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HPLC ESTIMATION OF QUERCETIN IN HYDROALCOHOLIC EXTRACT OF *FOENICULUM VULGARE*

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ABSTRACT

There is at present growing interest, both in the industry and in the scientific research, for aromatic and medicinal plants because of their antimicrobial, antidiabetic, anticancer and antioxidant properties etc. These properties are due to many active phytochemicals including flavanoids, terpenoids, carotenoids, coumarins, curcumines etc. These bioactive principles have also been confirmed using modern analytical techniques. *Foeniculum vulgare* Mill. (Fennel), a perennial herb with a characteristic aniseed flavor, belongs to the Apiaceae family and is cultivated worldwide. For centuries, fennel seeds have been used as a traditional herbal medicine in Europe and mainland China. A number of beneficial properties such as anti-inflammatory, analgesic, antibacterial, antioxidant and anti-depressant effect have been attributed to fennel seeds. The aim of the present study was to examine *F. vulgare* seeds for phytochemical profile and quercetin was detected in hydroalcoholic seed extract of *F. vulgare* under study by using RP-HPLC analysis. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The total phenolics content of seed hydroalcoholic extract was (0.271 mg/100mg), followed by flavonoids (0.543mg/100mg). For future studies, phytochemcials responsible for various activities can be isolated and modified for pharmacological purpose.

Key words: *Foeniculum vulgare*, Phytochemical profile, Quercetin, RP-HPLC, Flavonoids.

INTRODUCTION:

Medicinal plants are very good sources of drugs for traditional systems of medicine. Indian medicinal plants have lot of potential towards curing many diseases. Medicinal plant extracts contain various types of bioactive compounds known as phytochemicals. These phytochemicals can be used in treatment as anticancer, antimicrobial, antioxidant, anti-inflammatory agents etc (Wadikar and Patki., 2016). Recent studies show that these phytochemicals are safe, broadly effective and

have less adverse effects. However in vivo studies of these phytochemicals are necessary to demonstrate their efficacy, safety and to verify their bioavailability (Soni and Singhai., 2012). Spices are used all over the world to improve the taste and flavour of food products. In addition, they have medicinal properties, and can be beneficial in the prevention of different human diseases. Epidemiological and *in vitro* studies strongly suggest that phytochemical of spices have potential protective effects against many diseases. Therefore, they could be used as anti-mutagenic, antibacterial, antiviral and anti-inflammatory agents (Seneviratne et al., 2006). There is increasing evidence that consumption of phytochemical compounds present in spices may lower the risk of serious health disorders (Hertog et al., 1993; Surh 1999). In India, the spices such as cumin (*Cuminum cyminum* L.), fennel (*Foeniculum vulgare* Mill.) and Fenugreek (*Trigonella foenum-graecum* L.) are important ingredients used in the food. *Foeniculum vulgare* Mill. (Fennel), a perennial herb with a characteristic aniseed flavor, belongs to the Apiaceae (Umbelliferae) family and is cultivated worldwide. For centuries, fennel seeds have been used as a traditional herbal medicine in Europe and mainland China (Villarini et al., 2014). A number of beneficial properties such as anti-inflammatory, analgesic, antibacterial, and antioxidant (Aprotosoaie et al., 2008; Shahat et al.,2011), dyspeptic disorders (Bub et al., 2006), hepato-protective activity (Devika et al., 2013), anti-depressant effect (Singh et al., 2013) have been attributed to fennel seeds. Hypolipidemic effect of volatile oil of fennel was evaluated (Oulmouden et al., 2011; Al-Doghachi et al., 2012; Shahat et al., 2012). Fennel seeds are reported to contain phytochemicals such as polyacetylenes (falcarinol, falcarindiol, falcarindiol-3-acetate) and polyphenols (caffeic acid, gallic acid, apigenin-7-o-glucoside, ferulic acid, syringic acid, isovitexin, phloridzin) (Rawson et al., 2013). The study was designed to demonstrate the phytochemical constitute. Total phenolic and flavonoid content was determined spectrophotometrically as well as flavonoid characterized by using HPLC.

MATERIAL AND METHOD

Plant Material

Seeds of *F. vulgare* were purchased from local market of Bhopal MP.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade. Quercetin was kindly provided by Scan Research Laboratories, Bhopal (India). Methanol and acetonitrile were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade from Merck Ltd, New Delhi, India.

Extraction Procedure

Defatting of Plant Material

Powdered plant material of *F. vulgare* was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction

was continued till the defatting of the material had taken place

Extraction

100gm of dried plant material were exhaustively extracted with hydroalcoholic solvent using maceration method for 48 hrs. Then extract was filtered. The extract was evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extract (Mukherjee; 2007).

Qualitative phytochemical analysis of plant extract

The *F. vulgare* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate (Khandelwal., 2005; Kokate., 1994)). The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from seeds of *F. vulgare* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso et al., 2011. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [19]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Quantification of flavonoid compounds by HPLC technique

For HPLC investigation of flavonoid compounds the hydroalcoholic extracts of *F. vulgare* seed under study were used as a preliminary assessment of various compounds. The HPLC apparatus used for analysis was composed of a waters equipped with a UV dual detector and generated data were analyzed using Waters Ace software. For chromatographic separation Thermo C18 column (250 X 4.6 mm, 5µm) was applied. The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 ml/ min. A small sample volume of 20 µl was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm.

Sample volume (20 ul) and analysis time was 10min for both, standards and samples used for analysis. A quercetin was used as standards. A thermospectronic model of Labindia 3000+UV/VIS Spectrophotometer with 1cm. matched quartz cells were used for determination of λmax. The sample solution was chromatographed and a concentration of quercetin in extract sample was found out using regression equation.

RESULTS AND DISCUSSION

The crude extracts so obtained after the hot continuous percolation extraction process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant. The yield of extracts obtained is depicted in the Table 1.

Table 1: Percentage Yield of Extract of *F. vulgare*

S. No.	Solvents	Percentage Yield (%)
1.	Hydroalcoholic	4.3

Phytochemical analysis of hydroalcoholic extracts of seed sample of *F. vulgare* showed the presence of flavonoid, protein, diterpines and phenols. This study indicates the presence of flavonoids present in sufficiently enough quantity in extract so flavonoid is the phytochemicals that are present in hydroalcoholic extract Table 2.

Table 2: Phytochemical Screening of *F. vulgare* Extract

S. No.	Constituents	Extract
1.	Alkaloids	-
2.	Glycosides	-
3.	Flavonoids	+
4.	Diterpenes	+
5.	Phenolics	+
6.	Amino Acids	-
7.	Carbohydrate	-
8.	Proteins	+
9.	Saponins	-
10.	Oils and fats	-

(+ve)= Present, (-ve) = absent

The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of hydroalcoholic extract of *F. vulgare* seed showed the content values of 0.271 mg/100mg (GAE). The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of hydroalcoholic extract of *F. vulgare* seed showed the content values of 0.543 mg/100mg (QE). Results are provided in (Table 3 and Fig. 1, 2).

Table 3 Total phenolic and total flavonoid content of *F. vulgare*

S. No.	Extracts	Total Phenol (GAE) (mg/100 mg)	Total flavonoid (QE) (mg/100 mg)
1.	**Hydroalcoholic**	0.271	0.543

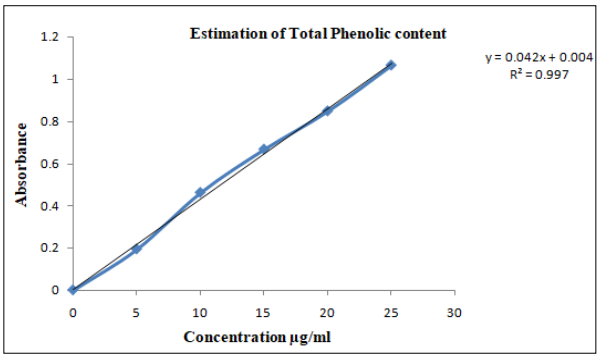


Figure 1 Graph of Estimation of Total Phenolic content

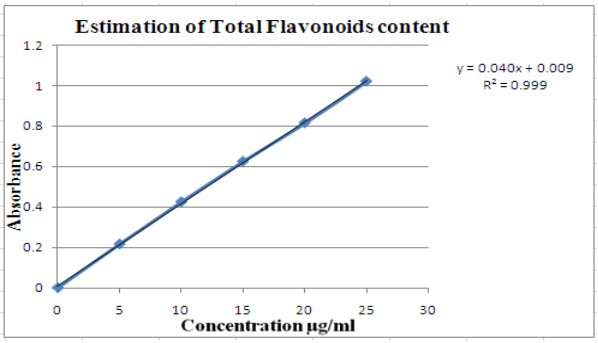


Figure 2 Graph of Estimation of Total Flavonoids content

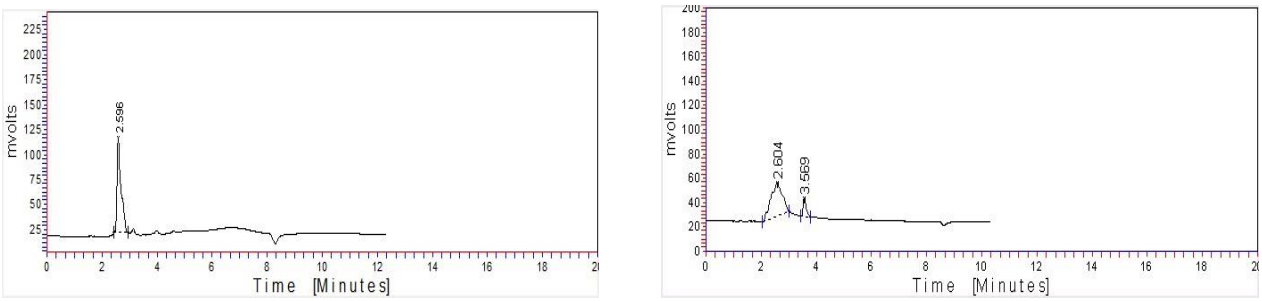
Flavonoids compounds are secondary metabolites in plants which play an immensely important role in human health and nutrition. The HPLC chromatogram of standard quercetin and hydroalcoholic extract are shown in Fig. 3 and the values are expressed in ppm. The retention time for standard and extract was found to be 2.596 min and 2.604 min respectively. Characteristics parameters for standard quercetin was given in table 4 and results of quantitative estimation of quercetin in hydroalcoholic seed extract were given in table 5.

Table 4 Characteristics of the analytical method derived from the standard calibration curve

Compound	Linearity range µg/ml	Correlation co-efficient	Slope	Intercept
Quercetin	5-25	0.999	94.39	-30.43

Table 5 Quantitative estimation of quercetin in Hydroalcoholic extract

S. No.	Extract	RT	Area	% Assay
1.	*Foeniculum vulgare*	2.604	766.011	0.843



(A) **(B)**

Figure 3 Chromatogram of (A) Standard Quercetin (B) Hydroalcoholic Extract of *Foeniculum vulgare*

CONCLUSION

The present study concluded that this medicinal plant viz. *Foeniculum vulgare* is a promising source of various activities and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity and to explore the existence of synergism if any, among the compounds.

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