

Phenolic Profile , Essential oil Composition , Purification of Kaempferol 3-arabinofuranoside and Antimicrobial Activity of Parsley Cultivated in Dakhalia Governorate

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ABSTRACT

The present study was conducted to identify and quantify the phenolic profile of parsley seeds and green parts methanolic extracts, to investigate the essential oil composition extracted from parsley seeds, to purify bioactive components from green parts of ethyl acetate fraction and to investigate the antimicrobial activity of this mentioned preparations. Results revealed that parsley seeds methanolic extract contained higher amount of polyphenols than leaves methanolic extract. Rosmarinic acid was the most detected compound in parsley seed methanolic extract carried out by HPLC technique (38777 µg/g) meanwhile in green parts methanolic extract (as 21468 µg/g). Apiole was the main constituent of the essential oil of parsley seeds (45.21 %). Ethyl acetate fraction of the green parts was employed to TLC chromatographic analysis. The component of the main band was purified with preparative thick layer chromatogram PTLC using ethyl acetate: methanol: water (100:16.5:13.5 by volume) as running system. The obtained data from NMR spectrum for the purified component showed the presence of Kaempferol 3-arabinofuranoside. Seeds and green parts methanolic extracts were evaluated as antimicrobial agent along Kaempferol 3-arabinofuranoside in a comparative study with seeds essential oil. Aromatic oil of *Petroselinum crispum* was the most effective against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Fusarium oxysporum*, and *Alternaria alternate* being with inhibition zones 25.6, 23.8, 30.0, 30.22 and 25.64 mm, respectively. Kaempferol 3-arabinofuranoside demonstrated moderated antibacterial activity against the examined microbes.

Keywords: *Petroselinum crispum*, phenolic profile, essential oil, ethyl acetate fraction, Kaempferol 3-arabinofuranoside, antimicrobial activity

INTRODUCTION

Petroselinum crispum (mill.) is a member of the Umbelliferae family, are commonly known as parsley. The plant has been employed in the food, pharmaceutical, perfume, and cosmetics industries (Kurowska and Galazka (2006). Parsley is found to have different bioactive phytochemicals such as tocopherol, flavonoids, apiole, phenyl propanoids, furano coumarins, terpenoids and carotenoids (Tunali et al.1999). Different bioactive flavonoids i.e. apiin, apigenin, 6-acetylapiin and kaempferol 3-O-β-D-glucopyranoside were isolated from aerial part of *Petroselinum crispum* (Yoshikawa et al. 2000; Chaves et al. 2011 and Gadi et al. 2012), Whole parts of the plant produce the essential oil particularly its seeds (Bruneton 1999.) Myristicin and apiole are the two main components of *Petroselinum crispum* essential oil which are responsible for its antioxidant activity (Zhang et al. 2006).

Parsley different extracts possess different therapeutic activities including hypoglycemic, anti-nephrotoxicity, hypolipidemic, anti-cancer and anti-inflammatory in different animals experiments (El-Kherbawy et al.2011; Rashwan 2012; El-Gazar 2013; El-Beltagi et al. 2010; Farshori et al. 2013; Alol et al. 2012 and Al-Howiriny et al. 2003).

Antimicrobial activity of parsley against wide board of microorganisms was well postulated for its essential oil or different organic solvent extracts (Vokk et al. 2011; Al-Hadi et al. 2013; Karimi et al. 2014; Farah et al. 2015 and Linde et al. 2016). The researchers still searching for novel phytochemicals rather than myristicin and apiole in parsley as antimicrobial agents. So the purification of bioactive

components from the ethyl acetate fraction was one of the potential strategies. Phenolic profile of parsley which varied due to several environmental factors may be affect on the antimicrobial potential of parsley.

So the objective of the current paper was to identify or quantify the phenolic profile of parsley green parts and seeds methanolic extract cultivated in Dakhalia governorate, Egypt, to identify the composition of seeds essential oil, to purify and identify bioactive components from the ethyl acetate fraction of the green parts and to evaluate the antimicrobial activity of parsley mother methanolic extracts, seeds essential oil and ethyl acetate bioactive fraction of green parts in a comparative manner.

MATERIALS AND METHODS

Plant samples

Fresh green parts and seeds of parsley were collected in February 2016 from the local market, Mansoura, Egypt. The samples were shade dried and coarsely powdered.

Extraction of plant samples

The powdered green parts and seeds of *Petroselinum crispum* were extracted by soaking in methanol for 24 hour then filtration process was done and the residues were extracted for three times. The combined methanol extract was evaporated using rotary evaporator till dryness to obtain sticky brown extracts.

Successive extraction of *P. crispum* green parts methanolic extract

Methanolic extract of *P. crispum* green parts was successively partitioned using organic solvents i.e. methylene chloride and ethyl acetate according to the method described in details by Taher et al. (2016).

Preliminary phytochemical tests

Preliminary phytochemical tests were carried out on parsley green parts and seeds methanolic extracts (*Gp.M.E and **S.M.E) to detect the presence of different categories of natural metabolites as described by Harborne (1988).

*GpME = green parts methanolic extract

**SME= seeds methanolic extract

Essential oil extraction

The powdered seeds firstly placed in Clevenger-type apparatus for steam distillation, then immersed in distilled water and heated to 100°C. The essential oil which, was evaporated together with water vapor collected, partitioned and dried over anhydrous sodium sulfate. Oil was stored in refrigerator until further analysis.

Determination of total flavonoids content

Total flavonoids content of methanolic extracts of parsley green parts and seeds (Gp.M.E and S.M.E) was calorimetrically estimated according the method described by Lin and Tang (2007). Standard curve was done by quercetin and total flavonoids content was expressed as mg quercetin / gram dry extract.

Determination of total Phenolics Content

Total phenolics of methanolic extracts were determined according to Folin-Ciocalteu method as described by Singleton et al.(1999). Gallic acid was chosen as a standard and total phenolics content was expressed as mg gallic / gram dry extract.

HPLC analysis

HPLC technique was used for the detection and quantification of different flavonoids and polyphenols in parsley green parts and seeds. HPLC analysis was conducted in the Laboratories of Food Technology Research Institute, Agric.Res.Center, Giza, Egypt.

Identification and quantification of flavonoids

Flavonoids of *Petroselinum crispum* green parts and seeds were identified and quantified by HPLC technique as described in details by Mattila et al. (2000).

Identification and quantification of polyphenols

Phenolic compounds of *Petroselinum crispum* green parts and seeds methanolic extracts were dissolved, then identified and quantified according to the method described by Goupy et al.(1999) using the reversed phase HPLC (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 1050) with a guard column Alltima C₁₈, 5 mm (Alltech).

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

Analysis of the essential oil was done using Agilent 6890 gas chromatography equipped with an Agilent mass spectrometric detector, with a PAS-5MS fused silica capillary column (30 m × 0.32 mm × 0.25 μm film thicknesses). The constituents of aromatic oil were identified by matching their relative retention times and mass spectra with those of WILEY and NIST 05 mass spectral database.

Chromatographic separation of ethyl acetate fraction containing compounds

Ethyl acetate fraction of parsley green parts was separated by chromatographic techniques in order to get pure compounds to be identified by ¹H-NMR spectra.

The preparative TLC was performed by using a readymade glass plates 20x20 cm, which were coated with silica gel G_{F254}; layer thickness 1 mm; made by MERCK, Germany, these plates were activated at 110°C for one hour before use. Ethyl acetate fraction was dissolved in methanol, then applied as a concentrated solution in a row of spots using capillary tubes, the mobile phase was ethyl acetate: methanol: water (100:16.5:13.5 by volume), separated compound which appeared as a band was identified using UV-light detection method. The band was then assigned and the assigned silica gel was scrapped out and collected in a beaker and mixed with hot DMF and then filtered. Silica gel on the filter paper was washed again with hot Dimethyl formamide (DMF). The DMF solution was evaporated to dryness under reduced pressure to give the corresponding precipitate which then recrystallized using aqueous DMF and maintained for NMR analysis.

Nuclear Magnetic Resonance (NMR)

Nuclear magnetic resonance analysis for isolated compound was done using JEOL Nuclear Magnetic Resonance Spectrometer ECA-500 equipped with an Aspect X-32 computer, using Delta NMR Data Processing Software. ¹H-NMR spectra were recorded at 500 MHz in dimethyl sulfoxide (DMSO). The same solvent was used as internal standard. Previous determination was achieved at Central Laboratory, Faculty of Science, Cairo University, Cairo, Egypt.

Antimicrobial activities

Bacteria

The bacterial strains were one of Gram negative, non-spore forming short rod bacterial namely *Escherichia coli* and one Gram positive, that potentially pathogenic such as, *Staphylococcus aureus* that conccoid shaped bacterial in clusters and the other one Gram negative, non spore forming short rod bacterial namely *Salmonella typhi*.

Fungal strains :

The phytopathogenic fungal strains used in this work was *Fusarium oxysporum*, and *Alternaria alternate*, The choice of these test organisms was based on their economic and hygienic roles for humanity in nature.

Cultivation media

Nutrient agar (NA) and potato dextrose agar (PDA) were used for cultivation of bacterial and fungal strains, respectively (Bagamboula et al. 2003).

The agar diffusion method

This method was carried out according to Bagamboula et al.(2003). In this method, examined extract(4mg/ml) along 10 μl of seeds essential oil were used to be tested on the surface of an agar plate freshly seeded with a standard in oculum of young culture, 12 hours old bacteria and seven days old fungi . The plates of bacterial strains were then incubated at 37°C for 24 hours, while those fungi were maintained at 28°C for 7 days. At the end of incubation period, the inhibition zones were measured and recorded.

RESULTS AND DISCUSSION

Preliminary phytochemical tests of methanolic extracts

Data of preliminary phytochemical screening of parsley methanolic extracts revealed the presence of alkaloids(+), flavonoids(+), terpenes(+), tannins(+), glycosides(+) and saponins(+).

Determination of total flavonoids and polyphenols

Data in table (1) showed that seeds methanolic extract contained higher amount of polyphenols as 91.29 mg gallic acid equivalents (GA/g) dry extract than green parts methanolic extract 64.48 mg gallic acid equivalents (GA/g) dry extract. Data of the present study are generally disagreed with the data obtained by *Farah et al. (2015)*. They noticed that parsley leaves extract have higher amounts of phenolic compounds 9.2 mg (GA/g) dry extract than the seeds 6.2 mg (GA/g) extract. Our results are on contrast with those obtained by *El-Tablawy et al. (2015)* who found that parsley aqueous extract contained 369.33 mg (GA/g) extract.

The results showed that green parts methanolic extract contained higher amount of flavonoids as 25.84 mg quercetin equivalents (Q/g) dry extract than seeds methanolic extract 11.85 mg (Q/g). It could be suggested that genetic and/or environmental factors could be affected on parsley total phenolic and flavonoids contents.

Table 1. Total polyphenols and flavonoids of parsley methanolic extracts

Components Sample	Total polyphenols mg G.A. /g	Total flavonoids mg Q. /g
GpME	64.48	25.84
SME	91.29	11.85

Identification and quantification of phenolic compounds by HPLC

From table (2) rosmarinic acid was the most detected compound in parsley aerial parts methanolic extract (as 21468 µg/g) followed by naringin, reversetrol, benzoic, pyrogallol, ellagic acid and salicylic acid as 4313.7, 2894.50, 1043.46, 1016.56, 970.46 and 434.9 (µg/g), respectively. These mentioned compounds were also the main detected phenolic compounds in seeds methanolic extract with some quantity differences, where the amounts of rosmarinic acid, reversetrol, naringin, pyrogallol, salicylic, benzoic acid and ellagic acid were 38777, 5646.70, 1985.8, 1843.51, 1483.04, 1238.76 and 846.99(µg/g), respectively.

No previous researchers dealt with the retention times of 35 standard phenolic compounds which were used in the present study to identify and quantify the individual phenolic compounds of the examined samples by HPLC technique. Furthermore, no published papers studied the phenolic profile of mother methanolic extract of different parts of parsley plant. So obtained data could be conflicted with those obtained in the literature. In this respect, *Muchuweti et al.(2007)* demonstrated the presence of just five phenolic compounds namely, gallic acid, caffeic, ferulic, p-coumaric and protocatechuic acid when parsley aqueous methanolic extract analysed by HPLC technique. *Stan et al.(2012)* identified four flavonoids such as apigenin, luteolin, quercetin and kaempferol from the chromatogram of parsley leaves extracted with ethanol-water in the ratio of (50:50, v/v). Obtained data revealed the presence of the previous mentioned flavonoids with amounts of 98.95, 112.52, 1095.1 and 235.85 (µg/g), respectively.

Table 2. Identification and quantification of phenolic compounds in the examined samples by HPLC

Compounds	Conc. µg/g		Compounds	Conc. µg/g	
	GpME	SME		GpME	SME
Gallic acid	23.61	56.58	p-coumaric	447.67	225.24
Pyrogallol	1016.56	1843.51	Cinnamic	68.06	20.65
4-aminobenzoic	13.30	22.31	3-OH-Tyrosol	12.87	165.46
Protocatechuic	79.65	130.21	3,4,5-methoxy-cinnamic	171.80	232.72
Chlorogenic	194.73	87.92	Naringin	4313.7	1985.8
Catechol	375.61	238.24	Rutin	185.85	257.82
Caffeine	45.97	49.37	Hisperdin	243.92	455.62
p-OH benzoic	36.63	108.32	Rosmarinic	21468	38777
Caffeic	28.80	195.60	Quercetrin	122.38	633.79
Vanillic	72.79	94.30	Quercetin	1095.1	772.61
Ferulic	223.50	130.00	Kampherol	235.85	213.09
Isoferulic	104.56	101.68	Hesperitin	337.81	177.87
Reversetrol	2894.50	5646.70	Apigenin	98.95	213.78
Ellagic	970.46	846.99	7-OH flavone	29.12	10.09
α- coumaric	102.31	209.68	Luteolin	112.52	618.65
Benzoic	1043.46	1238.76	Catechein	217.80	105.56
Salicylic	434.90	1483.04	Epicatechein	235.29	125.08
Coumarin	68.06	20.65			

Essential oil fingerprint

GC-MS analysis of parsley essential oil led to the documentation of 13 different constituents, representing 99.72% of the total oil as shown in Table (3). Apiole was the main constituent of the essential oil of parsley seed (45.21

%) followed by di-n-octyl phthalate, myristicin, carvone and 1,4- benzenediamine as percentage of 19.76, 18.46, 4.56 and 4.22, respectively. The obtained data are in agreement to a large extent with those reported by *Linde et al.(2016)*. They found that apiole was the predominant constituent of

the essential oil (50.3 %) followed by β -phellandrene and myristicin (14.6 and 14.0 %), respectively. On the contrary, obtained data are disagreed with those obtained by several researchers, for instance, Kurowska and Galazka (2006) found that α -Pinene was the main compound (32%) followed by β -Pinene (19%), myristicin (18.3%), 1-Allyl-2,3,4,5 tetra methoxybenzene(12.8%) and apiole (10.1 %).

In more recent paper, Vokk *et al.* (2011) showed that myristicin was the major constituent of the essential oil of parsley grown in different seasonal conditions with percentages ranged from 30.7 – 42.7 . They was also mentioned that detectable components with moderated percentage were β -phellandrene (21.8 – 35.9 %), P-1,3,8 – Menthatriene (5.4 – 10 %) and β -Myrcene (4.5 – 8.7 %).

Table 3. The composition of parsley seeds essential oil

No	Rt	Compound	Peak area %	Molecular formula
1	16.13	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2 α ,4 α ,8 α)]-	1.14	C ₁₅ H ₂₄
2	16.72	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	0.31	C ₁₀ H ₁₄ O
3	19.00	(-)-Carvone	4.56	C ₁₀ H ₁₄ O
4	19.72	3-Cyclohexen-1-one, 2-isopropyl-5-methyl-	0.63	C ₁₀ H ₁₆ O
5	25.4	2-Propenoic acid, 3-phenyl-, methyl ester	0.61	C ₁₀ H ₁₀ O ₂
6	28.15	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- (Myristicin)	18.46	C ₁₁ H ₁₂ O ₃
7	28.83	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	3.51	C ₁₂ H ₁₆ O ₃
8	32.16	Apiole	45.21	C ₁₂ H ₁₄ O ₄
9	34.69	Tributyryn	0.29	C ₁₅ H ₂₆ O ₆
10	37.08	1H-Indene, 2,3-dihydro-4-methyl-	0.15	C ₁₀ H ₁₂
11	38.70	1,4-Benzenediamine	4.22	C ₆ H ₈ N ₂
12	44.39	Di-n-octyl phthalate	19.76	C ₂₄ H ₃₈ O ₄
13	49.03	(Phenylthio)acetic acid, 1-adamantylmethyl ester	0.87	C ₁₉ H ₂₄ O ₂ S
		Total identified	99.72	
		Monoterpene hydrocarbons	1.29	
		Oxygenated terpenes	93.34	
		Other constituents		

¹H-NMR spectra

In the ¹H- spectra of the pure component, all signals are in agreement with the literature data for kaempferol 3-O-substituted and those of *Kaempferol 3-arabinofuranoside* as shown in Table (4) and Figure (1). In the ¹H-NMR spectra, arabinose had anomeric proton signal appeared in the form of a doublet at 5.43 ppm of a low coupling constant J = 0.4 Hz which is characteristic for α -arabinofuranose.

Table 4. ¹H-NMR data of *Kaempferol 3-arabinofuranoside* in CD₃ OD, δ (ppm), (JHz).

Aglycone proton						Sugar proton
2'	3'	5'	6'	8	6	
7.96 d	6.91 d	6.91 d	7.96 d	6.40 d	6.20 d	5.43 d
d(8.9)	(8.9)	(8.9)	(8.9)	(2.1)	(2.3)	(0.4)

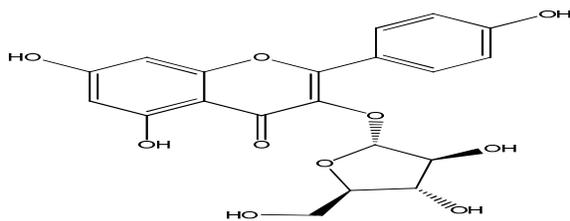


Figure 1. The structure of the purified compound *Kaempferol 3-arabinofuranoside*

Evaluation of antimicrobial activities of the examined plant extracts

The antimicrobial activities of parsley methanolic extracts (GpM, SM), green parts ethyl acetate fraction and

seeds essential oil were evaluated against five microorganisms. These microbes were three bacterial strains, one was G⁺ coccoid shaped bacterial namely *Staph. aureus*, the other were G⁻ short rods bacteria called *E.coli* and *Salmonella typhi* . Other two microbes were phytopathogenic fungal strains namely *Fusarium oxysporum*, and *Alternaria alternate*. These activities were assessed by the presence or absence of inhibition zones around the microbial growth on solid cultivation media and obtained diameter in which no growth observed were measured after incubation at 37°C/24h and 28°C/7 days for examined bacteria and fungi, respectively.

Applying the tested extracts of the examined plant against five microbial strains was carried out and obtained results were recorded. Results listed in Table (5) showing the effect of methanolic extracts, ethyl acetate of green parts (4mg/ml) of *Petroselinum crispum* and seeds essential oil of seeds(15 μ l) on the microbial strains either G⁺bacteria or G⁻ bacterial strains and fungal strains. Results illustrated that aromatic oil of *Petroselinum crispum* was the most effective against the three tested bacterial strains with inhibition zones being 23.8 , 25.6 and 30 mm for *Staph. aureus*, *E.coli*, and *Salmonella typhi*, respectively.

Data showed that bacterial strain namely *Staph. aureus*, coccoid shaped G⁺ was the most resistant bacterial strain to the examined samples as illustrated in Table (5).

Lipopolysaccharides in the outer membranes of G-bacteria are responsible for their high resistance to external agents (Suresh *et al.* 2010).

Antimicrobial agents that irreversible fatal action on bacteria are called as bactericidal whereas those with reversibly inhibit the bacterial growth are known as bacteriostatic (Rajesh and Rattan 2008). Antimicrobial agents may damage pathogens by inhibiting microbial

protein and nucleic acid synthesis, disrupting microbial membrane structure and function, hampering cell wall synthesis or inhibition of key enzymes responsible for metabolic pathways (Willey *et al.* 2008).

Table 5. Values of inhibition zones (mm) around the microbial growth on solid cultivation medium as a result of treatment by examined plant extracts

Tested microbial strains	<i>Petroselinum crispum</i>			
	Green parts methanolic extract	Green parts ethyl acetate fraction	Seeds methanolic extract	Seeds essential oil
<i>E.coli</i>	9.6	12.54	15.0	25.6
<i>Staph. aureus</i>	8.5	10.96	14.3	23.8
<i>Salmonella typhi</i>	12.22	15.47	17.56	30.0
<i>Fusarium oxysporum</i>	9.29	25.6	22.3	30.22
<i>Alternaria alternata</i>	10.53	15.32	18.42	25.64

Obtained results belonging to bactericidal and fungicidal properties of parsley essential oil are in agreement with those obtained by others, (Vokk *et al.* 2011; Karimi *et al.* 2014; Linde *et al.* 2016) who examined the antimicrobial activity of parsley essential oil extracted from different parts (i.e. leaves and seeds) against different bacterial and fungal strains. Obtained results are in conflict with those obtained by Viuda-Martos *et al.* (2011); Gutierrez *et al.* (2008), who observed that parsley essential oil extracted from aerial parts had no antibacterial activity. The composition of parsley essential oil which were widely varied worldwide due to genetic and/or environmental factors could be affected on their antimicrobial activity (Camilotti *et al.* 2015 and Linde *et al.* 2016). Although there are global variations in the major constituents of parsley essential oil, apiole and/or myristicin have always been among the main components. In this respect, obtained results showed that apiole (45.21%), di-n-octyl phthalate (19.76%) and myristicin (18.46%), were the most abundant components of parsley essential oil cultivated in Egypt. Therefore, the present study supported the belief that apiole and/or myristicin were the more actively antimicrobial agents in *Petroselinum crispum* essential oil.

No previous studies dealt with the antimicrobial activity of Kaempferol 3-*arabinofuranoside* (K-3-A) even in a pure form or crude fractions containing it. Chromatographic analysis for ethyl acetate fraction showed the presence of two bands and the main component was sequentially purified and identified as Kaempferol 3-*arabinofuranoside*. Parsley ethyl acetate fraction which is considered as a rich source for K-3-A showed a moderated antimicrobial activity and their highest inhibitory zone around the growth was 25.6 mm for *Fusarium oxysporum* as shown in Table (5). This may be attribute to their high phenolic content. Leaf ethyl acetate fraction of *P. koraiensis* rich with kaempferol-3-O-glucoside and kaempferol-3-O-arabinoside exhibited antibacterial activity against *P. acnes*, *S. aureus*, *P. ovale*, and *E. coli* with minimal inhibition concentration (MIC) values as 0.06 %, 0.25 %, 0.13 % and 0.50 %, respectively kim *et al.* (2010). In another study, Shafek *et al.* (2012) purified two high molecular weight kaempferol glycosides with antibacterial activity against both Gram positive and

Gram negative bacteria. Rosmarinic acid, the most detected phenolic compound in methanolic extracts in the present study was reported to possess strong antibacterial activity when purified from the hydromethanolic extract of *Hyptis atrorubens* (Abedini *et al.* 2013). It could be suggested that rosmarinic acid synergistically acts with other bioactive component in parsley methanolic extracts especially the essential oil components as antimicrobial agent.

In conclusion, this study demonstrates that antimicrobial activity of parsley products depending on the essential oil composition and the phenolic profile which may be affected by genetic and/or environmental factors. Apiole, myristicin and rosmarinic acid may be responsible for the antimicrobial activity of parsley cultivated in Dakahlia governorate, Egypt. Kaempferol 3-*arabinofuranoside* was isolated for the first time from ethyl acetate fraction of parsley green parts. Finally, ethyl acetate fraction which contained K-3-P as the main component; possessed moderated antimicrobial activity especially against *Fusarium oxysporum*.

REFERENCES

- Abedini, A.; Roumy, V.; Mahieux, S.; Biabiany, M.; Standaert-Vitse.; Rivière, C.; Sahpaz, S.; Bailleul, F.; and Hennebelle, T. (2013). Rosmarinic acid and its methyl ester as antimicrobial components of the hydromethanolic extract of *hyptis atrorubens* Poit. (Lamiaceae). Evidence-Based Complementary and Alternative Medicine Volume 2013 Article ID 604536, 1:11.
- Al-Howiriny, T. A.; Al-Sohaibani, M.O.; El-Tahir, K. H. and Rafatullah, S. (2003). Preliminary evaluation of the anti-inflammatory and anti-hepatotoxic activities of 'Parsley' *Petroselinum crispum* in rats. Journal of natural remedies vol. 3/1 54 – 62.
- Al-Hadi, A. ; Samiya, S. ; Al Rahbi, M. d.; Sohail, A. ; Sadri, S. and Afaf, W. Q. (2013) Phytochemical screening, antibacterial and cytotoxic activities of *petroselinum crispum* leaves grown in Oman. Iranian Journal of Pharmaceutical Sciences: 9 (1): 61-65.
- Alol, L. H. ; Kahtan , A. Al-M. and Shalal, M. H. (2012). The Promising anticancer efficacy of parsley seeds flavonoid (Apigenin) in induced mammary adenocarcinoma (AMN3) Mice. J Physiol Biomed Sci; 25(1):5-12.

- Bagamboula, c. ; Uytteendle, M. and Debevere, J. (2003). Antimicrobial effect of spices and herbs *Shigilla sonnei* and *Shigilla flexneri*. *J. Food Prol.*, 66: 668-673.
- Bruneton, J. (1999). *Pharmacognosy, Phytochemistry, Medicinal Plants*, 2nd ed. London: Intercept Ltd.; 519-520.
- Camilotti, J.; Ferrarrese, L.; Bortolucci, W. C. and Gonçalves, J. E. (2015). Essential oil of parsley and fractions to in vitro control of cattle ticks and dengue mosquitoes. *J. Med. Plants Res.* 9: 1021-1030.
- Chaves, D.S.; Frattani, F.S. and Assafim, M. (2011). Phenolic chemical composition of *Petroselinum crispum* extract and its effect on haemostasis. *Nat Prod Commun*; 6(7):961-964.
- El-Beltagi, H. S. and Abdel-Rahim, E.A. (2010). Constituents of apple, parsley and lentil edible plants and their therapy treatments for blood picture as well as liver and kidneys functions against lipidemic disease. *Ejefache*, 9 (6), issn:1579--4377.
- El-Gazar, A.F. and Alaa,O.A.(2013). Nephroprotective and diuretic effects of three medicinal herbs against gentamicin-induced nephrotoxicity in mal rats. *Pakistan journal of Nutrition* 12(8):715-722.
- El-Kherbawy, G.M.; Ibrahim, E.S. and Zaki, S.A. (2011). Effects of Parsley and Corianderleaves on hypercholesterolemic rats. The 6th Arab and 3rd International Annual Scientific Conference on: 1227 - 1240.
- El-Tablawy, N.A.; Hanan, A. S. and Mona, S. H. (2015). Antioxidant and antidiabetic role of *petroselinum crispum* against stz-induced diabetes in rats. *Journal of Biomedical and Pharmaceutical Research*. Volume 4, Issue 3, 32-45.
- Farah, H.; Elbadrawy, E. and Al-Atoom, A. A. (2015). Evaluation of antioxidant and antimicrobial activities of ethanolic extracts of Parsley (*Petroselinum erispum*) and Coriander (*Coriandrum sativum*) plants grown in Saudi Arabia. *International Journal*, 3(4), 1244-1255.
- Farshori, N.N.; Ebesam, S. Al-Sh.; Mai, M. Al-O.; Javed, M.; Abdulaziz, A. Al-Kh. and Maqsood .A.S. (2013). Anticancer activity of *Petroselinum sativum* seed extracts on MCF-7 human breast cancer cells. *Asian Pac J Cancer Prev*, 14 (10), 5719-5723.
- Gadi, D.; Bnouham, M. and Aziz, M. (2012). Flavonoids purified from parsley inhibit human blood platelet aggregation and adhesion to collagen under flow. *J Complement Integr Med*; 9: article 19. doi: 10.1515/1553-3840.1579.
- Goupy, P. ; Hugues, M. ; Boivin, P. and Amiot, M. J. (1999). Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and isolated phenolic compounds. *J.Sci. Food Agric.*; 79:1625.
- Gutierrez, J.; Rodriguez, G.; Barry-Ryan, C. and Bourke, P. (2008). Efficacy of plant essential oils against foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables: antimicrobial and sensory screening. *J. Food Prot.* 71: 1846-1854.
- Harbrne, J.B. (1988). *Phytochemical Method*, 2nd ed. Published in USA by Chapman and Hall 29, West 35th street, New York.
- Karimi, F.; Rezaei, M. ; Shariatifar, N. ; Sayadi, M. ; Mohammadpourfard, I. ; Malekabad, E. and Jafari, H. (2014). Running Head: Antimicrobial activity of Parsley antimicrobial activity of essential oil of Parsley (*Petroselinum crispum*) against food pathogenic bacteria. *World Applied Sciences Journal* 31 (6):1147-1150.
- Kim, JH.; Kwon, HJ.; Lee, KH.; Chun, SS.; Cho, YJ. and Cha, WS. (2006). Inhibitory effect against *Helicobacter pylori* and biological activity of Thyme (*Thymus vulgaris* L.) extracts. *J Korean Soc Appl Biol Chem.* 49:243-47.
- Kurowska, A. and Galazka, I. (2006). Essential oil composition of the parsley seed of cultivars marketed in Poland. *Flavour and Fragrance Journal* ; 21: 143-147.
- Lin, J.Y. and Tang, C.Y. (2007). Determination of total phenol and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food chem.* 101:140.
- Linde, G.A.; Gazim, Z.C. ; Cardoso, B.K. ; Jorge, L.F. Tešević, V. ; Glamočlija, J. ; Soković, M. and Colauto, N.B. (2016). Antifungal and antibacterial activities of *Petroselinum crispum* essential oil. *Genetics and Molecular Research* 15 (3): gmr.15038538
- Mattila, P.; Astola, J. and Kumpulainen, J. (2000). Determination of flavonoid in plant material by HPLC with diode-array and electro-array detections. *Journal of Agriculture and Food chemistry*, 48:5834.
- Muchuweti, M.; Kativu, E.; Mupure, C.H.; Chidewe, C.; Ndhlala, A.R. and Benhura, M.A.N. (2007). Phenolic composition and antioxidant properties of some Spices. *American Journal of Food Technology*. 2(5):414-420.
- Rajesh, H. and Rattan, F. (2008). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol.* 88: 308-316.
- Rashwan, N.M. (2012). Biological study on the effect of arginine parsley and on renal toxicity in rats. *World Journal of Medical Sciences* 7 (4): 264-269.
- Shafek, E.; Shafek, N. and Michael, H. (2012). Antibacterial and antioxidant activity of two new kaempferol glycosides isolated from *Solenostemma argel* stem extract. *Asian J of Plant Sci*, 11(3): 143-147.
- Singleton, V.L.; Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol*, 152: 299.
- Stan, M.; Soran, M.L. ; Varodi, C. and Lung, I. (2012). Extraction and identification of flavonoids from Parsley extracts by HPLC analysis. *AIP Conf. Proc.* 1425, 50-52.

- Suresh, C.V. ; Zaika, L.L. and Sahkyl ,A.C. (2010). Spices and herbs: their antimicrobial activity and its determination. J Food Saf.; 9: 97_118.
- Taher,M.A.; Dawood, H. D.; Mostafa, I.S. and Ramadan, A. H. (2016). Searching for anti-hyperglycemic phytomolecules of Tecoma stans. European Journal of Chemistry 7 (4) (2016) 397-404.
- Tunali, T.; Yarat , A.; Yanardag , R.; Ozcelik ,F.; Ozsoy, O.; Ergenekon, G. and Emekli, N. (1999). Effect of parsley (*Petroselinum crispum*) on the skin of STZ induced diabetic rats. Phytother. Res. 13: 138-141.
- Viuda-Martos, M.; Mohamady, M.A.; Fernández-López, J. and Abd ElRazik, K.A. (2011). In vitro antioxidant and antibacterial activities of essential oils obtained from Egyptian aromatic plants. Food Contr. 22: 1715-1722.
- Vokk, R.; Lõugas, T. ; Mets, K. and Kravets, M. (2011). Dill (*Anethum graveolens* L.) and Parsley [*Petroselinum crispum* (Mill.) Fuss] from Estonia: Seasonal differences in essential oil composition. Agronomy Research 9 (Special Issue II), 515-520.
- Wiley, J.M.; Sherwood, L.M. and Woolverton, C.J. (2008). Prescott, Harley and Klein's Microbiology, 7th Edn., Mc Graw Hill Companies, New York, USA.
- Yoshikawa, M.; Uemura, T. and Shimoda, H. (2000). Medicinal foodstuffs. XVIII. Phytoestrogens from the aerial part of *Petroselinum crispum* Mill. (Parsley) and structures of 6'-acetylapiin and a new monoterpene glycoside, petroside. Chem Pharm Bull; 48(7): 1039-1044.
- Zhang, H.; Chen, F. and Wang, X. (2006). Evaluation of antioxidant activity of parsley (*Petroselinum crispum*) essential oil and identification of its antioxidant constituents. Food Res Int; 39(8): 833-839.

التفريد الفينولي وتركيب الزيت العطري وتنقية كامفيرول ٣ - ارابينوفورانوزيد وتقييم القدرة المضادة لنشاط الميكروبات لنبات البقدونس المزروع بمحافظة الدقهلية
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اجريت الدراسة التالية للتقدير الكمي للمركبات الفينولية للمستخلصات الميثانولية لبذور البقدونس والأجزاء الخضراء ، التعرف على المكونات الأساسية للزيت العطري المستخلص من بذور البقدونس ، تنقية بعض المركبات النشطة بيولوجيا من مستخلص خلاص الأيثيل للأجزاء الخضراء للنبات ودراسة النشاط المضاد للميكروبات في المستخلصات السابقة. اوضحت النتائج ان المستخلص الميثانولي لبذور البقدونس تحتوي على كمية أعلى من الفينولات الكلية عن المستخلص الميثانولي للأجزاء الخضراء. وكان حمض روزمارينيك أعلى المركبات في المستخلص الميثانولي لبذور البقدونس بواسطة التحليل الكروماتوجرافي عالي الكفاءة وقدره ٣٨٧٧٧ ميكروجرام / جم وكذلك في المستخلص الميثانولي للأجزاء الخضراء بقدر ٢١٤٦٨ ميكروجرام / جم . وكان الأبيول المكون الرئيسي للزيت العطري من بذور البقدونس بنسبة (٤٥.٢١٪). تم استخدام مستخلص خلاص الأيثيل الناتج من التجزئة للمستخلص الميثانولي للأجزاء الخضراء لتحليل كروماتوجرافي الطبقة الرقيقة وتم تنقية المكون الرئيسي الناتج من التفريد باستخدام محلول سريان مكون من خلاص الأيثيل: الميثانول: الماء (١٠٠ : ١٦.٥ : ١٣.٥ بالحجم). وأظهرت البيانات التي تم الحصول عليها من طيف الرنين المغناطيسي النووي للمكون المنقى وجود كامفيرول ٣-أرابينوفورانوسيد. تم تقييم المستخلصات الميثانولية للبذور والأجزاء الخضراء لنبات البقدونس كعامل مضاد للميكروبات بجانب المستخلص الغني بكامفيرول ٣-أرابينوفورانوسيد في دراسة مقارنة مع الزيت العطري الناتج من البذور. وكان الزيت العطري من بذور نبات البقدونس (بييتروسيلينوم كريسبوم) الأكثر فعالية ضد الإشريشيا القولونية، سنافلوكوكوس أوربوس، السالمونيلا التيفية. الفيوزاريوم أوكسيسبورم، و التيرناريا الترنات مع مناطق تثبيط ٢٥.٦، ٢٣.٨، ٣٠.٠، ٣٠.٢٢ و ٢٥.٦٤ مم على التوالي. كما أظهر المستخلص الغني بالكامفيرول ٣-أرابينوفورانوسيد نشاط مضاد للبكتيريا معتدل ضد الميكروبات تحت الدراسة .