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RESEARCH ARTICLE

Preliminary Screening and Quantification of Flavonoids in Selected Seeds of Apiaceae by UV-Visible Spectrophotometry with Evaluation Study on Different Aluminium Chloride Complexation Reaction

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Abstract

Background : The objective of the study includes a preliminary phytochemical screening of selected plant seeds of Apiaceae for flavonoid content and developing a suitable method of quantification by a UV-Visible spectrophotometer and validation of the same. **Methods:** Selected dry seeds of Apiaceae were extracted using 70% ethanol at 60°C for 1h in the shaker. Procedures of different chemical compositions were designed to evaluate flavonoid-Aluminium chloride complexation reaction to determine the best suitable method for the quantification of total flavonoid content by UV-Visible spectrophotometer. The antioxidant activity was investigated by the phosphomolybdenum method and DPPH radical scavenging method. The developed method was validated by considering the parameters such as linearity, accuracy, precision, and limit of detection and quantification. **Findings:** The concentration of flavonoid varied from 246 μg QE/g - 2856 μg QE/g among the selected dry seeds of Apiaceae and showed promising antioxidant activities with IC_{50} values varying between 9.64-249.75 μg/ml. Evaluation study on different aluminum chloride complexation reactions involved in the flavonoid quantification analysis revealed that procedure 3 developed by the researcher was the accurate and suitable method. The developed method was showed to be the simple and rapid method compared to previously published methods. The developed method showed to be linear (R2 = 0.999), precise (R.S.D. < 2%), accurate (recovery of 103.03%), and inexpensive. **Novelty:** The extracts were shown to contain a significant amount of flavonoid and displayed promising antioxidant activity and can therefore be a potential source of flavonoid and natural antioxidant agents. This article data could serve as a reference against the improper use of $AlCl_3$ chelation methods for the quantification of flavonoids in food and plant samples.

Keywords: Apiaceae; Quercetin; Total Flavonoid Content; Antioxidant Activity; Flavonoid and Aluminium chloride Complex; UV-Visible spectrophotometer

1 Introduction

During ancient times, plants/plant extracts were given either orally or applied externally to the affected areas to alleviate pain and to treat various forms of illness. People are shifting their choice of treatment from synthetic to natural form, not because they are inexpensive but for the fact that, it has better compatibility with the human body and has negligible side effects [(1)], [(2)]. The prediction by the World Health Organization (WHO) suggests that about 80% of the population of the developing countries uses traditional method, particularly plants for their primary health care [(3)]. The modern drugs in the market, at least 25% of them contain compounds derived from plant origin and many other synthetic drugs are designed based on the prototype compounds isolated from the plants [(4)], [(5)]. The ceaseless and never-ending individual's enthusiasm for restorative plants created a new line of research, this brought about today's modern and sophisticated fashion of their processing and manufacturing a drug to cure illness [(6)]. Epidemiological investigations have given promising results that regular consumption of fruits and vegetables helps in lowering the risk of developing chronic diseases, such as cancer and cardiovascular disease. Flavonoids are shown to act as a potent anticancer agent by executing its preventive mechanism of action in a number of ways such as through its antioxidant activities, regulation of apoptosis pathways, regulation of cell cycle, inhibition of angiogenesis, and regulation of cell signal transduction pathways. A research study conducted by Liu, 2004 suggests that the additive substance and synergistic impacts of phytochemicals in fruits and vegetables are in charge of these powerful antioxidant and anticancer properties and that the advantage of an eating routine wealthy in leafy foods and fruits is credited to the intricate blend of phytochemicals present in whole foods [(7)]. Flavonoids found in fruits and vegetables take part in maintaining balanced cell proliferation and averting cell carcinogenesis [(8)]. Flavonoids have a potency to instigate apoptosis in malignant cells. Fatty acid snythase enzyme (FAS) is a key enzyme responsible for the synthesis of fatty acid which is over expressed in various forms of human cancers. Flavonoids inhibit this FAS enzyme and thereby causing a cell to undergo apoptosis [(9)]. Hence flavonoids are regarded as the anticancer agent which helps in reducing the chances of occurrence of cancer and the spread of tumor. Flavonoids exhibit an anti-inflammatory property. Several mechanisms of action explains in vivo flavonoid anti-inflammatory actions, such as antioxidative and radical scavenging activities, modulation of the production of other proinflammatory molecules, modulation of proinflammatory gene expression, regulation of cellular activities of inflammation [(10)]. Flavonoid content present in diets rich food and beverages has epidemiologically shown to have a positive role in reducing the risk of fatal cardiovascular disease [(11)]. Studies conducted further reveals that five flavonoid classes—anthocyanidins, flavan-3-ols, flavones, flavonols, and proanthocyanidins were individually imparted its action in lowering the risk of fatal cardiovascular disease [(12)], [(13)].

The Apiaceae (Umbelliferae) family includes several of the frequently eaten spices. They are an outstanding source of phenolic compounds [(14–18)] . Information concerning the flavonoid content of the commonly used Indian spices belonging to the Apiaceae is not completely explored.

Several methods for the determination of flavonoids in medicinal plants have been reported, including Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC) and spectrophotometer. Both, TLC and HPLC are comparatively time-consuming methods, either because of the extended run time or more solvent consumption [(19)]. Spectrophotometer has indispensable advantages for the

routine laboratory analysis such as simplicity, low operational cost and shorter run time.

The increasing potential health benefits of flavonoids and pharmaceutical supplement demands the development of alternative methods for the routine analysis of flavonoids from various plant sources. Within this context, the aims of this study was to explore some of the traditional medicinal plants of Apiaceae which are rich sources of flavonoids by developing suitable method of quantification by UV-Visible spectrophotometry with evaluation study on different Aluminium chloride complexation reaction.

2 Materials and methods

2.1 Sample collection and preparation

Eight seed varieties of Apiaceae (dry) family as shown in Figure 1 were selected for analysis based on the literature survey and were listed as "a to h". These seed samples were purchased from the local supermarket (Mysuru, Karnataka, India), cleaned and grounded to fine powder using a blender, sieved (80 mesh) and stored in airtight container at 4°C until analysis.

Fig 1. Selected dry seed samples ofApiaceae for analysis

2.2 Chemicals

The Quercetin standard and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Himedia (Mumbai, India). Extra pure Methanol, Ethanol, Aluminium chloride ($AlCl_3$), Sodium phosphate, Ammonium molybdate, Sulphuric acid (H_2SO_4), Potassium Acetate, Sodium hydroxide (NaOH) and Sodium nitrite were purchased from Merck (Mumbai, India). All the chemicals obtained are of analytical grade. Milli-Q water was generated using a Milli-Q purification system (Millipore, Bangalore, India) and used throughout the study.

2.3 Instrumental and analytical conditions

Absorbance measurements were made on UltraViolet/Visible (UV-Vis) 1800 spectrophotometer (Shimadzu, Japan) with a pair of matched quartz cells of 1 cm width. Aluminium chloride colorimetric method was used for flavonoid quantification in samples. After reaction with $AlCl_3$, the UV-Visible spectrum of $AlCl_3$ and Quercetin complex was recorded between 200 nm to 600 nm. In the spectral analysis, the wavelength 430nm was defined for the quantification of flavonoids in seed extract samples. The time interval was recorded to determine the ideal time of reaction to form an $AlCl_3$ and Quercetin complex and also to evaluate the complex stability. Hence spectrophotometric determination of Quercetin solutions was carried out immediately after the $AlCl_3$ addition and at a regular time interval of 10 minutes, until 60 minutes. The interval time of 10 minutes was defined as the ideal time for measuring the absorbance of solutions after $AlCl_3$ addition.

2.4 Solvent extraction

Solvent extraction was carried out by placing 1 g of ground seed sample in a total volume of 45 ml of 70% Ethanol in a conical flask at 60°C, 120 rpm for total 1 hour in a shaker. For every 20 minutes until 60 minutes, the flask was removed, extract

was centrifuged, filtered and solid was resuspended in a free solvent and kept for extraction again. A total of three trials were conducted in this way to maximize the yield of flavonoids. After 1 hour, the extraction apparatus was rinsed twice with additional extraction solvent and filtered with Whatman filter paper grade No.1. The Final volume of extraction solution was made up to 20 ml by evaporation in hot air oven maintained at 80°C. This extract was subjected to preliminary phytochemical screening and UV-Visible spectrophotometry for quantification of flavonoid.

2.5 Preliminary phytochemicals screening

2.5.1 Shinoda test

1 mL of ethanol and few drops of concentrated Hydrochloric acid were added to extract solution. Appearance of red color indicates the existence of aurones and chalcones. Magnesium chips were added in instances where no color change was observed. The formation of orange, red or magenta colours, respectively, stated the existence of flavones and flavonols [20].

2.5.2 Sodium hydroxide

Few drops of Sodium hydroxide (10%) were added to extract solution. Appearance of yellow-red, coffee-orange, purple-red or blue coloration indicates the existence of xanthones and/or flavones, flavonols, limons and anthocyanins respectively [20].

2.6 Development and Evaluation of Aluminium complexation reaction for estimation of Total flavonoid content (TFC)

Total flavonoid content was determined by Aluminium colorimetric method using an external calibration curve of a standard by spectrophotometry. Quercetin is the most commonly used standard for the quantification of flavonoids. Quantification of total flavonoid using Quercetin as a standard was done using Aluminium chloride and Potassium acetate method [21] [22,23] or Aluminium chloride, Sodium nitrite and Sodium hydroxide method [24] [25]. This study was designed to develop and evaluate the best suitable method for quantification of total flavonoids when expressed it in terms of Quercetin equivalents.

Procedure 1: The Aluminum chloride colorimetric method reported by Kamtekar et al., 2014 was followed for the quantification of TFC [26]. A clean test tube was taken, 1 ml of the Quercetin standard (20 μg/ml) was added, followed by 1.25 ml of water, then 0.3 ml of 5 % Sodium nitrite solution was added and allowed to stand for 5 minutes and then 0.3 ml of 10 % Aluminium chloride was added. After 6 minutes, 2 ml of 1.0 M Sodium hydroxide was added and the final volume was made up to 5 ml using water. Blank was prepared in the same manner where 1 ml of distilled water was used instead of the standard. The absorbance of the mixture was measured at 510 nm on the UV-Visible spectrophotometer immediately.

Procedure 2: The Aluminum chloride colorimetric method was modified from the procedure reported by Chia-Chi et al., 2002 and used for quantification of TFC [21]. 1 ml of the Quercetin standard (20 μg/ml) was taken and 1.5 ml of 80% Methanol, 0.3 ml of Aluminum chloride and 0.3 ml of 1 M Potassium acetate was added. Tubes were incubated at room temperature for 30 minutes and then the final volume was made up to 5 ml using water. The absorbance spectrum was taken between 200 nm to 600 nm and the absorbance was taken at a wavelength where maximum OD is obtained. In the spectral analysis, the wavelength 430 nm was defined for quantification of Quercetin.

Procedure 3: 1 ml of the Quercetin standard (20 μg/ml) was taken and 0.3 ml of 10% Aluminium chloride was added. After reaction with $AlCl_3$, the UV-Visible spectrum of Quercetin and $AlCl_3$ solutions were recorded in the range of 200 nm to 600 nm. In the spectral analysis, the wavelength 430 nm was defined as the wavelength yielding highest absorbance. The time interval for recording the UV spectrum after complex formation was also investigated to determinate the optimal time for reaction and to evaluate the complex stability. Hence, spectrophotometric determination of Quercetin and $AlCl_3$ complex was carried out immediately after the $AlCl_3$ addition and at regular intervals of 10 minutes, until 60 minutes. The interval time of 10 minutes was defined for measuring the absorbance of solutions after $AlCl_3$ addition.

2.7 Antioxidant activity

2.7.1 Total antioxidant activity

The Total Antioxidant Activity (TAA) of extracts of selected seeds of Apiaceae were determined based on phosphomolybdate method proposed by Prieto et al [27]. The procedure described by Subhasree et al was used for the study with minor modifications [28]. In brief, 0.2 mL of extract solution was mixed with 1.8 mL of phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM Sodium phosphate and 4 mM ammonium molybdate) in capped tubes and were incubated for 90 minutes at 90 °C on a water bath. After the samples had cooled to room temperature, the absorbance of solution of each was measured spectrophotometrically at 695 nm against a blank. TAA of each sample was expressed as ascorbic acid equivalent using the

following linear equation established using ascorbic acid as standard: [A = 0.0037C + 0.0343; R^2 = 0.998] where A is the absorbance and C the concentration expressed as ascorbic acid equivalent (μg/ml).

2.7.2 Radical scavenging activity using DPPH method

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, 2,2-diphenyl 1-picrylhydrazyl (DPPH). The method described by Barros et al was adapted to study the free radical scavenging capacity of the extracts of Apiaceae with minor modification [29]. 1 mL of extract of varying concentrations (0.3 -1.7 mg/mL) was taken in a test tube and mixed with 2.7 mL of freshly prepared DPPH solution (0.004% (w/v)). The test tubes were shaken vigorously and incubated at room temperature for 60 minutes in dark. The extent of reduction of the free radical, DPPH was determined spectrophotometrically by measuring the absorption at 517 nm. Ascorbic acid was used as the positive control. The radical scavenging activity (RSA) was calculated using the following equation 1:

$$RSA\ (\%) = \left(\frac{A_{C_0} - A_{C_t}}{A_{C_0}}\right) \times 100$$

Where A_{C_0} is the absorbance of the control at t=0 min

A_{A_t} is the absorbance of the antioxidant at t= 60 min

The radical scavenging activity were expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% inhibition of initial free radical (DPPH) concentration. IC_{50} value was calculated from the graph by linear regression analysis.

2.8 Validation of the spectrophotometric method

The analytical method was validated to meet the acceptance criteria of the International Council for Harmonization [30].

2.8.1 Linearity

To ascertain the linearity, the stock solution of the standard (160 μg/ml) was prepared. Seven portions of the standard solution were accurately removed (0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 ml, respectively) in seven test tubes. An aliquot of 0.3 ml of 10% (w/v) $AlCl_3$ in methanol was added to each diluted solution and final volume made up to 5 ml and absorbance of the individual in triplicate was measured in UV-Visible spectrophotometer. A calibration curve for concentration versus absorbance was plotted and the obtained data were subjected to regression analysis using the Least Square Method, where the square of the correlation coefficient $R^2 > 0.99$ is indicative of the measure of linearity.

2.8.2 Accuracy

Standard solutions of Quercetin, at three different concentration levels, were added to any one of the test sample solutions before reaction with $AlCl_3$. At each level, solutions were prepared in triplicate and the recovery percentage was determined.

2.8.3 Precision

The intra-day precision was determined by calculating the percentage of relative standard deviation (% R.S.D.) for three independent determinations at three different concentrations (6.4, 25.6 and 48.6 μg/ml). To determine the intermediate precision the same method was applied and the samples were analyzed for three consecutive days.

2.8.4 The limits of detection (LOD and quantification (LOQ)

Diluted Quercetin standard solutions were analyzed, at decreasing concentrations, in the range of 0.5 to 0.05 μg/ml. To estimate the limit of detection, absorbance values and UV-Visible spectra were evaluated, whereas the limit of quantification was taken as the lowest concentration which provided responses with the precision (R.S.D. < 2.0%).

3 Results

3.1 The Phytochemical screening and total flavonoid content (TFC

All eight seed samples were subjected to TFC analysis. The phytochemical screening of crude ethanolic extracts of all seed samples revealed the presence of flavonoids. Quantification of flavonoid was done by the designed method as procedure 3. The optimal time interval for the formation of Quercetin and the $AlCl_3$ complex was evaluated by a UV-Visible spectrophotometer.

The UV absorbance of the Quercetin-$AlCl_3$ complex reaches its maximum absorbance after 5 minutes of the time interval. The stability of the complex was observed to remain constant for 60 minutes with no significant variation in absorbance measurements. Based on this result finding, a time period of 10 minutes was defined as the optimal time of detection for quantification of TFC in seed samples after $AlCl_3$ addition.

TFC results were calculated, as Quercetin equivalents, using Equation 2, and it represents the average of three-determinations. The results were expressed as the amount of flavonoid (μg)/g of seed material.

$$TFC = \frac{TFC_{test\ solution} \times DF \times V}{W}$$

Where $TFC_{test\ solution}$ is the total concentration of flavonoids in the test solution (μg/ml) obtained from the graph by plotting concentration vs. absorbance, D F corresponds to the Dilution Factor, V is the volume of the stock solution (ml), W is the weight of seed material (g).

Table 1 shows the total flavonoid content of the eight crude seed extract. The total flavonoid contents in the different crude extracts varied from 246 μg/g - 2856 μg/g. The highest amount of flavonoid was found in ***Cuminum cyminum*** (2856 μg/g).

Table 1. The analytical results of total flavonoid content and DPPH radical scavenging activity of the selected seeds of Apiaceae

Sample Name	Species	Concentration of flavonoid (μg QE/g)	IC_{50} (μg/ml)
a	*Coriandrum sativum*	579.8	187.97
b	*Apium graveolens*	750	60.67
c	*Cuminum cyminum*	2856	49.91
d	*Foeniculum vulgare*	1300	48.35
e	*Vernonia anthelmintica*	1376	9.64
f	*Nigella sativa*	246	249.75
g	*Carum carvi*	1600	35
h	*Anethum graveolens*	1370	102.5

3.2 Evaluation of aluminium complexation reaction for total flavonoid quantification

The three procedures, procedure 1, 2 and 3 were studied to develop a best suitable method when Quercetin is used as a standard for total flavonoid quantification by external calibration curve method. Flavonoids containing 5-hydroxy-4-keto, 3-hydroxy-4-keto or o-dihydroxyl systems are able to chelate with $AlCl_3$ and the reaction is disclosed by a bathochromic shift of the bands in the UV Visible spectrum [31]. The absorbance peak of Quercetin alone is at 260 nm and 370 nm. According to the principle of quantification of flavonoid based on the $AlCl_3$ method, When $AlCl_3$ was added to Quercetin; it reacts with $AlCl_3$ and shows a shift in the peak band which is indicative of Quercetin and $AlCl_3$ complex formation. As shown in Figure 2, there was no proper shift in the peak observed with procedure 1 and hence making it an unsuitable method to quantify TFC when expressed it in terms of Quercetin equivalents. Procedure 2 shows a proper shift in the peak compared to procedure 1 but it gives a lesser absorbance of Quercetin-$AlCl_3$ complex compared to procedure 3 and it also involves the addition of Potassium acetate, whereas in procedure 3, it involves no other extra reagent, still it was able to give the highest absorbance and good results compared to procedure 1 and 2 and hence procedure 3 is considered as the simple, rapid, inexpensive and best suitable method to quantify TFC when it is expressed in terms of Quercetin equivalents.

3.3 Antioxidant activity

3.3.1 Total antioxidant activity

The phosphomolybdate method is based on the principle that, the reducing agent (antioxidant) causes the reduction of molybdenum (VI) to molybdenum (V) with the transfer of electron which results in formation of green phosphomolybdate (V) complex which can be measured spectrophotometrically at 765 nm. Higher the intensity of green color, higher the rate of reduction, indicating good antioxidant activity. Many natural sources, including phenols and flavonoids, can cause this reduction. Figure 3 shows the TAA of extracts of eight different species of Apiaceae at various concentrations in a dose-dependent manner. The extract of *Vernonia anthelmintica* gave the highest TAA, followed by the extracts of *Carum carvi*, *Cuminum cyminum, Apium graveolens*, *Foeniculum vulgare*, *Anethum graveolens*, *Nigella sativa*, *Coriandrum sativum*.

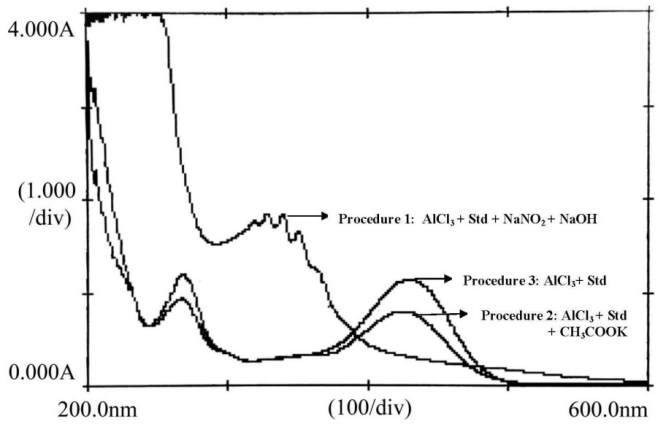

Fig 2. The absorption spectra of Quercetin in the presence of $AlCl_3$ and at different reaction environment obtained by UV-Visible spectrophotometer. ([Std: Standard (Quercetin)])

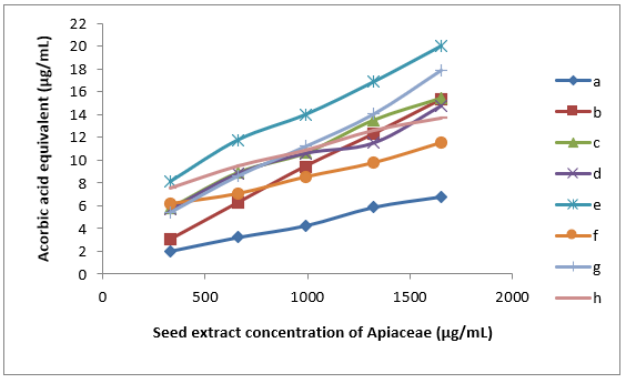

Fig 3. Total antioxidant activity of extracts of selected seeds of Apiaceae at various concentrations. (Values are mean of triplicate analysis)

3.3.2 Radical scavenging activity using DPPH method

The radical scavenging activities of different species of Apiaceae at various concentrations are shown in figure 3B and IC_{50} values for each species are tabulated in Table 1. Generally, the high values of RSA (%) and low values of IC_{50} indicate good antioxidant activity. Among the eight different species used in this study, *Vernonia anthelmintica* showed the highest Radical scavenging activity (134%) at the concentration 165 μg/mL with the lowest IC_{50} value, 9.64 μg/ml. As Figure 4 reveals, the antioxidant activity is concentration-dependent. The radical scavenging activities of different species of Apiaceae were in the following order: e>g>d>c>b>h>a>f. In general fruits and vegetables with higher amount of flavonoids will have high antioxidant activity. In the present study as shown by Figure 5, there was no correlation observed between total flavonoid contents (μg QE/g) and radical scavenging activities of extracts of selected species of Apiaceae. Since here, flavonoids contents are expressed as Quercetin equivalents, the sample with low flavonoid content (QE/g) showing higher radical scavenging activities might be due to the presence of other type of flavonoid other than Quercetin and radical scavenging activity mainly depends on structure of flavonoid and specifically, the position of hydroxyl molecule which can act as a proton donor [32].

3.4 Method validation

3.4.1 Linearity

The Linearity of calibration curve of analytical standard Quercetin was constructed by plotting concentration versus absorbance. The seven-point calibration curve was found to be linear over the concentration range of 6.4-44.8 μg/ml with a correlation coefficient of 0.999 (Figure 6).

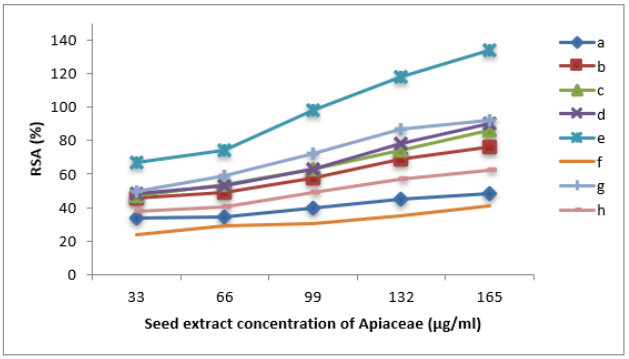

Fig 4. DPPH radical scavenging activity of extracts of selected seeds of Apiaceae at various concentrations. (Values are mean of triplicate analysis).

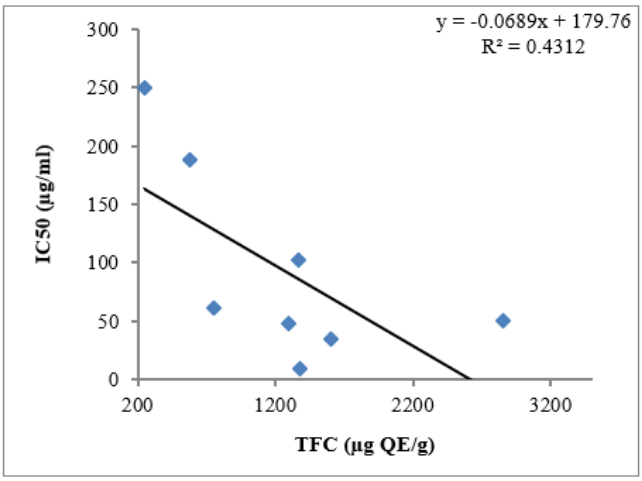

Fig 5. Correlation between antioxidant activity (DPPH method) and flavonoids content of extracts of selected seeds of Apiaceae

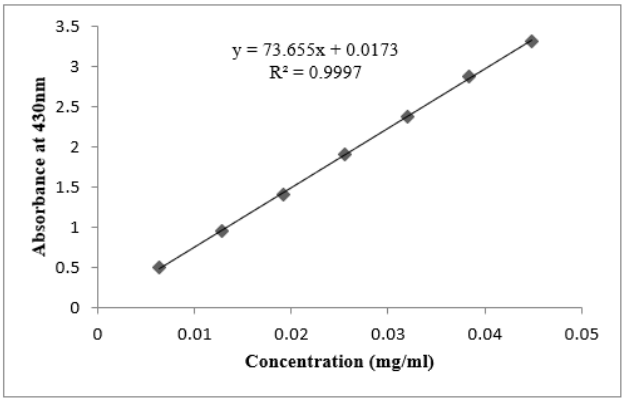

Fig 6. Linearity curve of Quercetin obtained by UV-Visible spectrophotometer

3.4.2 Precision

The precision of the method was evaluated with intra and inter assay tests. In the intra-day precision (n=9) and inter-day precision analysis (n = 9), the values found to be R.S.D. = 0.92% and RSD = 1.21% respectively. The obtained R.S.D. (%) values, lower than 2.0%, attested the precision of the method.

3.4.3 Accuracy

It was investigated by means of a standard addition experiment, at three concentration levels (50%, 75%, 100%) in triplicate (n=9). The mean recovery of 103.03% (R.S.D. = 0.78%) assured the method accuracy.

3.4.4 Limit of detection and quantification

In the spectrophotometric analysis of diluted solutions of Quercetin, it was possible to identify the absorption band at 430 nm in the concentration 0.10 μg/ml, with a corresponding absorbance value of 0.003 A. Quercetin standard solution of concentration 0.29 μg/ml provided absorbance values of 0.006 A, with adequate precision (R.S.D.<2.0%) and therefore, this value can be considered as the quantification limit. The low values of detection and quantification limits demonstrate the high sensitivity of the method.

Table 2. The validation data of UV-Visible spectrophotometer

Linear concentration range(μg/ml)	Regression coeffiecnt R^2	LOD (μg/ml)	LOQ (μg/ml)	Intra-day precision RSD (%) (n=9)	Inter-day precision RSD (%) (n=9)	Accuracy recovery (%)
6.4-44.8	0.999	0.10	0.29	0.92	1.21	103.03

4 Discussion

Flavonoids have several significant advantages among the different phytochemicals present in plants as it is involved in lowering the risk of major chronic diseases such as cancer and cardiovascular disease and neurodegenerative disorders. Drug discovery and research on food science are the two extensively studied research field to find safe, potent and affordable cures for an expanding spectrum of human health issues and to replace synthetic additives with natural additives as many synthetic additives are carcinogenic. Plants are considered as the promising source for variety of therapeutic molecules and other beneficial compounds. All the plants seeds studied in the present preliminary screening investigation showed positive results and found to contain significant amount of flavonoids. To the best of our knowledge, there are no previous studies dealing with the estimation of flavonoid content as Quercetin equivalents in methanolic extracts of seeds of Apiaceae.

Phenolic content and antioxidant properties of selected Indian spices of Apiaceae and TFC (Rutin equvivalent) of spices, namely, *Anethum graveolens,Apium graveolens*, *Carum carvi, Coriandrum sativum* and *Cuminum cyminum* have been found to be 18.159 mg/g, 13.242 mg/g, 12.812 mg/g, 45.262 mg/g, 38.364 mg/g, and 15.854 mg/g respectively[17]. TFC have been found in five species of Apiaceae as Quercetin equivalents by following procedure involving NaOH which gives inappropriate results as this procedure holds well when rutin, luteolin and catechins standards are used. TFC in *Anethum graveolens*, *Carum carvi*, *Coriandrum sativum*, *Cuminum cyminum*, have been estimated to be 58 μg/g, 140 μg/g, 30 μg/g, 32 μg/g, and 176 μg/g respectively[18].

The free radicals are highly reactive chemical species found in the body and over production of active oxygen radical have the potential to cause damage to the DNA, cells and other biomolecules, resulting in chronic diseases[33]. Plants are an excellent source of antioxidants and antioxidants from natural sources are the best choice to neutralize the free radicals as these are safe, inexpensive and readily available to everyone. This demands the discovery of new plant species which are potent to neutralize the action of free radicals. To collect useful data in an environment close to the real-life situation, DPPH radical scavenging method is preferred and this method is faster than β-carotene bleaching method and can be useful in rapid preliminary estimation of radical scavenging activity[34]. The method is sensitive, reproducible, and independent of the substrate polarity. The ability of selected species of Apiaceae to act as a natural antioxidant was assessed by means of DPPH radicals scavenging and phosphomolybdenum method. All the selected species of Apiaceae which are edible are shown to be an excellent source for antioxidants and also, this is the first study to explore the flavonoid content and antioxidant activity in ***Vernonia anthelmintica*** and ***Nigella sativa*** plant species by UV-Visible spectrophotometer. The inhibition concentration (IC_{50}) of ***Vernonia anthelmintica*** (9.64 μg/ml) was greater than the ascorbic acid (12.48 μg/ml) measured by DPPH radical scavenging method. Concentrated pyroligneous acid extract from a Rhizophora apiculata plant have shown superior free radical scavenging activity with EC_{50} value = 0.1235 mg/ml. The ferric reducing power of concentrated pyroligneous acid extract was

about 3.7, 5.1, 6.1, and 21.3 times higher than that of ascorbic acid, Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and alpha-tocopherol. Concentrated pyroligneous acid extract showed the highest antioxidant efficacy (A_{695} = 1.278) compared to different standards estimated by phosphomolybdenum assay[35]. The young fruit extract of mangosteen fruit rind have been shown to have better antioxidant capacity with EC_{50} value 5.56 μg/mL determined by DPPH radical scavenging method[36]. The ethanol extract of marshmallow (94%) have been shown to have better DPPH radical scavenging activity compared to BHT (61%) at the concentration of 120 μg/mL[37]. Many studies have shown that application of natural antioxidants in edible oils in order to substitute synthetic additives to prevent auto-oxidation of oils and fats is a better choice as most of the natural additives have strong antioxidants activity and thermal stability than synthetic additives in different edible oils with no side effects/minimal side effects. Natural antioxidants can be extracted from waste food materials such as green tea, sesame, olives etc and can be used in food processing industry with minimal expenses[38]. This shows antioxidants from plants origin are as powerful as synthetic antioxidants and the daily intake of foods rich in polyphenols will be beneficial against impairment of human body.

Spectrophotometric assay based on Aluminium chelation method is one of the wide spread procedure for determination of total flavonoid content in medicinal plants. Recently study was conducted to evaluate the two assay based on aluminium complex reaction for several compounds from different classes of flavonoid family. The research study suggests that the procedure involving $NaNO_2$ in alkaline medium can only be used for Rutin, Luteolin and Catechins and procedure performed in neutral media, without $NaNO_2$ can be only applied to determine the content of Flavonols and Luteolin (from Flavones family)[39]. This research study further evaluated the procedure involving only Quercetin and $AlCl_3$ without the addition of any reagents. Evaluation study on different Aluminium chloride complexation reaction provided procedure 3 as the best suitable and inexpensive method. A simple and rapid spectrophotometric method for the quantification of total flavonoid content in the seeds of selected plants of Apiaceae was successfully developed and validated. The developed method showed to be feasible, has a shorter run time and is of low cost. Hence, compounding pharmacies and small laboratories can rely on this devolved method for routine quality control analyses or may be used as a preliminary test to evaluate the flavonoid content in a large number of plant samples in a short duration of time. During the quantification of flavonoid, it is always recommended to run the spectrum analysis of standard and the $AlCl_3$ complex to determine the ideal wavelength showing maximum absorbance, instead of following a previously specified wavelength because instruments, chemicals and other analytical conditions used may be different from person to person.

5 Conclusions

This research study facilitated the determination of the TFC in the seeds of selected plants of Apiaceae. Among the selected species of Apiaceae, namely, *Cuminum cyminum, Carum carvi, Vernonia anthelmintica, Anethum graveolens, Foeniculum vulgare* were found to contain significant amount of flavonoids and can serve as an important source of flavonoids. The antioxidant activity was evaluated by two methods namely phosphomolybdenum method and DPPH radical scavenging method. Antioxidant activities of seed extracts of Apiaceae were found to be concentration dependent and there was no correlation found to exist between the total flavonoid content and antioxidant activity in selected seeds of Apiaceae. The seed extract of ***Vernonia anthelmintica*** displayed impressive antioxidant activity and can be a potential source of natural antioxidant agents. The present study might lead towards establishment of some compounds that could be used to investigate plant's new and powerful antioxidants. Further research on isolation and identification of active molecules from the crude extract and in vivo studies of such isolated compound is essential. This paper data gives guidance about the proper usage of $AlCl_3$ chelation methods for quantification of flavonoids in the plant samples.

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