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Identification of Garlic (*Allium sativum* L.) Cultivars by using Morphological and Chemical Characters.

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Abstract

At vegetable research farm, Horticulture and Landscape Design Dept., College of Agriculture and Forestry, Mosul Univ., Iraq, the current study was conducted with six garlic cultivars (Allium sativum L). The cultivars'morphological characters have been categorized based on the height of the plant,leaf length, leaf width, number of leaves in each plant, head length, foliage color, number of cloves per head, head diameter, clove length, clove diameter,the skin color of bulb (head) and cloves. These characters possessed a wide range of variation and a good value for identification between the cultivars.The investigation of chemical characters identified 7 Phenolic compounds (Benzoic acid,Quercetin, Para hydroxy, resorcinol, Catechol, Hydrogunion, and Gallic acid) in the leaves'extract with the use of high-performance liquid chromatography (HPLC) technique. Also, the cultivars showed variation interms of containing the compound and their concentration. The differences between the cultivars regarding the chemical content can be relied upon as a guideline and classification indicator in the identification and isolation of the cultivars studied.

Keywords: Garlic (*Allium sativum* L.), Cultivars, Morphology-oil,phenoliccompounds

Introduction

Garlic can be defined as one of the monocotyledonous vegetables with origins in Central Asia (Kazakhstan) and secondary diversification centers in Mediterranean region and China. Also, garlic is the 2nd most widely grown bulb vegetable, and it is applied as a flavoring and spice agent in a variety of dishes. Allium is the major representative genus related to the Alliaceae family, with 700 species found throughout the Northern Hemisphere, including North Africa, North America, Asia, and Europe (Tsiaganis etal., 2006). The Allium genus includes garlic (Allium sativum L.). For a long time, the taxonomic status of Allium and associated genera was a source of debates. (*Friesen andFritsch, 2002*). The next hierarchy has beenused (*Taktajan, 1997*): -

a)Class – Liliopsida
b)Subclass –Liliidae
c)Superorder-Liliianae
d)Order – Amaryllidales
e)Family – Alliaceae
f)Subfamily – Allioideae
g)Tribe- Allieae
h)Genus- Allium

In genus Allium, the estimate accepts approximately 750 species (Stearn, 1992). As a result, the total number of species exceeds 800, which may be a little high, yet a number of approximately 780 Allium species now appears to be a reasonable estimate (Friesen et al., 2006). Six sub-genera and 56 sections were used for dividing a large number of species (Hanelt *et al*., 1992). After onions, garlic is the 2nd commonly cultivated bulbous crop. Allium derives its name from Celtic word all that represents pungent. It comes from the Allium longicuspisprogenitor, with Central Asia as its origin (Tsaiganis*etal*., 2006). In addition, garlic is high in proteins (6.3%), carbohydrates (29%), essential oils (0.1-0.4%), and minerals (0.3%), as well as one of the fair sources of vitamin C, fat, and sulphur (Friesen, et al.,2006). Among bulbous crops, it has extremely high nutritional value (Hanelt et al., 1992). The existence of diallyl disulfide, an odoriferous sulphur compound, gives it its

distinctive pungency and flavor (McCollum, 1976). Because allicin is considered the most prominent flavor compound in garlic, it is also the most abundant thiosulphate, accounting for between (60-95)% of total thiosulphate (Memane et al.,2008). It also has antifungal (Natale et al., 2005), antibacterial (Pandey, 1997), antiprotozoal (Kaur and Arora, 1999), antiviral (Block et al., 1992), anticancer and antioxidant properties (Lawson,1991 and Hughes). The morphology of genus Allium is relatively uniform, with just some common morphological traits (Synapomorphies) used to distinguish natural groups within the genus. Based on the capability for producing color and scape of foliage, shape bulb, length, the color of the compound bulb, and the number of the cloves in each one of the bulbs, Stavelikova (2008) divides 613 genotypes into 3 major morphological groups. In research of molecular and morphological garlic characterization (Allium sativum L.), Sabir et al. (2017) found that the genotypic as well as thephenotypic co-efficients of variation for average cloves per bulb, clove width, plant height, and leaf length were all high. The morphological properties related to Japanese garlic clones are described by Shiga et al. (2015). Tarigue et al. (2016) use morphological traits to characterize 25 garlic (Allium sativum L.) genotypes.Plants abound the huge numbers of different chemical compounds which are increasingly being discovered as a result of the great progress in the field of multiple chemical analysis methods, the possibility of diagnosing and detecting these compounds, and considering the great benefits and importance of these chemical compounds in the field of plants taxonomy. Radford *et al.* (1974) indicated that adding chemotaxonomy information to other results information such as phenotypic, anatomical, and cellular information can provide us with a solid basis for botanical taxonomy decisions; they also indicated that phenolic compounds are well–known chemicals that are widely used in taxonomy because they are widespread and usually exist in leaves, flowers, fruit, wood, and cotyledons. Al-Mousawi (1987) reported that chemicals in the plant make an effective contribution to nature in determining the flavor and taste of the plants in which they are found, and in many cases, the species and the cultivars can be distinguished by the taste of these plants regardless of any other characteristics. Samuel and Luchsinger (1987) mentioned that Flavonoids have important uses in the chemical classification due to their absolute presence in almost all higher plants and their ease of separation and diagnosis no matter how little plant matter there is, in addition to their presence in varying concentrations, the presence of fixed genetic bases for these changes and their chemical stability that contributed to the possibility of adapting these compound as classification indicators in most lower and higher plant groups. Flavonoids are the largest known group among the more than 1,000 phenolic compounds in addition to lignin and tannins (Mullen *et al*., 2002). The values of flavonoids and phenolic compounds differ among species due to their genetic characteristics, environmental conditions, and stage of maturity (Ercisli and Orhan, 2007).

Al Aroussi and Weassifi (2007) showed that the basis of plant division has advanced in the use of biochemical properties due to the development of methods for accurate separation of compounds, the basis of which was the detection of certain compounds to distinguish between taxa. Compounds of low-presence in plants, such as flavonoids, have been used for this distinction. Nagella et al. (2014) qualified and identified a total of 33 polyphenolic compounds from garlic bulbs, such as hydroxyl cinnamic acid, hydroxybenzoic acid, flavonols, and other phenolic compounds.

The aim of the studied was to Identification of Garlic (*Allium sativum* L.) Cultivars Based on by using morphological and chemical characters which was planting under mosul condition , Iraq

Materials and Methods

*** Experimental Location**

Throughout the autumn's growing season, the experiment has been carried out at vegetable research center, Dept. of Horticulture and Landscape Designs, College of Forestry and Agriculture, Mosul Univ. The cloves regarding each one of the cultivars have been planted 15 cm apart in the two sides of ridges running east-west based on 6 garlic cultivars (table 1). All common horticultural practices for garlic management were followed as usual.

Table1- Garlic cultivarsources

	garlic cultivars	
		harket in Nineveh, Iraq
		ega, Mosul, Iraq
		dcultivars from Syria
	omany	omany, Duhok, Kurdistan Region, Iraq
		harket in Nineveh,Iraq
		Nineveh, Iraq

***Data for morphological characters:**

The data was collected on ten garlic cultivars that were chosen at random. Plant height (cm), leaf length, number of the leaves ineach one of the plants, foliage color, leaf width, head length, head diameter, number of cloves in each head, clove length, and diameter have been used to classify the cultivars.

***Extraction acid analysis and diagnosis of phenolic compounds**:

The technique of grand *et al*.has been followed in the preparation of ethanol extract modified from the primary method of Verporte*et al*. (1982). The acidic decomposition of the leaves extract for the cultivars understudy was conducted according to the Harborne method (1973).The phenolic compound was diagnosed according to the method of Mradu *et al*. (2012) using (HPLC) technology.Values of retention time for standard compounds were approved by matching them with the retention values for the compounds that were separated from the extract under study and that were injected into the apparatus under the same conditions as shown in table (2). The concentration of the samples was calculated according to the method of Sousse and Ali (2017).

Sample Concentration = (SampleArea) / area of standard X concentration ofstandard X Dilution factor

Table 2: Retention time and area for standard phenolic compounds setting with applying HPLC.

	rd compound	ion time (RT)	
	quinon		
	ol		
	cinol		
	ten		
	droxy		
	c acid		
	acid		

Results and Discussion

Morphological characters

The observation recorded in six garlic cultivars on (12) morphological traits is indicated from table3 comparison of the mean values for characters garlic exhibited differences between plant height have been recorded in Sinjary (75.5cm) and the minimum (50.6 cm) in Kany komany.Also, plant height variability was identified by(Tarigue *etal*., 2016;Radford *etal*., 1974;Al-Muswi, 1987); the investigations on the number of leaves in each plant showed that the highest (8.0) leaves /plant has been in Kany komany,whereas it was lowest (5.0) leaves /plant in Chines.Those findingshave been in agreement with (Radford *et al*., 1974; Al-Muswi, 1987; Meng *et al*., 1993,Shiga *et al*., 2015).leaf length indicated from the table (2) that different each other among the cultivars and maximum was recorded in Sinjary (45.0 cm) while minimum in Francy (24.2 cm), the range of leaf width was between (1.6- 3.5 cm) the minimum in Chines (1.6cm) and the maximum value in Kany komany (3.5cm).

The data pertaining to head diameter and head length among the cultivars in table (3) indicate that head diameter had a range of (4.4 cm) to (6.7 cm). The maximum was observed in Francy (6.7 cm), whereas the minimum (4.4) was observed with Sinjary cultivars, but the head length showed the lowest variation between cultivars; the maximum was observed in Francy (5.6 cm) and the minimum in local (4.2 cm). These results finding were quite similar as reported by (Samuel and Luchsinger,1987; Mullen *et al*., 2002;Al-Muswi, 1987;Ercisli and Orhan, 2007; Meng *et al*., 1993,Al-Aroussi and Weassfi, 2007, Esho,2015). The number of cloves per head also showed the lowest differences among the cultivars and the maximum observed in local (40.6), whereas minimum (31.1) was found in Kany komany cultivar.The data in the table (3) indicates that clove length variants among cultivars and the range for clove length were (1.9- 3.6 cm) with cultivar Kany komany (1.9 cm) showed minimum clove length, while Local cultivars (3.6cm) showed maximum clove length.The width of clove data indicated the lowest variation among the cultivars; the minimum was recorded with the Kany komany (1.3 cm), and the maximum was observed in Syrian cultivars, which value is (2.2 cm). (Esho, 2015).

Chemical traits.

The result of (HPLC) analysis showed the diagnosis of seven phenolic compounds, which are (Hydroquinone, Catechol, Resorcinol, Quriceten, Parahydroxy, Benzoic acid,and Gallic acid), which showed variation in founding and content of the leaves extract of garlic cultivars under the studied. Table 4 and 5, and Fig 1, 2 (63.294 conc., and compound are:

1) Hydrogunion is found in Chines (63.294 conc.), and francy (59.767) cultivars.
2) Catechol is found in Chines (77.492) and Kany komany (74.936).
3) Resorcino is found only in Sinjary (78.821)
4) Quriceten is found only in Kany komany (74.821)
5) Parahydroxy is found in Local (73.872), Sinjary (73.101), and Kany komany (75.984) cultivars.
6) Benzoic acid is diagnosed in all cultivars under the study with the same concentration nearly.
7) Gallic acid is found in Local (76.079), Syrian (64.339), and Francy (73.478).

To theresults, we found that the cultivars of the garlic species *Allium sativum*that are studied are much different in the terms of the containment and concentration on a variety of the phenolic compounds, which can gives a good identifications value.

*The Benzoic acid was diagnosed in all cultivars of the garlic species *Allium sativum* under study,and this constitutes an evolutionary phenomenozesignificance.

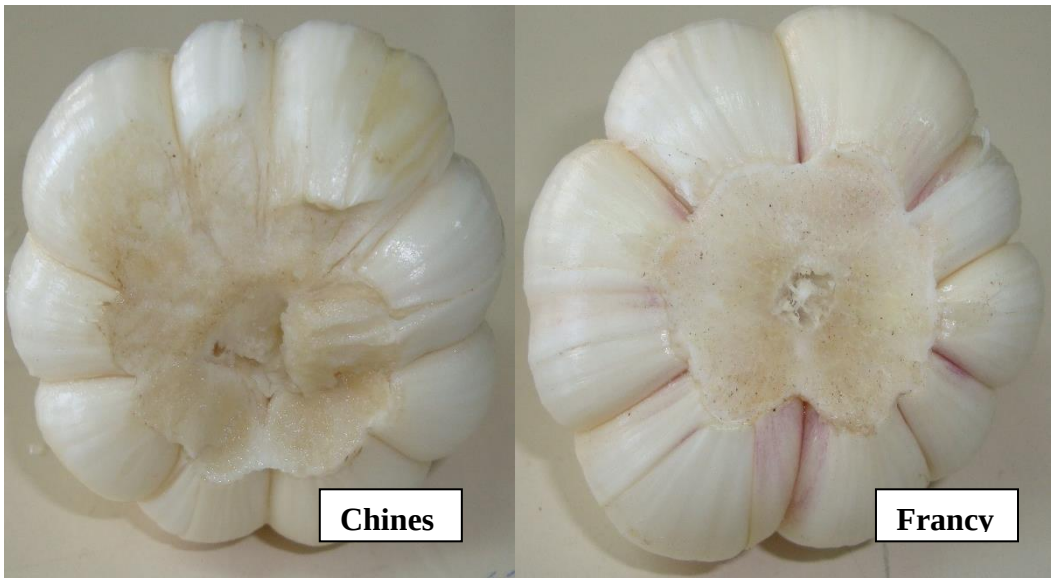
*It also agrees with Divis Iteywool (1963) and states that the study of the phenolic compound has ahigh identification value in isolating cultivars from each other.

Table -3-Qualitative and quantitative characters for the garlic (*Allium sativum*)cultivars.

eight (cm)	leaves /plant	dth (cm)	ngth (cm)	ength (cm)	iameter (cm)	cloves /head	ength (cm)	diamet
71.4)		1.7)	41.3)	(4.2)	(5.2)	40.6)	(3.6)	(1.9)
52.7)		1.6)	37.6)	5 (5.8)	(6.3)	38.0)	(3.2)	(1.85)
57.8)		1.7)	7 (38.6)	(5.3)	(5.3)	35.8)	(3.4)	(2.2)
75.5)		2)	45.0)	(4.3)	(4.4)	39.1)	(2.8)	(1.7)
		2.8)	29.2)	(5.6)	(6.7)		(3.3)	(1.8)
50.6)	0)	(3.5)	38.0)	(4.6)	(5.1)	31.1)	(1.9)	(1.3)

Continue table(3)

cultivars	colour	kin Colour	kin Colour
	reen		
	green		
	reen		
omany	reen		



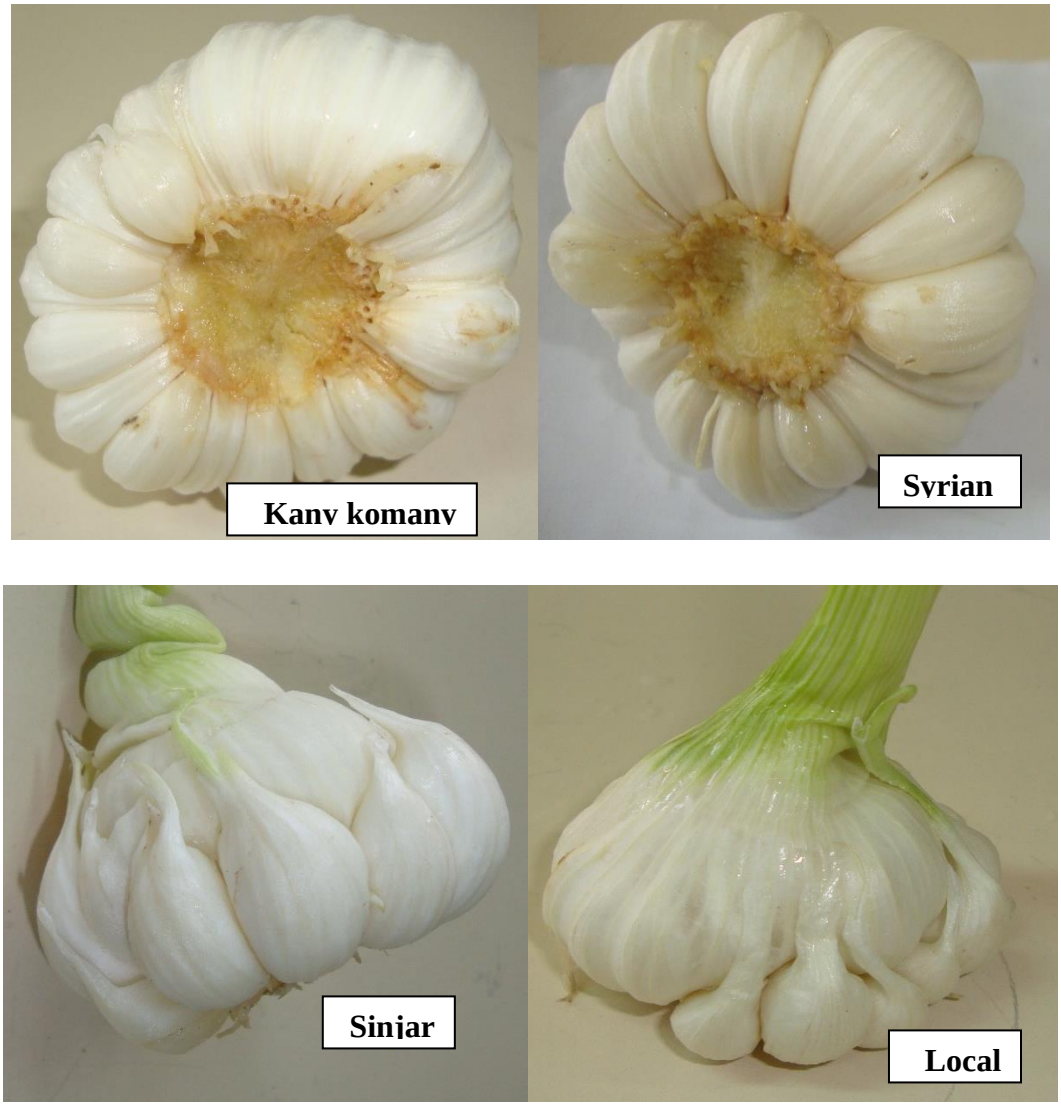


Figure 1: The shape of the garlic genotypes heads under the study

Table 4: Phenolic compounds separated from the leaves of garlic cultivars using (HPLC) technique.

s	quinone	hol	inol	ten	droxy	c acid	acid
omany							

Table 6: Concentration of phenolic compounds in leaves of garlic cultivars measured in Mg/g.

s	quinone	hol	inol	ten	droxy	c acid	acid

pmany							

<Chromatogram>

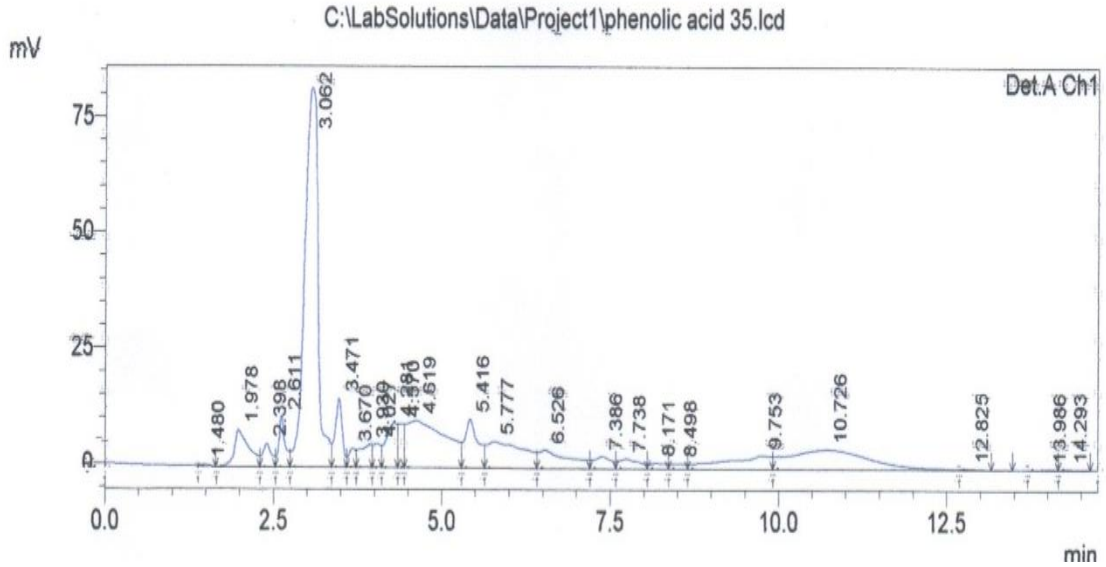


Figure -3-the compound of Guriceten in the garlic genotypes leaves .

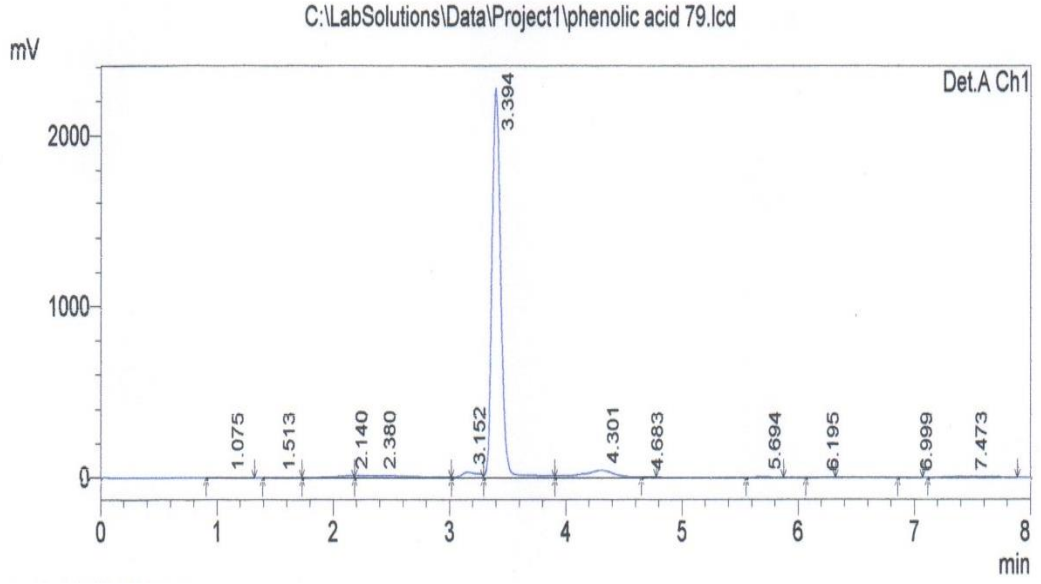


Figure 4: The curve of phenolic acid in leaves for genotype Local of garlic

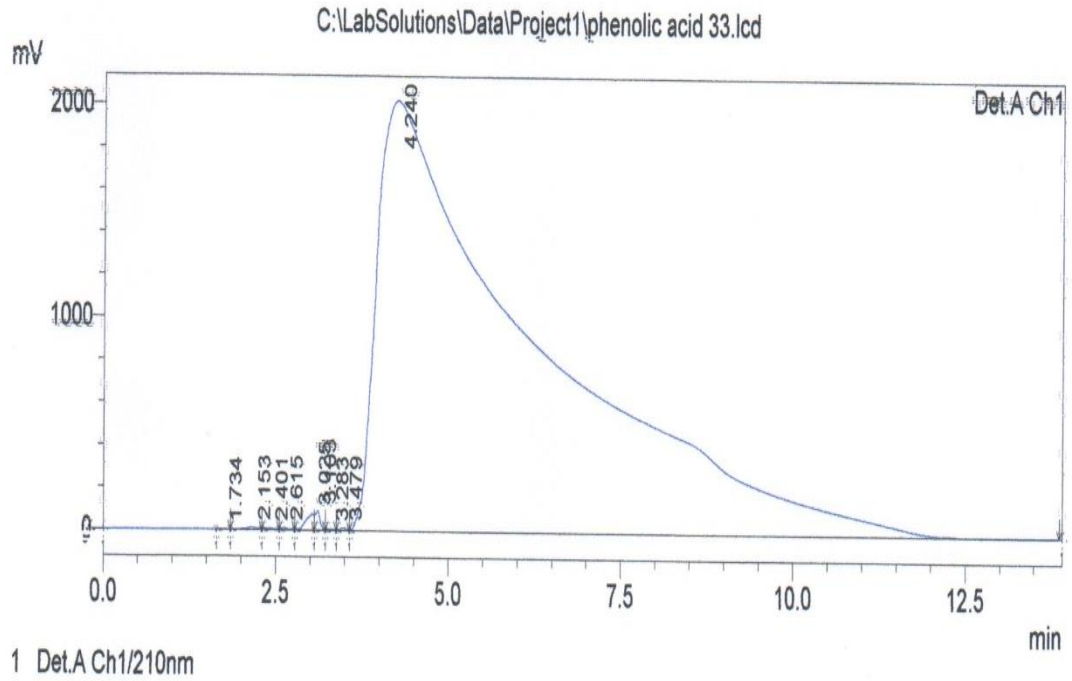


Figure 5: The curve of Gallic acid in leaves for genotypes of garlic

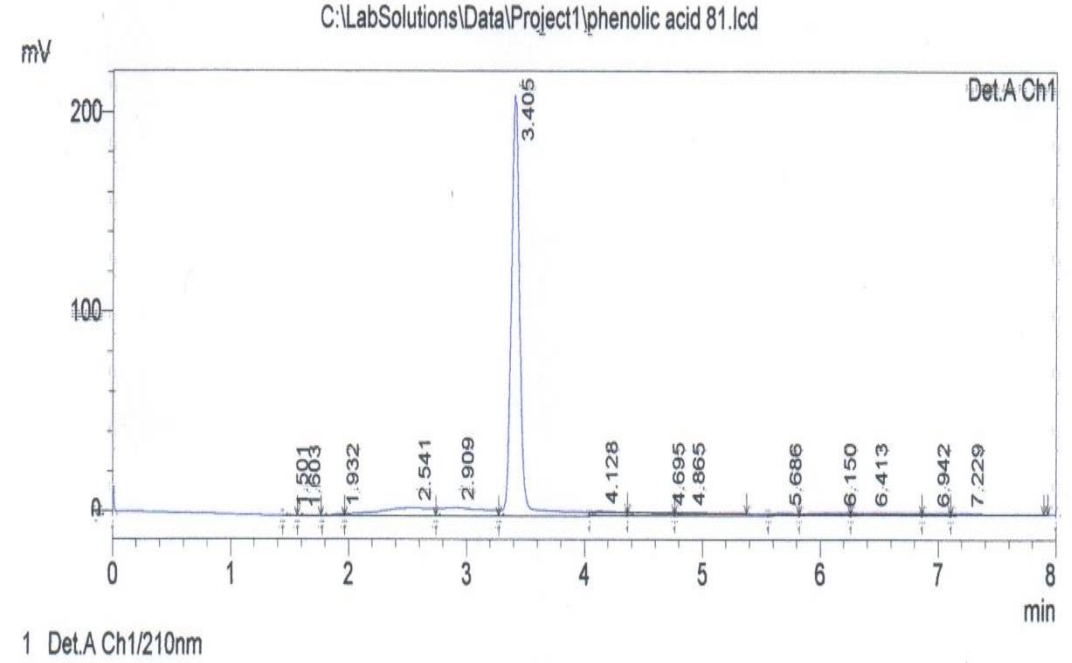
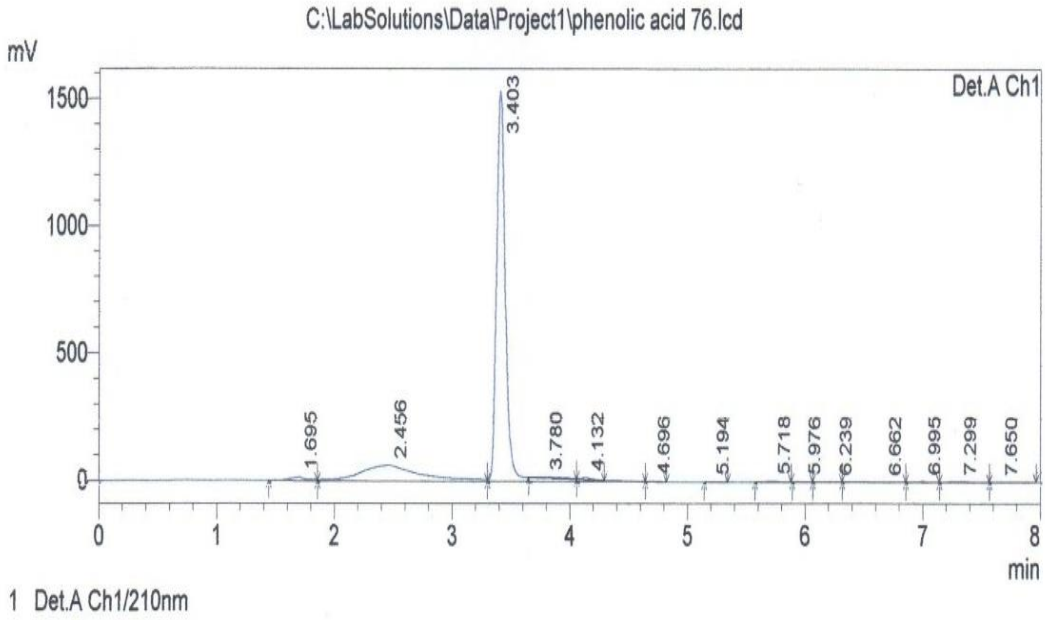


Figure 6: The curve of phenolic acid in leaves for genotype Chines of garlic

<Chromatogram>



Figur 7 : The curve of Benzaic acid in leaves for genotypes of garlic .

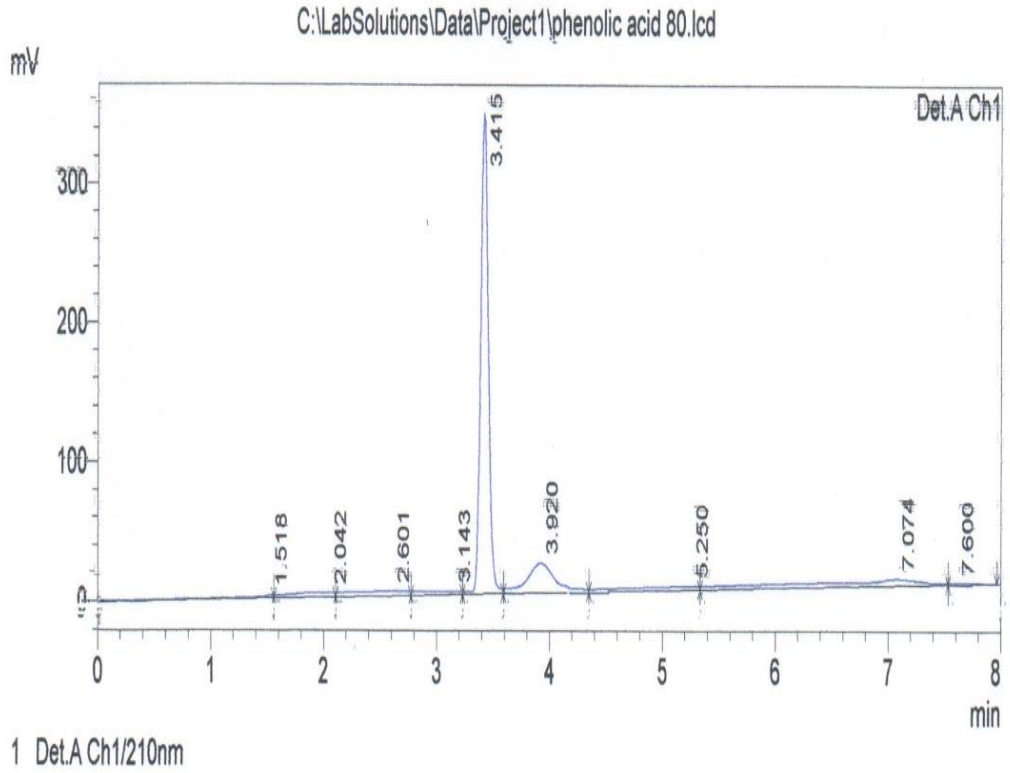


Figure 8: The curve of phenolic acid in leaves for genotype Syrian of garlic

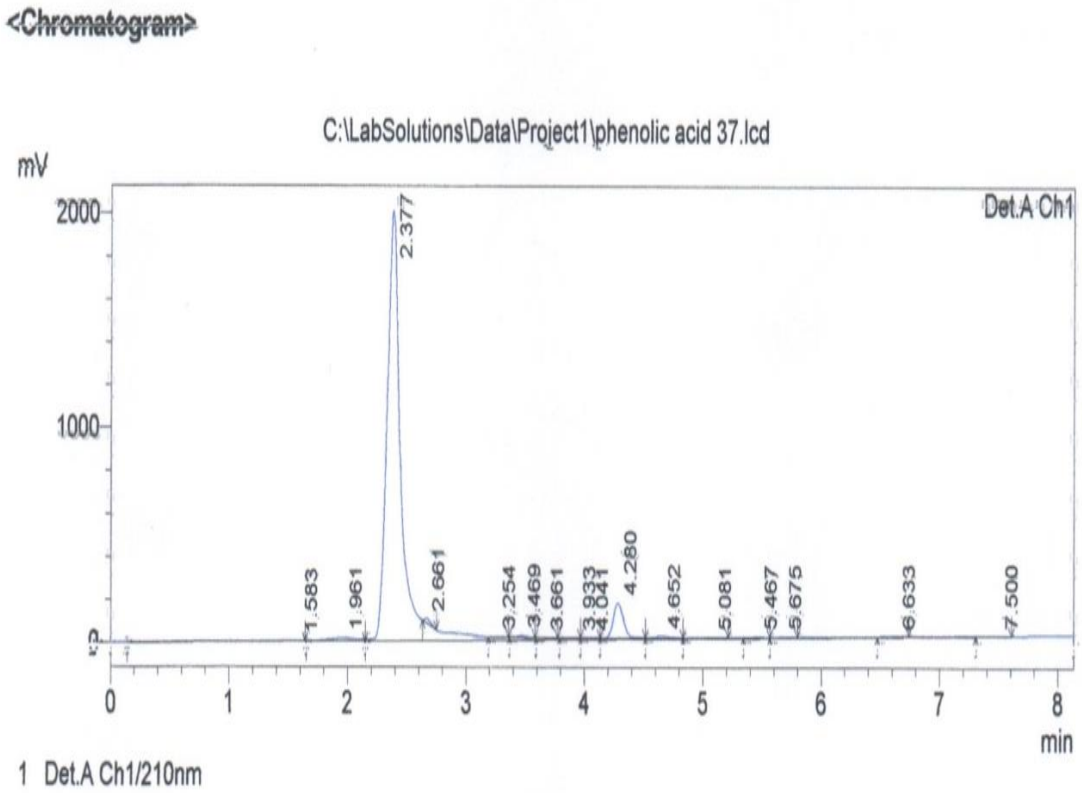


Figure 9: The curve of Katechol in leaves for genotypes of garlic

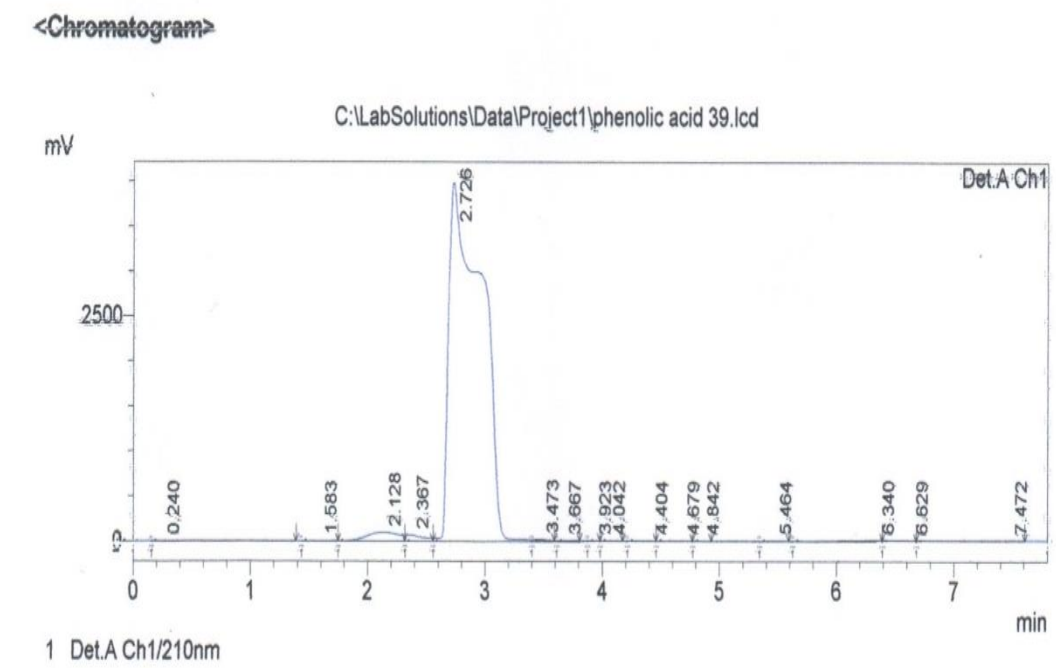


Figure 10 : The curve of Re sorcinol in leaves for genotype of garlic

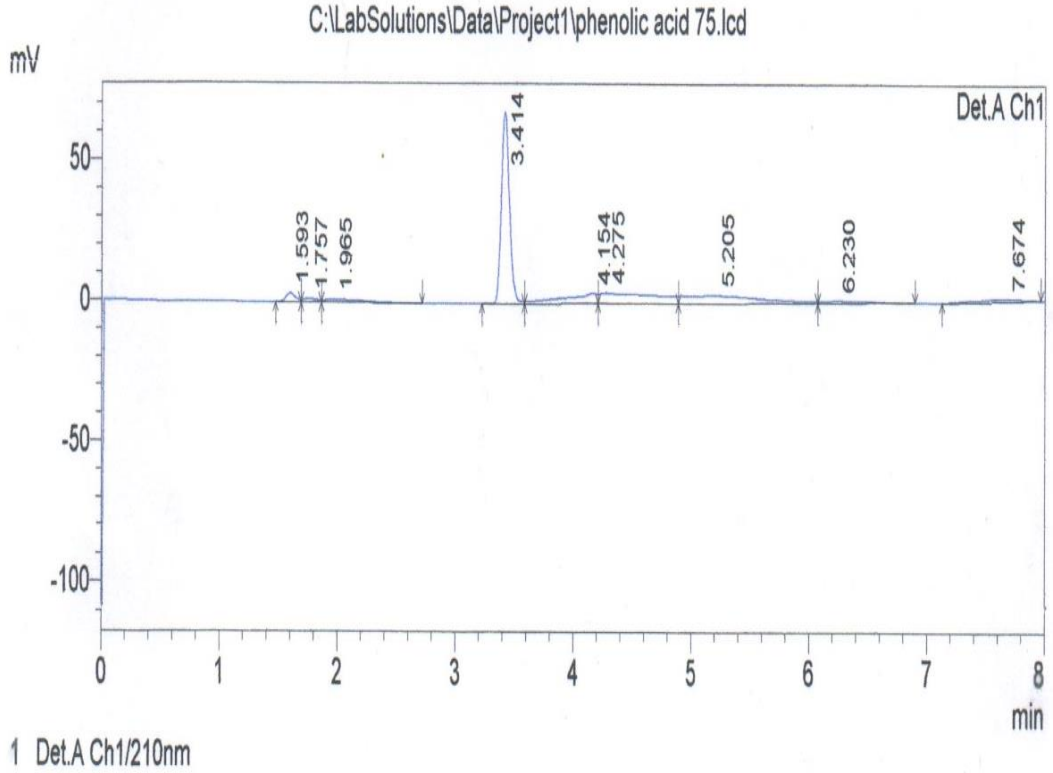


Figure 11: The curve of phenolic acid in leaves for genotype Francy of garlic

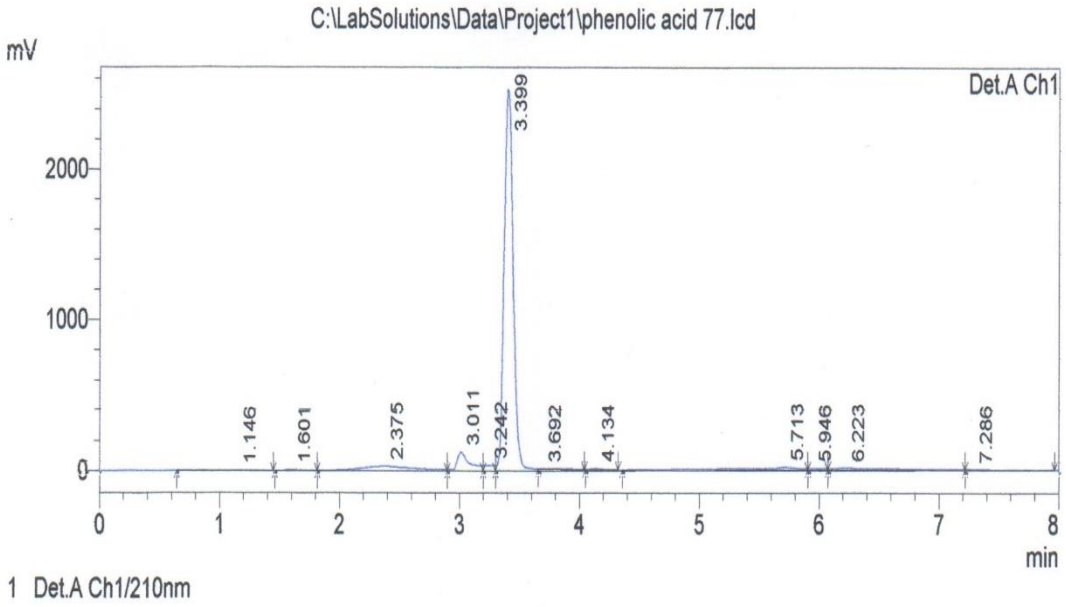


Figure 12: The curve of phenolic acid in leaves for genotype Kany komany of garlic

Conculusion

Through our study, we conclude that the genotypes of garlic varied in its morphological characteristics and content of phenolic compounds, the genotype Sinjary produced the highest plant height, the Local variety produced the highest number of cloves per onion, and the Syrian variety gave the highest content of benzoic acid.

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