 0.09 | 0.0026 | 0.0054 | 0.19 | 0.56 | 0.21 |
| 10 | NPD/307/ UW /16 | 0.37 | 0.73 | 0.02 | 0.0010 | 0.0029 | 0.08 | 0.34 | 0.05 |
| 11 | NPD/307/ W /16 | 0.31 | 0.68 | 0.02 | 0.0013 | 0.0029 | 0.07 | 0.42 | 0.04 |
| 12 | NPD/308/ W /16 | 0.41 | 0.64 | 0.10 | 0.0022 | 0.0035 | 0.12 | 0.55 | 0.16 |
| 13 | NPD/308/ UW /16 | 0.14 | 0.73 | 0.14 | 0.0023 | 0.0036 | 0.15 | 0.42 | 0.23 |
| 14 | NPD/309/ UW/ 16 | 0.19 | 0.62 | 0.07 | 0.0028 | 0.0049 | 0.18 | 0.65 | 0.18 |
| 15 | NPD/309/ W /16 | 0.08 | 0.52 | 0.01 | ND | 0.0019 | 0.03 | 0.38 | 0.02 |
| 16 | NPD/310/16 | 0.30 | 0.59 | 0.07 | 0.0044 | 0.0040 | 0.16 | 0.14 | 0.19 |
| 17 | NPD/311/ UW /16 | 0.17 | 0.68 | 0.07 | 0.0051 | 0.0048 | 0.16 | 0.55 | 0.17 |
| 18 | NPD/311/ W /16 | 0.09 | 0.61 | 0.04 | 0.0009 | 0.0024 | 0.07 | 0.46 | 0.09 |
| 19 | NPD/312/16 | 0.20 | 0.70 | 0.10 | 0.0018 | 0.0040 | 0.15 | 0.48 | 0.23 |
| 20 | NPD/313/IS | 0.40 | 0.98 | 0.10 | 0.0038 | 0.0126 | 0.25 | 0.43 | 0.33 |
| 21 | NPD/313/HIS/16 | 0.41 | 1.16 | 0.08 | 0.0040 | 0.0120 | 0.25 | 0.10 | 0.26 |
| 22 | NPD/313/ SD/ 16 | 0.38 | 0.95 | 0.09 | 0.0059 | 0.0074 | 0.24 | 0.11 | 0.26 |
| 23 | NPD/821/15 | 0.25 | 0.71 | 0.09 | 0.0049 | 0.0123 | 0.24 | 0.61 | 0.22 |
| 24 | NPD/822/15 | 0.12 | 0.70 | 0.06 | 0.0024 | 0.0086 | 0.16 | 0.33 | 0.21 |
| 25 | NPD/823/15 | 0.25 | 0.94 | 0.12 | 0.0039 | 0.0087 | 0.28 | 0.61 | 0.29 |
| 26 | NPD/823/B/15 | 0.41 | 0.62 | 0.03 | 0.0015 | 0.0036 | 0.13 | 0.45 | 0.10 |
| 27 | NPD/325/UW/16 | 0.32 | 0.81 | 0.09 | 0.0027 | 0.0066 | 0.21 | 0.40 | 0.24 |
| 28 | NPD/325/ W /16 | 0.19 | 0.47 | 0.00 | Not detected | 0.0012 | 0.03 | 0.54 | 0.02 |
| 29 | NPD/326/UW/16 | 0.32 | 0.56 | 0.03 | 0.0019 | 0.0030 | 0.10 | 0.36 | 0.08 |
| 30 | NPD/326/W/16 | 0.11 | 0.47 | 0.00 | Not detected | 0.0007 | 0.02 | 0.39 | 0.01 |
| 31 | NPD/327/16 | 0.33 | 0.76 | 0.06 | 0.0032 | 0.0028 | 0.11 | 0.26 | 0.15 |
| 32 | NPD/328/16 | 0.29 | 0.61 | 0.09 | 0.0028 | 0.0049 | 0.15 | 0.48 | 0.20 |

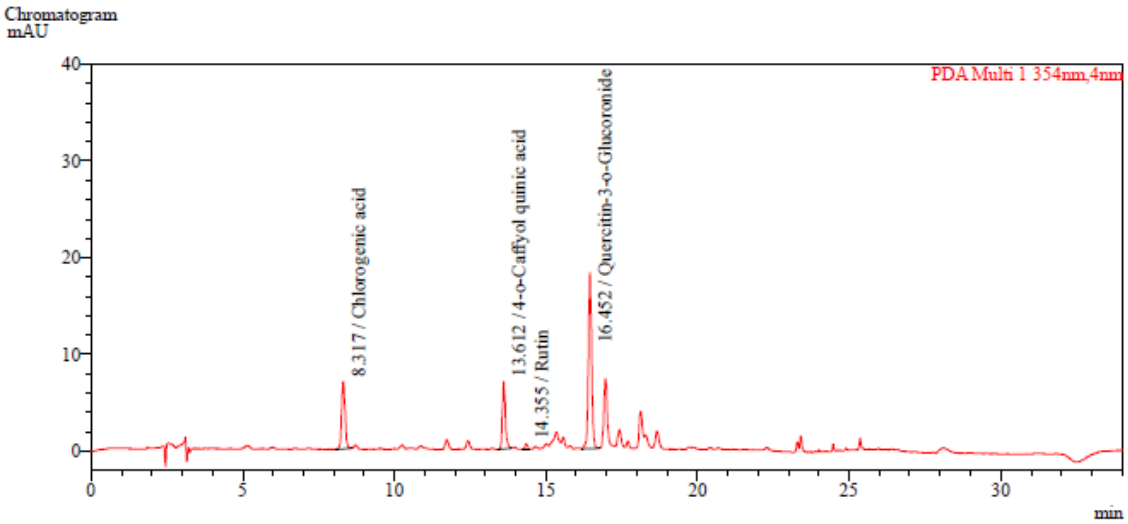



**Fig. 1a: HPLC profiling of Fennel Seed-Phenolic acids and flavonoids**

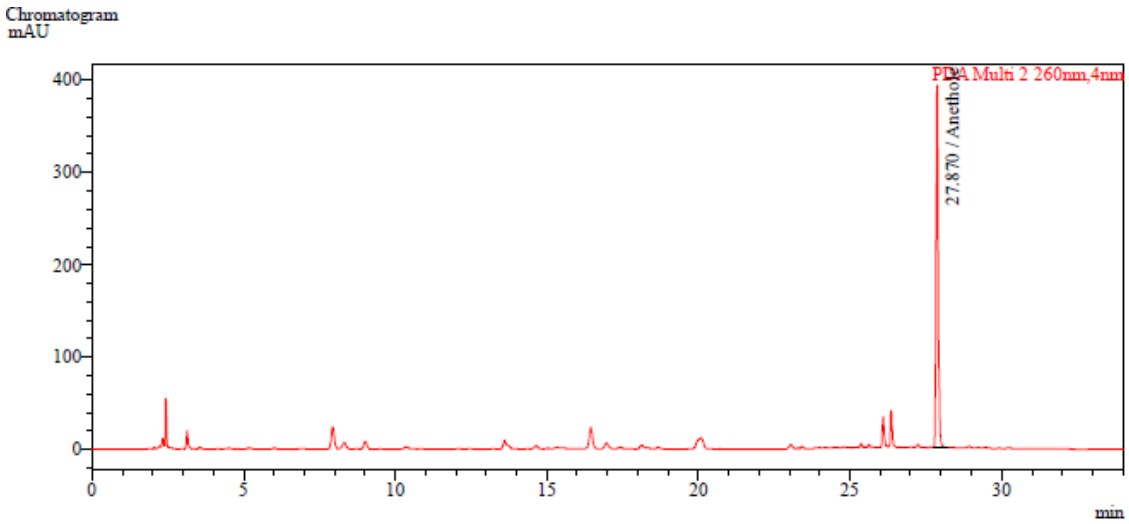
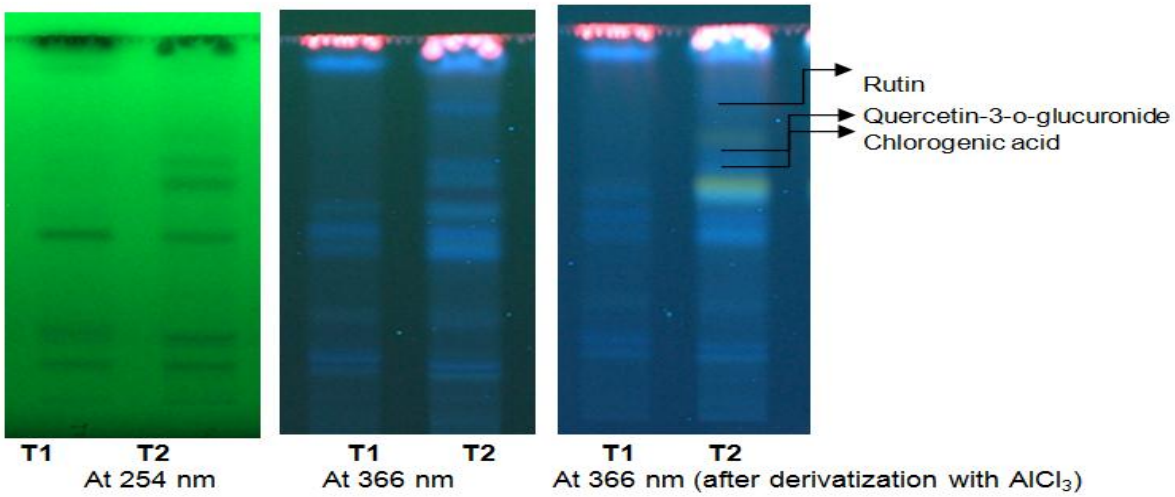
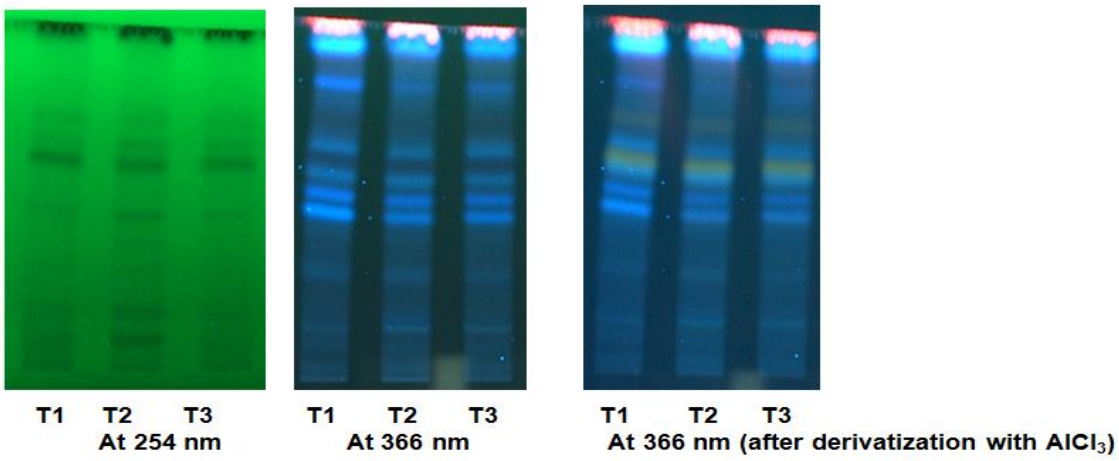



**Fig. 1b**: **HPLC profiling of Fennel seed-Anethole**




**T1:** Fennel Seed-Washed sample
**T2:** Fennel Seed- Un Washed sample
**Fig. 2: TLC fingerprint of Fennel seeds**




**T1:** Fennel Seed mixed with stalk
**T2:** Fennel Seed without any stalk
**T3:** Fennel Seed with hallow seed
**Fig. 3: TLC fingerprint of Fennel seeds with stalk and hallow seed**

**Seeds, Seeds with 2 to 5% stalk and 95% Stalk**

Based on the analytical results, the physico-chemical profile is same between seeds, seeds with 2 to 5% stalks and 95% stalks except anethole content. The anethole content in stalk is very less compared to seeds, the best quality of material of the seeds should be free from the stalk. As the percentage of stalk in the seeds increased, there is a decrease in the anethole content which leads to the reduction in the quality of material. From this, it is very difficult to identify the fennel seed admixed with few percentage of stalk in the powder form due to the same chemical profile by HPTLC or HPLC.

**Immature, mixed, and mature seeds**

The results revealed that immature seeds contain slightly rich polyphenols and flavonoids compared to mixed and matured seeds, the same is reflecting in the other individual markers such as chlorogenic acid, 4-o-caffeoylquinic acid, rutin and quercetin-3-o-glucuronide.

**Washed and unwashed seeds**

The unwashed seeds are green in colour due to the presence of chlorophyll pigments which is missing after water washing and sun drying due to which the seeds become brown in colour. The unwashed seeds are rich in polyphenols, flavonoids, chlorogenic acid, 4-o-caffeoylquinic acid, rutin and quercetin-3-o-glucuronide. During the process of washing, the water soluble components dissolved in water are washed away except anethole content. The analytical data indicates that there is a loss of water soluble phyto-constituents during the washing. After washing, content of anethole is impact as it is insoluble in water and due to its volatile nature.

## CONCLUSIONS

The phytochemical analysis is the preliminary step for the evaluation of medicinal plants to find out the authenticity and quality of crude drug. The analytical data is an important tool for the development of herbal raw material as a drug. The fennel seeds samples were widely studied for physico-chemical parameters such as loss on drying, total ash, water extractive value, alcohol extractive value, total flavonoids and polyphenols by UV, identification and quantification of chlorogenic acid, 4-o-caffeoylquinic acid, Rutin, quercetin-3-o-glucuronide by HPLC and identification by TLC fingerprint. The quality control of the raw material is achieved by the analytical data.

The physico-chemical analysis of different variants of fennel seeds, seeds with stem stalk (2 to 5%) and 95% stalk, immature, mature, mixed, water washed, and unwashed seeds were performed. As per the analytical data it is concluded that seeds without any stalk, immature, mature and mixed seeds has shown more phytoconstituents such as polyphenols, flavonoids, chlorogenic acid, 4-o-caffeoylquinic acid, rutin and quercetin 3-o- glucuronide compared to seeds with stalk and washed seeds.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Authors are thankful to management of Himalaya Wellness Company, Bengaluru, India for providing necessary resources to carry out this work.

## REFERENCES

1. Diaaz Maroto MC, Hidalgo IJD, Saa nchez-Palomo E and Pea rez-Coello MS. Volatile components and key odorants of fennel (Foeniculum vulgare Mill.) and thyme (Thymus vulgaris L.) Oil extracts obtained by simultaneous distillation–extraction and supercritical fluid extraction. J Agric Food Chem. 2005;53:5385–5389.
2. Agarwal R, Gupta SK, Srivastava S and Saxena R. Oculohypotensive effects of Foeniculum vulgare in experimental models of glaucoma. Indian J Pharmacol. 2008;52:77-83.
3. Tognolini M, Ballabeni V, Bertoni S, Bruni R, Impicciatore M and Barocelli E. Protective effect of Foeniculum vulgare essential oil and anethole in an experimental model of thrombosis. Pharmacol Res. 2007;56:254-260.
4. Dev S. Ayurveda and modern drug development. Proceedings of the Indian National Science Academy, Physical Science.1988;54:12-42.
5. Raynor DK, Dickinson R, Knapp P, Long AF and Nicolson DJ. Buyer beware. Does the information provided with herbal products available over the counter enable safe use. *BMC Med.* 2011:9:94-96.
6. Dua A, Garg G and Mahajan R. Polyphenols, flavonoids and antimicrobial properties of methanolic extract of fennel (Foeniculum vulgare Miller). European Journal of Experimental Biology. 2013;3:203-208.

7. Anna S, Cezary P and Max H. Influence of environmental abiotic factors on the content of saponins in plants. Phytochemistry Reviews. 2011;10(4):471-491.
8. Miura Y.K, Ogawa H, Fukui and Tabata M. Changes in the Essential Oil Components During the Development of Fennel Plants from Somatic Embryoids. Planta Medica.1986;52:95-96.
9. Arslan N, Bayark A and Akgul A. The Yield and Components of Essential oil in Fennels of different origin (Foeniculum vulgare Mill.) growing in Ankara conditions. Herba Hungarica. 1989;28:27-31.
10. Parejo I, Viladomat F, Bastida J, Schmeda-Hirschmann G, Burillo J and Codina C. Bioguided isolation and identification of the non-volatile antioxidant compounds from Fennel (Foeniculum vulgare Mill.). J Agric Food Chem. 2004;52:1890-1897.
11. Parejo I, Jauregui O, Schmeda-Hirschmann G, Viladomat F, Bastida J and Codina C. Separation and characterization of phenolic compounds in fennel (Foeniculum vulgare Mill.) using liquid chromatography- negative electro spray ionization tandem mass spectroscopy. J. Agric Food Chem. 2004;52:3679-3687.
12. Indian pharmacopoeia. 2018;1, 2.4.19.
13. Indian pharmacopoeia .2018;1, 2.3.19.
14. Ayurvedic Pharmacopoeia of India. part-1;1,2.2.7
15. Ayurvedic Pharmacopoeia of India. part-1;1,2.2.6
16. Faudale M, Viladomat F, Bastida J, Poli F and Codina C. Antioxidant activity and phenolic composition of wild, edible and medicinal fennel from different mediterranean countries. J Agric Food Chem. 2008;56:1912-1920.
17. Singh G, Maurya S, Lampasona MP and Catalan C. Chemical constituents, antifungal and antioxidative potential of Foeniculum vulgare volatile oil and acetone extract. Food control. 2006;17:745-752.