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Qualitative and Quantitative Analysis of *Anethum graveolens* L. Leaves in Various Extracts

Vrushali Kiran Jadhav*, Sushama Sunil Pawar

Department of Zoology, Yashwantrao Mohite College of Arts, Science and Commerce Bharati Vidyapeeth (Deemed to be) University, India

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Abstract *Anethum graveolens* L. (Dill) is an essential curative herb having a lot of therapeutic values. It is an annual herb of family Apiaceae having a distinctive smell. *Anethum graveolens* L. is traditionally used as an antioxidant, anticancer, antihyperlipidemic, antifungal, cardioprotective. *Anethum graveolens* L. leaves are used to reduce the level of cholesterolaemia and risk of cancer. Present study deals with physicochemical, phytochemical screening by using standard methods and estimation of total phenolic and flavonoid contents in various extracts like aqueous, acetone, ethanol, methanol hydroalcoholic and dichloromethane of *Anethum graveolens* L. leaves by using the spectrophotometric method. In aqueous, acetone, ethanol, methanol, hydroalcoholic and dichloromethane extracts of *Anethum graveolens* L. leaves tannins, saponins, glycosides, flavonoids, alkaloids, steroids, amino acids, proteins, carbohydrates were observed. The estimated total phenolic contents were 11.21 to 23.31 mg gallic acid equivalent per gm of extract. Flavonoid contents were 4.38 to 47.81 mg quercetin equivalent per gm of extract. In acetone, hydroalcoholic and dichloromethane extracts of *Anethum graveolens* L. leaves total phenolic and flavonoid constituents were highly significant ($P<0.0001$). The solvent gives an idea about the nature of phytochemical constituents present in *Anethum graveolens* L. material. These constituents are relevant to bioactive compounds as it may be important for therapeutic effects of *Anethum graveolens* L.

Keywords *Anethum Graveolens* L., Physicochemical, Phytochemical, Extractive Value, Phenols, Flavonoid

1. Introduction

Scientific and therapeutic potential of medicinal plants acquired big exposure in last few years [1, 2]. Like flavonoids, phenols, amino acids, terpenoids, alkaloids, tannins, steroids, carbohydrates, and carboxylic acid, these important bioactive constituents are present in medicinal plants [3]. These bioactive components of medicinal plants are used for different diseases [4]. Primary or secondary metabolites of plants are also considered as an essential product [5]. *Anethum graveolens* L. is a medicinal, annual plant from family Apiaceae commonly known as Dill [6, 7]. It grows through India chiefly in Maharashtra, Punjab, Rajasthan, Gujrat, Assam, Orrisa, Madhya Pradesh [8]. The ariform green part of *Anethum graveolens* L. plant &seed is largely used as nutritional edibles [9]. Dill leaves have been used as a condiment in salad, seafood, eggs, soup in bread and flavorings pickles [10, 11]. Dill is used to solve a gastric intestinal problem including stomach ache, colic to tract intestinal gas, indigestion and as diuretic [1]. *Anethum graveolens* L. has biological properties including antioxidant, anti-inflammatory, antisecretory, analgesic, antimicrobial, hyperlipidaemic, gastric mucosal protective [12-14]. Many scientific investigations confirm that *Anethum graveolens* L. has various phytochemicals such as alkaloids, terpenoids, steroids, phenols, tannins, saponnins, cardiac glycosides, terpenes, flavonoids, phenolic acid and cumarins [15, 16]. The prospects of natural compounds of

plants are utilized in therapy of distinct disorders [1]. Secondary metabolites, like flavonoids, phenolic, tannin, cardiac glycosides, saponins also terpenes have excellent antioxidant activity. Shekhawat and Jana [17] reported that various phytochemicals like flavonoids, phenolic acid, cumarins and α-phellandrene, triterpens are present in *Anethum graveolens* L. leaves and are used in therapy of distinct disorders. *Anethum graveolens* L. consists of spacious variations in vitamins, carotenoids, flavonoids, anthocyanins, internal metabolites and dietary glutathione molecules of free radical scavengers [6]. Phenolic compounds can compensate for precisely reactive oxygen species (ROS) attacks and discontinue free radical mediated oxidative reactions to defend the human body from some diseases [18]. Flavonoids, phenolic acid and tocopherols are plant based natural antioxidants used in preventive and therapeutic medicines, as well as in food. Through their antioxidant aspects, such essential substances show anticarcinogenic potential and various health benefit effects [19, 20]. Leaves, seeds and essential oil of *Anethum graveolens* L. show antioxidant activities [21, 22]. The phytochemical analysis was used to estimate the biomolecules like alkaloids, flavonoids, steroids, phenols, terpenoids, tannins, saponnins, terpenes, cardiac glycosides. In present study by consideration of therapeutic uses of *Anethum graveolens* L., we carried the analysis of phytoconstitents and evaluation of total phenolic and flavonoid in various extracts.

2. Materials and Methods

2.1. Plant Material

Anethum graveolens L. was cultivated during June at Katraj, Pune. Fresh leaves were collected in September by taking all the precautions. The identification of plant material was verified by Botanical Survey of India (BSI), Pune, Maharashtra (JVK-01). *Anethum graveolens* L. leaves were preparation of extract.

2.2. Preparation of Extract

Anethum graveolens L. leaves were washed and shade dried. By using mechanical grinder, fine powder was done. The crude powder of leaves was subjected to extract successively with aqueous, acetone, ethanol, methanol, hydroalcoholic and dichloromethane using maceration technique. The extract was filtered & collected at pleasant cool temperature. That was preserved at 5^0C into sealed bottle in consideration of further study. Generally polar solvents are used to carry plant extraction. Polar solvents are water, alcohol, acetone, dichloromethane etc., which are used to carry medicinal plant extraction. In our study, the polar solvents namely aqueous, acetone, ethanol, methanol, hydroalcoholic & dichloromethane to form various extracts of *Anethum graveolens* L. leaves, flowers and seed were used. Within these extracts we will select one extract for further research.

2.3. Phytochemical Screening

According to the pharmacopoeial method, such as extractive value was determined by using various solvents, total ash value, acid insoluble ash value, water soluble ash value, loss on drying. Phytochemical analysis of *Anethum graveolens* L. leaves in aqueous, acetone, ethanol, methanol, hydroalcoholic and dichloromethane solvents was carried out to detect secondary metabolites including alkaloids, phenolic compounds, flavonoids, terpenoids, tannins, steroids, saponnins, cardiac glycosides and so on by using the standard method [23, 24].

2.4. Estimation of Total Phenolic and Flavonoid Content

The total amounts of phenolic and flavonoid contents into different extracts were calculated by using standard curve of Gallic acid and Quercetin respectively (Figure 1 and Figure 2) [25, 26]. The expressed phenolic and flavonoid content were mg/g equivalent of gallic acid and mg/g equivalent of quercetin content respectively.

Total phenolic content by using gallic acid as standard was determined by Folin-Ciocalteu method in different extracts of *Anethum graveolens* L. leaves. Extract [0.2 ml] was assorted with [2.8 ml] distilled water then Folin-Ciocalteu phenol reagent and 20% NA_2CO_3 were added in test tube, keeping test solution for warming [1min]. Through the UV/visible spectrophotometer, the observation of extracts and standard solution was read against the blank at 650nm in triplicates.

Total flavonoid content of *Anethum graveolens* L. extract was determined by aluminum chloride colorimetric assay. The [0.2ml] extract was mixed with methanol to make 3ml volume, 10%aluminum chloride and Na-K tartarate were combined to the blend, and then 2.8 ml of D/W was mixed in each tube, shaked vigorously and set at normal temperature as 30 minute. Through the UV spectrophometer absorbance was calculated against the blank at 415nm in triplet.

3. Results

3.1. Physicochemical analysis of *Anethum graveolens* L. leaves

Table 1. Physicochemical analysis of *Anethum graveolens* L. leaves

No.	Physicochemical parameter	Concentration %
1.	Total ash value	14
2.	Acid insoluble ash value	1
3.	Water insoluble ash value	7
4.	Loss on drying	4

The Physicochemical analysis of *Anethum graveolens* L. leaves was determined and represented in Table 1

Total ash value in leaves was 14%, acid insoluble ash value was 1%, water soluble ash value was 7% and loss on drying was calculated as 4% of *Anethum graveolens* L. leaves.

3.2. The Extractive Values of *Anethum graveolens* L. leaves

Table 2. Extractive values of *Anethum graveolens* L. Leaves

Solvents(ml)	Weight of plant material (gm)	Extractive value%
Aqueous	2	15
Acetone	2	4.5
Ethanol	2	12.7
Methanol	2	12.5
Hydroalcoholic	2	15.2
Dichloromethane	2	2.1

The extractive values in aqueous, acetone, ethanol, methanol, hydroalcoholic and dichloromethane extracts of *Anethum graveolens* L. leaves were 15%, 14.5%, 12.7%, 12.5%, 15.2%, 2.1% respectively which were showed in Table 2. The extractive value is high in hydroalcoholic extract as compared to aqueous, acetone, ethanol, methanol and dichloromethane. Dichloromethane extract showed the lower extractive value than all other extracts.

3.3. Phytochemical Screening in Various Extracts of *Anethum graveolens* L. leaves

Phytochemical analysis of aqueous, acetone, ethanol, methanol, hydroalcoholic and dichloromethane extracts of *Anethum graveolens* L. leaves is presented in Table 3. It reveals that the phytochemical contents such as carbohydrates, proteins, flavonoids, steroids, glycosides, alkaloids, amino acids and tannins were present. The proteins, saponin glycosides, flavonoid and alkaloids were observed in all extracts of *Anethum graveolens* L. leaves. Steroids were present in acetone, methanol, hydroalcoholic and dichloromethane extracts. Cardiac glycosides were present only in dichloromethane extract. Saponin glycosides were present in all extracts. Tannins were present within aqueous, acetone, ethanol, methanol & hydroalcoholic extracts of *Anethum graveolens* L. leaves. Carbohydrates were absent in aqueous & methanol extracts and amino acids were absent only in dichloromethane extracts.

Table 3. Phytochemical screening of aqueous, acetone, ethanolic, methanolic, hydroalcoholic & dichloromethane extracts of *Anethum graveolen* L. leaves

Constituent test	**Aqueous**	**Acetone**	**Ethanol**	**Methanol**	**Hydro Alcoholic**	**Dichloro Methane**
Carbohydrate test (Molishs test)	_	+	+	_	+	+
Protein (Biuret test)	+	+	+	+	+	+
Amino acids test (Ninhydrintest)	+	+	+	+	+	_
Steroid						
Burchard reaction	_	+	_	+	+	+
Libermans reaction	_	_	_	_	_	_
Glycosides						
Cardiac	_	_	_	_	_	+
Antraquine	_	_	_	_	_	_
Saponingly	+	+	+	+	+	+
Flavonoid						
Lead acetate	+	+	+	+	+	+
NaOH	_	_	_	+	+	_
Alkaloids (Mayers test)	+	+	+	+	+	+
Tannins	+	+	+	+	+	_

3.4. Total Phenolic and Flavonoid Content

The high content of phenolics was observed in acetone extract (23.31 mg/GAE/g) and low content was observed in dichloromethane (0.210 mg/GAE/g) extract of *Anethum graveolens* L. leaves shown in Table 4. In aqueous, ethanol, methanol and hydroalcoholic extracts of *Anethum graveolens* L. leaves total phenolic contents were observed 11.21 mg/GAE/g, 16.91mg/GAE/g, 16.32mg/GAE/g, 21.93 mg/GAE/g respectively.

Table 5 shows the results of flavonoid contents in *Anethum graveolens* L. leaves extracts. The high content of flavonoid was observed in acetone extract (47.81 mg/QE/g). Aqueous extract (4.38 mg/QE/g) showed less content of flavonoids than all other extracts. Total flavonoid contents in ethanol, methanol, hydroalcoholic and dichloromethane extracts were observed 18.76 mg/QE/g, 15.85 mg/QE/g, 20.23 mg/QE/g, 35.26 mg/QE/g respectively in leaves of *Anethum graveolens* L.

Table 4. The total phenolic content of the different extracts of *Anethum graveolens* L. leaves (mg/GAE/g)

Extracts of leaves	Phenolic content (mg/GAE/g)
Aqueous	11.21 ±0.020
Acetone	23.31 ±0.023
Ethanol	16.91 ±0.023
Methanol	16.32 ±0.29
Hydroalcoholic	21.93 ±0.17
Dichloromethane	0.210 ±0.008

Table 5. The total flavonoid content of the different extracts of *Anethum graveolens* L. leaves (mg/QE/g)

Extracts of leaves	Flavonoid content (mg/QE/g)
Aqueous	4.38 ±0.043
Acetone	47.81 ±0.020
Ethanol	18.76 ±0.0233
Methanol	15.85 ±0.028
Hydroalcoholic	20.23 ±0.044
Dichloromethane	35.26 ±0.050

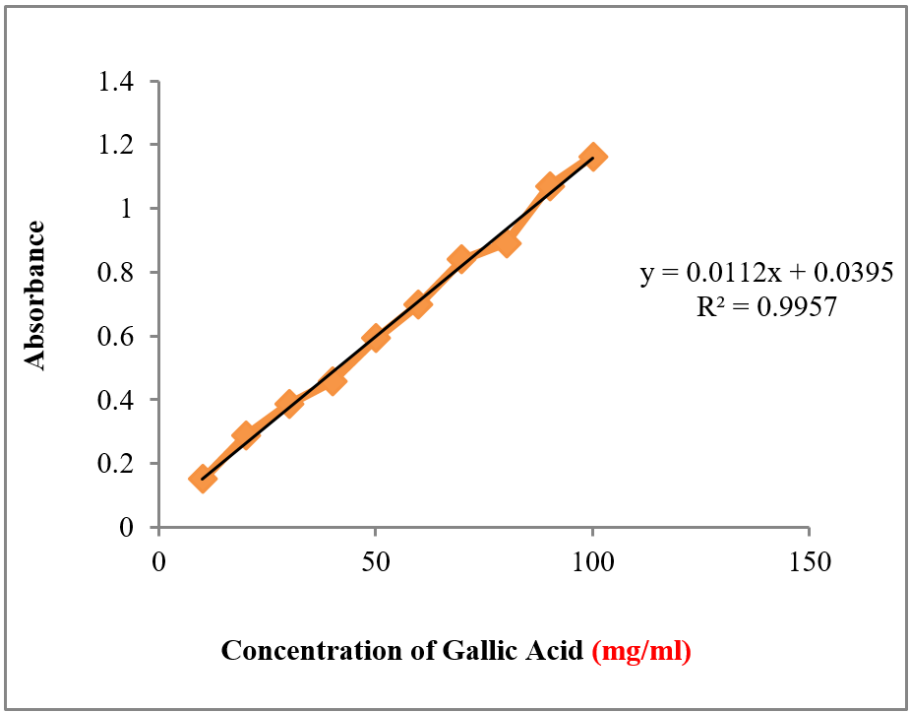

Figure 1. Standard Curve of Gallic Acid

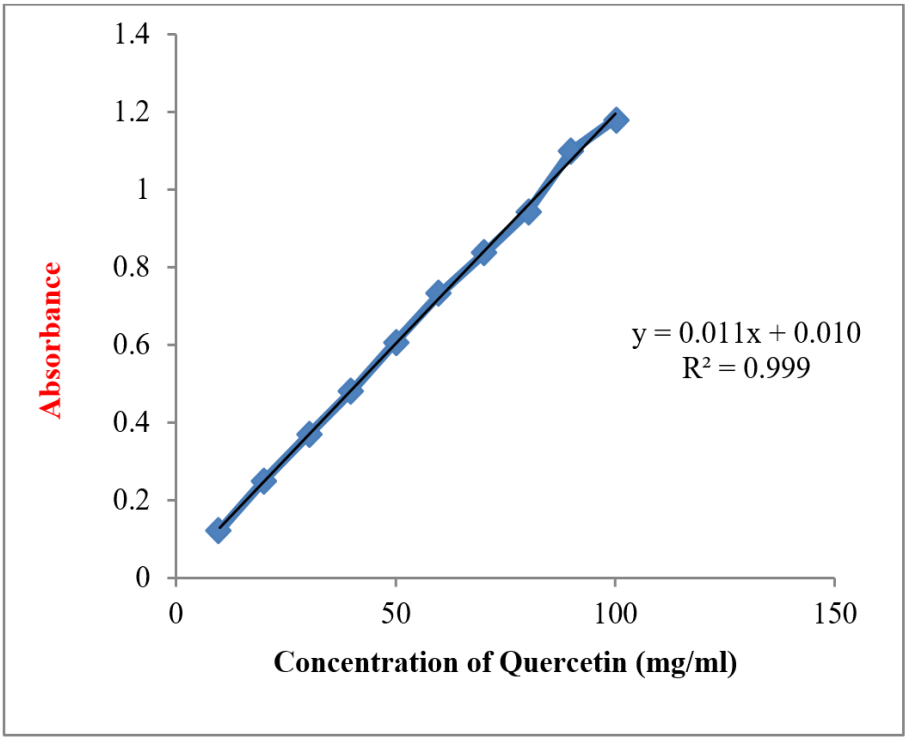

Figure 2. Standard Curve of Quercetin

4. Discussion

In present physicochemical study of *Anethum graveolens* L. leaves, it was observed that in different concentrations, total ash value, acid insoluble ash value, water soluble ash value and loss on drying were observed 14%, 1%, 7% and 4% in *Anethum graveolens* L. leaves respectively. In earlier studies of various physicochemical analysis of *Anethum sowa* L. seed, it has been observed that loss on drying, acid insoluble ash value, water insoluble ash value and total ash values were observed 7.86, 4.12, 6.63, 14.5% respectively and alcohol soluble extractive value (11.8%), water soluble extractive value (17.5%), n-haxane soluble extractive value (2.4%), chloroform soluble extractive value (19.6%), petroleum ether soluble extractive value (9.1%) were observed [27,28]. The phytochemical analysis of *Anethum graveolen* L. is found in aqueous, ethanol, ethyl acetate, petroleum ether, methanol and chloroform extracts, the presence of alkaloids, tannins, flavonoids, terpenoids, saponins, phenols, steroids, glycosides, cardiac glycosoides, proteins, coumarins [1,7]. Various activities like anticancer, anti-inflammatory, antioxidant, antiallergic, xanthine oxidase inhibitors, antibacterial activities are revealed by phenolic compounds. The alkaloids and saponins phytoconstituents indicate the antimalerial, central nervous system activities, antibiotic properties and also defend body from hypercholesterolemia. Bioactive components of plants are detected by experts and utilized in beauty products, food and directly used in medicine for diseases [1]. Many scientists proved that *Anethum graveolen* L. has the medicinal value utilizing the phytoconstituents [22,29]. In present study, results explained that different phytochemicals such as phenolic, flavonoids, glycosides, steroids, protein, carbohydrates, amino acids were present in various extracts like aqueous, acetone, ethanol, methanol, hydroalcoholic and dichloromethane of *Anethum graveolen* L. leaves. Total phenolic content of *Anethum graveolen* L. in different extracts varied form 0.210 to 23.31GAE/g. Higher content of total phenols 23.31mgGAE/g was observed in acetone extract and hydroalcoholic extract 21.93 mg GAE/g than other extracts. Flavonoid contents in *Anethum graveolens* L. leaves extract were showed as (mg/g) equivalent of quercetin (mg QE/g). Higher content of flavonoid was observed in acetone extract (47.81mgQE/g) coming after dichloromethane (35.26mgQE/g) and hydroalcoholic (20.23mg QE/g) extracts of *Anethum graveolens* L. leaves. By the polarity of solvent, total phenolic and flavonoid contents of extracts were greatly affected. Total phenolic and flavonoid contents of plant material are associated with antioxidant activity [15]. High constituents of total phenols and flavonoids were observed in high polarity of solvent used, hence the antioxidant prospect is high [30]. Phenol reacts with reactive oxygen species like hydroxyl radicals, superoxide anion radical and lipid paroxy radical, Folin – Ciocalteau reagent detects polyphenols and phenols compound [31]. Phenolic compounds help in absorbing & neutralizing free radicals and to quench singlet and triplet oxygen, to decompose peroxide [15]. Phytoconstituents like flavonoids show the significant role to protect cell degradation that should be concentrate for analysis of many

biological properties. Polyphenols are important phytochemicals to represent primary antioxidant [32]. These phytoconstituents of *Anethum graveolens* L. plant may show a role in medicine.

5. Conclusions

Present phytochemical study concludes that the presence of carbohydrates, proteins, amino acid, steroid, glycosides, flavonoids, alkaloids and tannins presents various extracts of *Anethum graveolens* L. leaves are completely secured. The leaves show viable utilization in food and medicinal industries as a secure source as an antioxidant. *Anethum graveolens* L. has natural compounds which are used as nutritional supplements in diet with antioxidant properties. This reveals that bioactive compounds of *Anethum graveolens* L. leaves can be valuable for various ailments and may be used as a potential source of medicine in future.

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