GC-MS Investigation of Essential oil and antioxidant activity of Egyptian White Onion (*Allium cepa* L.)

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ABSTRACT

Objectives: This study carried out to investigate the chemical constituents of white onion (*Allium cepa* L.) essential oil as well as evaluate the antioxidant activities, total phenolic and total flavonoid contents of their different extracts.

Methods: The Essential oil of white onion (*Allium cepa* L.) bulbs were extracted by hydro distillation method and analysed by gas chromatography coupled with mass spectrometry (GC–MS) to determine the chemical composition of the volatile compounds. The antioxidant activities of the methanolic extract of white onion and its derived fractions (*n*-butanol and water fractions) were determined by using two different spectrophotometric techniques as DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) scavenging assay and phosphomolybdenum assay. Also, the total phenolic and flavonoid contents were determined.

Results: In total, GC-MS analysis showed 40 compounds representing 93.89 % of the total oil composition were identified. The major compound in this essential oil was dodecane (28.69 %) and the other predominant constituents were represented 65.20% contains hydrocarbons, alkaloids and organosulfur compounds. The antioxidant results showed that the butanolic fraction has the highest antioxidant activity in the DPPH assay (SC_{50} = 59.21 ± 0.35 μg/ml) and phosphomolybdnum assay (127.92 ± 1.00 mg ascorbic acid eq. /g ext.). Also, the butanolic fraction has high total phenolic (41.05± 0.34 mg gallic acid eq. /g ext.) and flavonoid contents (3.82± 0.24 (mg rutin eq. /g ext.).

Conclusion: The essential oil content and the antioxidant results of white onion (*Allium cepa* L.) may be have great potential for their application in food preservation and natural health products.

Key words: plant extracts, essential oils, total phenolic, total flavonoids, GC-MS.

INTRODUCTION

Aromatic and medicinal plants were used from many centuries as remedies for human diseases because they contain chemical components of high therapeutic potential. In the last few years, plant products and their modified derivatives have been rich sources for clinically useful drugs. According to the World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [1, 2, 3]. Essential oils are used in traditional therapies especially aromatic oils which extracted by steam or hydro distillation methods of different plant parts (flowers, leaves, seeds, roots, bulbs, stems and bark) have been used to obtain essential oils. Essential oils and their components are widely used in medicine, in the food industry as flavoring additives and in cosmetics as fragrance [2, 4, 5].

The main constituents of essential oils are mono and sesquiterpenes, carbohydrates, phenols, alcohols, ethers, aldehydes and ketones which responsible for their biological activity as well as for their fragrance. In recent years, several researchers have reported that phenolic compounds, mono- and sesquiterpene hydrocarbons are the major components of essential oils inhibit microbial pathogens which attack the pathogens through cell wall and cell membrane. Thus, active phenolic compounds might have several invasive targets which could lead to the inhibition of human infectious bacterial and fungal pathogens [6, 7, 8, 9, 10].

Allium genus (family *Alliaceae*) includes about 700 species, they different in taste, form and colour but they close in biochemical, phytochemical and neutraceutical properties [11]. These species are widely distributed all over the world; mainly in Europe, North America, Northern Africa and Asia [12]. For many centuries, several of these species have been used as vegetables and spices, and as folk medicines for curing various diseases**.** Many researchers reported that essential oil and crude extract of several *Allium* species have antibacterial, antifungal, antioxidant and cytotoxic activities [13, 14, 15]. On the other hand, previous chemical investigations reported that a variety of compounds including thiosulfinates, organosulfur derivatives, steroidal and triterpenoidal saponins [16, 17].

The present study aimed to evaluate the antioxidant activity of white onion (*A. cepa* L.) methanolic extract and its derived fractions by using two spectrophotometric methods and the essential oil composition were characterized by GC-MS technique.

MATERIALS AND METHODS

Chemicals

DPPH was purchased from Fluka (Germany). Aluminum chloride, Folin-Ciocalteu reagent sodium carbonate, sodium phosphate, ammonium molybdate, ascorbic acid, rutin and gallic acid were purchased from Sigma Aldrich Chemicals (USA). Absorbance measurements were recorded using the ultraviolet-visible Spectrophotometer, Spectronic 601Milton Roy (USA).

Plant materials

The bulbs of white onion (*A. cepa* L.) were collected from Giza governorate - Egypt in May 2013. The collected bulbs were kindly identified by Prof. Dr. Waffa Amer, Professor of plant taxonomy, Faculty of Science, Cairo University. The fresh bulbs of white onion (*A. cepa* L.) were cut to small pieces and submitted to extraction process.

Extraction and fractionation

2 Kg of fresh white onion plant bulbs were cut into small species and extracted with methanol. The methanolic extract was evaporated under vacuum to dryness using rotatory evaporator. The methanolic extract was partitioned with n-butanol and water then these fractions was evaporated under reduced pressure till dryness. The dried extracts were kept in dry vials for antioxidant assay and determination of total phenolic and flavonoid contents.

Extraction of essential oil

3 Kg of white onion (*A. cepa* L.) fresh bulbs were cut into small pieces and submitted to hydrodistillation process using a Clevenger-type apparatus. The plant material immersed in 4 L of distilled water contained in a 5 L flask. The extraction process was carried out for 5 h after the first drop of distillate until complete exhaustion of the plant. The distillation started after a heating time of 40 min. The condensation was carried out continuously with water chilled to 10 °C. Trials were performed with three successive repetitions. The essential oils extracted were recovered and stored in a refrigerator at 4 °C in tightly closed amber vials, away from contamination sources and collected prior to use for GC-MS analysis.

Gas chromatography-Mass spectrometry (GC-MS) analysis

For identification and quantification of essential oil compounds: 9 µL of white onion (*A. cepa* L.) essential oil were diluted with 991 µL of ethyl acetate. 0.5 µL of this solution were directly injected into the gas chromatograph mod. 6890N Network GC System (Agilent Technologies Palo Alto, CA) coupled with a mass spectrometer mod. 5973 Network Mass Selective Detector (Agilent Technologies Palo Alto, CA) and equipped with a column HP-5MS (30 m length, 0.25 mm internal diameter; 0.25 µm film thickness Agilent Technologies, Palo Alto, CA). Helium gas at a flow rate of 1.2 mL/min (linear velocity: 33 cm/s) was used as carrier. Injection were made into a split-splitless injector (split ratio 50:1) at 250°C, the oven temperature program was the following: 45 °C for 5 min.; increase of 7 °C/min. up to 100 °C, held for 15 minutes, from 100 °C to 150 °C with an increment of 5 °C per minute, held for 20 minutes, from 150°C to 200°C with an increment of 15°C per minute, held for 5 minutes. The MSD transfer line was set at a temperature of 250°C; MSD temperature quadrupole was of 150°C and ionization temperature was of 230°C. Mass spectra were acquired at 70 eV energy and the scan acquisition was performed in the range between 35 and 300 m/z. The identification of the components of the essential oils was assigned by matching their mass spectra with those available in the libraries NIST 02 and WILEY.

Determination of total antioxidant capacity (phosphomolybdenum assay)

The total antioxidant capacity of each extract was determined according to the phosphomolybdenum method as reported by Prieto *et al.*, 1999 [17]. Briefly, 0.3 ml of each extract and ascorbic acid (1000 µg/ml) in methanol was mixed in dried tubes with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction mixture were capped and incubated in a boiling water bath at 95 °C min. After cooling the samples at room temperature, the absorbance was measured at 695 nm against a blank. The blank consisted of all reagents and solvents without the sample and it was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the extracts was expressed as the number of equivalents of ascorbic acid (AAE).

DPPH radical scavenging activity

The free radical scavenging activity of each extract or pure isolated compound was determined by using a stable (DPPH) according to the procedure described by Mensour *et al.*, 2001 [18]. Briefly, 1.5 ml of each plant extract in methanol was added to 1.5 ml of 0.1 mM DPPH in methanol. The reaction mixture was shaken and

left for 20 min at room temperature in the dark. The degree of reduction of absorbance was recorded at 517 nm. Ascorbic acid was used as a reference standard. Control was prepared containing the same solvents and reagents without any extract. All experiments were carried out in triplicate and the scavenging activity (antioxidant activity) of each extract was expressed as SC_{50} which is defined as the concentration of extract required for 50 % scavenging of DPPH radicals compared with that of ascorbic acid. The lower SC_{50} value corresponds to higher scavenging activity (higher antioxidant activity) of plant extract.

Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteu reagent, according to method reported by Miguel *et al.,* 2010 with little modification [19]. Briefly, (50 µl) of each dried extract dissolved in methanol (500µg/ml) was combined with 250µl of the Folin–Ciocalteu reagent and 0.750 ml of sodium carbonate (20%). The mixture was shaken, made up to 5 ml using distilled water and allowed to stand for 2 h. Then the absorbance was measured at 765 nm against a blank with distilled water and using gallic acid as standard. The results were expressed as mg gallic acid equivalent per gram dry weight extract (mg GAE/g extract). The results were the averages of triplicate analyses

Determination of total flavonoid content

Total flavonoid content (TFC) was determined spectrophotometrically according to the reported procedures by Kumaran and Karunakaran, 2006 [20]. 100 µl of each plant extract was mixed with 100 µl of aluminium trichloride in methanol (20%) and then add one drop of concentrated acetic acid and complete the total volume to 5 ml. After 40 min the absorbance of the mixture was read at 415 nm using spectrophotometer against the blank (the blank consisted of all reagents and solvents without any extract) and rutin was used as standard. All determinations were carried out in triplicate. The total flavonoid in each extract was determined as mg rutin equivalent (RE)/g extract.

Statistical analysis

All experiment were carried out in triplicate, and statistical analysis were performed using SPSS (13) software and Microsoft Excel program. The results were given as means ± standard deviation.

RESULTS AND DISCUSSION

Chemical composition of white onion (*A. cepa* L.) essential oil

The essential oil constituents of bulbs of white onion (*A. cepa* L.) were obtained by simultaneous steam and solvent distillation. The extract was subjected to GC-MS analysis and the results are represented in Fig. 1 and table 1. GC-MS analysis of the essential oils of white onion (*A. cepa* L.) bulbs revealed 40 compounds which representing more than 93.89 % of the total essential oils of this organ (bulbs) of the plant. The identification of the compounds were carried out based on their retention times, matching with the library and their mass spectra with literature data. The characteristic flavors of *Allium* species are attributed to thiosulfinates and related organosulfur compounds formed enzymatically from odorless precursors when the plants are cut or crushed [21]. The main components of onion oils were identified as dodecane (28.69 %), thiophene-2-carboxylic acid,3-methyl(methyl sulfonylamino) (8.96%), undecane (8.09%), undecane,2,6-dimethyl (6.86%) and 1,2,4-trithiolane 3.5-dimethyl (5.82%). In previous different studies on the composition of *A. cepa* essential oil mixtures of disulfide and trisulfate derivatives as well as mixture of hydrocarbons were recorded [14, 22, 23, 24, 25]. In the present study there is some compounds such as 2-piperidinone,N (4-bromo-n-Butyl) (2 %), Norleucine,2-butyl-N,N-dimethyl-methylester (2.71 %), Thiophene -2- carboxamide, N-(2-Furfuyl) (0.57 %) were detected for the first time in *Allium* species.

Antioxidant activity, total phenolic and total flavonoid contents

Medicinal plants and foods usually contain natural antioxidants that can scavenge free radicals. So, they prevent the radical chain oxidation which destroys cell membranes. The antioxidant activity of the plant extracts could be explained on the basis of their phenolic content. Also, it is known that flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [26, 27, 28]. The results in table 2 and table 3 revealed that the antioxidant of butanolic fraction derived from the methanolic extract of white onion has high activity with two assays DPPH scavenging assay and phosphomolybdenum method (SC_{50} = 59.21 ± 0.35 µg/ml and 127.92 ± 1.00 mg ascorbic acid equivalent / g extract) respectively. On the other hand it was appeared that the butanolic fraction has high phenolic content (41.05 ± 0.34 mg gallic acid equivalent/g plant extract) and flavonoid content (3.82± 0.24 mg rutin equivalent/g plant extract). the butanolic fraction higher antioxidant activity, total phenolic and total flavonoid contents more than methanol extract more than water fraction This study is in agreement with previous studies which reported that there is a high a correlation between total phenolic content and flavonoid contents and antioxidant capacities of a number of medicinal plant extracts [29, 30, 31].

CONCLUSION

The GC-MS analysis of white onion (*A. cepa* L.) showed that it contains hydrocarbons, alkaloids and organosulfur compounds which responsible for its antimicrobial activities. The antioxidant properties of the methanolic extract and its derived fractions of white onion (*A. cepa* L.) showed that the butanolic fraction has the highest antioxidant, total phenolic and total flavonoid contents which interested to isolate its active constituents in next study using different chromatographic techniques.

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Table 1. Chemical constituents of white onion (*A. cepa* L) essential oil.

Peak No	R.T.	% of Total	Match	MF	MW	Name
1	5.06	0.98%	653	C_6H_8S	112	3,4-Dimethyl thiophene
2	5.55	0.47%	488	$C_9H_{20}S_2$	192	n-butyln-pentyldisulfide
3	6.03	0.66%	689	$C_{10}H_{20}$	140	1,1,2,3-tetramethyl cyclohexane
4	6.31	1.07%	678	$C_2H_6S_3$	126	Dimethyl trisulfide
5	6.54	0.78%	660	$C_{10}H_{20}$	140	1-methyl-2-propyl cyclohexane
6	6.85	1.20%	816	$C_{10}H_{22}$	142	Decane
7	7.31	2.29%	789	$C_{11}H_{24}$	156	4-methyl Decane
8	8.04	1.99%	731	$C_{14}H_{26}$	194	11,11-dimethyl Bicyclo(8,2)dodecane
9	8.17	1.49%	724	$C_{11}H_{24}$	156	2-methyl Decane
10	9.03	8.09%	904	$C_{11}H_{24}$	156	Undecane
11	9.51	2.00%	688	$C_9H_{16}BrNO$	233	2-piperidinone,N(4-bromo-n-Butyl)
12	10.67	8.96%	586	$C_8H_{11}NO_4S_2$	249	2-carboxylic acid,3-methyl(methyl sulfonylamino)thiophene
13	10.96	3.59%	807	$C12H_{26}$	170	Undecane,2-methyl
14	11.205	2.18%	761	$C_{12}H_{26}$	170	Undecane,3-methyl
15	12.40	28.69%	929	$C_{12}H_{26}$	170	Dodecane
16	13.01	6.86%	880	$C_{13}H_{28}$	184	Undecane,2.6-dimethyl
17	20.70	5.82%	764	$C_6H_{12}S_3$	180	1,2,4-trithiolane,3,5-dimethyl
18	22.89	2.71%	457	$C_{13}H_{27}NO_2$	229	Norleucine,2-butyl-N-N-dimethyl-methylester
19	23.81	0.57%	486	$C10H_9NO_2S$	207	thiophene-2-carboxamide,N-(2-Furfuryl)
20	25.70	1.37%	422	$C_{11}H_{10}O_6$	238	1,2,4-Benzenetricarboxilic acid, 1,2-dimethyl
21	28.56	0.20%	474	$C_{10}H_9NO_2S$	207	thiophene-2-carboxamide,N-(2-Furfuryl)
22	28.77	0.28%	518	$C_{11}H_{18}O_2$	182	3(2H)-furanone,2-hexyl-5-methyl
23	29.17	0.40%	526	C_6H_8S	112	3,4-dimethylthiophene
24	29.90	0.94%	467	$C_8H_{18}S_3$	210	Propane,1.1`-thiobis (3-methylthio)
25	31.22	0.26%	497	$C_{24}H_{36}HO_2Si_2$	412	4-Methyl-2,4-bis (4`-trimethylsilyloxy phenyl -pentane
26	31.67	1.10%	790	$C_{12}H_{36}HO_4Si_5$	384	pentasiloxane,dodecamethyl
27	32.57	0.30%	508	$C_{10}H_{28}O4si_3$	296	Silicic acid, diethyl bis (trimethylsily) ester
28	33.43	1.02%	426	$C_{13}H_{36}O_4Si_4$	368	3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxyl)trisiloxane
29	33.63	0.41%	436	$C_{14}H_{24}O_3Si_2$	296	Benzoic acid,3-methyl-2-trimethylsiloxy-trimethylsilyl ester

30	33.99	1.23%	566	$C_6H_{12}S_3$	180	1,2,4-trithiolane,3,5-dimethyl
31	34.13	0.45%	435	$C_8H_{22}OSSi_2$	222	3-oxa-6-thia-2,7-disilaoctane,2,2,7,7-tetramethyl
32	34.90	0.38%	455	$C_7H_6F_6N_2OS$	280	Propane amide-3,3,3-trifluoromethyl-N-(2-thiazolin-2-yl)
33	35.00	0.42%	526	$C_{13}H_{22}OSi$	222	Silane,trimethyl-5-methyl-2-(1-methylethyl)phenoxyl
34	35.25	0.29%	449	$C_6H_{12}S_3$	180	trans-3,5-diethyl-1,2,4-trithiolane
35	35.98	1.00%	463	$C_3H_6S_3$	138	1,3,5-trithiolane
36	36.65	1.58%	511	$C_3H_6O_2S_2$	138	1,2-Dithiolane,1,1-dioxide
37	38.56	0.42%	628	$C_{14}H_{42}O_5Si_6$	458	hexasiloxane,tetradecamethyl
38	43.85	0.74%	490	$C_{11}H_{24}O$	172	2-methyl-3-decanol
39	55.80	0.12%	513	$C_7H_{22}O_2Si_3$	222	1,1,1,3,5,5,5,-heptamethyltrisiloxane
40	57.43	0.32%	445	$C_{12}H_{19}N_3OSi$	249	1-methyl-3-L-4-(1-trimethylsiloxyethlidene)-cyclohexa-3,5-dienyl

Table 2. Total phenolic and flavonoid contents of the methanolic extract of fresh *w*hite Onion *(Aillum cepa)* and butanolic and water derived from it.

Plant extract	**Total phenols (mg gallic acid equivalent/g plant extract)**	**Total flavonoids (mg rutin equivalent/g plant extract)**
MeOH ext.	17.02± 0.30	2.90± 0.09
BuOH fraction	41.05± 0.34	3.82± 0.24
H_2O fraction	36.44± 0.46	2.10± 0.10

Values are expressed as mean of triplicate determinations ± standard deviation.

Table 3. DPPH scavenging activity and total antioxidant and capacity assay of the methanolic extract of w*hite onion* (*A. cepa* L) and butanolic and water fraction derived from it.

Plant extract	**DPPH free radical scavenging activity SC_{50} [µg/ml]**	**Total antioxidant capacity [mg ascorbic acid equivalent / g extract]**
MeOH ext.	1603.13 ±15.8	102.80 ± 2.35
BuOH fraction	59.21 ± 0.35	127.92 ± 1.00
H_2O fraction	935 ± 7.82	124.95± 1.30
Ascorbic acid	8.06 ± 0.70	--

Values of SC_{50}, total antioxidant capacity assay are expressed as mean of triplicate determinations ± standard deviation.

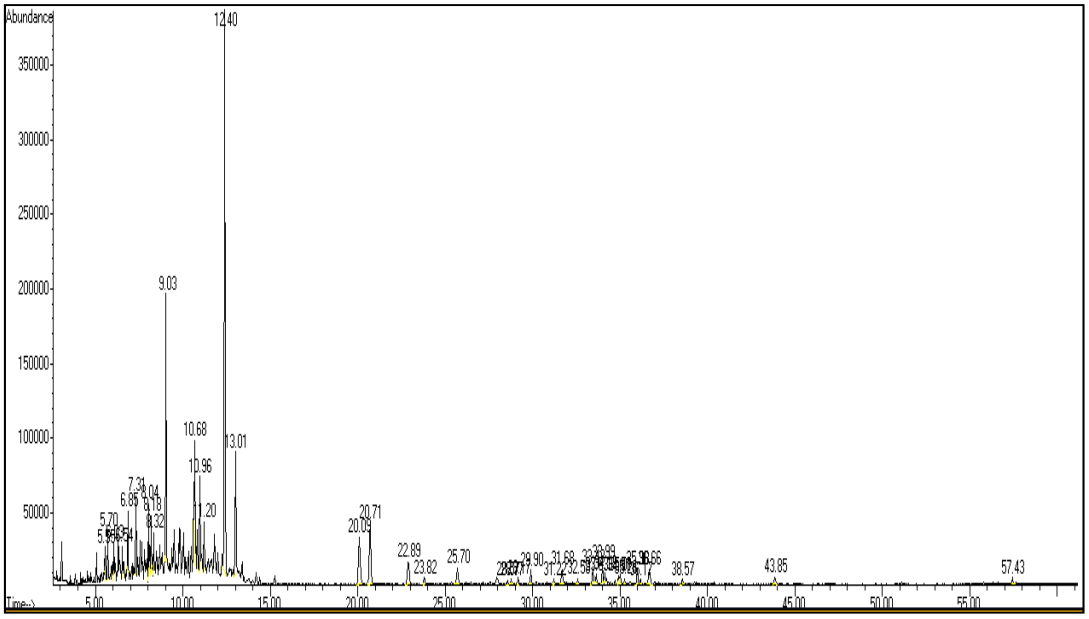

Figure 1. GC-MS chromatogram of essential oils from white onion (*A. cepa* L).