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Quantitative study of root the plant (*Anethum graveolens*) and evaluation of their antioxidant activity

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Abstract
The present work had two objectives: firstly, to determine the dosage of total phenolic compounds, flavonoids, condensed tannins and antioxidant activity content in the plant extracted successively by solvents of increasing polarity (hexane, dichloromethane (DCM), ethyl acetate and methanol using a Soxhlet extractor and by maceration. Secondly, determine whether the polarity of the solvents and the extraction method have an impact on these properties.
Our results revealed that hexane extracts of roots of *Anethum graveolens* are rich in total phenols (107 Gallic acid equivalent / 1g dry weight), ethyl acetate extracts rich in total flavonoids 52,269in mg of quercetin equivalent / g of dry weight). Also the highest content of condensed tannins observed in methanol extracts (3,4 in mg of catechin equivalent / 1 g dry weight). The extracts showed a slight antioxidant activity with the two methods. In turn, the results indicated that the antioxidant activity of the polar solvents were important for the methanol extract. The extracts tested in this study were found to be moderate in antioxidant capacity.

Keywords: Medicinal plants, *Anethum graveolens*, total phenol, quantitative survey, Antioxidant, DPPH.

Introduction
Morocco's native flora is filled of several or more species that have not been studied, or have had very little research concerning them and are said to be endowed with benecifical and theraputic properties.
In order to enhance the medicinal plants of our soil and their impact on health through their antioxidant and antiradical virtues, a quantitative study of phenols, total flavonoids, condensed tannins, antioxidant activity of extracts of the plant *Anethum graveolens* of the region of Ouazzane, Morocco, was carried out.
The species *Anethum graveolens* belongs to the family of the Apiaceae, and grows spontaneously in the rich and sunny lands of the region of Ouazzane,
This plant is widely used in traditional medicine it is gifted with carminative properties. It is slightly diuretic, energizing and stomachic and galactagogue. It is used by breastfeeding mothers to increase milk flow, helping prevent colic, bad breath, coughs, cold and flu, menstrual pain [23]

Materials and methods
Plant material
The roots of *Anethum graveolens*:
The roots constitute the part used of this plant in this study. The roots are freed from soiling using tap water and then washed with distilled water. Drying is then carried out in a ventilated chamber. As soon as the roots are well dried, they are cut into pieces and then ground in a mixer to obtain a powder form which is subsequently stored until use.
Two types of extraction methods were used to extract the organic solvents, maceration and soxhlet.

Maceration
In this first part, it has been chosen to extract the plant powder by simple maceration by varying the polarity of the solvent. Four solvents were chosen, hexane, dichloromethane, ethyl acetate and methanol. The extracts are prepared by adding 250 ml of the extraction solvent to 25 g of vegetable powder.
The mixture is kept to rest for 24 hours at room temperature and in the dark. The various extracts obtained are filtered with Wattman paper and evaporated to dryness using rota steam and at a boiling temperature. The extracts are adjusted to 2 ml each as a final volume. The filtrate is then stored at 4 ° C for subsequent analysis. This operation was repeated three times in succession.

Soxhlet
An amount of 25 g of the herbal powder are placed in a cartridge with 300 ml of the chosen solvent. The extraction lasted 2 hours. The extract is recovered filtered with filter paper and evaporated to dryness using a rotary evaporator and at a boiling temperature. The extracts are adjusted to 2 ml each as a final volume and then stored at 4 °C.

Determination of total polyphenols
Determining the content of polyphenols of the total phenolic compounds content of the roots of *Anethum graveolens* were obtained by the Folin-Ciocalteu method [21]

a. Principle
The Folin-Ciocalteu reagent consists of a mixture of Phosphotungstic acid (H3PW12O40) and Phosphomolybdic acid (H3PMo12O40). It is reduced during oxidation of the phenols to a mixture of blue oxides of tungsten, (W8O23), and molybdenum (Mo8O23) (Ribereau-Gayon *et al* (1976) cited by Ben Amara *et al* (2007).
This blue coloration shows the intensity of which is proportional to the levels of phenolic compounds present in the medium, giving a maximum absorption.

b. Expression of results
The concentration of total phenolic compounds is determined by position of the calibration curve of Gallic acid. The results are expressed in milligrams equivalent of Gallic acid per gram of the dry weight of the powdered plant by applying the following formula: C: (c * V) / m C: total polyphenol content (mg gallic acid / dry matter) c: Gallic acid concentration established from the calibration curve Solvent m: weight of the dry matter.

Dosage of flavonoids
The method used to estimate flavonoid levels in the underground part of *Anethum graveolens* is that described by Lamaison and Carnat (1991) and quoted [5]

a. Principle
The yellowish color given in this method is due to the formation of a complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids [16]

b. Expression of results
The absorbance of the extracts of the tubes of the second series are subtracted from those of the first series in order to avoid possible interference of the pigments such as the carotenoid. The concentration of flavonoids is determined by location of the calibration curve obtained using quercetin as a standard.

Determination of condensed tannins
The condensed tannin assay was carried out by the vanillin method described by [7] and the analysis was carried out by spectrophotometry at a wavelength of 500 nm.

a. Principle
Vanillin reacts with the free flavan 3-ols and the terminal units of the proanthocyanidins giving a red coloring. The intensity is proportional to the levels of flavanols present in the medium and which has a maximum absorption at wavelenghth 500 nm.
The results of this work explain the interest in phenolic compounds extended to all dill products. Phenolic compounds are present in all organs.

Results and Discussion
1. Determination of total polyphenols
The total polyphenol content was determined by the Folin-Ciocalteu colorimetric method. It is one of the oldest methods designed to determine the Polyphenols, medicinal plants.
Gallic acid is the standard most often used in this method. The results obtained are represented in a calibration curve (fig 1.) using the equation:
Y= 11,4919 + 0,0300 R^2 = 0.996
Data for the concentration of total phenols in dill root extracts are Expressed in milligrams of Gallic Acid Equivalent (EAG) per 1 g of dry mass (mgEAG / 1 gMS)

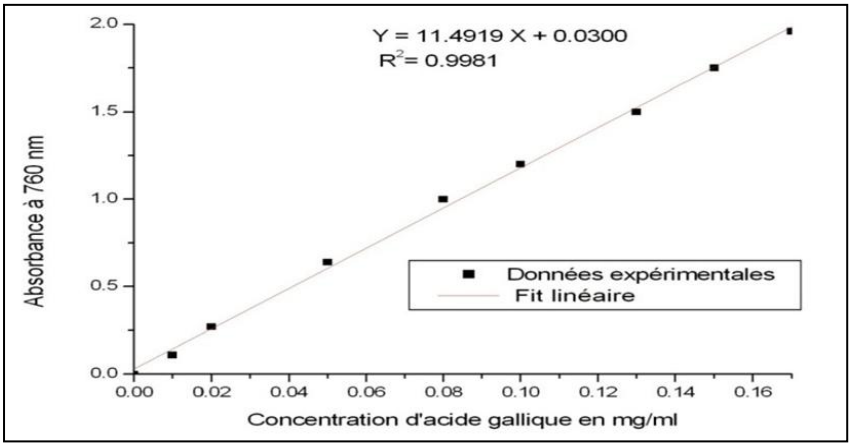

Fig 1: acid gallique Calibration Curve

Table 1: Content of total polyphenols in the root of *Anethum graveolens* extracted by soxhlet and maceration (mg eq AG / gMS)

Part of the plant	Extraction solvents	Total phenols content in mg GAE/g of dry plant material	
		Maceration	Soxhlet
Root	Hexane	90,71	107,139
	Dichloromethane	10,06	12,791
	Ethyl Acetate	1,89	6,554
	Methanol	11,575	22,284

The total polyphenol contents obtained by the two extraction methods presented in Table 1 shows a significant difference; However, soxhlet seems to be the best method of extracting total polyphenols, for example, a value of (107,139 mg eq AG / g PS) per soxhlet versus (90,71mg eq AG / g mS) for maceration: in the hexane extract with a value of (22,284mg and AG / g MS) by soxhlet and with a value of 11.575mg eq AG / g mS) by maceration in the methanolic extract.

These results are close to those of the research conducted by [15]. which reports that the phenolic compound content in the *Deverra scoparia* plant hexane extract of the Apiaceae family is high (18.87 ± 0.59 mg EAG / Extract), unlike the methanolic extract with the lowest value (1.74 ± 0.25 mg EAG / g extract).

Dosage of flavonoids

The concentration of the total flavonoids of the roots of *Anethum graveolens* was measured by the colorimetric method of Aluminum chlorite. The method used to estimate total flavonoid contents is that described by House and Carnet (1991) and quoted [5]

Data for the concentration of total flavonoids in dill root extracts were expressed in milligrams of catechin equivalent (EC) per 1 g dry mass (mg EC / 1 g ms in Table 2).

The concentration of the total flavonoids of *Anethum graveolens* determined by referring to the calibration curve using quercetin as a standar

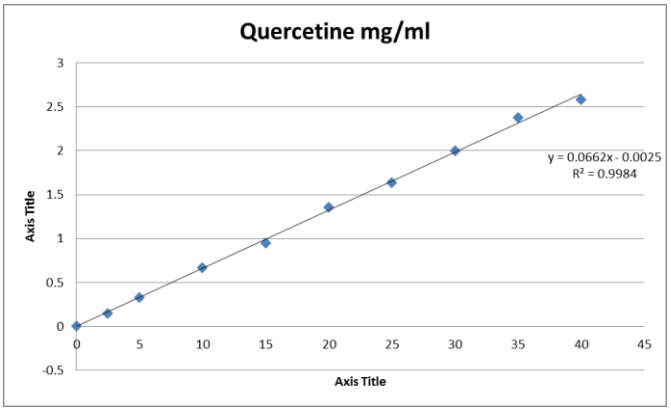

Fig 2: Quercetin Calibration Curv

Table 2: Content of total flavonoids in the root of *Anethum graveolens* extracted by soxhlet and maceration (mg QE/ g).

Part of plant	Extraction solvents	Flavonoid content in mg QE/ g of dry plant material	
		Maceration	Soxhlet
Root	Hexane	6,846	30,01
	Dicloromethane	1, 76	1, 76
	Ethyl Acetate	1,203	52,269
	méthanol	1,147	3,638

The amount of flavonoids in the various extracts is lower than that of the total phenols.

The ethyl acetate extracts are the richest in flavonoids, the amounts vary from (52.269 mg ER / g MS) by soxhlet at (4.94 mg ER / g MS) in maceration, followed by the hexane extract (30.01 mg EC / g).

Their quantities range from (30.01 mg EC / g MS) per soxhlet to (1.203 mg ER / g MS) by maceration. It is important to note that extracts of methanol and dichloromethane are less rich in flavonoids compared to the two other extracts, whatever the extraction method. This is consistent with the study done on the *Deverra scoparia* plant with a value of (12.81 ± 0.16 mg ER / g extract). The ethyl acetate extract of this was larger than those of the extracts methanol and hexane

Determination of condensed tannins

The condensed tannin assay was produced by the method of Vanillin described [7] and the analysis was performed by spectrophotometry at a wavelength of 500 nm.

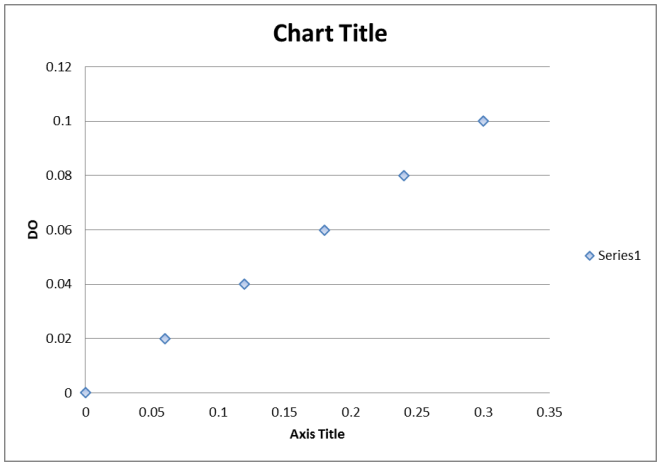

Fig 3: Catechin Calibration Curve

Table 3: Content of condensed tannins in the *Anethum graveolens* extracted by soxhlet and maceration (mg eq AG / g PS).

Part of the plant	Extraction Solvents	Tannin content in mg CE/ g of dry plant material	
		Maceration	Soxhlet
Root	Hexane	0,922	6,003
	Dicloromethane	0,001	2
	Ethyl Acetate	0,004	0,226
	Methanol	**3,4**	0,02

The results obtained from Table 3 show that hexane and dichloromethane soxhlet extraction method gave higher contents of tannins, (6.003 mg eq AG / g PS), (2 mg eq AG / g PS) respectively.

Tannin concentrations of hexane extracts, dichloromethane, ethyl acetate by maceration were very low. These contents correspond to traces, which is in agreement with the results obtained by [21]

Study of antioxidant activity calculation

The study of the antioxidant power of the algae extracts was carried out by spectrophotometry. This analysis is based on the reaction between the 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH °) and the antioxidants contained in the *Anethum graveolens* extracts. The reaction produces an oxidized form of the antioxidant and reduces the radical to 1,1-diphenyl-2-picryl-hydrazyne or DPPH-H.

The reaction was followed by spectrophotometry at 517 nm corresponding to the maximum absorbance of DPPH °. The percentage of the remaining DPPH is calculated by the following formula:

% DPPH ° r = ((A sample × 100) / A blank)

Also, the percentage inhibition (reduction) of DPPH (I%) is calculated according to the following formula:

I% = (A blank -A sample) / A white × 100

Thus, the antioxidant power of the extracts evaluated by this technique is inversely proportional to the disappearance of the violet color of the DPPH°.

1) The curves of the inhibition percentages of differentextracts

The obtained values allowed to plot curves having a logarithmicshape, (FIGS. 67 and 68). From these curves we can determine the percentage inhibition obtained as a function of the concentrations used.

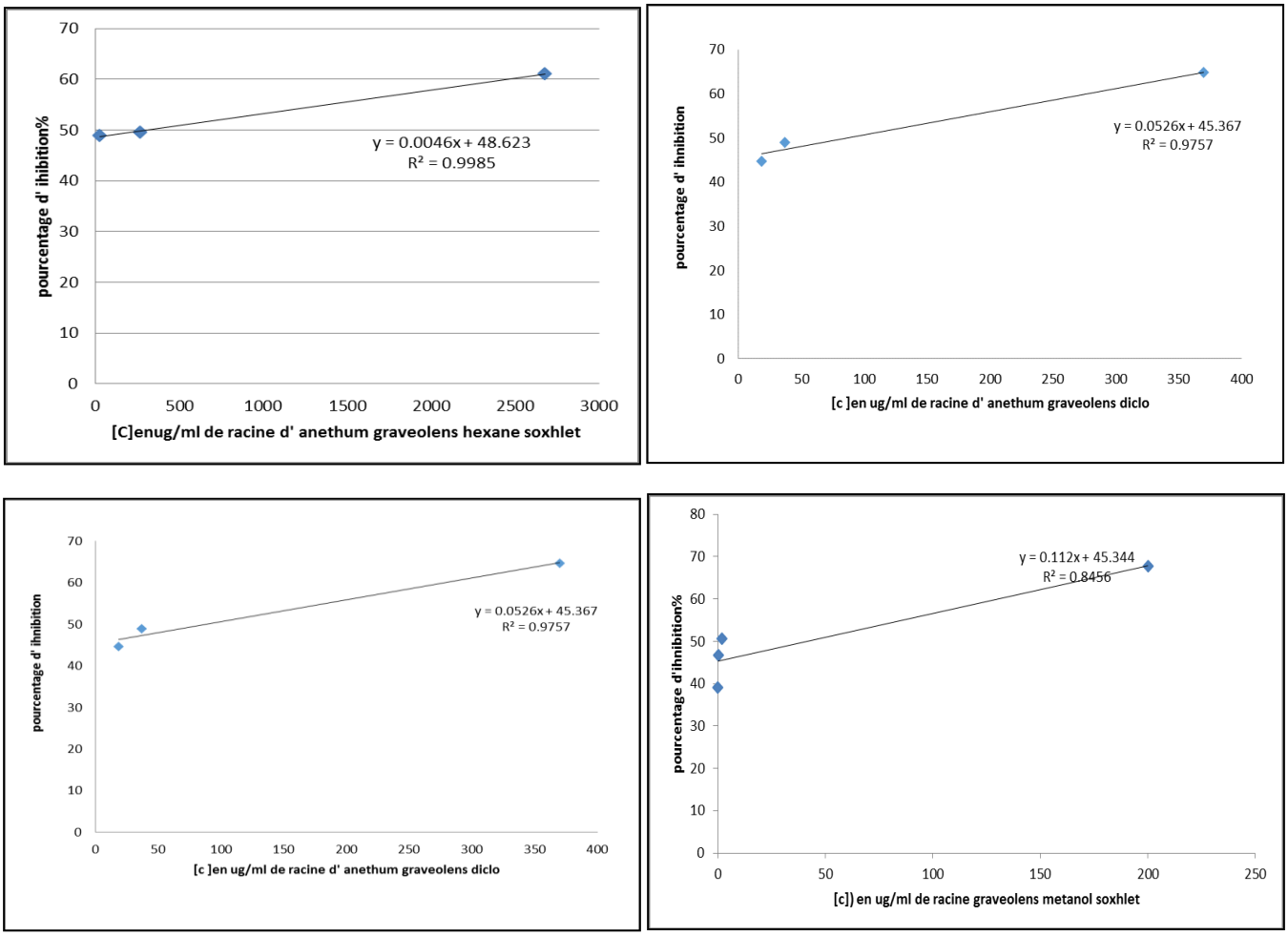

Fig 4: Curves of the inhibition percentages as a function of different concentrations of the soxhlet

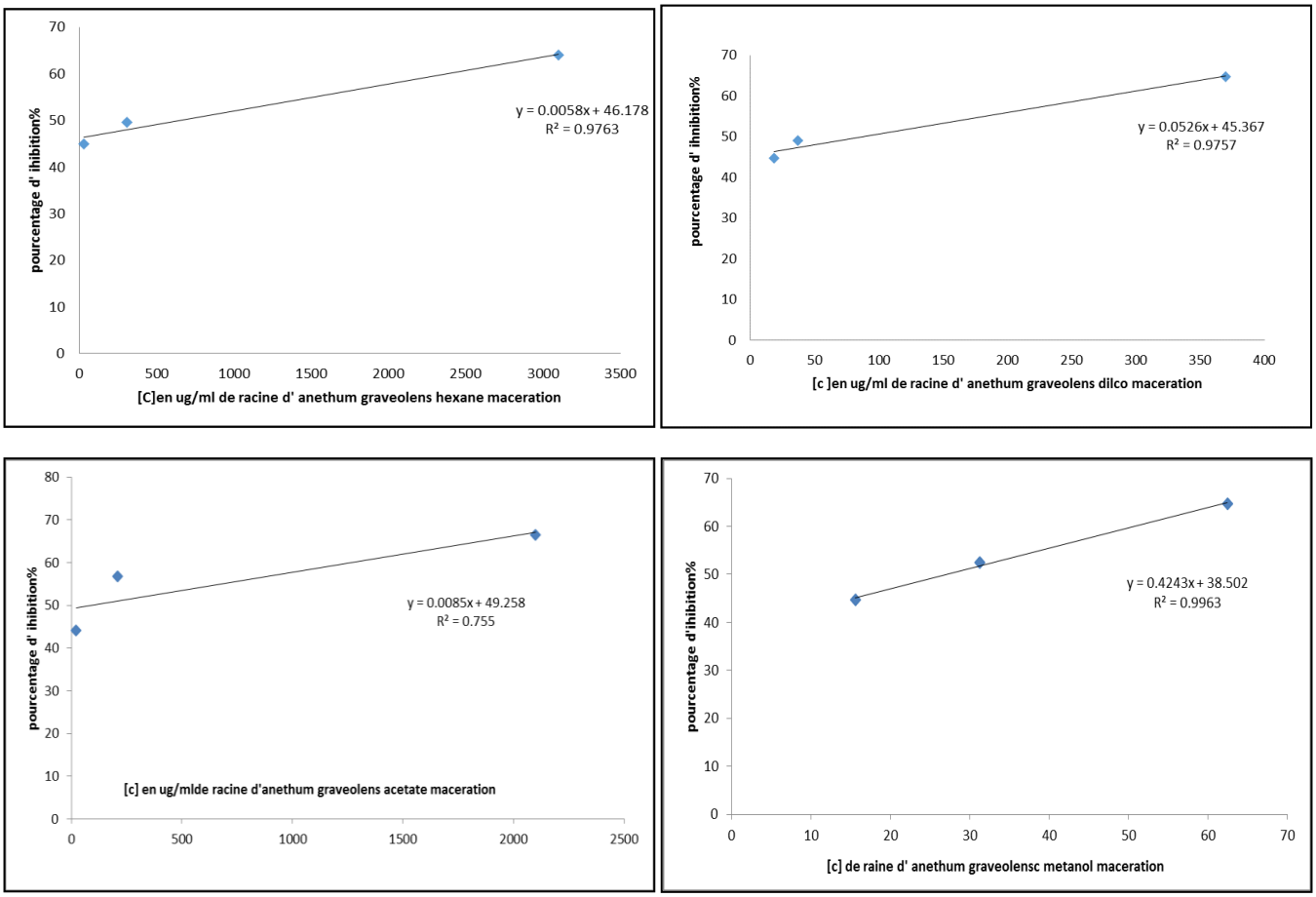

Fig 5: Curves of the inhibition percentags as a function of different concentrations of the maceration

The values obtained allowed for curves to be drawn. From these curves we can determine the percentage of inhibition obtained as a function of the concentrations

2) Calculation of IC50

IC50 or 50% inhibitory concentration (also known as EC50 for efficient concentration 50) is the concentration of the test sample needed to reduce 50% of the DPPH radical. The IC50s are calculated graphically by the linear regressions of the plotted graphs; percent inhibition as a function of different concentrations of the fractions tested.

(Figure 4, 5) shows the IC50 values after contacting the various extracts of *Anethum graveolens* with the DPPH solution (8.88 µM).

Table 4: IC50 in the root of anethum graveolens extracted by soxhlet and by maceration (ug/ml)

Part of the plant	Extraction Solvents	IC50 expressed in µg / ml	
		Maceration	**Soxhlet**
Roots	Hexane	658,965	299,347
	Dicloromethane	189,13	89,09
	Ethyl Acetate	104	65,312
	Methanol	41,571	27

All the extracts showed a lower anti-free radical power than that of the reference compound (Table 4). The anti - radical activity of the root extracts of *Anethum gravelons* by the DPPH method was determined by reference to ascorbic acid, a standard antioxidant (IC50 = 4 µg / ml). Extract of methanol by soxhlet was found to be the most active (27ug / ml) followed by Ethyl acetate extracts (65,312 ug / ml), Dichloromethane (189.13 µg / ml) and Hexane(299,347 µg / ml)

Methanol extracts by maceration also showed the most activity (41.571 µg / ml), based on the extract, of ethyl acetate (104 µg / ml), dichloromethane (189.13 µg / ml) and hexane (658,965 µg / ml). Previous studies by Tanruean *et al* (2014) also showed that the methanolic extracts of *Anethum graveolens* presented good free radical scavenging activity with an IC50 value of (22.3 µg / ml)

In conclusion, it can be pointed out that the amount of total phenols, flavonoids and tannins is variable according to the extraction method and the solvent used.

The concentration of these bioactive constituents depends on climatic conditions and biological variability.

Conclusion

The results of this study suggest that the root extract of *Anethum graveolens* contains phytochemical constituents capable of giving hydrogen to a free radical to eliminate potential damage.

The antioxidants of Apiaceae plants can be used to inhibit the harmful and pathological effect of free radicals. Christova-Bagdassarian *et al*., (2013). The same conclusions are reported by other authors Oktay *et al*., (2003), Gülçın *et al*., (2003), Ramadan *et al*., (2013), Darougheh *et al*., (2014). Free radicals could damage biomolecules that could lead to serious diseases. Coriander has potential for prevention of oxidative stress diseases. It would be useful as a supplement in combination with conventional drugs to improve the treatment of diseases such as cancer [23] while cari improves the immune functions [Dill can be used as a hepatoprotectant. [1] whereas fennel exhibits inhibitory effects against acute and subacute inflammatory diseases and type IV.

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