Influence of some the solvents on the extraction of major phenolic compounds and their antioxidant activities in Egyptian garlic (*Allium sativum L.)*

Abstract

Garlic *Allium sativum L.* belongs to the family Alliaceae.Garlic has many nutritional and medicinal uses.There are several solvents for extraction of bio active i.e. Acetone, Ethanol...etc. The present study is aimed to compare the four solvents (acetone, ethanol, hexane and methanol) to extract the phenolics from local garlic varieties (Seeds 40 and Sids 50) and its effect on antioxidants.The results showed the distinction of methanol over the rest of the other used solvents.It was found that they gave the highest content of total phenols estimated at 402.64 and 383.90 µg/g for both varieties, Sids 40 and Sids 50 respectively.The methanol as extract solvent has more potential to recover a higher amount of Gallic acid, Syringic acid derivatives, (+)-Catechin, *p*-Coumaric acid, *p*-Hydroxy-benzoic acid and Epicatechin content from garlic powder for both varieties.In general, that the garlic variety (Sids 40) was distinguished by its content of phenols compared to garlic variety (Sids 50). The highest radical scavenging activity was observed for the extract from methanol garlic extraction, i.e. 4.81 and 4.71 µg/mL for DPPH and 0.52 and 0.51 µg/mL for $ABTS^{+}$ (Sids 40 and Sids 50 respectively).

Keywords: *Allium sativum L.*; Sids 40; Sids 50; solvents; GC-MS; total phenolics; DPPH; $ABTS^{+}$.

Introduction

Garlic *Allium sativum L.* belongs to the family Alliaceae same family as onions, leeks, and shallots which are grown for spices/condiment. Most of the garlic is sold to the Egyptian fresh market as a whole green garlic or fresh bulbs. Processed products such as chopped garlic or garlic spreads are also sold, but to a lesser extent. Garlic is the highest in nutritional value and the 2nd most widely used member of the *Alliums species*[1]. Garlic are widely found in South East Asia, South Asia and some countries in Middle East, such as Egypt and Iran [2].The major garlic areas are ElMinia and Beni-Sueif governorates [3].Garlic (*Allium sativum L.*) is one of the most important crops in the world for both its cooking and health characteristics. Garlic is considered a valuable source of bioactive substances for human functional foods, natural health products, cosmetics and some medicines of peoples and Animals [4, 5].Other than being a vital flavor in numerous societies, garlic cloves (*Allium sativum L.*) is additionally well known for its great therapeutic

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properties [6]. Many previous studies have shown that the most important active ingredients in garlic cloves are sulphur-containing compounds such as alliin, allicin, ajoene, S-allylcysteine, diallyl disulphide, and dithiin.

On other hand there is evidence that its medicinal properties are primarily due to the content of organosulfur compounds (OSCs)[7].Some of these OSC-induced biological activities are antitumor andantibacterial, antifungal, antiviral, antiplatelet, blood pressure and blood Glucose modulator and immune booster [8].These compounds give garlic the distinctive taste and pungent odor garlic [9].Allicin is concentrated up to 70% of the overall thiosulphates in fresh garlic [10].This compound has effective antimicrobial, anti-microbial, anticancer and other properties.

Many food supplement products made from garlic cloves are available in plenty, including, for example, dehydrated garlic powder, garlic essential oil macerate, garlic essential oil capsules, and aged garlic extract [11, 9]. Fundamental oil of garlic ~~moreover~~ contains many numbers of sulfides such as dilly trisulphide and diallyl disulphide; but, allicin, can be totally eliminated amid chemical or thermal extraction processes [12].

Recently, the return to nature is increasing, which is represented in many industries to produce foodstuffs and pharmaceuticals of natural origin. On the other hand garlic cultivations in Egypt are constantly increasing, depending on the baladi variety, and finally two varieties, namely Sids 40 and Sids 50were produced a few years ago in Sids Horticultural Research Station, Beni-Suef governorate, Egypt.

The extraction of most bioactive compounds from medicinal plants sources is considered a major step, and solvents are an essential element in the extraction and recovery of bioactive compounds. Several types of solvents, i.e., non-polar and polar, extract the bioactive and phenolic compounds from the medicinal plants. ~~On other hand,~~ However, ~~N~~non-polar and weakly polar solvents are commonly used to extract pigments and lipophilic compounds frommedicinalplants[13].

Solvent extraction is widely used to extract phenolic compounds from fruits and vegetables, all the variables investigated to ensure extraction efficiency (sample pretreatment, solvent / sample ratio, solvent type, extraction time and temperature), solvent type was the most studied factors. Solvent polarity plays an important role in the extraction process, as changes in solvent polarity change the ability to dissolve a particular group of antioxidant compounds and affect the estimation of antioxidant activity. Due to the different chemical profiles of plant materials, it is not possible to develop a suitable universal solvent for extracting all types of

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antioxidant compounds from the plants. Therefore, a screening process is important to justify the optimal solvent for extracting antioxidant compounds so that the maximum antioxidant activity of a particular sample can be determined(14).

The purpose of this study is to investigate the effects of several solvents on the bioactivecontent of both varieties of Egyptian garlic extraction, as important process in the pharmaceutical industry and natural food, and to show how much bio active ingredient is affected by Egyptian garlic for each solvent.

Materials and Methods

Plant material

Garlic bulbs (*Allium sativum L.*) were supplied by Sids Horticultural Research Station, Beni-Suef governorate, Egypt. Garlic cultivar, commonly known asvarieties Sids 40 and Sids 50.

Preparation of Garlic

Fresh garlic was cleaned and dried in a tray dryer at 45°C until the weight was constant. Next, the dried garlic was crushed into powder and stored in a closed container before use.

Solvent extractions

Garlic (Sids40 and Sids50) were extracted with four different solvents including acetone 80%, ethanol 80%, hexane 80% and Methanol 80%. Samples were mixed with each solvent by using 1:5 ratio (g/mL).The mixtures were soaked at 120 rpm, 25°C for 48 h in an incubator shaker. After 48 h, the mixtures were filtered by using Whatman filter paper No.4. The crude extract was then concentrated using a rotary evaporator (BUCHIR205) at 45°C until a constant weight was obtained [15]. The concentrated crude extracts were diluted with DMSO (dimethyl sulfoxide) and kept at –20°C before use.

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Extraction of the volatile oil of garlic by hydro-distillation

Fresh Garlic (Sids 40 and Sids 50) were washed with tap water and cut into small size before mixing with distilled water by using 1:3 ratio (by the weight). After that, petroleum ether was added to the mixture at the ratio of 10:1 (mL/L) of water. The mixture was distilled at 100°C for 2.5 h. After distillation, the water fraction was removed. Then, sodium anhydrous was added in order to remove the excess water. [16].

Gas chromatography–mass spectrometry analysis (GC-MS)

Chemical composition of Garlicoils (Sids 40 and Sids 50) were analyzed by using Gas Chromatography-Mass Spectrometry (GC-MS) atCentral Laboratories Network, National Research Centre, and Cairo, Egypt. The analysis was performed using Agilent Technologies 7890A GC System, 5975C inert XL EI/el MSD with triple-axis detector and GC sampler 80 using HP 5 MS Ultra Inert type (30 m × 250 μm × 0.25 μm) column, using He as carrier gas, sample injection volume was 0.1 μl. The oven temperature was 60°C hold for 4 min, 250°C at 20°C/min hold for 1 min.The identification of the various components was determined by comparing the spectral fragmentation patterns with those stored in the Wiley and NIST mass spectral library databases.

Measurement of the total phenolic content

The total phenolic compound content in the garlic extract was determined using the Folin-Ciocalteu reagent according to the method of Singleton and Rossi [17]. The absorbance of sample was measured at 740 nm. The total phenol content was calculated based on GA (Gallic acid) standard curve. The total phenol content was expressed as mg / ml gallic acid equivalent (GAE).

Quantitative and qualitative analysis of total phenolics content by HPLC

Garlic extract samples were analyzed with a Varian Pro-Star HPLC according to [18]. The mobile phase consisted of 4.5% acetic acid (solvent A) and 50% acetonitrile (solvent B); the solvents were applied at a flow rate of 0.8 mL/min. The column was washed with 50% acetonitrile at the end of the gradient and equilibrated to the initial state for 10 minutes. The gradient elution was used as follows: 0 min, 92% A; 30 min, 70% A; 45 min, 60% A; 80 min, 60% A; 82 min, 0% A; 85 min, 0% A; 86 min, 92% A; and 90 min, 92% A. Detection was done at 270 and 370 nm. Spectral analysis and comparison of spectral retention times allowed to identify the phenols in the sample. The quantitative determinations were carried out with the external standard calculation, using calibration curves of the standards.

DPPH% radical-scavenging activity

DPPH radical scavenging was analyzed according to [19].Each garlic extract (1 mL, concentrations 2.5, 5.0, 10.0, and 20.0 mg / mL) was mixed with 1 mL of 25 mmol / LDPPH solution in 96% ethanol. After incubating for 30 minutes at room temperature, the absorbance of the sample was measured at λ = 515 nm using 96% ethanol as a blank of sample.

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Ascorbic acid (AA) was used as a positive control. % DPPH was calculated for each sample based on the equation:

$$DPPH\% = [1 – (AS/AC)] \times 100\ \%$$

(AS) absorbance of the sample; (AC) absorbance of the control (DPPH solution). The IC_{50} value was defined as an effective concentration of total phenolics that is required to scavenge 50% of radical activity.

$ABTS^+$ radical-scavenging activities

Scavenging of $ABTS^+$ free radical was evaluated according to[20]. The garlic extract (20 μL, conc. 0.125, 0.5, 1.0, 1.5 and 2.5 mg/mL) was mixed with 980 μL of a diluted $ABTS^+$ solution and incubated for 10 min. The decrease in $ABTS^+$ absorbance was measured at λ=734 nm using distilled water as a blank. Ascorbic acid (AA) was used as a positive control. The percentage of $ABTS^+$ scavenging was calculated based on the equation:

$$\%\ \text{of}\ ABTS^+ = [(1 – (AS/AC)] \times 100\ \%$$

Where: (AS) absorbance of the sample; (AC) absorbance of the control ($ABTS^+$ solution). IC_{50} value was defined as an effective concentration of total phenolics that is required to scavenge 50% of $ABTS^+$ radicals.

Fe2+ chelation assay

The chelation of iron (II) ions by garlic extracts was measured according to [21]. Follow, the absorbance was measured at λ = 562 nm. Used as a positive control was EDTA. Chelation activity was estimated as the rate % of inhibition of ferrosin-Fe^{2+} complex formation using the following formula:

$$\%\ \text{of}\ Fe^{2+} = [1 – (AS/AC)] \times 100\ \%$$

where: (As) absorbance of the sample; (Ac) absorbance of the control. The IC_{50} value was defined as an effective concentration of total phenolics in the extract from 1 g of raw garlic which is required to chelate 50% of Fe^{2+} ions.

Cu2+ chelation assay

Copper chelating activity was measured according to the method[22]. Used as a positive control was EDTA. Copper ion chelating capability was calculated according to the formula:

$$\%\ \text{of}\ Cu^{2+} = [1 – (AS/AC)] \times 100\ \%$$

where: (AS) absorbance of sample; (AC) absorbance of control. The IC_{50} value was defined as the amount of total phenolics in the extract from 1 g of raw garlic that is required to chelate 50% of Cu^{2+} ions.

Statistical analysis and experimental design

All measurements were conducted in three replicate. Data were reported as mean standard deviation (± SD). Analysis of significant differences among means were tested by one way ANOVA followed by L.S.D to compare treatment means at a probability level of 0.05 as illustrated by [23].

Results

Chemical composition of garlic oil and phenol content (Sids 40 and Sids 50)

The results in Table (1) indicate that the garlic variety(Sids50) had a higher percentage of most active compounds in the essential oil compared to the garlic variety(Sids 40) (Methyl2-propenyldisulfide 27.28%, 1,3-Dithiane 12.33%, Dimethyltrisulfide 38.09%, Diallyldisulphide 4.31%, 1-Oxa-4,6-diazacyclooctane-5-thione 3.18%, (Methylthio)-acetonitrile 8.04%).The results in Table 2 show, in general, that the garlic variety(Sids 40) was distinguished by its content of phenols compared to garlic variety(Sids 50).The results indicate that garlic extract of both varieties of the solvent methanol is distinguished by its higher content of phenols compared to the other solvents used in the experiment. On other hands the results indicated that the phenol content of the three garlic extracts for the other solvents were arranged in terms of their phenol content from highest to lowest, as follows: ethanol, acetone, and hexane.The results also indicate that the highest content of phenols for both garlic varieties (Sids 40 and Sids 50) were as follows p-Hydroxy-benzoic acid (ranged from 198.57 to 158.37 μg/g), (+)-Catechin (95.08 - 42.11 μg/g), Syringic acid derivatives (83.33 - 49.54 μg/g), Epicatechin (14.37 - 9.22 μg/g), Gallic acid (8.30 - 1.96 μg/g) and *p*-Coumaric acid (3.01 - 1.01 μg/g).

Free radical scavenging capacity

The free radical scavenging activity was compared with the content of total phenolic compounds and expressed as the IC_{50} value, defined as μg of phenolics in the extract from 1 g of raw garlic which are able to scavenge 50% of the analyzed free radicals (Table 3). The antioxidant potential of garlic extracts was evaluated using DPPH and $ABTS^{+}$ stable free radical scavenging assays. In both assays, the highest radical scavenging activity was observed for the extract from methanol garlic extraction, i.e. 4.81 and 4.71 μg/mL for DPPH and 0.52 and 0.51 μg/mL for $ABTS^{+}$(Sids 40 and Sids 50 respectively,Table3). In addition, the lowest IC_{50}values

for DPPH scavenging were noted for hexane garlic extracts (6.32 and 5.92 µg/mL) and (0.76 and 0.64 µg/mL) for $ABTS^{+\cdot}$(Sids 40 and Sids 50 respectively, Table3).The results for both varieties (Sids 40 and Sids 50) indicated that the IC_{50} value for the four solvents was arranged as follows: methanol, followed by ethanol, acetone and hexane. Ion chelation activity the ability to chelate Fe^{2+} and Cu^{2+} ions was compared among the analyzed extracts of four solvents for both Garlic (Sids 40 and Sids 50). The obtained data are displayed as the IC_{50} values, defined as the concentration of phenols in each extract from garlic extracts that is required to chelate 50% of Fe^{2+} or Cu^{2+} ions (Table 3). The highest Fe^{2+} chelation ability was noted for garlic extract by methanol, with the lowest IC_{50} values of 0.54 and 0.51 µg/mL, Sids 40 and Sids 50 respectively. The highest Cu^{2+} chelating ability was noted for garlic extract by methanol for both Garlic (Sids 40 and Sids 50) (33.28 and 31.43 µg/mL, respectively).

In general, the results show that the IC_{50} value was arranged for the four solvents as follows, the highest was methanol, followed by ethanol, then acetone, and finally hexane for both Varieties of garlic (Sids 40 and Sids 50).

Discussion

Garlic (*Allium sativum L.*) is a popular plant cultivated all over the world for its unique spicy flavor and medicinal properties, and has been recognized for thousands of years. In the ranking of total phenolics content of 23 commonly consumed vegetables garlic has been ranked second[24]. Phenolic compounds and other plant components such as plant proteins and peptides show significant biological activity [25, 26, 27, 28].Although most of the antioxidant effects of plant extracts are related to phenolic compounds. Although most antioxidant activities of plant extracts are connected with phenolic compounds. The methanol solvent are significantly accounted to recover a higher amount of gallic acid from garlic powder than respective others solvents. The results have shown that the maximum gallic acid was obtained with methanol as a solvent followed by ethanol, acetone and hexane, in both varieties (Sids 40 and Sids 50).The results indicated that the methanol solvent as extraction method has significant potential for recovering the higher amounts of gallic acid compared to other solvents methods. Gallic acid, Syringic acid derivatives, (+)-Catechin , p-Coumaric acid, p-Hydroxy-benzoic acid and Epicatechin content were significantly higher obtained with methanol as solvent followed by ethanol in both garlic varieties (Sids 40 and Sids 50). The results also indicated that garlic powders of variety Sids 40 had a higher recovery of *p*-Hydroxy-benzoic acid significantly as compared to Sids 50. Methanol and ethanol solvents are considered to be more suitable for extracting quercetin compounds than other solvents due to their high polarity and efficiency. Overall results of the present investigation are supported by [13, 29, 14]. Also, the methanol extract from garlic

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(Sids 40 and Sids 50) effectively chelated both Fe^{2+} and Cu^{2+} ions, compared with other solvent.

Assessment of antioxidant capacity of garlic extract using DPPH and ABTS + scavenging assays. In both DPPH and ABTS+ scavenging assays the lowest IC50 values, indicating the highest antiradical activity,Both Collens (Sids40 and Sids50) were observed in methanol-garlic extracts. It was one of the highest levels of p-hydroxybenzoic acid derivatives.The lowest free radical scavenging potential was manifested by hexane garlic extract (DPPH assay) and (ABTS+).Interestingly, the antioxidant capacity of the garlic extract detected by the ABTS + scavenging assay was significantly higher than that of the DPPH assay.The earlier study has demonstrated that higher scavenging of ABTS+ was observed for phenolics with three or more hydroxyl groups [31]. The antioxidant activity depends on the number and position of the hydroxyl groups of the aromatic ring binding site and the type of a substituent. These data suggest that the $ABTS^{+}$ assay may be more sensitive to detect and compare the free radical scavenging potential of garlic extracts [32].

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They also confirmed that the methanol as extract solvent has more potential to recover a higher amount of Gallic acid, Syringic acid derivatives, (+)-Catechin, *p*-Coumaric acid, *p*-Hydroxy-benzoic acid and Epicatechin content from garlic powder due to its high polar nature of methanol [13,14]. The non-polar solvent such as hexane cannot recover the bioactive contents from garlic powders. Therefore, the study concluded that the methanol as extractive solvent are potential to extract the phenolic compounds from garlic extract compare with other solvents (acetone, ethanol and hexane).The further study and practical implication of garlic waste phenolic compounds should be explore in food and pharma sector.

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CONCLUSION

Solvents play an important role in the pharmaceutical and food industries of natural origin. In this study, we concluded that methanol as an extraction solvent can extract phenolic compounds from garlic compared to other solvents.

Table 1: Chemical composition of garlic oil (Sids 40 and Sids 50) analyzed by GC-MS

RT(min)	CompoundName	Sids 40 Compound %	Sids 50 Compound%
3.933	(Z)-3-Hexen-1-ol	0.38	0.40
5.505	Methyl2-propenyldisulfide	26.15	27.28
6.11	1,3-Dithiane	12.29	12.33
8.35	Dimethyltrisulfide	36.97	38.09
11.03	Diallyldisulphide	3.96	4.31
11.35	2-Vinyl-1,3-dithiane	0.44	0.39
11.71	1-Oxa-4,6-diazacyclooctane-5-thione	4.89	3.18

12.10	Nonanal	0.65	0.75
12.81	Methyl(methylthio) methylDisulfide	0.34	0.38
13.45	(Methylthio)-acetonitrile	7.86	8.04
13.91	(Z)-1-(methylthio)-1-Propene	0.71	0.73
14.27	AllylmethylSulfide	0.59	0.67
16.03	DimethylTetrasulfide	2.31	1.56
17.26	N,N-dimethyl-Methanethioamide	0.75	0.28
19.07	Di-2-propenylTrisulfide	0.23	0.29
20.88	tert-Butlymethylsulfoxide	0.24	0.27
23.64	S-Methylmethanethiosulfinate	0.44	0.21
33.79	2,2,7,7-tetramethyl-3-Oxa-6-thia-2,7-disilaoctane	0.80	0.84
Total	-	100	100

Table 2. Content of phenolic compounds (μg/g) in aqueous extracts from garlic (Sids 40 and Sids 50).

Garlic Extracts	**Gallic acid**	**Syringic acid derivatives**	**(+)-Catechin**	***p*-Coumaric acid**	***p*-Hydroxy-benzoic acid**	**Epicatechin**	**Total (sum)**
Sids 40							
Acetone 80%	4.10±0.89	65.09±0.46	71.33±0.49	2.09±0.15	178.34±0.18	11.18±0.37	332.13
Ethanol 80%	5.31±0.41	78.71±0.14	81.61±0.26	3.01±0.12	192.69±0.19	11.91±0.43	373.24
Hexane 80%	2.46±0.41	52.19±0.39	49.67±0.65	1.55±0.11	164.27±0.29	10.24±0.42	280.38
Menthol 80%	8.30±0.04	83.33±0.27	95.08±022	2.99±0.21	198.57±0.67	14.37±0.15	402.64
Sids 50							
Acetone 80%	3.81±0.21	64.14±0.35	66.37±0.44	1.74±0.04	166.54±0.29	9.02±0.29	311.62
Ethanol 80%	3.98±0.43	71.21±0.38	80.27±0.31	2.36±0.07	172.81±0.21	9.34±0.25	339.97
Hexane 80%	1.96±0.19	49.54±0.37	42.11±0.45	1.01±0.08	158.37±0.27	9.22±0.33	262.21
Menthol 80%	7.24±0.37	80.73±0.45	90.53±0.52	1.98±0.05	190.48±0.39	12.94±0.54	383.90
L.S.D $_{0.05}$	2.59	4.25	4.79	2.49	5.37	2.02	

All values are expressed as mean ±SD (standard deviation) for three replicates.

Table 3. IC_{50} value for antiradical activity against DPPH and ABTS+ and the ability tochelate $Fe^{2+}$and Cu^{2+} ions determined for garlic extracts (Sids 40 and Sids 50) (µg/mL).

Garlic Extracts	DPPH	$ABTS^{+}$	Fe^{+2}	Cu^{+2}
	IC^{50} (µg/mL)			
Sids 40				
Acetone 80%	5.81±0.04	0.66±0.02	0.60±0.02	36.71±2.06
Ethanol 80%	5.38±0.03	0.55±0.02	0.57±0.02	34.09±2.11
Hexane 80%	6.32±0.07	0.76±0.04	0.61±0.03	39.82±2.08
Menthol 80%	4.81±0.02	0.52±0.03	0.54±0.01	33.28±2.10
Sids 50				
Acetone 80%	5.43±0.02	0.57±0.05	0.54±0.04	34.34±2.04
Ethanol 95%	5.06±0.04	0.54±0.03	0.52±0.03	32.43±2.11
Hexane 80%	5.92±0.05	0.64±0.04	0.56±0.02	36.22±2.08
Menthol 80%	4.71±0.03	0.51±0.03	0.51±0.02	31.43±2.02
Positive control	[1] 0.73±0.06	[1] 0.12±0.05	[2] 0.39±0.02	[2] 0.24±0.33
$L.S.D_{0.05}$	0.0081	0.0046	0.0063	0.0721

1 ascorbic acid (AA) was used as a positive control; 2 ethylenedinitrilotetraacetic acid (EDTA) was used asa positive control.All values are expressed as mean ±SD (standard deviation) for three replicates.

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