MULTIVARIATE ANALYSIS OF POLYPHENOLIC CONTENT AND IN VITRO ANTIOXIDANT CAPACITIES OF DIFFERENT FRACTIONS OF *LEPIDIUM SATIVUM* L. AERIAL PARTS AND ROOTS FROM ALGERIA

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Abstract. Despite the great therapeutic and industrial values of *Lepidium sativum* L., all scientific reports have mainly focused on studying its seeds. For the first time, the aim of this study is to determine the effects of extraction methods and solvents on the phenolic contents and the antioxidant capacity of *Lepidium sativum* L. aerial parts and roots. Different techniques such as maceration, Soxhlet and ultrasound-assisted extraction were applied. The antioxidant effects were examined by four tests; 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), reducing power and phenanthroline assays. The greatest content of total phenolic content was obtained for the methanolic fraction of aerial parts prepared by maceration while the chloroformic fraction of roots extracted by the Soxhlet gave the highest content of total flavonoids. *L. sativum* fractions demonstrated good antiradical and chelating activities. High-performance liquid chromatography (HPLC) profiles revealed the presence of flavonoids, phenolic acids and other phenolic compounds. However, the fraction obtained by Soxhlet was the richest in bioactive compounds compared to that prepared by maceration. Multivariate analysis showed that *L. sativum* fractions belong to three distinct types in terms of their polyphenolic content and antioxidant activity.
Keywords: *Lepidium sativum L., principal component analysis, phenolic compounds, HPLC-DAD, antiradical activity, chelating capacity*

Introduction

Recently, the use of bioactive compounds from medicinal plants as therapeutic agents has been an important area in biomedical and natural products research (Mgbeahuruike et al., 2017). Typically, bioactive compounds of plants are produced as secondary metabolites. These compounds can be divided into three major categories; terpenoids, alkaloids and phenolic compounds. Taking into consideration the great variations among bioactive compounds and the huge number of plant species, it is necessary to build up a standard and integrated approach to screen out for the compounds carrying human health benefits (Azmir et al., 2013). This approach is initiated by extraction and followed by the determination of the quantity and quality of

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bioactive compounds. Technically, various procedures are used in the extraction of medicinal plants including maceration, infusion, decoction, hydrodistillation, Soxhlet, microwave and ultrasound-assisted extraction (Azwanida, 2015). The choice of an appropriate extraction method depends on many factors such as stability to heat, nature of the solvent, duration of extraction, the required final volume and the intended use of extract either for consumption by humans or for experimental testing (Abubakar and Haque, 2020). Phenolic compounds and flavonoids are responsible for several pharmacological properties. However, these compounds have been investigated for their antioxidant potential which is mediated by their hydroxyl groups that are able to scavenge free radicals and chelate metals (Tungmunnithum et al., 2018).

Oxygen is strongly involved in the initiation of oxidative stress which, although useful to the body, can lead also to various pathologies in certain situations by the formation of very reactive free radicals which are grouped under the term Reactive Oxygen Species (ROS). These free radicals are generally very unstable and will therefore react quickly with the biological molecules that are nearby (Navarro-Yepes et al., 2014). Thus, oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of ROS in cells and tissues and the ability of a biological system to detoxify these reactive products (Pizzino et al., 2017). However, the use of medicinal plants with antioxidant properties has been exploited for their ability to treat or prevent several human pathologies in which oxidative stress seems to be one of the causes (Sharifi-Rad et al., 2020).

Lepidium sativum L. belongs to the Brassicaceae family, it is locally known as Hab Elrachad and as a garden cress all over the world. This plant is an edible, glabrous, erect and annual herb (Ahmad et al., 2015). It is cultivated as a culinary vegetable worldwide, particularly for its comestible seed oils. In addition, different parts of this plant, especially the seeds, have been shown to have a wide range of biological and pharmacological properties (Vinti Dixit et al., 2020). Young leaves are eaten raw as a salad or cooked, while their seeds are used, fresh or dried, as a seasoning with a peppery flavor (Zia-Ul-Haq et al., 2012). The seeds are bitter, thermogenic, depurative, rubefacient, galactogogue, tonic, aphrodisiac, ophthalmic, antiscorbutic and antihistaminic (Diwakar et al., 2010). They are valuable in the treatment of lung problems such as asthma, coughs and bronchitis as well as skin disease, diarrheas, dyspepsia and lumbago (Gokavi et al., 2004). Furthermore, the seed paste is applied to rheumatic joints to relieve pain and swelling (Zia-Ul-Haq et al., 2012). Moreover, it was assumed that seeds of *L. sativum* can be a functional food. Some studies showed that adding the extracts of *L. sativum* to fruits can elevate and improve their nutritive value and consequently the quality of food. Similarly, other works on seeds revealed the possibility of using them as nutraceutical food ingredients in the dietary formulation and as food preservatives (John et al., 2020). Leaves of the same plant were reported to have antibacterial, diuretic and stimulant properties (Wright et al., 2007). Kassie et al. (2002) have revealed that the juice of an eight-day-old of the whole plant has chemoprotective properties against 2-amino-3-methylimidazo [4.5-f]quinoline)-induced genotoxic effects and colonic neoplastic lesions in rats. Phytochemical studies demonstrated that *L. sativum* is a rich source of polyphenols as well as essential oils that have huge medicinal and pharmaceutical applications (Eddouks et al., 2005). On the other hand, despite the great therapeutic value of *L. sativum,* it is observed that all scientific reports have focused mainly on studying its seeds and not the other parts (leaves, stem, flower and roots).

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To the best of our knowledge, there are no reports describing the chemical composition and the antioxidant potential of the aerial parts and roots of *L. sativum.* Therefore, this first study was carried out to elucidate the effect of using different extraction methods (maceration, Soxhlet and ultrasound-assisted extractions) and different extraction solvents (hexane, chloroform, methanol and water) on the chemical composition (particularly phenol, flavonoid and tannin contents) and also on the antioxidant activity of aerial parts and roots of *L. sativum* which was assayed by different models (DPPH, ABTS, reducing power and phenanthroline tests). Also, this study aimed to analyze the relationship between polyphenols and the antioxidant capacities of the tested fractions. Furthermore, the methanolic fractions of the aerial parts obtained by different extraction methods were analyzed by High-Performance Liquid Chromatography (HPLC-DAD) to determine their phenolic composition profile.

Materials and methods

Plant sampling and preparation

The cultivated *Lepidium sativum* L. samples were collected in March 2021 from El Outaya region situated between 34°57’391”N and 05°30’289”W. This region is located in the Northwest of Biskra (Algeria) and it is characterized by an arid climate. Thirty different plant samples were cleaned and separated into aerial parts (leaves and stems) and roots, and then left to dry at room temperature. After dryness, the two different organs were ground separately using a mechanical grinder (Sayona Electric Coffee Spices Grinder, Australia) to obtain a fine powder and then preserved in the refrigerator at 4 °C for further studies.

Chemicals and reagents

All reagents were of analytical grade. Hexane, methanol,1, 10- phenanthroline, Folin-Ciocalteu reagent, sodium carbonate, aluminum chloride, hydrochloric acid, potassium ferricyanide, sodium acetate, ferric chloride, potassium persulfate and gallic acid were purchased from Biochem Chemopharma (France). Other chemicals namely chloroform, quercetin, vanillin, acetic acid, acetonitrile, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azino-bis-3-ethyl benzthiazoline-6-sulfonic, trichloroacetic acid were bought from Sigma-Aldrich Gmbh (Germany).

Extraction method

Three extraction techniques were applied for the extraction of bioactive compounds from different parts of *L. sativum* in order to compare their efficiency following the protocols of (Karoune et al., 2015; Dall’Acqua et al., 2020). These techniques are divided into conventional (maceration and Soxhlet extractions) and unconventional (ultrasound-assisted extraction).

Maceration

Ten grams (10 g) of aerial parts and roots were placed in Erlenmeyer flasks and then exhausted successively with various solvents of increasing polarity; started with hexane (to remove pigments and fatty components), followed by chloroform, methanol and

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ended with distilled water. Each mixture was left to macerate under stirring in the dark at room temperature for 24 h. The extraction process was performed by using 100 mL of each solvent.

Soxhlet-assisted extraction

In a Soxhlet apparatus, 10 g of each organ of the plant were extracted first with 100 mL of hexane. Then, an exhaustion extraction of the remaining plant materials with chloroform, methanol and distilled water was assessed successively in order to allow the release of maximum active compounds. The extraction cycle lasted 6 h for every solvent. The temperature was fixed at 60 °C for the solvents except for distilled water (100 °C).

Ultrasound-assisted extraction

The ultrasound-assisted extraction was performed in a Bandelin Sonorex Digetec model (Type: DT 514, Germany) using 250 W of power and 35 kHz of frequency. Aerial parts and roots were placed in a beaker separately and subjected to 1 h extraction with 100 mL of hexane, chloroform, methanol and distilled water successively at a constant temperature of 60 °C.

Phenolic composition determination

Total phenols content (TPC)

Following the method of Muller et al. (2010), the total phenolic content of the studied fractions was measured using the Folin-Ciocalteu reagent. One hundred microliters (100 µL) of Folin-Ciocalteu (10-fold dilution) and 75 µL of sodium carbonate (Na_2CO_3, 7.5%) were added to 20 µL of each fraction in methanol or distilled water (1 mg/mL, 3 replicates per sample). Then, this mixture was incubated for 2 h at room temperature in the dark. The absorbance was measured at 765 nm using a 96-well microplate reader (Thermo Scientific^{TM} Multiskan Sky, Singapore, ref: 5111700DP). Contents results were expressed in micrograms of gallic acid equivalents per milligrams of dry extract (µg GAE/mg DE). The calibration curve was performed using gallic acid at different concentrations of 25, 75, 100, 125, 150, 175 and 200 µg/mL.

Total flavonoids content (TFC)

The content of flavonoids was evaluated using the aluminum chloride colorimetric method (Topçu et al., 2007). Fifty microliters (50 µL) of each extract were mixed with 50 µL of aluminum chloride ($AlCl_3$, $6H_2O$, 2%) and 150 µL of 5% sodium acetate in wells of a 96-well microplate. The microplate was then incubated in dark at room temperature for 2.5 h. After incubation, absorbance was determined at 440 nm. The results are expressed in µg equivalent of quercetin/mg (µg EQ/mg) of the extract with reference to the calibration curve for quercetin (obtained at different concentrations from 25 to 200 µg/mL).

Condensed tannin content (CTC)

Tannin content was performed using the vanillin colorimetric method as described by (Julkunen-Tiito, 1985). Ten microliters (10 µL) of each plant extract (1 mg/mL) were mixed with 155 µL of 4% vanillin solution (in methanol) and 80 µL of concentrated

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hydrochloric acid. After 20 min of incubation, the absorbance was obtained at 550 nm. Tannin contents were presented as µg equivalent of catechin/mg of dry extract (µg EC/mg) using the catechin standard curve.

HPLC-DAD analysis

To study the phenolic compounds profile from the methanolic fractions of *L. sativum* aerial parts obtained by maceration and Soxhlet extraction methods, HPLC analysis was performed using Agilent HPLC 1100 apparatus, coupled with UV detector and diodes array system (DAD), equipped with a quaternary rapid separation pump and Hypersil BDS-C18 column (5 µm, 250 × 4.6 mm). The mobile phase consisted of acetic acid (0.2% in water) as solvent A and acetonitrile as solvent B in a linear gradient for 30 min at a flow rate of 1.5 mL/min. This phase started with 95% of solvent A and ended with 100% of solvent B. The column temperature was set to 30 °C and the injection volume was 15 µL. The wavelength was set to 280 nm for the detection of phenolic compounds. Identification of phenolic compounds was carried out by comparing their retention times with that of standard injected in the same conditions.

Antioxidant activity evaluation

DPPH radical scavenging assay

The scavenging abilities of *L. sativum* fractions obtained from different extraction methods were determined according to Bouchoukh et al. (2019). This assay consists in estimating the reduction of DPPH with a discoloration generated by the antioxidant to be examined. Briefly, 40 µL of each extract at different concentrations (12.5, 25, 50, 100, 200, 400 and 800 µg/mL) dissolved in methanol or distilled water were blended with 160 µL of 0.1 mM DPPH radical solution in methanol. After 30 min of incubation in the dark, the decrease of DPPH absorption was measured at 517 nm using a microplate reader. BHT was used as a positive control. The scavenging effect of DPPH radical was calculated following *Equation 1:*

$$DPPH\ Scavenging\ effect\ (\%) = \frac{AC - AF}{AC}\ X100 \qquad \text{(Eq.1)}$$

where AC and AF are the absorbances of control and fraction at 30 min, respectively.

This activity was expressed as IC_{50} (µg/mL) which corresponds to the dose of extract that causes 50% of inhibition.

ABTS radical scavenging assay

ABTS radical scavenging effect was carried out following the protocol of Re et al. (1999). $ABTS^{+}$ was prepared by mixing 5 mL of 7 mM ABTS and 5 mL of 2.45 mM potassium persulfate and kept in the dark at room temperature for 16 h. Then the mixture was adjusted with distilled water until getting an absorbance of 0.700 ± 0.020 at 734 nm. After that, 160 µL of $ABTS^{+}$ solution were added to 40 µL of each extract at various concentrations (12.5, 25, 50, 100, 200, 400 and 800 µg/mL). Methanol and BHT were used as negative or positive controls respectively. After 10 min of incubation, the absorbance was determined at 734 nm. $ABTS^{+}$ scavenging activity was expressed as IC_{50} (µg/mL). The inhibition of ABTS free radical was determined as below in *Equation 2.*

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$$\text{ABTS scavenging effect} = \frac{A0 - A1}{A0} \, X100 \qquad \text{(Eq.2)}$$

where A0 and A1 are the absorbances of control and fraction at 10 min, respectively.

Reducing power activity (RP)

The reducing power effect was assessed according to the method of Oyaizu (1986). Ten microliters (10 µL) of each extract at different concentrations (3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL), 40 µL of phosphate buffer (pH = 6.6) and 50 µL of potassium ferricyanide (1%) were added for each well of the microplate. After 20 min incubation at 50 °C, 50 µL of trichloroacetic acid (10%), 40 µL of distilled water and 10 µL of ferric chloride ($FeCl_3$, 0.1%) were then added respectively to the first mixture. For comparison, methanol and ascorbic acid were used as negative and positive controls, respectively. Absorbance was measured at 700 nm using a microplate reader. In this activity, higher absorbance values mean a greater reducing power effect.

Phenanthroline activity (Phen)

The phenanthroline assay was determined by the method of Szydłowska-Czerniaka et al. (2008). Ten microliters (10 µL) of the tested extract at different concentrations (3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL), 50 µL of $FeCl_3$ (0.2%), 30 µL 1,10-phenanthroline (0.5%) and finally 110 µL of methanol were placed into a 96-well microplate. After 20 min incubation at 30 °C, the absorbance was then measured at 510 nm. Methanol and BHT were used as negative and positive controls, respectively.

Statistical analysis

All data are expressed as Mean ± standard deviation (SD). Three replicates were conducted for each test. Statistical analysis was performed using ANOVA one-way followed by Tukey's HSD for multiple comparisons between studied groups. Correlations between all variables were determined. Moreover, hierarchical cluster analysis (HCA) and principal component analysis (PCA) were performed to identify groupings, similarities, or differences among all analyzed plant fractions according to statistically independent variables. This analysis was based on the results of TPC, TFC, CTC, DPPH, ABTS, RP and Phen tests. Results were considered statistically significant when *P*-values were below 0.05. Statistical tests were performed using Statistica software (version 6.0, 2001).

Results and discussion

Effect of extraction technique and solvents on extraction yields

The effect of different extraction techniques using water, hexane, methanol and chloroform solvents on the extraction yield of *L. sativum* aerial parts and roots is shown in *Table 1*. Globally, results showed that the methanolic fraction of the aerial parts prepared by maceration gave significantly ($P < 0.05$) the highest extraction yield by 15.65%. The comparison of the three-extraction methods showed that in general, maceration was more effective than Soxhlet and ultrasound-assisted extraction.

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Conversely in roots, methanolic and chloroformic fractions obtained by Soxhlet, were more yielding productively than other techniques.

For the effect of the used parts on extraction yield, results showed that aerial parts of the plant recorded the highest values than roots. Moreover, for these two parts, extraction using methanol as a solvent achieved the greatest yield values compared to other solvents. On the other hand, the evaluation of the extraction yield was less efficient in ultrasound-assisted extraction. In this study, the extraction yield found for *L. sativum* aerial part fractions was higher compared to that of seed extracts reported by Aydemir and Becerik (2011).

Table 1. *Extraction yields of L. sativum fractions obtained by different extraction methods*

	Yields (%)							
Extraction method	**Aqueous fraction**		**Hexane fraction**		**Methanolic fraction**		**Chloroformic fraction**	
	Aerial parts	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**
Maceration	9.03 ± 0.02^{a}	3.08 ± 0.0008^{a}	2.47 ± 0.01^{a}	0.80 ± 0.0002^{a}	15.65 ± 0.1^{a}	2.46 ± 5.6^{c}	6.29 ± 0.01^{a}	0.501 ± 0.002^{a}
Soxhlet	0.64 ± 0.01^{c}	0.53 ± 0.01^{b}	1.98 ± 0.01^{b}	0.39 ± 0.003^{b}	12.59 ± 0.03^{b}	8.27 ± 0.004^{a}	3.903 ± 0.3^{ab}	0.57 ± 0.03^{a}
Ultrasound	1.14 ± 0.1^{b}	0.302 ± 0.02^{c}	2.12 ± 0.002^{b}	0.267 ± 0.01^{c}	7.69 ± 0.7^{c}	3.95 ± 0.3^{b}	1.82 ± 0.04^{b}	0.28 ± 0.2^{a}

Data are expressed as Mean ± SD, 3 replicates per sample. Means in each column followed by a different letter are significantly different $P < 0.05$, one-way ANOVA followed by Tukey's test)

Extraction efficiency generally depends on the polarity of solvents, temperature, extraction time and the composition of extracts. It has been reported that extraction in highly polar solvents resulted in a high extract yield (Nawaz et al., 2020). In this study, extraction yields obtained by maceration were found to be high in methanol and water, two polar solvents, then in no-polar ones (hexane and chloroform), but this relative was not accurate in the case of using Soxhlet and ultrasound-assisted extraction methods where the fractions prepared using water yielded less than those from no-polar solvents. However, it is necessary to mention that these extraction yields values are not straightly connected to the phenolic composition of the studied samples or their antioxidant effects because there could be different compounds with antioxidant effects apart from polyphenols (Jiménez-Moreno et al., 2019).

Effect of extraction technique and solvents on TPC, TFC and CTC

The amounts of phenolic compounds in all tested fractions of *L. sativum* aerial parts and roots are presented in *Figure 1A*. Among all samples, the methanolic fraction of the aerial parts prepared by maceration contained the highest quantity of phenolic compounds followed by the hexane fraction obtained by Soxhlet (149.84 ± 2.1 and 125.7 µg GAE/mg, respectively). Despite the high polarity of water, the aqueous fractions were found to be less efficient compared to other solvents with low polarity. This result can be explained by the high chemical structure varieties of phenolic compounds which make them react differently towards the Folin-Ciocalteu reagent (Abu et al., 2017). It is observed that the amounts of phenolic compounds in the aqueous fraction were remarkably enhanced when using sonification. These results are in accordance with other studies which revealed an improvement in the photochemical contents extracted after sonification (Lanez and Ben Haoua, 2017). Soxhlet was recorded to be more efficient than maceration and ultrasound-assisted extraction in the hexane fraction. On the other hand, methanolic and chloroformic fractions extracted by

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maceration exhibited higher phenolic contents than those obtained by Soxhlet or ultrasound-assisted extraction methods. Similar results were found for *L. sativum* seeds which revealed that methanol extract prepared by maceration has maximum total phenolic content compared to water and ethanol extracts (Aydemir and Becerik, 2011). However, the efficiency of the maceration method could be related to the extraction time which represents an important parameter. According to Fan et al. (2020), the degradation or polymerization reaction of the phenolic compounds could be provoked by extraction duration and the longer extraction time increased the total phenolic content. Furthermore, higher TPC of root fractions were obtained by Soxhlet compared to the other two applied methods.

The total flavonoid contents of *L. sativum* fractions obtained by three different extraction methods are reported in *Figure 1B*. Findings showed that the highest content of flavonoids was achieved for the chloroformic fraction of roots (26.04 µg QE/mg), while it is lower for the aqueous fraction of aerial parts prepared by maceration. Similar results reported that the chloroformic extract of *Merremia borneensis* leaves had the highest TFC compared to hexane and water extracts (Amzad Hossain and Shah, 2015). Both for aerial parts and roots, there were no significant differences *($P > 0.05$)* between the content of flavonoids and the studied extraction methods in the hexane fraction. On the other hand, the total flavonoid contents were higher in root fractions than in aerial parts fractions. Results also indicated that fractions from roots prepared by Soxhlet exhibited higher contents than those prepared by maceration or ultrasound-assisted extraction methods. On the contrary, these two last techniques were the most effective methods of extracting flavonoids from aerial parts than Soxhlet. Possibly, these findings could be related to the effect of the temperature factor as it had a significant effect on TPC and TFC according to Alide et al. (2020) who reported that the TFC of aqueous garlic extracts increased in cooking temperature. On the opposite, some studies revealed that excessive temperature might also degrade and decompose some phenolic compounds, causing lower contents of these compounds (Hasni et al., 2021). Thus, this might explain the significant reduction of TFC in aerial part fractions when using the Soxhlet method. Moreover, from these findings, it is estimated that flavonoids present in aerial parts were more sensitive to heat than those present in roots which might confirm the variations in the phenolic composition between them.

For the determination of the contents of condensed tannin, the results revealed that in almost all *L. sativum* fractions obtained with maceration, Soxhlet or ultrasound-assisted extraction methods were statistically similar ($P > 0.05$) (*Fig. 1C*) which means that the extraction technique had no effect on the concentration of CTC. Moreover, the tannin contents in the hexane and chloroformic fractions (9.2 ± 7.2 and 8.04 ± 0.1 µg CE/mg, respectively) obtained from aerial parts were important compared to other analyzed fractions. From the obtained results it can be observed that the CTC are not dependent on the solvent polarity. In addition, the tannin contents of different fractions from aerial parts and roots of *L. sativum* were very low compared to TPC and TFC contents. Based on the used parts, results indicate that the high content of tannin was recorded by aerial parts fractions compared to root fractions where it was very low.

In view of TPC, TFC and CTC results, extraction of *L. sativum* using different parts, solvents and extraction methods extracted different and diverse groups or classes of phenolic compounds.

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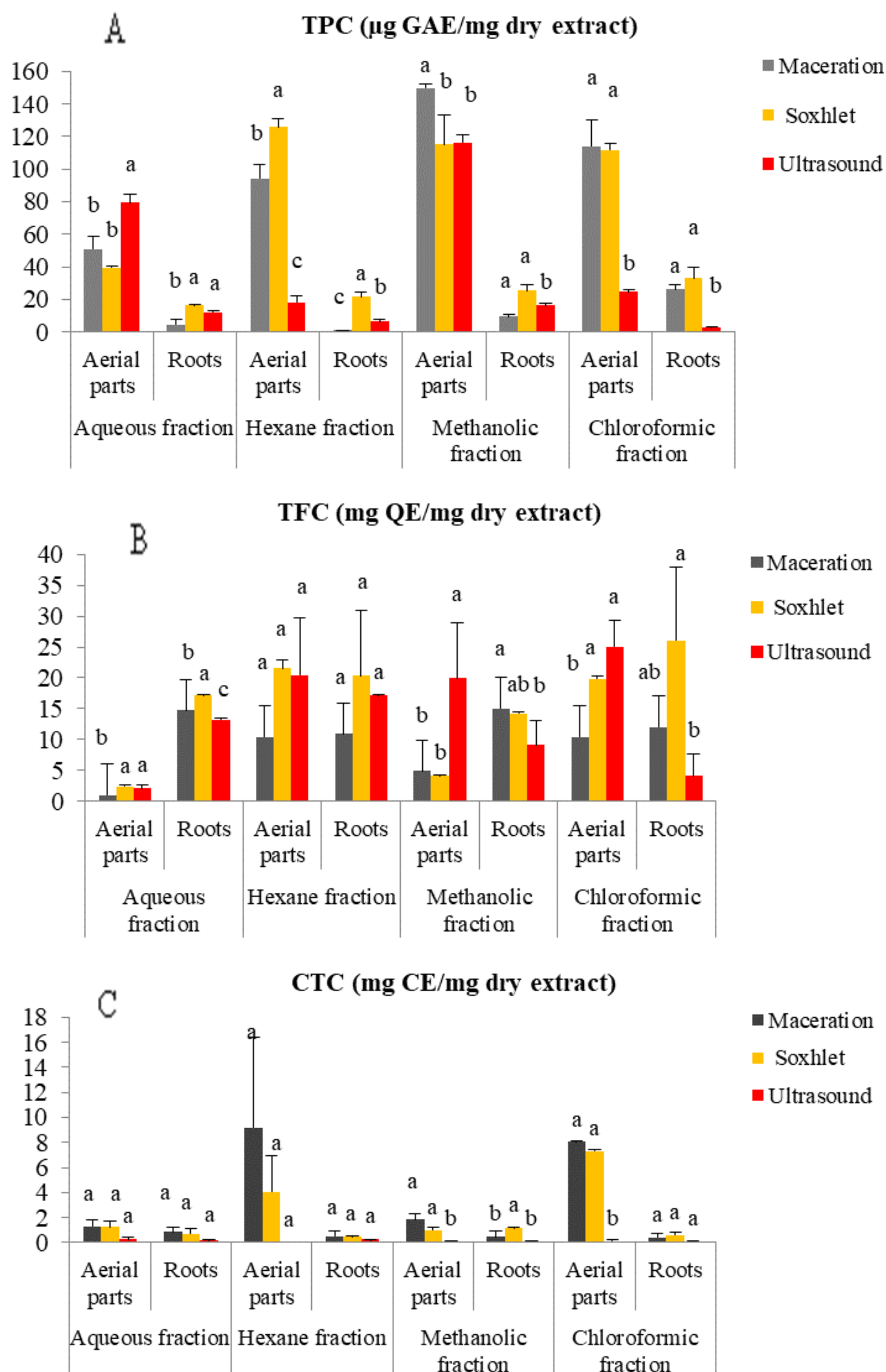

Figure 1. *Effect of extraction techniques and solvents on (A): the total phenolic content (TPC), (B): the total flavonoids content (TFC) and (C): the condensed tannin content (CTC). Data are expressed as Mean ± SD, 3 replicates per sample. For each fraction, means in bars followed by a different letter are significantly different (P < 0.05, one-way ANOVA followed by Tukey's test)*

HPLC-DAD analysis

Methanolic fractions of *L. sativum* aerial parts obtained via maceration and Soxhlet extraction methods were analyzed by HPLC-DAD. Overall, thirty

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compounds were detected and identified, which belonged mainly to phenolic acids and flavonoids classes. In general, the methanolic fraction obtained by Soxhlet was the richest in bioactive compounds compared to that prepared by maceration, as it had a variable profile. These results showed a difference from those obtained from total phenolic content determination which indicated that fractions prepared by maceration have high phenolic content than those prepared by Soxhlet. This can be explained by the fact that HPLC analysis is more specific, while spectrophotometric tests are more general and practically any compound could react with the reagent (Prokopiou et al., 2021).

Among these detected compounds, eight phenolic acids including syringic, iso-vanillic, resorcylic, vanillic, dinitrosalicylic, n-hydroxycinnamic, salicylic and 3,4,5-trimethoxy benzoic acids, five flavonoids namely catechin, orientin, vitexin-2-O-rhamnoside, rutin, quercetin-3-B-D-glycoside as well as aesculetin (6,7-dihydroxycoumarin) as coumarin derivatives and resorcinol as biphasic phenolic compounds were detected in both fractions analyzed whether prepared by maceration or Soxhlet.

On the other hand, 1,2-di-hydroxy-benzene and cinnamic acid were discovered only in the methanolic fraction obtained by maceration (*Fig. 2 A*). Compounds such as gallic acid, hydroxyquinone, vanillin and o-anisic acid as benzoic acid derivatives as well as di-hydroxycinnamic, caffeic, sinapic, 3-hydroxy-4-methoxycinnamic acids as cinnamic derivatives, plus, luteolin-7-glycoside, quercetin 4′-glucoside, myrecetin and caffeine were identified only in the fraction prepared by Soxhlet (*Fig. 2 B*).

By comparing the two HPLC profiles, the results showed a qualitative variability between the analyzed methanolic fractions obtained by different extraction techniques. Thus, it is suggested that the extraction method could influence the phenolic composition. Moreover, this variation may be also affected and influenced by the temperature factor which is applied in the Soxhlet technique. According to De Silva et al. (2010), structural variation of secondary metabolites can be achieved through enzymatic transformations such as methylation and hydroxylation due to the temperature effect. In their study, Kim et al. (2012) reported also that the content of caffeic acid derivatives from *Ligularia fescheri* increased when extracted with hot water.

By comparing our results to the literature, we found that eight of the detected compounds including gallic, caffeic, sinapic, cinnamic, salicylic, vanillic and hydroxycinnamic acids as well as quercetin have been previously identified in *L. sativum* seeds and callus extracts (Zia-Ul-Haq et al., 2012; Nayak et al., 2012; Elguera et al., 2013; Asad Ullah et al., 2019). Whereas, seventeen compounds namely syringic, iso-vanillic, resorcylic, dinitrosalicylic, 3,4,5-trimethoxy benzoic, 3-hydroxy-4-methoxycinnamic acids, catechin, orientin, vitexin-2-O-rhamnoside, rutin, aesculetin, resorcinol, 1,2-di-hydroxy-benzene, luteolin-7-glycoside, myrecetin, caffeine and vanillin, were identified and recorded for the first time in *L. sativum* aerial parts.

In general, HPLC-DAD analysis showed that the percentage of phenolic acids in the methanolic fractions obtained by maceration or Soxhlet methods in the total content of phenolic compound was higher than that of flavonoids. These obtained results are in accordance with those obtained by quantitative determination of the TFC which revealed low contents.

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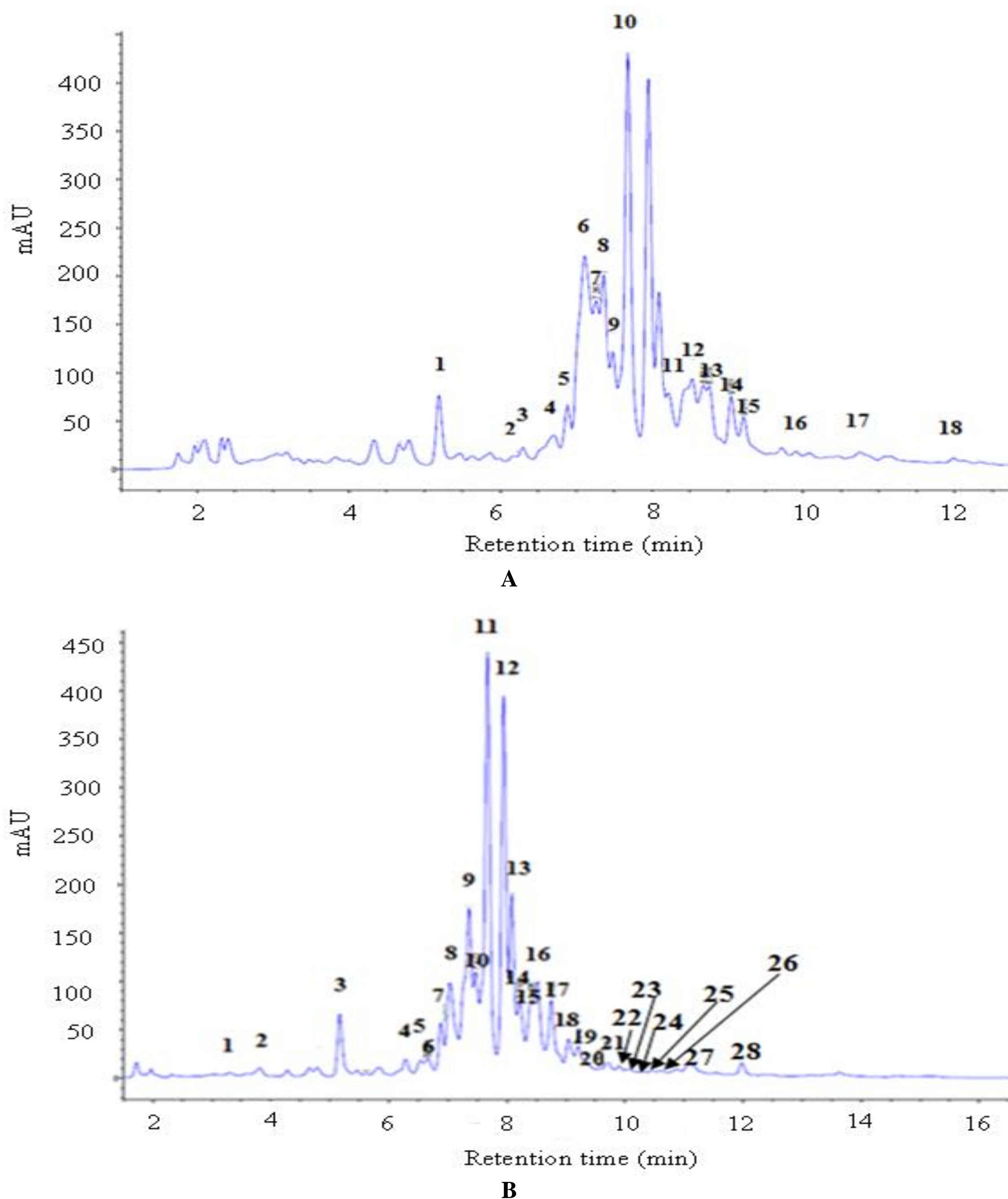

Figure 2. *HPLC profile of methanolic fraction of L. sativum aerial parts obtained by (a) the maceration and (b) Soxhlet assisted-extraction methods. (A) 1: resorcinol, 2: 1,2-di-hydroxy-benzene, 3: catechin, 4: resorcylic acid, 5: vanillic acid, 6: aesculetin, 7: syringic acid, 8: iso-vanillic acid, 9: dinitrosalicylic acid, 10: p-hydroxybenzaldehyde, 11: orientin, 12: vitexin-2-O-rhamnoside, 13: n-hydroxycinnamic acid, 14: rutin, 15: quercetin-3-B-D-glycoside, 16: salicylic acid, 17: 3,4,5-trimethoxybenzoic acid, 18: cinnamic acid. (B) 1: gallic acid, 2: hydroxyquinone, 3: resorcinol, 4: catechin, 5: caffeine, 6: resorcylic acid, 7: aesculetin, 8: di-hydroxycinnamic acid, 9: caffeic acid, 10: vanillic acid, 11: iso-vanillic acid, 12: syringic acid, 13: dinitrosalicylic acid, 14: p-hydroxybenzaldehyde,, 15: orientin, 16: vitexin-2-o-rhamnoside, 17: vanillin, 18: n-hydroxycinnamic acid, 19: rutin, 20: sinapic acid, 21: quercetin-3-b-d-glycoside, 22: luteolin-7-glycoside, 23: salicylic acid, 24: 3-hydroxy-4-methoxycinnamic acid, 25: o-anisic acid, 26: quercetin 4'-glucoside, 27: 3,4,5-trimethoxybenzoic acid, 28: myrecetin*

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Antioxidant activity evaluation

The DPPH inhibition percentages of *L. sativum* fractions obtained with different organs and extraction techniques are reported in *Figure 3*. Overall, results showed that the percent of inhibition increases with increasing fractions concentration. For aerial parts, results indicated that fractions obtained by the ultrasound-assisted extraction exhibited greater inhibitory effects than those prepared by maceration and Soxhlet methods. In contrast, the Soxhlet extraction demonstrated the maximum scavenging effect of DPPH compared to the maceration method for roots while the ultrasound method recorded the lowest scavenging percentages. However, the aqueous fraction of the roots showed nearly the same scavenging effect for all tested techniques. On the other hand, the highest inhibition of DPPH radical was obtained with methanol.

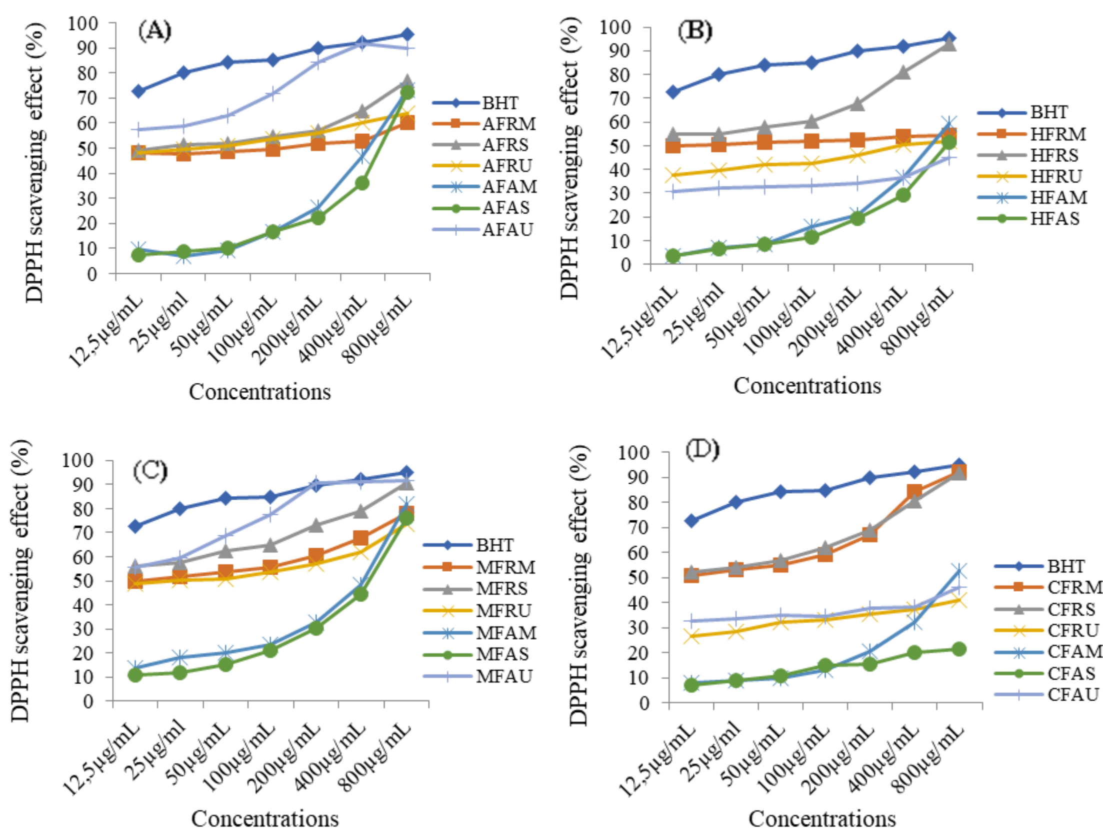

Figure 3. *DPPH scavenging effect of L. sativum (A) aqueous, (B) hexane, (C) methanolic and (D) chloroformic fractions from aerial parts and roots obtained with different extraction techniques. AF: aqueous fraction, HF: hexane fraction, MF: methanolic fraction, CF: chloroformic fraction, A: aerial parts, R: roots, M: maceration, S: soxhlet, U: ultrasound. 3 replicates per sample. All data was significant when compared to standard*

Similar to DPPH test results, the ABTS scavenging effect percentages of *L. sativum* different fractions raised with increasing concentrations of each fraction (*Fig. 4*). Results showed that the aqueous fraction from aerial parts was the most effective in inhibition ABTS free radicals for all tested methods, and it was even higher than the positive control BHT (87.31, 89.85 and 93.48%, respectively at 200, 400 and 800 µg/mL) in the case of using ultrasound-assisted extraction technique (91.51, 92.79

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and 95.19%, respectively at the same tested concentrations). Contrariwise, the aqueous fraction from roots exhibited weak efficiency compared to BHT. However, extraction with maceration and Soxhlet recorded an important scavenging effect in hexane fraction from aerial parts compared to this obtained with ultrasound-assisted extraction which also showed a low and poor effect. Likewise, this last method exerts the weakest ABTS scavenging capacity for the hexane fraction obtained from roots while the maceration method achieved the highest capacity. In addition, for all tested methods, the scavenging ability of the methanolic fraction was remarkable and almost similar for both aerial parts and roots at the highest studied concentrations. Moreover, the effectiveness of chloroformic fractions from both aerial parts and roots to inhibit ABTS radicals was higher with maceration and Soxhlet whereas it was lesser and weaker with the ultrasound-assisted extraction method.

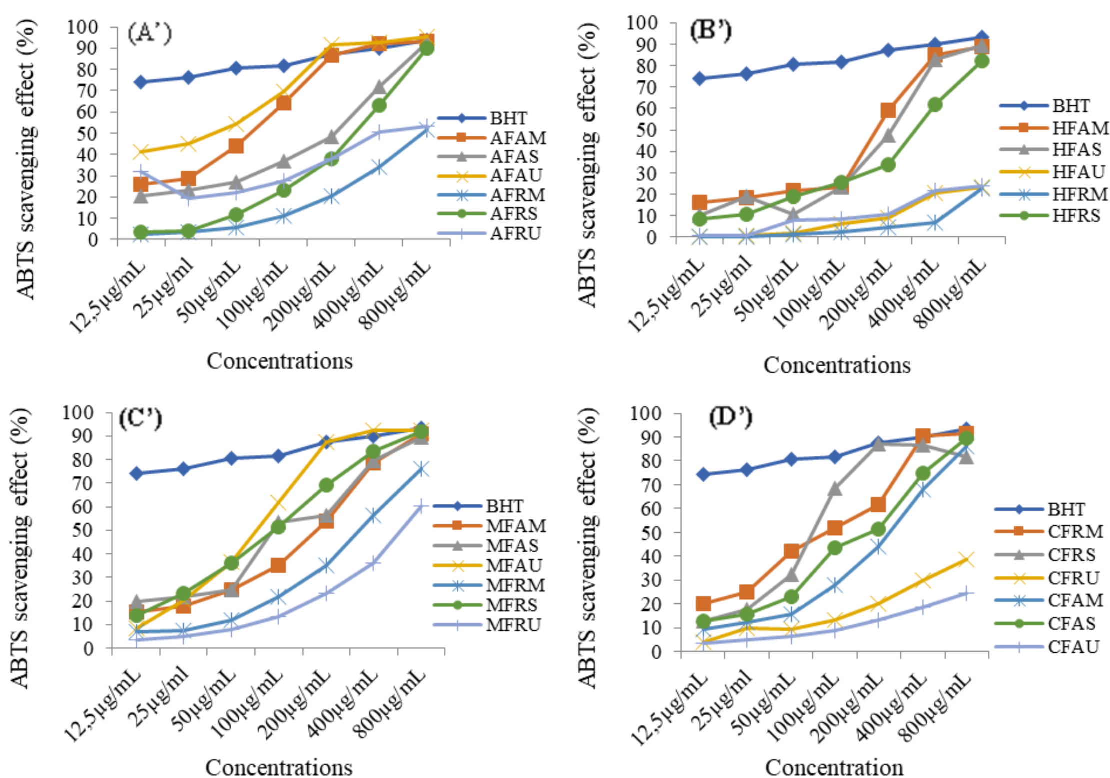

Figure 4. *ABTS scavenging effect of L. sativum (A') aqueous, (B') hexane, (C') methanolic and (D') chloroformic fractions from aerial parts and roots obtained with different extraction techniques. AF: aqueous fraction, HF: hexane fraction, MF: methanolic fraction, CF: chloroformic fraction, A: aerial parts, R: roots, M: maceration, S: soxhlet, U: ultrasound. 3 replicates per sample. All data was significant when compared to standard*

Phenanthroline and reducing power assays were tested to investigate the capability of plant fractions to reduce metallic irons.

The results of the phenanthroline assay showed that the absorbance values of *L. sativum* various fractions were increased with raising concentrations of fractions (*Fig. 5*). As shown in this figure, the positive control BHT was more effective than all tested fractions. For aerial parts, methanolic and chloroformic fractions obtained by ultrasound-assisted extraction and Soxhlet methods respectively gave the highest

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absorbance values compared to other fractions. Furthermore, extraction with Soxhlet recorded also great absorbance values for all root fractions whereas extraction with ultrasound showed the lowest values.

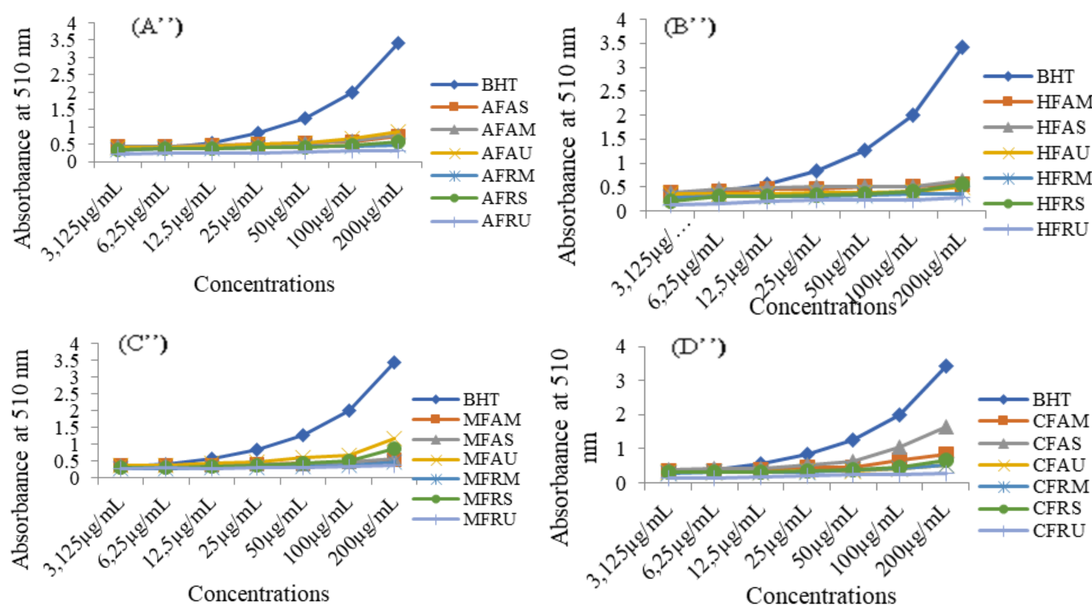

Figure 5. *Absorbance values of L. sativum (A'') aqueous, (B'') hexane, (C'') methanolic and (D'') chloroformic fractions in phenanthroline assay at 510 nm. AF: aqueous fraction, HF: hexane fraction, MF: methanolic fraction, CF: chloroformic fraction, A: aerial parts, R: roots, M: maceration, S: soxhlet, U: ultrasound. 3 replicates per sample. All data was significant when compared to standard*

The reducing power of plant extracts was determined using Fe^{+3} to Fe^{+2} reduction assay, whereby the yellow color of the test solution changes to various shades of green which depends on the reduction capacity of the tested extract (Qingming et al., 2010). The results revealed that ascorbic acid as a positive control was more effective than all the tested fractions. In addition, aqueous, methanolic and chloroformic fractions from aerial parts prepared respectively by maceration, Soxhlet and ultrasound-assisted extraction exhibited the highest absorbance values at 200 µg/mL compared to the other fractions (*Fig. 6*).

Over and above, IC_{50} values were determined from the inhibition percentage of DPPH and ABTS radicals curves while $A_{0.5}$ values were calculated from the linear regression graph of absorbance resulting from phenanthroline and reducing power tests (*Table 2*). Meanwhile, plant fraction with low IC_{50} or $A_{0.5}$ values is considered to have potent antioxidant activity.

For the DPPH assay, hexane, chloroformic and methanolic root fractions followed by aqueous and methanolic aerial parts fractions showed the lowest IC_{50} ($IC_{50} < 12.5$ µg/mL) values referring to strong antioxidant activity. Additionally, these fractions present the same capacity as the positive control BHT. Moreover, methanolic and hexane root fractions obtained with maceration exhibited also great antioxidant capacities which were characterized by the low IC_{50} values ($IC_{50} = 13.9 \pm 0.67$ µg/mL and $IC_{50} = 17.73 \pm 0.6$ µg/mL respectively). It is observed also that fractions from roots

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generally have a higher DPPH scavenging effect than those from aerial parts for all examined extraction techniques. From the obtained results of the TPC, TFC and CTC analysis, it is suggested that the DPPH scavenging activity was associated with the TFC which was found to be higher in root fractions.

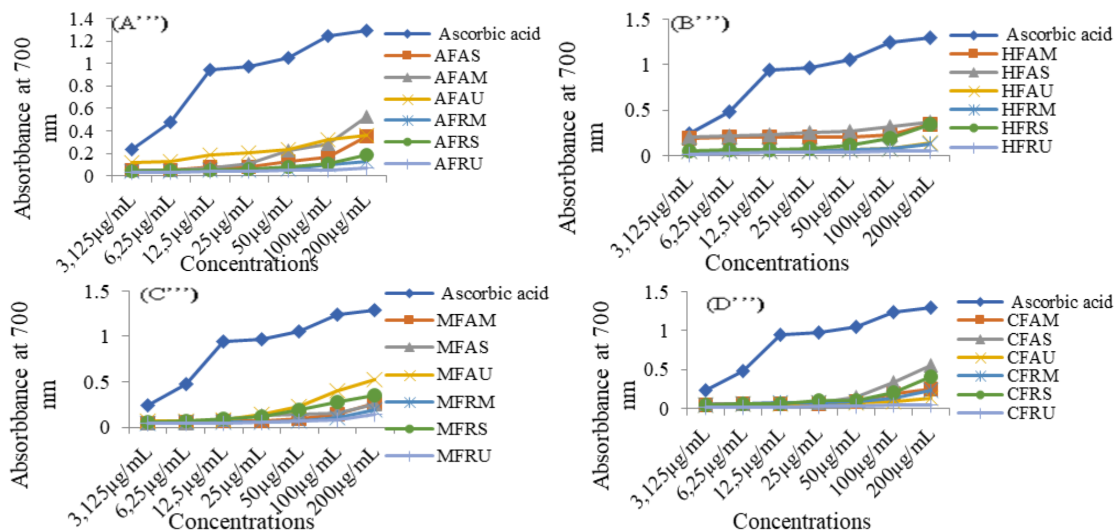

***Figure 6.** Absorbance values of L. sativum (A''') aqueous, (B''') hexane, (C''') methanolic and (D''') chloroformic fractions in reducing power assay at 700 nm. AF: aqueous fraction, HF: hexane fraction, MF: methanolic fraction, CF: chloroformic fraction, A: aerial parts, R: roots, M: maceration, S: soxhlet, U: ultrasound. 3 replicates per sample. All data was significant when compared to standard*

The scavenging activity assessed by ABTS showed that the aqueous aerial parts fraction extracted by the ultrasound-assisted extraction was the most powerful (IC_{50} = 38.18 ± 2.05 µg/mL) compared to other tested fractions but its capacity was lower than BHT as a positive control with IC_{50} < 12.5 µg/mL. In addition, aqueous, chloroformic and methanolic aerial parts fractions extracted respectively by maceration, Soxhlet and ultrasound-assisted extraction showed good antiradical activity (IC_{50} = 67.50 ± 1.05, 72.44 ± 2.8, 77.29 ± 1.4 µg/mL, respectively). Conversely to DPPH test findings, results revealed in the main that aerial parts fractions have higher ABTS scavenging activity than those obtained from roots. The obtained results are in accordance with the amount of TPC and CTC present in the aerial parts fractions. On the other hand, aerial parts and root fractions display different activity against DPPH and ABTS tests. This difference may due to the fact that ABTS radicals detect the antioxidant capacity of both hydrophobic and hydrophilic substances, while DPPH detects primary hydrophobic antioxidants (Rafińska et al., 2019).

Globally, the chelating effect investigation evaluated by phenanthroline showed that aerial parts were more efficient than roots for all tested techniques. The lowest values of $A_{0.5}$ were obtained by aqueous fraction prepared with ultrasound ($A_{0.5}$ = 22.11 ± 0.9 µg/mL), followed by chloroformic and hexane fractions prepared with Soxhlet ($A_{0.5}$ = 23.20 ± 1.7 µg/mL and $A_{0.5}$ = 23.55 ± 0.9 µg/mL, respectively). Furthermore, the methanolic fraction obtained by the ultrasound-assisted extraction that records the highest absorbance presents also a good antioxidant capacity. BHT exhibited excellent antioxidant activity ($A_{0.5}$ = 9.71 ± 0.9 µg/mL) and it is very high

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than the tested fractions. Concerning the reducing power assay, results revealed that all fractions of *L. sativum* had low and weak antioxidant activities where the $A_{0.5}$ values were very high compared to ascorbic acid ($A_{0.5} = 6.52 \pm 0.07$ µg/mL). Although the reduction activities from *L. sativum* fractions were less than those observed by the positive standard, this study revealed that the *L. sativum* aerial parts and roots have primarily antioxidant capacity.

Table 2. *IC_{50} and $A_{0.5}$ values of antioxidant activity of L. sativum fractions obtained with different extraction techniques*

Extraction technique	Samples	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	Phen $A_{0.5}$ (µg/mL)	RP $A_{0.5}$ (µg/mL)
Maceration	BHT	< 12.5	< 12.5	9.71 ± 0.9^{k}	-
	Ascorbic acid	-	-	-	6.52 ± 0.07^{c}
	AFA	481.82 ± 9.48^{de}	67.50 ± 1.05^{m}	26.2 ± 0.3ij	189.41 ± 0.7^{a}
	AFR	106.3 ± 11.07^{g}	754.38 ± 1.9^{a}	> 200	> 200
	HFA	675.67 ± 6.12^{b}	174.20 ± 5.2^{i}	42.06 ± 2.2^{i}	> 200
	HFR	17.73 ± 0.6^{h}	> 800	> 200	> 200
	MFA	414.41 ± 20.9^{f}	178.70 ± 9.1^{i}	144.67 ± 6.2^{de}	> 200
	MFR	13.9 ± 0.67^{h}	341.12 ± 5.7^{d}	> 200	> 200
	CFA	778.74 ± 46.3^{a}	90.44 ± 0.3^{kl}	51.08 ± 1.6^{h}	> 200
	CFR	< 12.5	259.27 ± 1.6^{f}	161.66 ± 1.9^{cd}	> 200
Soxhlet	AFA	543.01 ± 11.9^{c}	213.11 ± 2.2^{h}	26.32 ± 8.5^{ij}	> 200
	AFR	21.34 ± 5.2^{h}	298.65 ± 1.4^{e}	123.99 ± 0.3^{ef}	> 200
	HFA	773.06 ± 13.1^{a}	226.96 ± 1.9^{g}	23.55 ± 0.9	> 200
	HFR	< 12.5	310.21 ± 6.4^{e}	158.80 ± 2.7^{cd}	> 200
	MFA	463.14 ± 3.7^{ef}	96.28 ± 3.9^{jk}	125.78 ± 0.8^{ef}	> 200
	MFR	< 12.5	107.27 ± 2.1^{j}	85.65 ± 2.7^{g}	> 200
	CFA	> 800	72.44 ± 2.8^{m}	23.20 ± 1.7^{ij}	169.38 ± 1.2^{b}
	CFR	< 12.5	178.22 ± 2.3^{i}	111.91 ± 1.2^{f}	> 200
Ultrasound	AFA	< 12.5	38.18 ± 2.05^{n}	22.11 ± 0.9^{j}	> 200
	AFR	36.8 ± 3.1^{h}	594.31 ± 13.1^{c}	> 200	> 200
	HFA	> 800	> 800	174.18 ± 2.2^{ab}	> 200
	HFR	526.17 ± 49.06^{cd}	> 800	> 200	> 200
	MFA	< 12.5	77.29 ± 1.4^{lm}	30.05 ± 1.01^{ij}	177.51 ± 9.9^{b}
	MFR	25.79 ± 8.8^{h}	631.26 ± 5.8^{b}	> 200	> 200
	CFA	> 800	> 800	167.61 ± 2.6b^{c}	> 200
	CFR	> 800	> 800	> 200	> 200

Data are shown as mean ± DS, $n = 3$, Means in each column followed by different letters (a-n) are considered significant ($P<0.05$, one-way ANOVA followed by Tukey's test), AFA: aqueous fraction from aerial parts, AFR: aqueous fraction from roots, HFA: hexane fraction from aerial parts, HFR: hexane fraction from roots, MFA: methanolic fraction from aerial parts, MFR: methanolic fraction from roots, CFA: chloroformic fraction from aerial parts, CFR: chloroformic fraction from roots

Aydemir and Becerik (2011) reported that the methanolic extract of *L. sativum* seeds showed chelating effects on Fe^{+2} ($IC_{50} = 137.19$ µg/mL) and DPPH scavenging activity ($IC_{50} = 318.91$ µg/mL). Moreover, our results were in agreement with those reported by Rafińska et al. (2019) who showed that seed extracts had the lowest inhibitory

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concentrations for DPPH and ABTS methods and the most effective extraction technique was maceration and ultrasound-assisted extraction.

Correlation between antioxidant activities, yields and polyphenolic contents

Pearson's coefficients were calculated to identify the correlative relationship between the antioxidant effects assayed by DPPH, ABTS, phenanthroline and reducing power tests, and the total phenolic compounds contents as well as extraction yields in *L. sativum* fractions obtained with different extraction techniques (*Table 3*).

Table 3. *Matrix of correlation between antioxidant assays, extraction yields, TPC, TFC and CTC*

	Yield	TPC	TFC	CTC	DPPH	ABTS	Phen
TPC	0.64*						
TFC	-0.35	-0.14					
CTC	0.13	0.58*	-0.01				
DPPH	0.09	0.35	-0.02	0.52*			
ABTS	-0.45*	-0.69*	0.21	-0.42*	0.10		
Phen	-0.24	-0.68*	0.14	-0.55*	-0.26	0.80*	
RP	-0.21	-0.39	-0.17	-0.28	-0.13	0.35	0.46*

*Significant correlation with P<0.05. TPC: total phenolic content, TFC: total flavonoids content, CTC: condensed tannin content, Phen: phenanthroline, RP: reducing power

Results showed a significant and positive correlation between the extraction yield and the total phenol contents ($r = 0.64$, $p < 0.05$), and a negative correlation with ABTS scavenging activity ($r = -0.45$, $p < 0.05$). The total phenol content was positively correlated with the tannin content ($r = 0.58$, $p < 0.05$) and DPPH scavenging activity ($r = 0.35$) while it correlated negatively with ABTS ($r = -0.69$, $p < 0.05$) and phenanthroline ($r = -0.68$, $p < 0.05$) assays. Although many studies reported a strong association between the total flavonoid contents and antioxidant activity (Bhardwaj et al., 2020), our results indicated that the correlation coefficients between the total flavonoid contents and antioxidant activities measured by DPPH, ABTS, phenanthroline and reducing power were very low and weak. The tannin contents were correlated positively with DPPH ($r = 0.52$, $p < 0.05$) and negatively with ABTS ($r = -0.42$, $p < 0.05$) and phenanthroline ($r = -0.55$, $p < 0.05$). A strong, significant and positive correlation was observed between phenanthroline and ABTS ($r = 0.80$, $p < 0.05$). Also, there was a positive and significant correlation between phenanthroline and reducing power ($r = 0.46$, $p < 0.05$).

Based on the correlation results, the DPPH scavenging activity of *L. sativum* fractions obtained by different solvents and extraction methods was influenced by the extraction yield, total phenolic as well as by the condensed tannin contents while the antioxidant capacity evaluated using ABTS and phenanthroline assays was found to be more affected by the TFC. According to Odabasoglu et al. (2005), the antioxidant activity determination can be influenced also by the role of non-phenolic secondary metabolites because the applied antioxidant activity methods are not specific to the phenolic group compounds only. Also, individual phenolic compounds may have different antioxidant activities, and their interactions with macromolecules such as carbohydrates and proteins may be synergistic or antagonistic.

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Multivariate analysis

Principal component analysis (PCA) and cluster analysis (CA) were conducted to reveal groupings, similarities or differences among all tested studied plant fractions extracted by three extraction techniques according to their antioxidant capacities assayed by DPPH, ABTS, phenanthroline and reducing power assays, extraction yield, total phenol, total flavonoids and condensed tannin contents. PCA is a multidimensional descriptive statistical method that shows the distribution of variables (loading plot) and samples (score plot). However, CA is performed to define how studied samples clustered together depending on the variables.

In PCA plots, the first two principal components axis account for 61.90% of the total variance (*Table 4*). PC1 describes 44.23% of total variability with the highest eigenvalue (3.53) and had high positive loadings of phenanthroline (0.45) and ABTS (0.43) as well as negative loadings of total phenol content (-0.48), tannin content (-0.37) and extraction yield (-0.30). Since their loadings had the opposite sign on PC1, the combination of these variables suggested that the content of polyphenolic compounds and extraction yield had no influence on antioxidant activity assayed by phenanthroline or ABTS methods. PC2 accounted for 17.67% of the total variance and displays high positive loadings of extraction yield (0.43) and high negative loadings of TFC (-0.56), tannin content (-0.37) and DPPH (-0.48). Thus, it seems that the DPPH scavenging capacity is related to the flavonoids and tannin contents not to the extraction yield.

Table 4. *Loadings values of principal component analysis for the polyphenolic contents and antioxidant activities of L. sativum fractions obtained by different extraction techniques*

Variables	Loadings							
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Yield	-0.30	0.44	-0.16	-0.62	0.24	0.07	0.43	0.25
TPC	-0.48	0.04	-0.07	-0.18	0.30	-0.12	-0.79	-0.03
TFC	0.12	-0.56	0.47	-0.25	0.57	-0.17	0.16	0.04
CTC	-0.37	-0.37	-0.22	0.28	0.18	0.73	0.12	0.14
DPPH	-0.20	-0.48	-0.61	-0.17	-0.16	-0.40	0.21	-0.31
ABTS	0.43	-0.25	-0.31	-0.30	-0.16	0.08	-0.28	0.67
Phen	0.46	0.06	-0.17	-0.37	0.16	0.46	-0.16	-0.60
RP	0.29	0.23	-0.45	0.43	0.64	-0.23	0.05	0.09
Eigenvalues	3.54	1.41	1.14	0.78	0.57	0.34	0.14	0.07
% variance	44.23	17.68	14.28	9.76	7.11	4.22	1.80	0.91
% cumulative variance	44.23	61.91	76.19	85.95	93.06	97.28	99.09	100.00

TPC: total phenolic content, TFC: total flavonoids content, CTC: condensed tannin content, Phen: phenanthroline, RP: reducing power

From the PCA loadings plot (*Fig. 7A*), the studied fractions of different parts of *L. sativum* obtained by three different extraction methods were alienated in PC1 based on the differences in their total phenolic content, tannin content and also their capacities to inhibit ABTS radicals and metallic irons. While PC2 was associated with the extraction yield, the TFC, the tannin content and the DPPH scavenging effect.

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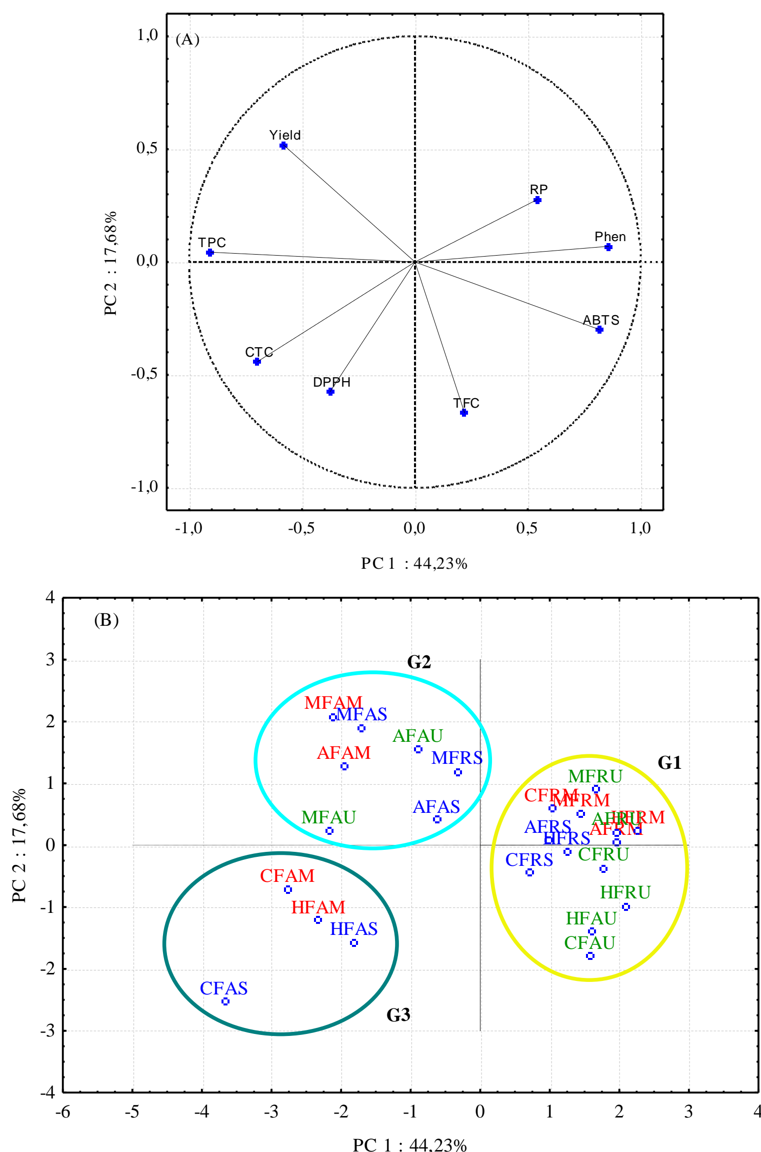

Figure 7. *Principal component analysis loading plot (A) and score plot (B) for L. sativum fractions prepared from aerial parts and roots extracted by maceration (with red color), Soxhlet (with blue color) and ultrasound (with green color) methods based on antioxidant capacities, extraction yields and phenolic compounds contents. AF: aqueous fraction, HF: hexane fraction, MF: methanolic fraction, CF: chloroformic fraction, A: aerial parts, R: roots, M: maceration, S: soxhlet, U: ultrasound*

The projection of studied samples in the score plot revealed three groups (*Fig. 7B*) and as general observations, these groups of samples obtained from different plant parts and prepared by various extraction methods were not well separated. In addition, it sounds that generally these tested samples were grouped depending on their similarity in polyphenolic contents or on their antioxidant activities not according to the applied extraction methods. Furthermore, this plot generally showed that root fractions are separated from aerial parts fractions which means that they are different in their phenolic compositions and antioxidant abilities. Moreover, the PCA plots showed that group 1 (G1) was distributed on the positive side of PC1 and the negative side of PC2. The

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comparison based on the response variables presented in the loading plot showed that the samples of this group could be divided into two sub-groups; the 1st one included all the fractions from roots obtained by maceration, Soxhlet or ultrasound-assisted extraction techniques except the methanolic fraction obtained by Soxhlet is characterized by high ability in scavenging ABTS radicals and chelating metallic irons, while the 2nd one which including hexane roots and aerial parts fractions as well as chloroformic aerial parts fraction is characterized by high TFC. Group 2 (G2) including the aerial parts methanolic and aqueous fractions as well as roots methanolic fraction which were extracted by maceration and Soxhlet methods were scattered on the negative side of PC1 and positive side of PC2 and were expressed by high extraction yields and total phenol contents. These observations showed that using methanol and water as extraction solvents could increase the extraction yields and phenolic concentrations. The third group (G3) contained the aerial parts hexane and chloroformic fractions which were positioned on the negative sides of both PC1 and PC2. This group was characterized by high tannin contents and DPPH scavenging capacities. Thus, these results indicated that tannin content contributed very much to DPPH scavenging activity.

Cluster analysis (CA) using Ward's and Euclidean distance as measurements of similarity between studied samples was employed. CA dendrogram revealed that *L. sativum* different fractions were divided into three clusters at 50% of similarity (*Fig. 8*). Cluster 1 was formed generally by root fractions as well as by aerial parts aqueous and methanolic fractions obtained by the ultrasound-assisted extraction method. Moreover, this cluster could be divided into two sub-clusters; a and b. Cluster 2 was included both aerial parts and root fractions extracted by ultrasound using hexane and chloroform solvents. All fractions from plant aerial parts obtained by maceration and Soxhlet extractions techniques were clustered together (Cluster 3), indicating that these two techniques have some similarities and the same effect on the phenolic contents and antioxidant activities. Additionally, this cluster can be also separated into two sub-clusters (c and d). As illustrated, sub-cluster (c) was composed of fractions prepared using water and methanol, whereas sub-cluster (d) was formed by those prepared using hexane and chloroform. Thus, the CA results confirmed the clustering presented in the PCA score plot.

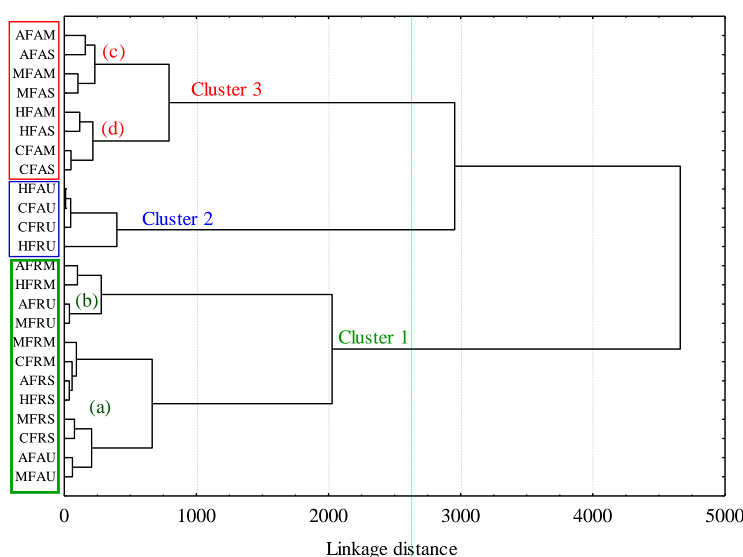

***Figure 8.** Cluster analysis of L. sativum aerial parts and root fractions obtained with different extraction methods and solvents*

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Conclusion

The effect of different extraction techniques and solvents on the contents of bioactive compounds, as well as on the antioxidant activity have been investigated. Our study indicated that *L. sativum* aerial parts and roots are good sources of phenolic compounds and they showed interesting antioxidant properties. Thus, it can be recommended to use them as a natural food preservative. Furthermore, HPLC analysis revealed for the first time the presence of seventeen compounds that were recorded and identified in the aerial parts of this species. Globally, the results of PCA and CA indicated that the quantitative variations in phenolic compounds contents and antioxidant activity were significantly related to the used parts of plant, solvents and extraction methods.

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Declaration of competing interests. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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APPENDIX

ANOVA tables

Extraction yields of L. sativum fractions obtained by different extraction methods

Extraction method	Yields (%)							
	Aqueous fraction		Hexane fraction		Methanolic fraction		Chloroformic fraction	
	Aerial parts	Roots	Aerial parts	Roots	Aerial parts	Roots	Aerial parts	Roots
Maceration	9.03±0.02[a]	3.08±0.0008[a]	2.47±0.01[a]	0.80±0.0002[a]	15.65±0.1[a]	2.46±5.6[c]	6.29±0.01[a]	0.501±0.002[a]
Soxhlet	0.64±0.01[c]	0.53±0.01[b]	1.98±0.01[b]	0.39±0.003[b]	12.59±0.03[b]	8.27±0.004[a]	3.903±0.3[ab]	0.57±0.03[a]
Ultrasound	1.14±0.1[b]	0.302±0.02[c]	2.12±0.002[b]	0.267±0.01[c]	7.69±0.7[c]	3.95±0.3[b]	1.82±0.04[b]	0.28±0.2[a]

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DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
AAM	3	2.70993	0.90331	0.000370057		
AAS	3	0.18838	0.062793333	8.95745E-05		
AAU	3	0.34296	0.11432	2.88889E-34		

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between groups	1.33162848	2	0.665814242	4345.744968	3.28301E-10	5.14325285
Within groups	0.00091926	6	0.000153211			
Total	1.33254775	8				

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
ARM	3	0.92526	0.30842	3.249E-07		
ARS	3	0.16033	0.05344333	0.0001049		
ARU	3	0.0905	0.03016667	3.3333E-09		

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between groups	0.14297982	2	0.07148991	2038.08767	3.18E-09	5.14325285
Within groups	0.00021046	6	3.5077E-05			
Total	0.14319028	8				

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance	
HAM	3	0.74246	0.247486667	0.000294799	
HAS	3	0.59619	0.19873	0.000147502	
HAU	3	0.63697	0.212323333	1.24633E-07	

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability
Between groups	0.00379845	2	0.001899226	12.87828099	0.006744555
Within groups	0.00088485	6	0.000147475		
Total	0.0046833	8			

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
HRS	3	0.11838	0.03946	4.6683E-06		
HRU	3	0.0791	0.02636667	3.3333E-07		

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between groups	0.00472921	2	0.00236461	1407.04721	9.6308E-09	5.14325285
Within groups	1.0083E-05	6	1.6805E-06			
Total	0.00473929	8				

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
MAM	3	4.691	1.563666667	0.015016333		
MAS	3	3.76981	1.256603333	0.000536032		
MAU	3	2.30859	0.76953	0		

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VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	0.96218137	2	0.481090684	92.80080551	3.07083E-05	5.14325285
Within groups	0.03110473	6	0.005184122			
Total	0.9932861	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
MRM	3	0.73208	0.24402667	1.2162E-05		
MRS	3	2.47567	0.82522333	3.0928E-05		
MRU	3	1.18605	0.39535	9.25E-08		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	0.5454794	2	0.2727397	18948.035	3.967E-12	5.14325285
Within groups	8.6365E-05	6	1.4394E-05			
Total	0.54556576	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CAM	3	1.88751	0.62917	7.77259E-05		
CAS	3	1.17103	0.390343333	0.061357768		
CAU	3	0.54723	0.18241	0		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	0.29986895	2	0.149934473	7.321556097	0.024554338	5.14325285
Within groups	0.12287099	6	0.020478498			
Total	0.42273993	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CRM	3	0.1506	0.0502	4E-08		
CRS	3	0.172719	0.057573	0.00061675		
CRU	3	0.08688	0.02896	0		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	0.0013242	2	0.0006621	3.22038737	0.11217898	5.14325285
Within groups	0.00123358	6	0.0002056			
Total	0.00255778	8				

Effect of extraction technique and solvents on TPC

Extraction method	**TPC µg GAE/mg dry extract**							
	Aqueous fraction		**Hexane fraction**		**Methanolic fraction**		**Chloroformic fraction**	
	Aerial parts	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**
Maceration	50.98±7.6b	4.56±3.1b	94.35±8.2b	0.93±0.09c	149.84±2.1a	9.27±1.5a	114.01±16.5a	26.12±2.6a
Soxhlet	39.5±0.9b	16.47±0.7a	125.70±5.6a	21.76±2.7a	115.03±18.3b	25.55±3.4a	111.86±3.7a	32.82±6.5a
Ultrasound	79.47±.3a	12.16±0.7a	18.2±4.03c	6.38±1.04b	116.48±4.9b	16.38±1.04b	24.58±1.3b	2..91±0.01b

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DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
AAM	3	152.94	50.98	59.1996		
AAS	3	118.5	39.5	0.8848		
AAU	3	238.42	79.4733333	28.5637333		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	2541.52782	2	1270.76391	43.0047604	0.000277304	5.14325285
Within groups	177.296267	6	29.5493778			
Total	2718.82409	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
ARM	3	13.68	4.56	10.0828		
ARS	3	49.42	16.4733333	0.61853333		
ARU	3	36.5	12.1666667	0.56333333		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	218.336267	2	109.168133	29.0735989	0.00081832	5.14325285
Within groups	22.5293333	6	3.75488889			
Total	240.8656	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
HAM	3	283.06	94.3533333	67.2401333		
HAS	3	377.12	125.706667	31.3665333		
HAU	3	54.6	18.2	16.2948		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	18340.0451	2	9170.02253	239.42312	1.89514E-06	5.14325285
Within groups	229.802933	6	38.3004889			
Total	18569.848	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
HRM	3	1.88	0.62666667	0.28093333		
HRS	3	65.28	21.76	7.8388		
HRU	3	19.16	6.38666667	1.09293333		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	716.134756	2	358.067378	116.600564	1.5782E-05	5.14325285
Within groups	18.4253333	6	3.07088889			
Total	734.560089	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
MAM	3	449.52	149.84	4.6272		
MAS	3	322.18	107.393333	343.696133		
MAU	3	320.1	106.7	131.1804		

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VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	3663.25982	2	1831.62991	11.4595348	0.008931015	5.14325285
Within groups	959.007467	6	159.834578			
Total	4622.26729	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
MRM	3	27.82	9.27333333	2.48253333		
MRS	3	76.66	25.5533333	11.9817333		
MRU	3	320.1	106.7	131.1804		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	16341.7753	2	8170.88764	168.30457	5.371E-06	5.14325285
Within groups	291.289333	6	48.5482222			
Total	16633.0646	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CAM	3	327.92	109.306667	203.020133		
CAS	3	335.58	111.86	13.7872		
CAU	3	73.74	24.58	1.7812		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	14802.926	2	7401.46298	101.580758	2.36052E-05	5.14325285
Within groups	437.177067	6	72.8628444			
Total	15240.103	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CRM	3	78.38	26.1266667	6.86093333		
CRS	3	98.46	32.82	43.4992		
CRU	3	8.74	2.91333333	0.00013333		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	1478.06827	2	739.034133	44.0248344	0.00025965	5.14325285
Within groups	100.720533	6	16.7867556			
Total	1578.7888	8				

Effect of extraction technique and solvents on TFC

	TFC							
	Aqueous fraction		**Hexane fraction**		**Methanolic fraction**		**Chloroformic fraction**	
	Aerial parts	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**
Maceration	0.98±0.06b	14.74±0.4b	10.45±0.3a	10.95±0.3a	4.90±0.6b	15±0.3a	10.45±1.2b	12.08±1.5ab
Soxhlet	2.41±0.2a	17.21±0.1a	21.52±1.3a	20.30±10.6a	4.19±0.1b	14.22±0.2ab	19.81±0.5a	26.04±11.8a
Ultrasound	2.12±0.5a	13.27±0.3c	20.44±9.2a	17.19±0.2a	19.90±9.1a	9.13±4.01b	25.11±4.2a	4.16±3.6b

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DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
AAM	3	2.9552	0.98506667	0.00401186		
AAS	3	7.23207	2.41069	0.06027589		
AAU	3	6.366	2.122	0.30566233		

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between groups	3.40836121	2	1.7041806	13.8195448	0.00567441	5.14325285
Within groups	0.73990017	6	0.1233167			
Total	4.14826138	8				

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
ARM	3	44.232	14.744	0.19661131		
ARS	3	51.6427	17.2142333	0.03404464		
ARU	3	39.8123	13.2707667	0.1216764		

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between groups	23.8233985	2	11.9116993	101.4244	2.3711E-05	5.14325285
Within groups	0.70466471	6	0.11744412			
Total	24.5280632	8				

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
HAM	3	31.366	10.4553333	1.61101964		
HAS	3	64.5624	21.5208	1.76977177		
HAU	3	61.34815	20.4493833	86.4825356		

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between groups	223.473522	2	111.736761	3.73022337	0.08856769	5.14325285
Within groups	179.726654	6	29.9544423			
Total	403.200176	8				

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
HRM	3	32.8659	10.9553	0.10770268		
HRS	3	60.92858	20.3095267	113.753674		
HRU	3	51.5982	17.1994	0.05622483		

VARIANCE ANALYSES

Source of variation	Sum of squares	Degree of freedom	Average of squares	F	Probability	Critical value for F
Between groups	136.163229	2	68.0816146	1.79291735	0.24522455	5.14325285
Within groups	227.835202	6	37.9725337			
Total	363.998432	8				

DETAILED REPORT

Groups	Number of samples	Sum	Mean	Variance		
MAM	3	14.723	4.90766667	0.39878282		
MAS	3	12.58921	4.19640333	0.02582288		
MAU	3	59.7142	19.9047333	83.3622673		

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VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	472.169536	2	236.084768	8.45304614	0.0179721 8	5.14325285
Within groups	167.573746	6	27.9289577			
Total	639.743281	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
MRM	3	45	15	0.12677539		
MRS	3	42.6874	14.2291333	0.0722676		
MRU	3	27.41954	9.13984667	16.1406369		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	60.8364713	2	30.4182356	5.58485281	0.04267418	5.14325285
Within groups	32.6793597	6	5.44655995			
Total	93.515831	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CAM	3	31.366	10.4553333	1.61101964		
CAS	3	59.4373	19.8124333	0.34031186		
CAU	3	75.3391	25.1130333	18.4458577		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	330.49985	2	165.249925	24.3048084	0.00132631	5.14325285
Within groups	40.7943783	6	6.79906305			
Total	371.294228	8				

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CRM	3	36.2499	12.0833	2.25794881		
CRS	3	78.1427	26.0475667	141.344881		
CRU	3	12.4909	4.16363333	13.1546881		

VARIANCE ANALYSES

Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	736.628402	2	368.314201	7.04873755	0.02660904	5.14325285
Within groups	313.515036	6	52.252506			
Total	1050.14344	8				

Effect of extraction technique and solvents on CTC

	CT							
	Aqueous fraction		**Hexane fraction**		**Methanolic fraction**		**Chloroformic fraction**	
	Aerial parts	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**	**Aerial parts**	**Roots**
Maceration	1.31±0.5a	0.86±0.4a	9.20±7.2a	0.52±0.4a	1.85±0.5a	0.48±0.4b	8.04±0.1a	0.42±0.3a
Soxhlet	1.26±0.5a	0.70±0.4a	4.05±2.9a	0.53±0.05a	1.01±0.2a	1.21±0.06a	7.31±0.1a	0.59±0.2a
Ultrasound	0.26±0.2a	0.18±0.01a	0.01±0.01a	0.28±0.001a	0.10±0.05b	0.11±0.007b	0.09±0.1b	0.13±0.02a

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DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
AAM	3	3.9316	1.31053333	0.27063481		
AAS	3	3.8681	1.28936667	0.28138917		
AAU	3	0.8038	0.26793333	0.04461634		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	2.13078884	2	1.06539442	5.35696818	0.04626127	5.14325285
Within groups	1.19328066	6	0.19888011			
Total	3.3240695	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
ARM	3	2.595	0.865	0.20335107		
ARS	3	2.1169	0.70563333	0.19500622		
ARU	3	0.5456	0.18186667	0.00015125		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	0.76640041	2	0.3832002	2.88475773	0.13248844	5.14325285
Within groups	0.79701709	6	0.13283618			
Total	1.5634175	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
HAM	3	27.6047	9.20156667	52.6258538		
HAS	3	12.1608	4.0536	8.61182119		
HAU	3	0.04673	0.01557667	0.00014343		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	127.189606	2	63.5948028	3.1154671	0.11805241	5.14325285
Within groups	122.475637	6	20.4126061			
Total	249.665242	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
HRM	3	1.5754	0.52513333	0.16383776		
HRS	3	1.6144	0.53813333	0.00286085		
HRU	3	0.8595	0.2865	2.59E-06		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	0.1204342	2	0.0602171	1.08368324	0.39646753	5.14325285
Within groups	0.33340241	6	0.05556707			
Total	0.45383662	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
MAM	3	5.5604	1.85346667	0.32176945		
MAS	3	3.0575	1.01916667	0.06801342		
MAU	3	0.30628	0.10209333	2.88E-05		

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VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	4.60438854	2	2.30219427	17.7177422	0.00303624	5.14325285
Within groups	0.77962335	6	0.12993723			
Total	5.3840119	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
MRM	3	1.4681	0.48936667	0.19658841		
MRS	3	3.6438	1.2146	0.00473692		
MRU	3	0.3481	0.11603333	5.0573E-05		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	1.87218989	2	0.93609494	13.9454857	0.00554883	5.14325285
Within groups	0.40275181	6	0.0671253			
Total	2.2749417	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CAM	3	24.1219	8.04063333	0.0251401		
CAS	3	21.9462	7.3154	0.39048192		
CAU	3	0.29985	0.09995	0.02275593		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	115.643134	2	57.8215669	395.696678	4.2603E-07	5.14325285
Within groups	0.87675591	6	0.14612599			
Total	116.51989	8				

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
CRM	3	1.2877	0.42923333	0.12683669		
CRS	3	1.795	0.59833333	0.07868233		
CRU	3	0.4145	0.13816667	0.00042356		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	0.32506798	2	0.16253399	2.36765966	0.17458577	5.14325285
Within groups	0.41188518	6	0.06864753			
Total	0.73695316	8				

DPPH scavenging activity

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
AAEM	3	1445.48	481.826667	96.8746333		
AAES	3	1629.03	543.01	142.3575		
AAEU	3	37.4	12.4666667	0.00333333		
RAEM	3	318.9	106.3	61.3089		
RAES	3	53.73	17.91	48.9108		
RAEU	3	110.41	36.8033333	9.65663333		
AHEM	3	2026.94	675.646667	18.7505333		

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AHES	3	2319.2	773.066667	171.630433		
AHEU	3	2403	801	3		
RHEM	3	53.2	17.7333333	0.39263333		
RHES	3	37.4	12.4666667	0.00333333		
RHEU	3	1578.51	526.17	2406.9981		
AMEM	3	1243.25	414.416667	437.867033		
AMES	3	1389.42	463.14	13.8033		
AMEU	3	37.4	12.4666667	0.00333333		
RMEM	3	41.7	13.9	0.4564		
RMES	3	37.6	12.5333333	0.00333333		
RMEU	3	77.37	25.79	78.8947		
ACEM	3	2255.83	751.943333	3227.40163		
ACES	3	2401	800.333333	0.33333333		
ACEU	3	2401	800.333333	0.33333333		
RCEM	3	37.6	12.5333333	0.00333333		
RCES	3	37.7	12.5666667	0.01333333		
RCEU	3	2401	800.333333	0.33333333		
VARIANCE ANALYSES						
Source of variation	*Source of variation*	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*
Between groups	7912521.12	23	344022.657	1228.77427	7.384E-59	1.75675938
Within groups	13438.6665	48	279.972218			
Total	7925959.79	71				

ABTS scavenging activity

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
AAEM	3	202.52	67.5066667	1.10903333		
AAES	3	639.354	213.118	4.901772		
AAEU	3	114.54	38.18	4.2348		
RAEM	3	2263.14	754.38	3.7429		
RAES	3	895.95	298.65	2.1027		
RAEU	3	1782.93	594.31	173.0239		
AHEM	3	522.61	174.203333	27.6270333		
AHES	3	680.88	226.96	3.9459		
AHEU	3	2400	800	0		
RHEM	3	2400	800	0		
RHES	3	930.65	310.216667	41.9289333		
RHEU	3	2400	800	0		
AMEM	3	536.12	178.706667	83.9304333		
AMES	3	288.85	96.2833333	15.5532333		
AMEU	3	231.89	77.2966667	2.06333333		
RMEM	3	1023.38	341.126667	32.9086333		
RMES	3	321.82	107.273333	4.54423333		
RMEU	3	1893.79	631.263333	33.6966333		
ACEM	3	271.32	90.44	0.1273		
ACES	3	217.32	72.44	7.9657		
ACEU	3	2400	800	0		
RCEM	3	777.83	259.276667	2.66083333		
RCES	3	534.68	178.226667	5.70403333		
RCEU	3	2400	800	0		

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VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	5898228.68	23	256444.725	13623.426	6.5403E-84	1.75675938
Within groups	903.542677	48	18.8238058			
Total	5899132.23	71				

Phenanthroline activity

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
AAEM	3	75.2	25.0666667	3.91583333		
AAES	3	78.96	26.32	73.8604		
AAEU	3	66.34	22.1133333	0.94823333		
RAEM	3	600	200	0		
RAES	3	387.21	129.07	77.3175		
RAEU	3	600	200	0		
AHEM	3	126.2	42.0666667	5.13403333		
AHES	3	70.65	23.55	0.8175		
AHEU	3	563.51	187.836667	561.616233		
RHEM	3	600	200	0		
RHES	3	463.53	154.51	59.1631		
RHEU	3	600	200	0		
AMEM	3	434.03	144.676667	39.5936333		
AMES	3	382.37	127.456667	8.83053333		
AMEU	3	90.15	30.05	1.0333		
RMEM	3	600	200	0		
RMES	3	256.96	85.6533333	7.63103333		
RMEU	3	600	200	0		
ACEM	3	153.25	51.0833333	2.87163333		
ACES	3	69.62	23.2066667	3.11663333		
ACEU	3	509.02	169.673333	16.3441333		
RCEM	3	484.98	161.66	3.7119		
RCES	3	335.74	111.913333	1.59203333		
RCEU	3	600	200	0		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	365069	23	15872.5652	439.126904	3.5351E-48	1.75675938
Within groups	1734.99533	48	36.1457361			
Total	366803.995	71				

Reducing power activity

DETAILED REPORT						
Groups	*Number of samples*	*Sum*	*Mean*	*Variance*		
AAEM	3	568.24	189.413333	0.58023333		
AAES	3	600	200	0		
AAEU	3	600	200	0		
RAEM	3	600	200	0		
RAES	3	600	200	0		
RAEU	3	600	200	0		
AHEM	3	600	200	0		
AHES	3	600	200	0		

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AHEU	3	600	200	0		
RHEM	3	600	200	0		
RHES	3	600	200	0		
RHEU	3	600	200	0		
AMEM	3	600	200	0		
AMES	3	600	200	0		
AMEU	3	600	200	0		
RMEM	3	600	200	0		
RMES	3	600	200	0		
RMEU	3	600	200	0		
ACEM	3	600	200	0		
ACES	3	534.26	178.086667	228.175033		
ACEU	3	600	200	0		
RCEM	3	600	200	0		
RCES	3	600	200	0		
RCEU	3	600	200	0		
VARIANCE ANALYSES						
Source of variation	*Sum of squares*	*Degree of freedom*	*Average of squares*	*F*	*Probability*	*Critical value for F*
Between groups	1644.78382	23	71.5123399	7.50276128	2.5779E-09	1.75675938
Within groups	457.510533	48	9.53146944			
Total	2102.29435	71				

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