Comparative analysis of genetic variation and chemical variability in commonly available Egyptian and Chinese garlic

Abstract

Three garlic varieties (Sids 40, Sids 50 and Chinese) comparisons of oil composition and genetic variation of three garlic cultivars (*Allium sativum L.*) commonly used for cooking, pharmaceutical industries and essential oil production in the Egyptian market. Garlic essential oils were obtained by hydrodistillation and then analyzed for chemical components by GC-MS. Also, Phenolic content, Alliin content and DPPH% radical-scavenging activity (IC_{50}) and determine the genetic diversity in different of the three garlic varieties and to investigate the genetic distances between them were evaluated. The results, in general, indicated that the variety of Sids 40 was distinguished in the studied traits. IC_{50} value was arranged as follows: Sids 40, Sids 50 and Chinese. The phenol content of the three garlic varieties were arranged in terms of their phenol content from highest to lowest, as follows: Sids 40 (396.50 µg/g), Sids 50 (366.58 µg/g) and Chinese (296.92 µg/g). The highest of Alliin content, was Sids 40 with a content of 91.53 mg 100/ml, followed by the Chinese variety at a content of 88.70 mg 100/ml, and the least of them was Sids 50 with a content 60.43 mg 100/ml. The highest similarity value (1.00) was recorded between Sids 40 genotypes and Sids 50 genotypes, while the lowest similarity value (0.610) was recorded between China genotypes and Sids 50 genotypes. On the other hand there was no similarity between China genotypes and Sids 40 genotypes.

Keywords: Garlic (*Allium sativum L.)*, Essential oil, GC-MS, IC_{50}, DNA isolation, Genetic similarity, SCoT primers, dendrogram.

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Introduction

Garlic (*Allium sativum L.*) like onions, leeks and shallots cultivated for spices belongs to the family Alliaceae. Most garlic is sold as whole green garlic or fresh bulbs in the Egyptian fresh market. Processed products such as chopped garlic and garlic spreads are not often sold. Garlic is the most nutritious and the second most commonly used member of the Allium species [1].

Functionally, garlic bulbs (*Allium sativum L.)* are very important products due to their many functional uses as cooking ingredients and medicines. Biochemically, garlic bulbs contain compounds of allicin and scodinin, which are antibiotics and can boost the body's immune system [2, 3]. Garlic is also known as a cooking ingredient from a culinary point of view and is very popular. Garlic bulbs usually contain methylallyl disulfide compounds, which makes the dish more delicious and spicy and fragrant [2].

About 300 types of garlic are cultivated all over the world, mainly in hot and dry places. Today, garlic is one of the 20 most important vegetables in the world. The FAO database indicated in 2019, that the total garlic production worldwide (TGPW) reached 30.7 million tons and the recorded land area reached 1.6 million hectares. The Asian continent's production is 90% of TGPW, follow the African continent's production is production is 2.7% of TGPW and the Americas is about 2.6% of TGPW. On the other hand, the database (FAO) in 2019 indicated that Egypt occupies the fifth place in the global production of garlic, reaching 318.8 thousand tons, where China ranked first among garlic producers with 23.3 million tons, followed, India, where its production reached 2.91 million tons, followed by Bangladesh with a productivity of 466.39 thousand tons, followed the republic of Korea, with a productivity of 387.67 thousand tons [4].

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Garlic has a historical background, as the writings of the ancient Egyptian temples indicated that the pyramids' builders (workers) were eating garlic daily. In the modern time, in World War I and II it was known that the soldiers also eating garlic as an antibiotic that prevents contamination of wounds and prevents gangrene from occurring [5].Now, taking garlic is known to have many health benefits. Lowers blood pressure and cholesterol to help prevent atherosclerosis (plaque buildup in the arteries causing blockage and possibly leading to heart attack or stroke), and reduce colds, coughs, and bronchitis and helps reduce the risk of cancer [6, 7, 8, 9]. Also, alliin is a bioactive compound that shows high anti-human colon cancer anti-stomach cancer activities and stimulates peripheral blood cell immune functions [10, 11].

Several studies have indicate that garlic is rich in antioxidants that can fight free radicals that accumulate with age. On other hand,

antioxidants work to reduce or prevent many free radical damage such as heart disease, cancer and Alzheimer's [12].

Molecular markers have been defined by Food and Agriculture Organization (FAO) as a DNA sequences exist in specific genome locations associated with linked gene or trait inheritance [13]. There are several applications of DNA markers in plant molecular genetic studies [14, 15]. The popular DNA marker methods random amplified polymorphic DNA (RAPD) [16, 17]. Inter simple sequence repeat (ISSR) [18, 19, 20, 21] and amplified fragment length polymorphism (AFLP) [22] have been used in genetic diversity analysis and relationships determination among garlic induced clones, as the environmental conditions do not affect them.[23]. [24, 25, 26, 27]. In recent years, many new promising dominant molecular marker techniques have been developed such as SCoT [28] and SRAP [29, 30] because of their simplicity, inexpensive, and reproducibility. In this study, marker systems called, Start Codon Targeted (SCoT) polymorphism [31] have recently become the best choice of molecular markers used in genetic diversity studies. SCoT was developed based on the short conserved sequence around the Translational Start Site (TSS) that is conserved in all plant genes. It is simple because its PCR products were resolved by performing agarose gel electrophoresis compared to arbitrary markers such as RAPD, it is highly reproducible due to the use of longer primers, these primers were designed following the short conserved region flanking the initial codon (ATG), it is a targeted molecular marker technique with one part of a functional gene markers generated from SCoT marker technique and their corresponding traits [32]. The present study aims to compare three varieties of garlic (Sids 40, Sids 50 and Chinese) generally available in Egyptian markets based on their morphology, bioactive components, volatile profiles of essential oils, DPPH% radical-scavenging activity (IC_{50}) and determine the genetic diversity in different of the three garlic varieties and to investigate the genetic distances between them.

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Materials and Methods

Plant material

Garlic bulbs (*Allium sativum L.*) were supplied by Sids Horticultural Research Station, Beni-Suef governorate, Egypt at 2020. Garlic cultivar,

commonly known as varieties Sids 40 and Sids 50, and Chinese variety from local market (Table.1).

Table (1): The morphological descriptions of three garlic varieties bulb

Garlic varieties	Bulb (cm)	Weight (g)	Shape	Colour of tunic	No.of bulblets	Clove size (cm)	Weight (g)
Sids 40	3.7 - 4.3	32.51 - 53.84	Round	Opaque white purple	12 - 15	1.33 x 3.72	2.67 - 5.08
Sids 50	3.1 - 3.9	31.06 - 46.11	Round	Pinkish white	8 - 11	1.04 x 2.34	2.02 - 3.25
Chinese	1.9 - 2.8	8.12 - 9.07	Oval	white	6 - 9	0.91 x 1.76	0.82 - 1.03

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.

Extraction of the volatile oil of garlic by hydro-distillation

Fresh Garlic (Sids 40, Sids 50 and Chinese) 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 remove. Then, sodium anhydrous was added in order to remove the excess water [33].

Analysis of chemical composition of garlic essential oil by (GC-MS)

Chemical composition of Garlic oils (Sids 40, Sids 50 and Chinese) were analyzed by using Gas Chromatography-Mass Spectrometry (GC-MS) at Central 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.

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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 [34]. 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 [35]. 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: (from 0 – 90 min), 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.

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Analysis of alliin content by spectrophotometry

The alliin content was analysed using the spectrophotometry method with some adaptations [36]. In short, garlic essential oil (4 µl) was incubated with 1.1 × 10-4 M of mercaptopyridine (4- MP) in 50 mM buffer of sodium phosphate buffer, pH 7.2, and containing alliinase. The decrease in the optical density (OD) at 324 nm was determined after 30 min of incubation at room temperature. The alliin concentration was calculated using the following equation:

$$[\text{alliin}] = \Delta A_{324} \times \text{dilution} \times [\varepsilon_M]^{-1}$$

where ΔA_{324} = [OD without extract] − [OD with extract]

ε_M = Molar extinction coefficient at 324 (4MP) = 19,800

DPPH% radical-scavenging activity

DPPH radical scavenging was analyzed according to [37].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 / L DPPH 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.

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 [38]. 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:

$$\%\ of\ ABTS^+ = [(1 - (AS/AC)] \times 100\ \%$$

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

Fe^{2+} chelation assay

The chelation of iron (II) ions by garlic extracts was measured according to [39]. Follow, 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 this formula:

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

where: Absorbance of the sample (As); Absorbance of the control (Ac). 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.

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Cu^{2+} chelation assay

Copper chelating activity was measured according to the method [40]. Used as a positive control was EDTA. The ability to chelate copper ions was calculated using the following 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 [41].

DNA isolation

Genomic DNA was isolated from fresh leaves by DNeasy plant mini kit (biobasic). DNA quality was checked using spectrophotometer and agarose gel electrophoresis.

Polymerase chain reaction

Genomic DNA was used as a template for PCR amplification using 10 SCoT primers which were designed by [31], depending on the consensus sequence derived from the previous studies by [42] and [43] **(Table.2)** and procured from Biobasic Com. All SCoT primers used in this study were 18-mer and were from Dataset-I which based on highly expressed gene. Dataset-I were first identified by comparing 36 genes, typically known for a high level of expression in plants on the basis of several individual studies using expressed sequence tags (ESTs) [44]. They were reported based on the abundance of their transcripts and the encoded protein products in plant tissues as described by [43]. For SCoT primers design, the start codon ATG (+1, +2, and +3), 'G' at position +4, 'C' at position +5, and A, C, C and A at positions +7, +8, +9 and +10, respectively, were fixed (5´———ATGGCTACCA—3´).Amplification reactions for SCoT techniques were performed as described by [45] and were carried out in Techni TC-512 Thermal Cycler as follows: One cycle at 94°C for 4 min followed by 40 cycles of 1 min at 94°C, 1 min at annealing temperature 57°C and 2 min at 72°C, followed by 72°C for 10 min, the reaction was finally stored at 4°C.

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Table (2): List of the primer names and their nucleotide sequences used in the study for SCoT procedures.

No	Primer code	Sequence	No	Primer code	Sequence
1	SCoT 2	5´ CAACAATGGCTACCACCC 3´	6	SCoT 8	5´CAACAATGGCTACCACGT3`
2	SCoT 3	5´ CAACAATGGCTACCACCG 3´	7	SCoT 9	5´ACA ATG GCT ACC ACT GCC3`
3	SCoT 4	5´ CAATGG CTA CCA CTA GCG 3´	8	SCoT 10	5´ACA ATG GCT ACC ACC AGC3`
4	SCoT 5	5´ CAATGG CTA CCA CTA GCG 3´	9	SCoT 12	5´ACGACATGGCTACCAACG3`
5	SCoT 6	5´ CAATGG CTA CCA CTA CAG 3´	10	SCoT 15	5´CCATGG CTA CCA CCG GCT3`

Gel electrophoresis

Amplified products were separated on a 1.5% agarose gel with ethidum bromide and 100 bp to 1.5 kb ladder markers.The run was carried out for about 30 min at 100 V in mini submarine gel BioRad.

Gel reading and analysis

DNA banding pattern photos were photographed using Bio-1DGel Documentation system and were analyzed by Gel Analyzer 3 software. Clear bands were scored as present (1) or absent (0) for each primer and entered in the form of a binary data matrix to calculate genetic similarity and to construct dendrogram tree among the studied apricot strains A dendrogram showing the genetic relationships between accessions based on the unweighted pair group method using the arithmetic mean was created using (UPGMA). Calculation was achieved using Dice similarity coefficients [46] as implemented in the computer program SPSS-10.

Results

Chemical composition of garlic oil, phenol content (µg/g) and Alliin content (mg 100/ml):

The results in Table (3) indicate that the main components (Methyl 2-propenyl disulfide, 1,3-Dithiane, Dimethyl trisulfide, Diallyl disulphide, (Methylthio)-acetonitrile and Dimethyl Tetrasulfide) of garlic oil were as follows: 91.53% of Sids 50 variety, followed by the Chinese variety 91.01%, then 90.38% for Sids 40 variety. It is clear from these results that

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Sids 50 variety gave the highest percentage the main components compared to both varieties Sids 40 and Chinese.

The results in Table (4) show, in general, that the garlic variety Sids 40 was distinguished by its content of phenols compared to both varieties Sids 50 and Chinese. On other hands the results indicated that the phenol content of the three garlic varieties were arranged in terms of their phenol content from highest to lowest, as follows: Sids 40 (396.50 µg/g), Sids 50 (366.58 µg/g) and Chinese (296.92 µg/g). The results also indicate that the highest content of phenols for the three garlic varieties (Sids 40, Sids 50 and Chinese) were as follows *p*-Hydroxy-benzoic acid (ranged from 171.39 to 187.44 µg/g), (+)-Catechin (51.14 to 96.15µg/g), Syringic acid derivatives (83.33 to 49.54 µg/g), Epicatechin (12.33 to 17.36µg/g), Gallic acid (4.33 to 7.21µg/g) and *p*-Coumaric acid (2.06 to 3.54 µg/g).

The results showed that the Alliin content in garlic oil extracts to the three varieties in Table (5). The highest of Alliin content, was Sids 40 with a content of 91.53 mg 100/ml, followed by the Chinese variety at a content of 88.70 mg 100/ml, and the least of them was Sids 50 with a content 60.43 mg 100/ml.

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Table (3): Chemical composition of garlic oil (Sids 40, Sids 50 and Chinese) analyzed by GC-MS

RT (min)	Compound Name	Sids 40 Compound %	Sids 50 Compound%	Chinese Compound%
3.91	(Z)-3-Hexen-1-ol	0.36	0.42	0.37
5.58	Methyl 2-propenyl disulfide	26.21	28.14	27.07
6.15	1,3-Dithiane	12.18	12.26	12.19
8.39	Dimethyl trisulfide	37.50	37.34	37.48
11.21	Diallyl disulphide	4.04	4.22	4.01
11.38	2-Vinyl-1,3-dithiane	0.41	0.38	0.40
11.75	1-Oxa-4,6-diazacyclooctane-5-thione	4.01	3.21	3.25
12.21	Nonanal	0.68	0.70	0.77
12.87	Methyl (methylthio) methyl Disulfide	0.35	0.37	0.35
13.51	(Methylthio)-acetonitrile	7.91	7.86	7.89
13.98	(Z)-1-(methylthio)-1-Propene	0.77	0.72	0.76
14.33	Allyl methyl Sulfide	0.56	0.65	0.66
16.14	Dimethyl Tetrasulfide	2.54	1.71	2.37
17.32	N,N-dimethyl-Methanethioamide	0.72	0.34	0.69
19.14	Di-2-propenyl Trisulfide	0.25	0.27	0.31
20.91	tert-Butly methyl sulfoxide	0.26	0.27	0.29
23.68	S-Methyl methanethiosulfinate	0.41	0.33	0.35
33.85	2,2,7,7-tetramethyl- 3-Oxa-6-thia-2,7-disilaoctane	0.84	0.81	0.79
Total	-	100	100	100

Table (4). Content of phenolic compounds (µg/g) in aqueous extracts from garlic (Sids 40, Sids 50 and Chinese).

Garlic varieties	Gallic acid	Syringic acid derivatives	(+)-Catechin	*p*-Coumaric acid	*p*-Hydroxy-benzoic acid	Epicatechin	Total (sum)
Sids 40	7.21±0.29	85.23±0.28	96.15±0.34	3.11±0.22	187.44±0.39	17.36±0.25	396.50
Sids 50	5.78±0.39	79.67±0.16	84.57±0.24	3.54±0.15	179.59±0.27	13.43±0.27	366.58
Chinese	4.33±0.31	55.14±0.35	51.67±0.66	2.06±0.13	171.39±0.31	12.33±0.32	296.92
L.S.D $_{0.05}$	1.04	5.35	5.93	0.83	6.82	2.69	

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

Table (5). Comparisons of Alliin content (mg 100/ml) in garlic bulbs varieties (Sids 40, Sids 50 and Chinese).

Garlic varieties	Alliin content (mg 100/ml)
Sids 40	91.53 ± 0.11
Sids 50	60.43 ± 0.18
Chinese	88.70 ± 0.20
L.S.D $_{0.05}$	2.54

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

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 6). 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 Sids 40 garlic extraction, i.e. 5.26 µg/mL for DPPH and 0.83 µg/mL for $ABTS^{+}$, 0.62 µg/Ml for Fe^{2+} and 34.51 µg/mL for Cu^{2+}. In addition, the lowest IC_{50} values for DPPH scavenging were noted for Chinese garlic. Next of variety Sids 40, the two varieties came Sids 50, then the Chinese variety, and the Chinese gave the lowest value for IC_{50}. The results for the three varieties (Sids 40, Sids 50 and Chinese) indicated that the IC_{50} value were arranged as follows: Sids 40,

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Sids 50 and Chinese. Ion chelation activity the ability to chelate Fe^{2+} and Cu^{2+} ions was compared among the analyzed extracts of Garlic varieties (Sids 40, Sids 50 and Chinese). 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 6). The highest Fe^{2+} chelation ability was noted for garlic extract by methanol, with the lowest IC_{50} values of 0.62, 0.69 and 0.83 µg/mL, Sids 40, Sids 50 and Chinese respectively. The highest Cu^{2+} chelating ability was noted for garlic extract for Garlic varieties (Sids 40, Sids 50 and Chinese) (34.51, 38.27 and 41.82 µg/mL, respectively).

In general, the results show that the IC_{50} value were arranged for the three varieties as follows, the highest was Sids 40 followed by Sids 50 and Chinese.

Table (6). IC_{50} value for antiradical activity against DPPH and ABTS+ and the ability to chelate 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	5.26±0.04	0.83±0.03	0.62±0.01	34.51±2.16
Sids 50	5.83±0.03	0.75±0.02	0.69±0.03	38.27±2.21
Chinese	6.57±0.07	0.96±0.04	0.83±0.04	41.82±2.08
Positive control	[1] 0.78±0.02	[1] 0.14±0.05	[2] 0.42±0.02	[2] 0.38±0.33
L.S.D $_{0.05}$	0.0083	0.0054	0.0073	0.0721

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

Molecular genetic identification

Start Codon Targeted (SCoT) Technique:

Ten primers were used for amplifying the SCoT-PCR patterns listed in Table (2). A total of 121 amplified products were scored. In which 73 were polymorphic exhibiting 60.33% polymorphism. The molecular sizes of patterns profile were ranged between 150 to 1464 bp scored in Table (7). Fig. (1) Showed comparative DNA profile in which the three different genotypes of *Allium sativum L.,* showed common and differentiating patterns with different primers. The primers SCoT2, SCoT15 and SCoT4 exhibited low level of polymorphism (25.0%, 33.3% and40%) respectively. On the other hand the primers SCoT8, SCoT10, SCoT3 and

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SCoT5 exhibited moderate levels of polymorphism (58.4%, 64.7%, 75% and 75%) respectively. However SCoT12 (83.3%) and SCoT9 (82.4%) primers exhibited high levels of polymorphism. The primer SCoT6 has no polymorphism.

Table (7) showed some SCoT primers revealed specific patterns. One specific band sized1300 bp in clone 3 was produced using MPST11 primer. One specific band sized 650 bp was produced by MPST12 primer in clone 2. Three specific bands were obtained in clone 2 at 550 bp, 650 bp, and 1700 bp by using MPST14. These results exhibit the potential use of SCoT marker to recognize *Allium sativum* genotypes and differentiate between them.

Table (7): Molecular data estimated from banding patterns of SCoT technique.

Primers code	Range of M.S.	TAF	MF	PF	SM	Polymorphism (%)
SCoT primers						
SCoT 2	150-635	12	9	3	1 (414)-(0)-(0)bp	25.0
SCoT 3	167-456	8	2	6	3 (422,198)-(410)-(0)bp	75.0
SCoT 4	174-532	10	6	4	1 (0)-(417)-(0)bp	40.0
SCoT 5	176-952	12	3	9	5 (952,446)-(907,475,315)-(0) bp	75.0
SCoT 6	232-303	3	3	0	0 (0) bp	0
SCoT 8	163-540	12	5	7	5(271)-(489,377,294)-(298)bp	58.3
SCoT 9	187-900	17	3	14	9(494,404,293)-(481,432,305)-(520,340,313)bp	82.4
SCoT10	153-1185	17	6	11	6 (0)--(0)(1113,833,640,324,212,153) bp	64.7
SCoT 12	174-1464	18	3	15	10(0)-(1464,807,704,544,480,422,275)-(783,371,188) bp	83.3
SCoT 15	158-1069	12	8	4	3 (700)-(273)-(1069)bp	33.3
Total		**121**	**48**	**73**	**43**	

SCoT4 primer produced ten fragments with molecular size ranging from174 to 532 bp (Fig. 1). Four fragments were polymorphic (40%) and one of them was species - specific markers at (417) bp for Sids 40 genotype, while the other six fragments were present in all genotypes which are considered as common fragments.SCoT5 primer produced twelve fragments with molecular size ranging from176 to 952 bp (Fig. 1). Nine fragments were polymorphic (75%) and five of them was species - specific markers at (952,446) bp for China genotype and at (907,475,315) bp for Sids 40, while the other three fragments were present in all genotypes which are considered as common fragments. SCoT 6 primer produced three fragments with molecular size ranging from 232 to 303 bp (Fig.1). None fragments were polymorphic (0%) and three fragments are considered as common fragments. SCoT 8 primer produced twelve fragments with molecular size ranging from163 to 540 bp (Fig.1). Seven fragments were polymorphic (58.3%) and five of them was species - specific markers at (271) bp for China genotype, at (489,377,294) bp for Sids 40 and (298)bp for Sids 50 , while the other five fragments were present in all genotypes which are considered as common fragments. SCoT 9 primer resulted in seventeen fragments with molecular size ranging from187 to 900 bp (Fig.1). Fourteen fragments were polymorphic (82.4%) and nine of them was species - specific markers at (494,404,293) bp for China genotype, at (481,432,305) bp for Sids 40 and (520,340,313)bp for Sids 50 , while the other three fragments were present in all genotypes which are considered as common fragments. SCoT 10 primer resulted in seventeen fragments with molecular size ranging from 153-1185bp (Fig.1). Eleven fragments were polymorphic (64.7%) and six of them was species - specific markers at (1113, 833,640,324,212,153) bp for Sids 50, while the other six fragments were present in all genotypes which are considered as common fragments. SCoT 12 primer resulted in eighteen fragments with molecular size ranging from 174-1464bp (Fig.1). Fifteen fragments were polymorphic (83.3%) and ten of them was species - specific markers at (1464, 807,704,544,480,422,275) bp for Sids 40 genotype and at (783,371,188) bp for Sids 50, while the other three fragments were present in all genotypes which are considered as common fragments. SCoT 15 primer resulted in twelve fragments with molecular size ranging from 158-1069 bp (Fig.1). Four fragments were polymorphic (33.3%) and eight of them was species - specific markers at (700) bp for China genotype, at (273)bp for Sids 40 genotype and at (1069) bp for Sids 50, while the other

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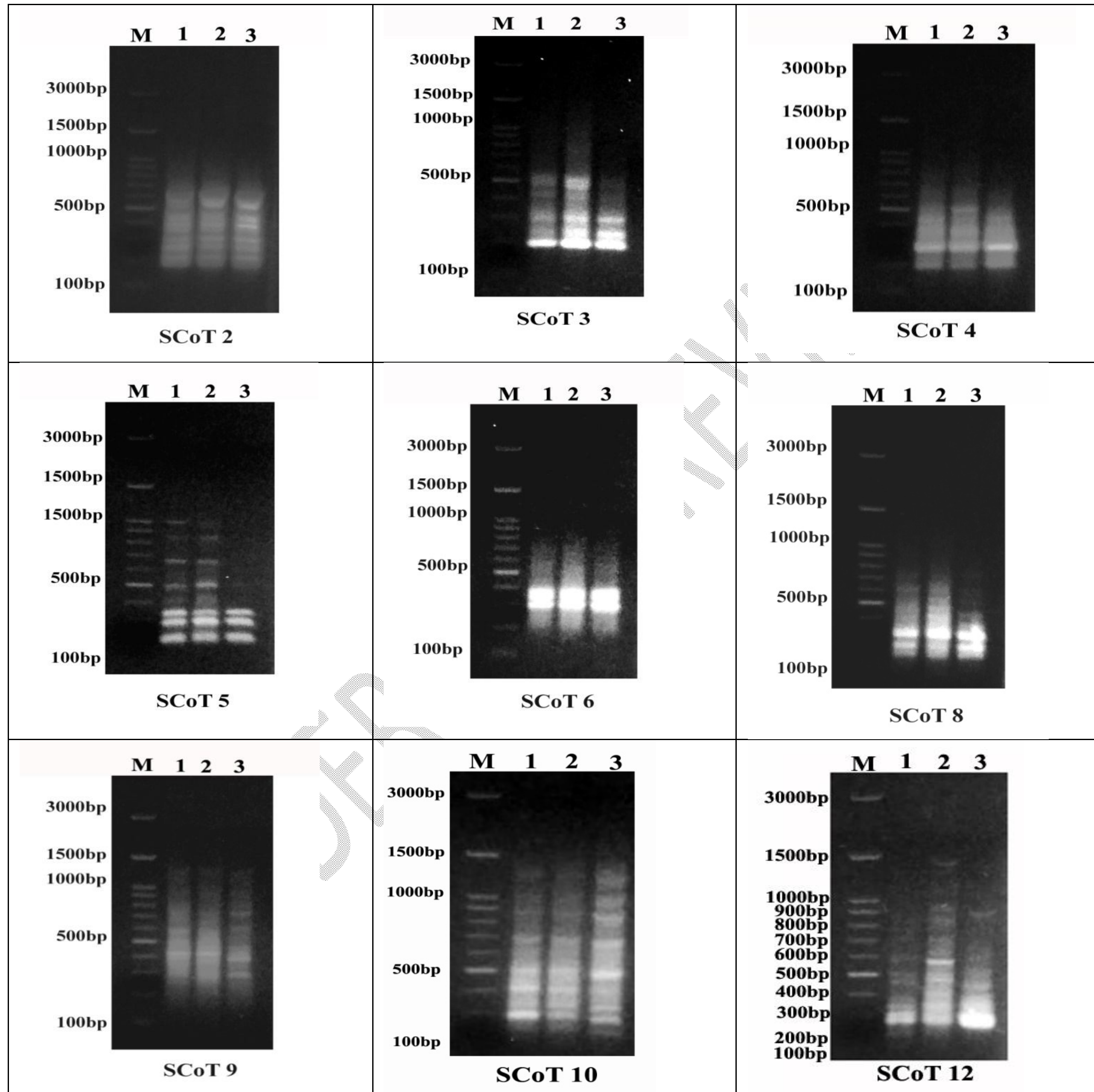
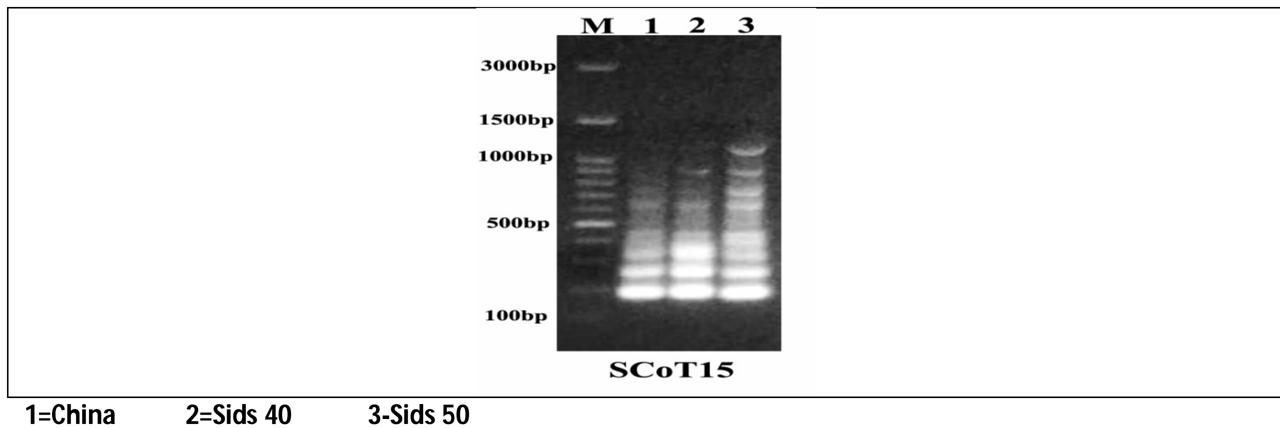
eight fragments were present in all genotypes which are considered as common fragments.





1=China 2=Sids 40 3-Sids 50

Fig. (1): SCoT-PCR analysis of different garlic verities cultivated under Egyptian condition. (second season)

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Genetic similarity and cluster analysis based on SCoT markers

The SCoT data were used to estimate the genetic similarity values among the three genotypes of Garlic by using UPGMA computer analysis (Table 8 and Fig.2). The highest similarity value (1.00) was recorded between Sids 40 genotypes and Sids 50 genotypes, while the lowest similarity value (0.610) was recorded between China genotypes and Sids 50 genotypes. On the other hand there was no similarity between China genotypes and Sids 40 genotypes.

Table (8): Similarity value (Pairwise comparison) of Garlic verities genotypes based on SCoT data

	China	Sids 40
China	1.000	
Sids 40	0.000	1.000
Sids 50	0.610	1.000

A dendrogram for the genetic relationship among the three genotypes of Garlic genotypes is exhibited in Fig. (2), which separated them into two major groups. The first group included China and Sids 40 genotype, while the second group included only Sids 50 genotype.

C A S E 0 5 10 15 20 25
Label Num
China 1
Sids 40 2
Sids 50 3

Fig. (2): A dendrogram illustrates the genetic distance for Garlic verities verities genotypes based on SCoT data

Discussion

The results indicate that Sids 40 variety was distinguished in its content of bioactive substances in the essential oil, compared to the other two varieties, Sids 50 and the Chinese variety, as well as distinguished in its content of alliin, and this is in agreement with the results (47). The highest radical scavenging activity was observed in the extract from garlic extract Sids 40. It is 5.26 µg / mL for DPPH, 0.83 µg / mL for $ABTS^{+}$, 0.62 µg / mL for Fe^{2+}, and 34.51 µg / mL for Cu^{2+}. In addition, the lowest IC_{50} value for DPPH scavenging was found in Chinese garlic, next to the Sids 40 variety. These results come as a reflection of the high total content of phenolic substances in the garlic variety Sids 40. The current study confirms the results of several studies that showed the relationship of total phenols content in the plants to the activity and level of antioxidants (48, 49). In this study, the results showed significant differences between the three genotypes of cultivated *Allium sativum*. The highest percentage of polymorphism was 83.3 % with SCoT 12 primers and the lowest was 25.0% with SCoT 2 primers. The level of polymorphism was comparatively higher than previous studies 82.5% in buck wheat (50).The genetic similarity percentage of genomic DNA using SCoT marker ranged from 61% to 100% as were showed in Table (8). This DNA marker technique considered as a useful tool for identifying population of the same species on a wide range of plants (31). It was validated in the model rice species *Oryza sativa* to fingerprint a small diverse set of rice genotypes. The percentage of polymorphic loci generated by using this effectiveness marker system in Dendrobiumnobile Lindl as an endangered medicinal orchid species was ranged from 25 to 56.82% and cluster analysis revealed high genetic variation among the genotypes according to (32). DNA profiling was revealed best results in *M. piperita* as compared to other Mentha species using SCoT marker analysis for correlating their similarity

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and distances between species (51). They estimated the genetic similarity between them ranged from 25.7% to 45.5% based on previous studies, their results proved the accuracy of SCoT marker system in estimating the genetic diversity

Conclusions

Garlic variety (Sids 40) crude extracts exhibited very promising in content bioactive content and antioxidant activity. This study indicated that genotype gave different profiles of bioactive compounds and this is reflected in the content of garlic varieties of bioactive active substances. However, considering the higher content of bioactive alliin, garlic (Sids 40) can be considered as the potential cultivar that can be used as raw material in food supplements and for further study. The study demonstrated SCoT markers were accurate and sensitive in differentiating and identification between varieties. Our results found that garlic variety (Sids 40) had 19 specific markers, it may be explain why this variety was distinguished in its content of bioactive substances in the essential oil, compared to the other two varieties

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