Qualitative Study of Bioactive Components of Dill *(Anethum graveolens L.)* From Northern Morocco

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Abstract

This work aims to extract and identify bioactive molecules of the plant Anethum graveolens, commonly known as Dill, which occupies an important place in Moroccan vegetation. In order to optimise their later use in the field of herbal medicine and nutrition, a focus on the phytochemical analysis of an extract from different parts of the plant were examined by using preliminary tests and staining reaction precipitation by the use of specific reagents, or by thin layer chromatographic analysis (TLC). Phytochemical tests, different organs, aerial and underground part (on different areas of the plant), revealed the richness of this plants tannins, reducing compounds, alkaloids, coumarins, terpenoids, sterols, flavonoids, irridoides, and this screening has allowed us to show that for the extraction of polyphenols, methanol is the best extraction solvent.

Keywords: Anethum graveolens, phytochemical screening, polyphenols, (TLC) Thin layer chromatography

Résumé

Le présent travail a pour objectif d'extraire et d'identifier les molécules bioactives de la plante *Anethum graveolens,* qui occupe une place importante dans la végétation Marocaine. En vue de l'optimisation de leur usage ultérieur dans les domaines de la phytothérapie et la nutrition, on a mis l'accent sur des analyses phytochimiques d'extrait de différentes parties de la

plante, soit par des tests préliminaires, réaction de coloration et précipitation, par l'utilisation des réactifs spécifiques, soit par des analyses chromatographiques sur couche mince (CCM).
Les tests phytochimiques, des différents organes, partie aérienne et souterraine, ont révélé la richesse de cette plante en tanins, composés réducteurs, alcaloïdes, coumarines, terpénoïdes, stérols, flavonoïdes, des irridoides etc…et ce criblage, nous a permis de montrer que, pour l'extraction des polyphénols par macération, le méthanol est le meilleur solvant d'extraction.

Mots clés : Anethum graveolens, *screening phytochimique, polyphénols,* La chromatographie sur couche mince

Introduction

Since ancient times, humans have used countless resources found in the environment to treat and cure all kinds of diseases (Athamena, 2009). Plants are still used as an alternative source of medical care in developing countries with an absence of a modern medicinal system (Tabuti, 2003), not only for the high cost of drugs and the inability of many developing countries to provide them, these plants baptized " medicinal " were able to show true and effective results which were deemed culturally acceptable. (Benaissa & ADEFA, 2011).

Consequently, the developments of the medical and aromatic plants (MAP) have experienced a significant increase in the lack of modern medicine on both the national and international scale. MAP's are now being used in many fields including pharmaceutical, cosmetic and food industries.

Morocco is one of the mediterranean countries that share a long history of medicinal knowledge and traditions based on plants (Scherrer et al., 2005). Among the big range of existing aromatic plants in Morocco, particular attention was given to the formerly named Umbelliferae family now known as Apiacea, with particular focus and attention being placed on *Anethum graveolens*.

Dill grows wild in the rich earth and sunny region of Ouezzane. Uses of dill seeds are carminative, stomachic and diuretic. It can also be used to increase milk production for mothers who breastfeed, helped prevent colic, bad breath, coughing, colds, flu and menstrual pains (Setorki & Rafieian - Kopaei , 2013). In this context and in order to illustrate the therapeutic effects of *Anethum graveolens,* a qualitative phytochemical screening completed by technical analytical separation on CCM was carried out on different parts of this plant in order to identify secondary metabolites characterizing this plant.

Materials and Methodology

1. Plant materials

Anethum graveolens (Dill) is widespread and spontaneous in the Mediterranean region and western Asia. It is a herbaceous plant that thrives in exposure to the sun, and it is grown in a sufficiently moist and nutrient-rich soil.

The plant used in this study was randomly harvested in the region Ouezzane during the month of April 2013. When it reaches a height of 25 to 30 cm, the whole plant is torn off by hand. The seeds are obtained during the flowering of the umbels, and then shaken to make them fall. The leaves, stems, and roots of *Anethum graveolens* are washed and dried in the shade, away from moisture and stored carefully. The different parts of the plant were then grounded to powder.

2. Phytochemical screening

a) Tubes characterization reactions

The alkaloids

20 g of powder were moistened in 150 ml of distilled water for 24 hours in a tube. After filtration, the filtrate was left in 2 test tubes to be tested by two reagents. The Dragendorff reagent test appears as an orange-red precipitate proving the presence of alkaloids.

The tannins

Infused in 5% is prepared from 5g of powder in 100ml of boiling water for 15 minutes , afterwards the suspension is filtered and rinsed to produce 100ml. Hydrolysable Gallic tannins are highlighted by adding 15ml of reagent stiansy to 30ml of 5% infused. After 15 minutes of heating in a water bath at 90° C, the mixture is filtered and saturated with 5g of sodium acetate; then, 1ml of a 1% solution of FeCl3 is added. The appearance of a blue - black color indicates the presence of Gallic tannins. The non-hydrolysable catechol tannins are characterized by the addition of 1ml of concentrated HCl in 5ml of previously prepared infusion. The mixture is boiled for 15 minutes. The formation of a red precipitate insoluble in isoamylalcohol affirms the presence of catechin tannins (Trease & Evans, 1987).

The flavonoids

The compounds belonging to the group of flavonoids have been highlighted by the reaction to cyanidin : In a test tube 5ml of infused , add 5ml of hydrochloric acid; 1ml of isoamyl alcohol, then some magnesium shavings. There is a precipitation reaction for several minutes.

The appearance of an orange pink color indicates the presence of flavones. Pink – purple indicates flavanones and red indicates the presence of flavanones and flavanonols.

The same reaction is carried out without adding magnesium chips. The solution is heated for 15 minutes in a water bath , allowing the detection of the presence of leucoanthocyanins in case of an appearance of cherry red or violet with Catechols giving a brownish-red tint (Harborne, 1998).

The anthocyanins

Anthocyanins are revealed by adding 5ml of 10% H2SO4 to 5ml and 5ml infused 5% NH4OH 50%. If the brewed color deepens by acidification and then turns blue in basic environment, one can conclude the presence of anthocyanin (Debrayb et al., 1971) (Paris et al., 1969).

The anthracene derivatives

An extract is prepared from 1g of powder in 10 ml solution of chloroform; followed by heating for 3 min in a water bath, the solution is filtered. The residue of the powder by CHCl3 serves to highlight forms heteroside (O- glycoside , and C - glycoside) .

Free anthracenes are emphasised by adding 1 ml of diluted solution NH4OH of chloroform extract, followed by stirring. The appearance of a more or less red color indicates their presence.

Search for O- Glycosides

5ml of hydrolysate, prepared above, was stirred with 5 ml chloroform , the organic phase is removed , introduced into a tube and then stirred with in 1ml of diluted NH4OH . The aqueous phase is kept. The presence of anthraquinone is revealed by a more or less intense red coloration. The O-glycosides with reduced genins are searched for in case of negative reaction.

Search for O- glycosides with reduced genins

5ml of the hydrolyzate (see above) is added to 3 to 4 drops of ferric chloride 1% , it is heated for 5 min in a water bath and left to cool under a stream of water , it is then stirred with 5 ml of chloroform and racked phase chloroform to which is added 1 ml of diluted NH4OH followed by stirring .
In the presence of oxidation products or anthranols anthranones, the red color is more intense than before.

Search for C- Glycosides

Resume with the aqueous phase (10 ml) which has been preserved and add 1ml of ferric chloride to 10%. The mixture is kept in a boiling water

bath for 30 min and then cooled, stirred with 5 ml of chloroform and 1 ml of NH4OH. A relatively intense red color indicates the presence of C-glycosides (Mogods, 2005).

The saponins

A 1ml decoction is prepared with 1g of powder in 100ml of boiling water for 15 min and then f iltered. The filtrate is stirred vigorously in a test tube. The appearance of foam at least 2 cm in height lasting for at least 10 minutes indicates the presence of saponins. (Trease & Evans, 1987)

The sterols and terpenes

1g of powder is stirred with 20ml of ether. The solution is left in the refrigerator for 24 hours; filtered, then made up to 20ml with ether. The ethereal extract is then evaporated to dryness and taken up with acetic anhydride and then chloroform. In case of positive reaction, it forms a brownish-red or purple ring to the two liquid contact zones and the supernatant is green or violet.

Thecarotenoids

20g of powder of the plants are infused in 150ml of distilled water, the mixture is then filtered. 3 ml of the filtrate is added 3 ml of HCl and then 3 ml of H2SO4. The appearance of a blue - green coloration is demonstrated by the presence of carotenoids (Étds.Rwandaises 1977).

The mucilage

1 ml of 10 % aqueous decoction is introduced into a test tube. 5 ml of absolute alcohol is added, after 10 minutes. A flaky precipitate is revealed indicating the presence of mucilage. (Badiaga2011).

The quinones

1g of milled dry plant material is placed in a tube with 15 to 30 ml of petroleum ether. After stirring and standing for 24 hours, the extracts are filtered and concentrated in a rotary evaporator. The presence of free quinones is confirmed by the addition of a few drops of NaOH 1/10, when the aqueous phase turns yellow - red (Mogods 2005).

The coumarins

1 g of the plant powder sample was placed in a test tube in the presence of a few drops of distilled water. The tube is covered with a paper, moistened with NaOH and heated in a water bath for a few minutes, then added 0.5 ml of diluted NH 4 OH (10%). The two fluorescence spots of this

mixture on a filter paper under ultraviolet light confirms the presence of coumarins (Rizk, 1982).

The essential oils

2ml of extract is added to 0.1 ml of sodium hydroxide (10%) and a few drops of diluted HCl 10%. The formation of a white precipitate indicates the presence of volatile oils (Mojab et al., 2003).

The lipoids

20g of powder of the plant is macerated in 150 ml of petroleum ether for 30 min and then filtered. The filtrate is evaporated on a hot plate to obtain an oily residue; 3 drops of H2SO4 are then added. An intense violet coloration shows the presence of lipoids (Étds.Rwandaises 1977).

The Iridoids

1 ml of concentrated hydrochloric acid to the extract decoction. The formation of black precipitate after heating the extract characterizes the presence of iridoids. (Paris & Moyse , 1965)

Drugs

Weigh 0.5 g of powder and introduce it into a test tube. Add 5 ml of petroleum ether and stir for 15 min. Decant the petroleum ether phase in a capsule. Evaporate to dryness in a water bath. Add 3 to 4 drops of 5% KOH in alcohol. The violet color indicates a positive reaction Beam (A. Diallo 2005).

b) ***Thin layer chromatography (TLC)***

Coumarines detection test

The chloroform extract is subjected to TLC, the elution system: Toluene / EtAc (93:10). Visualization of the chromatogram, after migration, is made at 365 nm in the absence and in the presence of NH3. (Rizk , 1982).

Flavonoids test

The methanolic extract is subjected to TLC, the migration of solvent is: EtAc / MeOH / NH4OH 50% (9: 1: 1). Revelation is made at 365 nm after spraying the plate with reagent Neu 1 % (2 -aminoethyl-diphénylboratedans pure MeOH (Dohou & Yamni , 2003).

Terpenoids test

The migration solvent used is benzene. After migration, the plate is sprayed with antimony chloride, and then placed in an oven at 110 ° C for 10

min. Any fluorescence detected after this treatment proves that the tested material contains terpenoids. (Randerath, 1971).

Saponosides test

The methanol filtered extract is subjected to TLC; migration solvents are chloroform / MeOH / H2O (30 : 5: 2) . The developer is antimony chloride (Louiz & Sellem , 2003).

Tannins and alkaloids test

15ml of acetone is added to 2 g of powder mixture and is placed in a decoction for 1 hour at 70 ° C; the extracts are filtered and evaporated to dryness. The residues are taken up in a few ml of pure methanol. (Dohou & Yamni, 2003).

Alkaloids test

The migration solvent is chloroform / diethylamine (45: 5). After migration, the fluorescent spots at 365 nm are sprayed with Dragendorff reagent. The appearance in visible light orange stain indicates the presence of alkaloids. (Sy & Barbosa , 2008).

Tannins test

TLC is performed, using as solvent ethyl acetate / methanol / H2O (40 : 8: 9). After drying, any fluorescence at 365 nm indicates the presence of tannins (Sy & Barbosa, 2008).

Carotenoids test

0.2 g of plant material is added to à1ml (ether / dichloromethane / ethanol) and stir until colour extraction; the extract is filtered and evaporated on a water bath, after the filtrate is dissolved in 1 ml of dichloromethane. The eluent used is petroleum ether / ether (40/60).

The appearance in visible yellow stains is proved by the presence of carotenoids generally. A blue green spot indicates the presence of chlorophyll a, if the colour is yellow-green is chlorophyll b the two successive yellow spots indicate the presence of xanthophyll (violoxanthine and neoxanthin) .

Anthraquinones test

The mobile phase: ethyl acetate / methanol / water (81 : 11: 8) was revealed by spraying a potassium hydroxide solution 10% in ethanol (Okombe , 2011).

Results

The experimental results of a phytochemical screening, based on reactions of coloration and precipitation (Table 1), or on analysis by TLC with chromatograms observed under UV at 365 nm (results shown in Table 2) of different parts our plant has enabled us to highlight the presence of a number of secondary metabolites in these tissues.

We see under Table 1, the leaves, stems, flowers and seeds are all rich in tannins, carotenoids, coumarin, sterols, polyterpenes, flavonoids and irridoides. For alkaloids these were found in stems, flowers and seeds. Flavones are contained in the leaves, flowers and seeds against absent in stems and roots. On anthocyanins and leucoanthocyanes, which represent one of the subclasses of flavonoids, are present only in the leaves and stems.

Genins the O-glycosides and C-glycosides genins, anthracene derivative components, are observed only in leaves, stems and roots. However there was a total absence of flavanones, free anthracene derivatives, lipoids, mucilage and drugs in all parts of the plant.

Table 1: Identification by staining different chemical groups (GC) in different parts of the plant

GC \ Parts		Leaf	Stem	Root	Flower	Seed
Tanins	free	+++	+	-	+++	++
	Galic tanin	+++	+++	-	+++	+++
	Catechins	-	+++	-	-	-
Flavonoïdes	Flavones	+++	-	-	-	+++
	Flavonones	-	-	-	-	-
	Anthocyanins	+++	+++		- -	- -
	Leucoanthocyanins	+++	++	-	- -	-
Alkaloids		-	+++	-	++	++
Sterols and poly terpenes		+	+++	+++	+++	+++
Mucilage		-	-	-	-	-
Quinones		-	-	-	+	+
Saponines		-	-	-	-	+
Anthracene derivative	free	-	-	-	-	-
	O- glycosides	-	-	-	-	-
	O- glycosides genins	++	++	++	-	-
	C- glycosides	+	++	+++	_	_
Reducing sugar		-	-	-	+++	+++
Coumarines	Test of NaOH	+	-	+	+	-
	Test of NH4OH et HCL	+++	+++++	+++++	+++	++
Carotenoids	H2SO4 and HCL	-	-	-	-	-
	antimony trichloride	+++	++	-	+++	+++
Drugs		-	-	-	-	-
Protein		-	-	-	-	-
Lipoïds		+	-	-	-	-
Essential oils		+	-	-	+	+
Irridoids		+++	+++	++	-	+++

(+++ Strong positive reaction ; ++ moderate positive reaction ; + weak positive reaction; - Negative reaction)

Table2: TLC confirmation of the presence of different chemical groups (GC) in different parts of the plant

	Leaf		Stem		Root		Flower		Seeds		S. of migration	Developer
	Rf	**Spots**	**Rf**	**Spots**	**Rf**	**Spots**	**Rf**	**Spots**	**Rf**	**Spots**		
Tanins	0,95		0,95	Red	0,95	Red	0,90	Red	-	-	ethyl acetate / Methanol/H2O	Ferric chloride
	0,90								-	-		Acetic acid
Flavonoïds	0,08	Yellow	0,06	Blue			0,12	Yellow	-	-		
	0,09	Blue					0,81	Blue	-	-		
	0,24	Red					0,8	Yellow	-	-	Ethyl acetate / Methanol / NH4OH	Reagent NEU
	0,84	Red							-	-		
	0,96	Red							-	-		
			0,27	Blue	0,27	Blue	0,01	Blue	-	-		
Terpeno Alkaloids	0,91	Jaune	0,96	Red			0,27	Blue	-	-	Chloroforme/ Diethylamin	Dragendorff
	0,83	Bleu							-			
	0,07	Red							-	-		
	-	-	0,90	Blue	0,46	Blue	-	-	-	-	Benzene	Antimony trichloride
	0,97	Blue	0,98	Blue	-	-	-	-	-	-		
	0,95	Red			-	-	-	-	-	-	Ethyl acetate / Méthanol/H_2O	KOH/ethanol
Saponos Quinons	0 ,92	Red			-	-	0,86	Blue	-	-		
	0,4	Red	0,50	Red			0,93	Yellow			Chloroform/ Methanol/H2O	Antimony trichloride
	0,18		0,20		0,19				0,85			
Coumarines	0,26		0,85								Chloroform / MeOH / H2O	Antimony trichloride
	0,7											NH3

Caroténoïdes	2 0,76 0,92 0 ,53 0,73 0,97	 Green - Yellow Green Blue Yellow	 0,57 0,77 0,97	 Green Yellow Green Blue Yellow	 0,84 0,97	 Green Blue Yellow	0.13 0,86 0,97	 Yellow Yellow Green Yellow Green Blue Yellow	 0,13 0,2 0,57 0,86 0,97		Toluenes / ethyl acetate Ether / petroleum ether	

Tannins detection

The disclosed chromatograms were visualized at 365 nm showed fluorescence of various colors in different parts of the plant; leaves red spots of Rf = 0.95 to 0.9; the flowers of red spots of Rf = 0.95 to 0.9 and blue Rf = 0.81 and 0.88 and the stalks of blue f = 0.058.

Alkaloids detection

The detection of alkaloids by TLC revealed with Dragendorff reagent gives five clearly visible bands (Rf = 0.96; 0.91; 0.83; 0.3 and 0.071) of a red color; blue and yellow in the leaves and strips (f = 0.2 to 0.9) of blue and red color to the stalks. The major spots obtained for the roots (Rf = 0.27) is still blue coloring.

Detection of flavonoids

In favor of the existence of flavonoids: the yellow spot leaves (Rf = 0.08); flowers of yellow and blue spots respectively Rf = 0.88 0,81et and stems a blue (R = 0.058).

Detection of saponosides

CCM saponosides revealed with antimony trichloride has detected four visible bands (Rf = 0.93; Rf = 0.86) of a yellow and blue color in flowers and red for the leaf and stem of the same Rf 0.42.

Detection of Coumarins

Several bands were observed on TLC different organs. After exposure to NH3 vapor and visualization under UV / 365, yellowish and violet fluorescence were observed in favor of the existence of coumarin. For the sheets (Rf = 0.26 and 0, 72); the rods (Rf = 0.19 and 0.85); roots (Rf = 0.19) and seeds (Rf = 0.85).

Detection of terpenoids

Terpenes fluorescèrent UV / 365 nm yellow blue, green and violet (Lagnika, 2005).

The revelation of the chromatogram to antimony chloride allowed to highlight two bands of blue color at a rod (Rf = 0.9) and the other at the root level (Rf = 0.46).

Detection of carotenoids

Several bands were observed on TLC different organs. After visualization under UV / 365, different colored fluorescences are observed; the leaves of green-yellow spots; -Blue green and yellow (Rf = 0.53, 0, 73 and 0.97); the stems of color spots (Rf = 0.57 and 0.84); roots (Rf = 0, 97et 0.84); Rf flowers (0.1, 0.73, 97) and seeds (Rf = 0.97).

Discussion

After the tube reactions that are often difficult to interpret, it was necessary to confirm our results by chromatographic tests, based on bibliographic data, which also was proven by phytochemical tests of *Anethum graveolens*, the presence of tannins, terpenoids, steroids and flavonoids in leaves, roots and seeds (Jana & Shekhawat, 2010). Similarly, tests carried out by Gurinder and Daljit (2009) also showed that the leaves contain tannins, flavonoids, terpenoids and saponins. Other tests performed by H.O Edeoga, D.E Okwuand, B.O Mbaebie, (2005) showed the presence of alkaloids, flavonoids , saponins in the seeds. This is comparable to our results, with few exceptions, it may due to several intrinsic or extrinsic factors including climatic conditions related to the collection area, cultural practices, storage conditions and stage of plant development at harvest time (Falleh & al., 2008) (Podsedek, 2007). And also could perhaps be explained by the effect of treatment of pre-extraction and extraction solvent of total phenolics in extracts as the case of rosemary (Perez & al, 2007). It notes the absence of any compounds lipoids mucilage; in all parts of the plant (this is not determined by previous work). This failure may be due to the fact that these substances are fragile and rapidly degradable.

Taking into account the observations on saponins, phenols and amino acids (Paris & Moyse, 1965 Paris & Moyse, 1965); it seems that the

different compounds found in the different organs of Dill could justify the use of the latter.

Conclusion

In order to value medicinal plants, the *Anethum graveolens* of the area of Ouazzane was chosen. The study consisted of making a phytochemical screening of five organs of this plant. These results showed that Dill contains flavonoids, coumarins , sterols terpenes, tannins , saponins, alkaloids, reducing compounds , anthracene derivatives O -glycosides , C-glycoside and irridoides .

By following this qualitative characterization, it remains to make a quantitative assessment of the identified secondary metabolites and determine the antimicrobial and antioxidant of Anethum graveolens.

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