Phytochemical Analysis of Some Apiaceae Plants

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Abstract: The preliminary phytochemical screening to investigated alkaloids, glycosides, cardiac glycosides, saponins, phenol, sterol, tannins, flavonoids, and volatile oil present in plants under investigation. Alkaloids, glycosides and flavonoids present in all plants under investigation, while cardiac glycosides are absent in *Foeniculum vulgare, Petroselinum crispum, Pimpinella anisum* and *Carum carvi*. Also, saponins were absent in *Petroselinum crispum, Pimpinella anisum* and *Carum carvi*, while phenol was absent in *Petroselinum crispum,* and sterol was absent in *Cuminum cyminum, Foeniculum vulgare* and *Petroselinum crispum*. Also, tannins not found in *Petroselinum crispum, Anethum graveolens* and *Petroselinum crispum.* Volatile oils were absent in *Cuminum cyminum* and *Foeniculum vulgare.* Quantitative assay of the plants under investigation, show that total flavonoids and total phenolic acids have a maximum value in *Pimpinella anisum* and *Coriandrum sativum,* respectively, while minimum value in *Petroselinum crispum.* On the other hand both of total tannins and alkaloids show maximum value in *Carum carvi* while minimum values in *Cuminum cyminum* and *Foeniculum vulgare*, respectively, at the end, total saponins have a maximum values in *Anethum graveolens* and *minium value* in *Petroselinum crispum* and *Coriandrum sativum*.

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1. Introduction

Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The chemical constituents present in the plant play a significant role in the identification of crude drugs (Akindele & Adeyemi, 2007).

Medicinal plants play a major role in the treatment of human diseases and have various effects on living systems, Plants belonging to the family Apiaceae are extensively used for food and medicinal purposes. Some plants of this family such as carrots, parsley, and celery are common vegetable crops, while other members like anise, coriander, cumin, fennel, and dill are famous for their medicinal and aromatic properties. Different plant taxa of the family Apiaceae have been used as wild edible plants, besides these crop plants. To date, around 101 genera including 451 species under the family Apiaceae have been reported (OZhatay *et al.,* 2009).

The Egyptian habitats are very rich in medicinal plants belonging to many families. Family Apiaceae or Umbelliferae, commonly known as the celery, carrot or parsley family, are a family of mostly aromatic plants with hollow stems. Apiaceae is large, with more than 3,700 species spread across 434 genera; it is the 16th-largest family of flowering plants (Boulos, 2009). So, it is of interest to choose some plant species belong to the apiaceae family to make further investigation.

2. Material and Methods

2.1. Plant Materials

The fresh parts of some apiaceae plants were collected from Sinai.

2.2. Preliminary phytochemical screening

2.2.1. Test for Alkaloids (Woo *et al.*, 1977)

The alcoholic extract of each plant under investigation was concentrated under vacuum till dryness. The dried extracts were dissolved in 2N-hydrochloric acid pH= 1.5 on a water bath, shaken and filtered, the obtained filtrates was shacked with chloroform to remove undesirable matters.

The acidic aqueous layer was adjusted to alkaline pH= 9 with ammonia and the liberated alkaloid bases were extracted by chloroform till exhausted and then tested by Mayer's and Dragendorrf's reagents. The presence of color or precipitation indicated the presence of alkaloids.

Reagents for Alkaloids (Balbaa *et al.*, 1981)

a) Wagner's reagent (Potassium tri-iodide)

Iodine: 1.3g, Potassium iodide: 2.0g, and Water to make: 100ml

b) Dragendorrf's reagent (Potassium bismuth iodide)

Solution (A): 1.7g of bismuth sub nitrate and 20g tartaric acid were dissolved in 80ml water.

Solution (B): 16g potassium iodide was dissolved in 40ml water.

Stock solution: 1:1 (v/v) mixture of (A) and (B) was freshly prepared for spraying.

Spray reagent: 5ml of stock solution was added to a solution of 10g tartaric acid in 50ml water.

2.2.2. Test for Glycosides

2.2.2.1. Glycosides Test (Treare & Evans, 1985)

To small amount of extract, add 1 ml water and shake well. Then the aqueous solution of NaOH was added. Yellow color appeared that indicated the presence of glycosides.

2.2.2.2. Modified Borntrager's Test (Treare & Evans, 1985)

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

2.2.3. Test for Cardiac Glycosides

Legal's Test (Treare & Evans, 1985)

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

2.2.4. Test for Saponins

2.2.4.1. Foam Test (Kokate *et al.*, 2001)

The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam indicated the presence of saponins**.**

2.2.4.2. Haemolysis Tests (Kokate, 1994)

Leaves extract was added to one drop of blood placed on glass slide. Hemolytic zone appears.

2.2.5. Test for phenols

Ferric Chloride Test (Ahmad *et al.*, 2005)

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.2.6. Test for Sterols and Terpens

Three ml of the alcoholic extracts were evaporated till dryness. The residue was dissolved in 2ml chloroform and filtered. The filtrate was subjected to:

2.2.6.1. Salkwski Reaction's (Brieskorn &Klinger-Hand Polonius, 1961)

To one ml chloroform extract, conc. Sulfuric acid was added slowly down the side of the test tube. Positive reaction was indicated by the formation of yellow colored ring changing to bloody red.

2.2.6.2. Libermann-Burchard's test (Fieser and Fieser, 1959)

One ml anhydrous acetic acid was added to one ml chloroform extract, followed by few ml of conc. sulphuric acid, poured carefully down the side of test tube, where blue, green, red, or orange colors that change with time will indicate a positive reaction.

2.2.7. Test for Tannins (Treare & Evans, 1985)

2.2.7.1. Lead Acetate Test

To 5 ml of extract, add few drops of 10% lead acetate solution were added. Formation of a yellow or red precipitate indicated the presence of tannins.

2.2.7.2. Gelatin Test

To the extract, gelatin (gelatin dissolves in warm water immediately) solution was added. Formation of white precipitate indicated the presence of tannins.

2.2.8. Test for Flavonoids

2.2.8.1. Shinoda's Test (Geissmann, 1962)

Half ml of hydrochloric acid was added to an aliquot of aqueous extracts followed by few mg of magnesium turnings. A pink color indicated the presence of flavonoids.

2.2.8.2. NaOH Test (Khandeal, 2008)

To 2-3 ml of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow color that became colorless on addition of a few drops of dilute HCl indicated the presence of flavonoids.

2.2.9. Test for Anthraquinones

Borntrager's Test (Yadav *et al.*, 2013)

To 200 mg of each extract, dil. H_2SO_4 was added and boiled. Then, it was filtered and cooled. To the cold filtrate, 3 ml of benzene was added and mixed. The benzene layer was separated and to it, ammonia (2 ml) was added and ammonical layer was observed.

2.2.10. Detection of proteins and amino acids

Xanthoproteic Test

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

2.2.11. Steam Distillation of Volatile Oils (Balbaa *et al.*, 1981)

Fifty grams of each plant under investigation subjected to steam distillation to extract volatile oils.

2.3. Investigation of Total Active Materials

2.3.1. Estimation of Total Phenolic Content (TPC)

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as µg/mg gallic acid equivalent to (GAE). Concentrations of 2, 4, 6, 8 and 10 µg/ml of gallic acid were prepared in methanol. Concentration of 1mg/ml of plant extract was also prepared in methanol and 0.5 ml of each sample were introduced into test and mixed with 2.5ml of a 10 fold dilute Folin Ciocalteu reagent and 2ml 0f 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrophotometrically. All determination was performed in triplicate. The Folin Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue color upon reaction. This blue color was measured spectrophotmetrically

(Chun *et al.,* 2013 & Maurya and Singh, 2010). Line of Regression from Gallic acid was used for estimation of unknown phenol content. From Standard curve of Gallic acid line of Regression was found to be y = 0.0913x + 0.03 & R_2 = 0.9976 where (**y**) was the absorbance and (**x**) was the µg GAE/mg of the extract. Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned GA.)

2.3.2. Estimation of Total Flavonoid Content (TFC)

The amount of Total Flavonoid content in extracts was determined aluminum chloride assay through Colorimetric. A 0.5ml aliquot of appropriately diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15ml of a 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total Flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. All samples were analyzed in three replications (Samatha *et al.,* 2012 and Han and May, 2012). y = 0.0029x + 0.0034 & R_2 = 0.9935 where (**y**) was the absorbance and (**x**) was the µg rutin/mg of the extract Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned rutin)

2.3.3. Estimation of Total Tannins

Gravimetric Method (Copper Acetate Method)

This method depends on quantitative precipitation of tannin with copper acetate solution, igniting the copper tannate to copper oxide and weighing the residual copper oxide (Ali *et al.,* 2011). Two grams of each plant under investigation were separately extracted for about one hour with two successive quantities, each of 100ml of acetone-water (1:1) and then filtered. The combined extract, in each case, was separately transferred into 250ml volumetric flask and adjusted to volume with distilled water. Each extract was quantitatively transferred to a 500ml beaker and heated till boiling, then 30ml of 15% aqueous solution of copper acetate was added with stirring. The precipitate of copper tannate was collected on ashless filter paper and the precipitate was ignited in a porcelain crucible (the crucibles were previously ignited to a constant weight at the same temperature). Few drops of nitric acid were added to the residue and reignited to constant weight. The weight of copper oxide was determined and the percentage of tannin was calculated according to the following correlation: Each 1g of Cuo = 1.305g tannins.

2.3.4. Estimation of Total Saponins

20g of each plant under investigation were dispersed in 200 ml of 20 % ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorous. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage according to Obadoni & Ochuko (2001) and Okwu & Ukanwa (2007).

2.3.5. Estimation of Total Alkaloids (Gravimetric Method)

About (10g) of each plant under investigation were extracted with 90% ethanol till exhaustion (tested with Mayer's reagent). The alcoholic extract of the plant was concentrated under reduced pressure until dryness at a temperature not exceeding 40°C, acidified with HCl (3%), and filtered; the filtrate obtained was extracted with chloroform to remove acid alkaloid portion. The acidic aqueous layer was adjusted to alkaline media with ammonia and the liberated alkaloid base portion was extracted with chloroform till exhaustion (tested by Mayer and Dragendorrf's reagents). The chloroform extract was filtered over anhydrous sodium sulfate and evaporated under reduced pressure till dryness, then weighed it to calculate the percent w/w (Woo *et al.,* 1977).

3. Results and Discussion

Qualitative and quantitative assay are summarized in table (1 and 2). Results indicated that, preliminary phytochemical screening indicated present or absent special class of compound and according to table (1) we note that each of alkaloids, glycosides and flavonoids were present of all plants under investigation (*Cuminum cyminum, Foeniculum vulgare, Petroselinum crispum, Pimpinella anisum, Carum carvi, Coriandrum sativum, Anethum graveolens* and *Petroselinum crispum),* The cardiac glycosides are an important class of naturally occurring drugs which actions include both beneficial and toxic effects on the heart, and have played an

outstanding role in the therapy of congestive heart failures (CHF), from table (1) we note that cardic glycosides were present in *Cuminum cyminum, Coriandrum sativum, Anethum graveolens* and *Petroselinum crispum,* while saponins Low-molecular-weight antimicrobial molecules within plants is one component of defense against pathogens. Among them, preformed antimicrobial compounds (phytoanticipins) are the first biochemical barriers against pathogens.

Table 1. preliminary phytochemical screening of plant some apiaceae plants

Group	Test	*Cuminum cyminum*	*Foeniculum vulgare*	*Petroselinum crispum*	*Pimpinella anisum*	*Carum carvi*	*Coriandrum sativum*	*Anethum graveolens*	*Petroselinum crispum*
Alkaloids	Wagner's test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
	Dragndorrf test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Glycosides	Glycosides test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
	Modified borntrager's test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Cardiac glycosides	Legal's test	+ ve	- ve	- ve	- ve	- ve	+ ve	+ ve	+ ve
Saponins	Foam test	+ ve	+ ve	- ve	- ve	- ve	+ ve	+ ve	+ ve
	Hemolysis test	+ ve	+ ve	- ve	- ve	- ve	+ ve	+ ve	+ ve
Phenols	Ferric chloride test	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
Sterols	Salkawskis test	- ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
	Libermann burchard's test	- ve	- ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
Tannins	Lead acetate test	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve	- ve
	Gelatin test	+ ve	+ ve	- ve	+ ve	+ ve	+ ve	- ve	- ve
Flavonoids	Shinoda's test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
	NaOH test	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Volatile oil	Stem distillation	- ve	- ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve

Table 2. Quantitative assay of some apiaceae plants {Mean ±SE (n= 3 replicate per plant)}

Items / Plants	Total flavonoid	Total phenolic	Total saponins	Total tannin	Total alkaloid
Cuminum cyminum	218.7±1.86	283.3±1.20	2.26±0.09	1.76±0.09	1.80±0.06
Foeniculum vulgare	237.7±1.45	292.3±1.20	1.73±0.07	1.80±0.06	1.63±0.09
Petroselinum crispum	183.7±3.18	245.3±1.85	1.51±0.02	0.00±0.00	1.70±0.06
Pimpinella anisum	297.0±2.30	211.0±0.57	2.26±0.09	2.27±0.14	2.06±0.09
Carum carvi	240.7±2.18	216.7±2.33	1.73±0.07	2.57±0.09	2.37±0.09
Coriandrum sativum	232.7±0.88	305.3±2.33	1.51±0.02	2.16±0.07	1.67±0.14
Anethum graveolens	182.7±2.96	265.0±2.31	2.27±0.09	0.00±0.00	1.76±0.09
Petroselinum crispum	166.0±1.73	202.0±1.53	1.73±0.07	0.00±0.00	2.10±0.06
F ratio	297.159	502.630	23.958	226.911	8.773
Sig.	***	***	***	***	***

*** = significant at $P < 0.001$

Saponins (a group of phytoanticipins) are present constitutively in plants and play important roles in plant defense according to (Turk., 2005), saponins were absent in *Petroselinum crispum, Pimpinella anisum* and *Carum carvi*, phenol which know also Plant phenolics are secondary natural metabolites arising biogenetically from either the shikimate/phenylpropanoid pathway, which directly provides phenylpropanoids, or the "polyketide" acetate/malonate pathway, which can produce simple phenols, or both, thus producing monomeric and polymeric phenols and polyphenols, which fulfill a very broad range of physiological roles in plants, phenol absent in *Petroselinum crispum.* Sterols found

in all eukaryotic organisms are membrane components which regulate the fluidity and the permeability of phospholipid bilayers. Certain sterols in minute amounts, such as campesterol in Arabidopsis thaliana, are precursors of oxidized steroids acting as growth hormones collectively named brass into steroids according to (Schaller, 2003), sterol were absent in *Cuminum cyminum, Foeniculum vulgare* and *Petroselinum crispum.* Tannin are present in *Cuminum cyminum, Foeniculum vulgare, Pimpinella anisum, Carum carvi* and *Coriandrum sativum,* tannins is nature is a unique source of structures of high stereochemical diversity, many of them possessing interesting biological activities and medicinal properties. In the context of the world-wide spread of deadly conditions such as AIDS and a variety of cancers, an intensive search for new lead compounds for the development of novel pharmacological therapeutics is extremely important (Karamali and Teunis, 2001). *Volatile oil were absent in Cuminum cyminum* and *Foeniculum vulgare.* Quantitative assay in table (2), show that total flavonoids have a maximum value in *Pimpinella anisum* (297 mg/eq. gm rutin) and minimum value in *Petroselinum crispum* (166 mg/eq. gm rutin) also total phenolic acids have a maximum value in *Coriandrum sativum* (305.3 mg/eq. gm gallic acid) and minimum um value in *Petroselinum crispum* (202 mg/eq. gm gallic acid) phenolic compounds(Flavonoids and phenolic acids) possess many biochemical properties, but the best described property of almost every Group of flavonoids is their capacity to act as antioxidants. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and chelation ability according to Pandey *et al.* (2012) on another hand total saponins have a maximum value in *Cuminum cyminum* and *Pimpinella anisum* (2.26%) and minimum value in *Coriandrum sativum* (1.51%) Properties of saponin containing herbs are many & varied and may include alterative, diuretic, expectorant, anti-catarrhal, anti-inflammatory, antispasmodic, aphrodisiac, antioxidant, emmenagogue, cardiac stimulant, hormone modulating, hepatoprotective, and adrenal adaptogenic effects. Possibly their most important property is to accelerate the body's ability to absorb other active compound**,** while total tannin have amaximum value in *Carum carvi* (2.57%) and minimum value in *Cuminum cyminum* (1.51%), Many carcinogens and/or mutagens produce oxygen-free radicals for interaction with cellular macromolecules. The antcarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds (Chung *et al.,* 1998). Alkaloids and other plant phenolic are reported to have multiple biological activities in addition to their antioxidants or free radical terminators activity (Bendii *et al.,* 2006).

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