**Chemical Composition, Oxidative Stability, and Antioxidant Activity of Allium ampeloprasum L. (Wild Leek) Seed Oil**

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Abstract: *Allium ampeloprasum* L., commonly known as wild leek, is an edible vegetable that has been cultivated for centuries. However, no detailed studies have been undertaken to valorize *A. ampeloprasum* seed oil. This study aims to evaluate the physicochemical properties, chemical composition, and antioxidant activity of *A. ampeloprasum* seed oil. The seed oil content was found to be 18.20%. Gas chromatography-mass spectrometry (GC-MS) showed that linoleic acid (71.65%) was the dominant acid, followed by oleic acid (14.11%) and palmitic acid (7.11%). *A. ampeloprasum* seed oil exhibited an oxidative stability of 5.22 h. Moreover, γ- and δ-tocotrienols were the major tocols present (79.56 and 52.08 mg/100 g oil, respectively). The total flavonoid content (16.64 µg CE /g oil) and total phenolic content (62.96 µg GAE /g oil) of the seed oil were also determined. The antioxidant capacity of the oil, as evaluated using the ABTS assay (136.30 µM TEAC/g oil), was found to be significant. These findings indicate that *A. ampeloprasum* seeds can be regarded as a new source of edible oil having health benefits and nutritional properties.

Key words: *Allium ampeloprasum* seed oil, antioxidant properties, oxidative stability, thermal profile, fatty acid composition, tocopherols and tocotrienols

1 Introduction

*Allium ampeloprasum* Linn. (*A. ampeloprasum*), known as wild leek or kurath (in Arabic), is a robust herbaceous biennial plant belonging to Amaryllidaceae family. It is a species closely related to leek (*Allium porrum* L.). *Allium* is a bulbous vegetable genus, containing more than 800 species⁴. Onion and garlic are among the members of this family. Cultivated leeks are plants that are about 30 cm tall, with a small bulb, producing a long white stem of superimposed layers of green flat leaves, and are said to have a flavor with a shallot fragrance, but subtler and sweeter⁵. It is a hardy, cold-resistant plant. The leaves and stem are edible and used in cooking⁶. In folk medicine, *A. ampeloprasum* has been used since ancient times due to its pharmacological properties⁷. A literature survey revealed some notable health benefits for *A. ampeloprasum*, including antibacterial, antifungal, antioxidant, antihelmintic, antihypertensive, and diuretic properties⁸. It can decrease the risk of gastrointestinal diseases, as well as protect the skin against damage⁹. *A. ampeloprasum* is native to the Mediterranean region but has been introduced in other regions of the world¹⁰. Leek is in flower from July to August¹¹. As the season progresses, the flowering head full of seeds dries on the stalk. Once the dry head opens, ripe seeds (which look like black pyramids) fall to the ground. Wild leek is known to have a high content of phenolic acids but has been shown to have a low antioxidant activity, as measured by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay¹². It has been found that linoleic acid (53.45%), palmitic...
acid (26.42%), and oleic acid (7.39%) are the major fatty acids present in the lipid fraction of wild leek. The content of tocopherols in this fraction has been reported to be negligible (0.05 mg/100 g). To the authors’ knowledge, a detailed study of the chemical, physical, and antioxidant properties of wild leek seed oil has not been reported in the literature. Therefore, the aim of this work is to characterize *A. ampeloprasum* seed oil using the following parameters: fatty acid and tocopherol profiles, physicochemical properties, UV-Visible analysis, thermal and oxidative stabilities, as well as antioxidant properties, including total phenolic content (TPC), total flavonoid content (TFC), and ABTS assay. The results presented here may improve our knowledge about the food and non-food applications of *A. ampeloprasum* seed oil.

### 2 Materials and Methods

#### 2.1 Plant material and extraction

Mature seeds of *A. ampeloprasum* (Kurath) were purchased from “Bin Mengshash” Herbs, Spices & Seeds Store, Riyadh (Saudi Arabia). Kurath seeds were collected from the Riyadh region. Before oil extraction the seeds were oven-dried at 40°C for 24 h, then milled. The hexane extraction was made by a Soxtec 8000 extraction unit (Foss Analytical Co., Suzhou, China) under the following conditions: boiling temperature of 100°C, boiling time of 5 min, rinsing time of 20 min, recovery time of 60 min, powder mass of 15 g, and hexane volume of 60 mL. The extracted oil was stored in a refrigerator at −15°C until analysis. Fatty acid methyl ester (FAME) standards were obtained from Sigma-Aldrich Corporation (Steinheim, Germany). Tocopherols and tocotrienols (tocols) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The chemicals used in this investigation were of analytical grade or chromatographic purity.

#### 2.2 Physicochemical properties of *A. ampeloprasum* seed oil

We used International Organization for Standardization (ISO) standards to determine the acidity (ISO 660) and peroxide value (ISO 3960). Carotenoid and chlorophyll contents were determined from the absorption spectra of the extract according to the method described by Nehdi et al. Carotenoids and chlorophylls were extracted from the seeds with methanol. The absorption was measured at 290–400 and 400–800 nm using UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The refractive index of the oil was determined using an Abbe 60 refractometer (Bellingham and Stanley Ltd, Kent, England). The kinematic viscosity was determined at 40°C using an Ubbelohde viscometer size 2 (Koehler Instrument Co., Inc., Bohemia, New York, USA).

### 2.3 Antioxidant assays (total phenolic content (TPC), total flavonoid content (TFC), and ABTS assay)

In order to perform these assays, the oil samples were extracted using the method described by Fuentes et al., with slight modifications. In short, the oil sample (2 g) was dissolved in 4 mL of hexane, and the compounds of interest were extracted with 3 mL of methanol/water (60:40, v/v), by means of vortexing for 2 min. Both phases were then separated using centrifugation at 3,500 rpm for 10 min. The hexane phase was re-extracted using the same steps as described above. The methanolic extracts were then used for the determination of TPC and TFC, as well as the ABTS assay.

#### 2.3.1 Determination of TPC

TPC was determined using the Folin-Ciocalteu (FC) assay as described by Tay et al. The methanolic extract (0.32 mL) obtained from the oil sample was mixed with 1.6 mL of FC reagent (diluted 10-fold). After 5 min, 1.28 mL of sodium carbonate (7.5% w/v) was added and the mixture was vortexed and allowed to stand in the dark at room temperature for 30 min. A blank was prepared by replacing the methanolic extract with methanolic phase (methanol/water, 60:40, v/v). Absorbance was measured against the blank at 765 nm. TPC was calculated based on a gallic acid calibration curve and expressed as gallic acid equivalents (GAEs) in (µg GAE/g oil).

#### 2.3.2 Determination of TFC

TFC was estimated according to the procedure described by Thoo et al. Methanolic extract (0.25 mL) was added to 1.25 mL of deionized water and 75 µL of 5% sodium nitrite. After 6 min, 150 µL of 10% aluminum chloride was added. After 5 min, 0.5 mL of 1 M sodium hydroxide and 275 µL of deionized water were added and the mixture vortexed. Then, the absorbance of the mixture was immediately measured at 510 nm against a blank. The TFC was calculated based on a calibration curve using catechin as a standard, and was expressed as catechin equivalents (CEs) in (µg CE/g oil).

#### 2.3.3 ABTS Assay

The ABTS (2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical-scavenging capacity was determined according to the method described by Thoo et al. The ABTS radical solution was prepared by mixing 10 mL of 7 mM ABTS solution with 10 mL of 2.45 mM potassium persulfate and allowed to stand in the dark at room temperature for 16 h. This ABTS radical solution was adjusted with ethanol to achieve an absorbance of 0.7 (±0.01) at 734 nm. Methanolic extract (0.1 mL) was added to 3.9 mL of the adjusted ABTS radical solution and allowed to react for 6 min. A negative control was prepared by replacing the methanolic extract with the methanolic phase (methanol/water, 60:40, v/v). The percentage of ABTS radical-scavenging activity was calculated based on the expression [1 –...
(As/As) × 100%, where As and Ac are the absorbance values of the test sample and control, respectively. Measurements were calibrated to a standard curve of prepared trolox. The trolox equivalent antioxidant capacity (TEAC) was expressed in terms of (μM TEAC/g oil).

2.3.4 DPPH Assay
Antioxidant capacity was determined based on the methods by Tay et al.\textsuperscript{13} and Thoo et al.\textsuperscript{14}, with slight modifications. Briefly, the oil sample was dissolved in acetone at a ratio of 1:1 (v/v). The dissolved oil sample (0.1 mL) was then added to 3.9 mL of ethanolic DPPH (60 μM). The mixture was vortexed and placed in the dark at room temperature. After 30 min, absorbance was measured at 517 nm. Negative control was prepared by replacing the dissolved oil sample with 0.1 mL of acetone. The percentage of DPPH radical-scavenging activity was calculated based on the expression \[1 - \frac{As}{Ac} \times 100\%\]. Measurements were also calibrated to a standard curve of prepared trolox and expressed as millimoles of trolox equivalent antioxidant capacity (TEAC) per 1 g dry weight (mM TEAC/g DW).

2.4 GC/MS analysis
The FAMEs were prepared following the protocol described by Nehdi et al.\textsuperscript{15}. A GC-MS (QP2010 Ultra, Shimadzu, Kyoto, Japan), and an RT-2560 column (100 m length, 0.25 mm internal diameter, 0.25-μm film thickness) were used for FAME analysis. Helium was used as a carrier gas at a flow rate of 1.50 mL/min. The temperature of the oven was increased from 115°C to 240°C at a rate of 2°C/min and then maintained at 240°C for 5 min. The NIST 11 library and NIST analysis software were used for the identification of each FAME, as well as interpretation of the mass spectra. In addition, a Shimadzu software (Cat. No. 225-21731-92) was used for chromatogram analysis.

2.5 Analysis of tocols (tocopherol and tocotrienol)
The tocol profile and content of A. ampeloprasum seed oil were determined according to the standard ISO 9936 procedure. A 0.5 g aliquot of the A. ampeloprasum oil was dissolved in 25 mL of hexane, and 20 μL of the solution was injected into a Shimadzu LC-20AT high-performance liquid chromatography (HPLC) pump (Kyoto, Japan). A Hypersil silica column (15 cm × 3 mm I.D., 3-μm particle size; Thermo Scientific) was used for the tocol separation. A mixture of hexane/2-propanol (99.5:0.5; v/v) at a flow rate of 0.5 mL/min was used for tocol elution. Tocols were identified using a fluorescence detector set at emission and excitation wavelengths of 330 nm and 295 nm, respectively. Authentic standards α, β, γ, δ isomers of tocopherols and tocotrienols (Sigma Chemical Co., St. Louis, MO, USA) were used for tocol identification.

2.6 Oxidative stability
The oxidative stability or induction time (IT) of A. ampeloprasum seed oil was analyzed with a 743 Rancimat analyzer (Metrohm AG, Herisau, Switzerland). Briefly, 3 g of the oil was incubated in the measuring tube and heated up to 110°C under an air flow rate of 20 L/h to obtain the IT of the oil. The oxidative stability was also measured at 90 and 100°C. The oxidative stability at 25°C of the oil being studied was estimated by extrapolation of the induction times obtained at 90°C, 100°C, and 110°C.

2.7 Thermal analysis
A TGA-50 (Shimadzu, Kyoto, Japan) thermogravimetric analyzer was used to determine thermogravimetric analysis (TGA) and differential thermogravimetric analysis (DTGA) curves. A 5 mg oil sample placed in an alumina crucible was heated up to 600°C at a heating rate of 10°C/min under a synthetic air atmosphere (100 mL/min). Three independent measurements were made and then analyzed using a Shimadzu TA-60WS (2.20) software.

Differential scanning calorimetry (DSC) curves were recorded with a PerkinElmer (Norwalk, CT, USA) model DSC-7 that had been previously calibrated with indium. Samples weighing between 10 and 12 mg were packed in aluminum pans and covers were sealed. The samples were subjected to the following temperature program: 60°C isothermal for 1 min, cooled to −60°C at a rate of 10°C/min and held for 1 min. The samples were heated and cooled under a constant flow of nitrogen (99.9999% purity).

2.8 Statistical analysis
All measurements were performed in triplicate. The values of the various parameters were expressed as the mean ± standard deviation (SD).

3 Results and Discussion
3.1 Fatty acid composition
The results of GC/MS analysis of A. ampeloprasum seed oil (Table 1) revealed that the key fatty acids (FA) present were linoleic acid (LA) (71.65%) and oleic acid (OL) (14.11%) which together represented more than 86% of the total FA. Palmitic acid and stearic acid were present to the extent of 7.11%, and 3.14%, respectively. A. ampeloprasum seed oil and safflower seed oil showed the same fatty acid profiles.\textsuperscript{16} In fact, safflower seed oil is composed of LA (68-83%), OL (10-20%), palmitic (6-7%), and stearic acid (2-3%). A. ampeloprasum seed oil can be classified in the linoleic-oleic group of oils such as safflower and sunflower oils.\textsuperscript{15, 16} Linoleic-oleic oils can be used to make salad cream and mayonnaise, for seasoning salads and to coat food products.\textsuperscript{17} Furthermore, owing to its negligible content of linolenic acid oil, A. ampeloprasum seed oil may be used for deep and shallow frying purposes.\textsuperscript{17}

Concerning health benefits, LA plays a crucial role in the

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α-tocotrienols were the major tocols present to the extent of 3.27 mg/kg, while γ-tocotrienol was present at a content of 18.20 mg/kg. Other pigments such as carotenoids were also present in the oil with a content of 3.58 mg/kg. The quality indices of the oil were determined, and the results showed that the oil was of high quality. The peroxide value (Ip) was 2.47 mg O₂/kg, and the acid value was 9.52 mg KOH/g. The ratio of polyunsaturated to saturated fatty acids (PUFA/SFA) was 6.15, and the ratio of unsaturated to saturated fatty acids (UFA/SFA) was 8.46. The oil was found to have potent biological functions such as anti-inflammatory and antioxidant properties. These properties are attributed to the presence of tocotrienols, which possess potent biological activities.

### 3.3 Physicochemical properties

*A. ampeloprasum* seed oil was found to have a high oil content of 18.20% (Table 2). In fact, this oil content is comparable to that of olive (18 – 22%) and soybean (17 – 27%) oils. The oil was observed to be greenish in color, which was attributed to the presence of chlorophylls, which were present to the extent of 2.47 mg/kg. Other pigments such as carotenoids were also present in the oil with a content of 3.58 mg/kg. The quality indices of *A. ampeloprasum* seed oil, namely, peroxide value (Ip) and acidity, were found to be 9.52 mg O₂/kg and 4.26%, respectively. These values indicate that this oil is unlikely to have a rancid flavor and that the hydrolysis reaction in the seeds during their growth is not intense.

The *A. ampeloprasum* oil also showed a low kinematic viscosity (26.38 mm²/s) compared to that reported for conventional oils such as corn (30.75 mm²/s), sunflower (35.86 mm²/s), and sesame (36.00 mm²/s). Previous studies have suggested that the observed decrease in the oil viscosity can be correlated with a higher percentage of LA content, and a decreasing percentage of OL content. The ratio of polyunsaturated FA to saturated FA (PUFA/SFA) of *A. ampeloprasum* oil showed the highest LA content, and the lowest OL content. Regarding the refractive index, *A. ampeloprasum* oil showed a high value of 1.4745, as compared to other oils such as olive oil (1.4678). Refractive index is known to be proportional to the degree of unsaturation of the oil. The ratio of polyunsaturated FA to saturated FA (PUFA/SFA) of *A. ampeloprasum* oil (6.15) is found to be higher than that of olive oil (0.99). Figure 1 shows that *A. ampeloprasum* can absorb hazardous substances more efficiently than other oils.
UV-B radiation (290-320 nm) that can cause sunburns \(^{(3)}\). However, *A. ampeloprasum* exhibits a low absorbance in the UV-C and UV-A ranges (200-290 nm, 320-400 nm, respectively).

### 3.4 Oxidative stability

Oxidative stability was evaluated through the determination of induction time in hours by the Rancimat test performed at 110°C. As shown in Fig. 2, *A. ampeloprasum* seed oil exhibited a lower oxidative stability of 5.5 h, as compared to the corresponding values for palm olein (18.8 h) and date seed oil (21.1 h) \(^{(24)}\). The substantial difference between these values is attributed to the high content of polyunsaturated fatty acids in *A. ampeloprasum* seed oil (nearly 72% of C18:2), which are very susceptible to lipid oxidation. However, the percentages of C18:2 content in date seed oil and palm olein were considerably smaller (8.13%, and 9.01%), and, additionally, the predominant fatty acids were monounsaturated (MUFA). Also, the PUFA/SFA ratio for these oils (0.20-0.21) were markedly less than the corresponding value for *A. ampeloprasum* seed oil (6.15).

Lipid oxidation occurs very slowly at room temperature. To estimate the oxidative stability (induction time) of *A. ampeloprasum* seed oil at 25°C, we measured the induction times at two other temperatures and observed values of 15.75 h at 90°C, and 7.82 h at 100°C. The autoxidation reaction is more rapid at high temperatures. The unstable

### Table 2  Physicochemical properties and tocols composition of *A. ampeloprasum* seed oil.

| Parameter                        | Unit          | *A. ampeloprasum* seed oil |
|----------------------------------|---------------|---------------------------|
| Yield                            | % (w/w)       | 18.20 ± 1.22              |
| Peroxide value                   | meq O₂/kg oil | 9.52 ± 0.83               |
| Free fatty acid                  | as oleic %    | 4.26 ± 0.11               |
| Color                            |               | Greenish                  |
| State at ambient temperature     |               | Liquid                    |
| Chlorophylls                     | mg/kg         | 2.47 ± 0.08               |
| Carotenoids                      | mg/kg         | 3.58 ± 0.10               |
| Oxidative stability (110°C)      | h             | 5.50 ± 0.21               |
| Oxidative stability (25°C)       | h             | 1223 ± 4.4                |
| Refractive index                 |               | 1.4745 ± 0.0001           |
| Viscosity (40°C)                 | mm²/s         | 26.38 ± 0.58              |

#### Tocols

| Tocols               | mg/100 g | 7.65 ± 0.33 |
|----------------------|----------|-------------|
| α- Tocopherol        | 2.12 ± 0.08 |
| β- Tocopherol        | 3.71 ± 0.12 |
| γ- Tocopherol        | 0.39 ± 0.06 |
| δ Tocopherol         | 2.18 ± 0.08 |
| α- Tocotrienol       | 79.56 ± 1.43 |
| β- Tocotrienol       | 52.08 ± 1.03 |
| γ- Tocotrienol       | 148.98    |
| δ- Tocotrienol       | 6.65 ± 1.01 |
| Total                | 5.50 ± 0.21 |

#### Antioxidant properties

| TPC                  | µg GAE/g oil | 62.96 ± 1.01 |
|----------------------|-------------|--------------|
| TFC                  | µg CE/g oil | 16.64 ± 0.77 |
| ABTS                 | µM TEAC/g oil | 136.30 ± 2.40 |
| DPPH                 | mM TEAC/g DW | 4.16 ± 0.12  |

The values of all parameters are the average ± SD of three replicates.
primary oxidation products such as peroxides (ROOR) and hydroperoxides (ROOH) convert to aldehydes, ketones, alcohols, and carbonic acids. The temperature and the rate of the oxidation reaction are exponentially related. Figure 3 shows the extrapolation curve and parameters. It is clear from Fig. 3 that there is a linear relation between 1/T and induction time ($t_i$) with good correlation, with $R^2$ being 0.979. The estimated oxidation stability at 25°C is 1223 h. This implies that A. ampeloprasum seed oil can be stored without deterioration for about 0.13 year. This low oxidative stability can be increased by limiting the exposure of the oil to oxidation-inducing factors like light or heat.

3.5 Antioxidant properties

TPC, TFC, and ABTS and DPPH radical-scavenging capacities of the crude A. ampeloprasum seed oil were found to be 62.96 µg/g, 16.64 µg/g, 136.30 µmol/g, and 4.16 mmol/g, respectively (Table 2). The TFC reported in this study was comparable to that of Chamaerops humilis seed oil (18 mg/g). A. ampeloprasum seed oil exhibited a suitable content of phenolic compounds (136.30 µmol/g), which may contribute to its oxidative stability. Furthermore, the values of free radical scavenging capacities (ABTS and DPPH) of this oil were positively correlated to the high tocot content (155 mg/100 g).

3.6 Thermal profiles

The DSC curves for the A. ampeloprasum seed oil are given in Fig. 4. The oil has a melting point of ~16.01°C. Safflower oil, with almost the same FA composition, is composed mostly of trilinolein (LLL) (49%), followed by dilinoleoyl-oleoyl-glycerol (OLL), and dilinoleyl-palmitoyl-glycerol (PLL). Safflower oil has a melting point of ~14°C. The melting curves of A. ampeloprasum seed oil showed two melting peaks at ~47.54 and ~16.01°C. These peaks can be attributed to the melting of LLL and PLL triacylglycerols (TAGs).

The crystallization curves consisted of three exothermic peaks at ~12.62°C, ~32.81°C, and ~44.62°C. These crystallization temperatures can be attributed to the solidification of TAGs such as PLL, OLL, and LLL, respectively. Both crystallization and melting curves showed a peak at about 18°C, corresponding to a reversible transformation of unknown origin.

The TGA curve of A. ampeloprasum seed oil (Fig. 5) is comparable to that of cress, olive and sunflower oils. A. ampeloprasum oil was thermally stable and was found to lose only 5% of its mass at 214.5°C. At high temperature, the oil decomposed in three stages, with rapid decomposition being observed at 361.6°C, 416.3°C and 538.0°C. The initial stages are attributed to the boiling of the different
groups of triacylglycerols. The final stage that ends at 600°C, is ascribed to the combustion of the residual carbonaceous material from the previous stages.

4 Conclusion
The current study mentioned that *A. ampeloprasum* is a valuable source of plant seed oil with a high yield (18.20%). The results also showed that *A. ampeloprasum* oil is rich in unsaturated fatty acids (89.42%). Furthermore, the oil has a high level of polyunsaturated fatty acids (72.62%), which is important in terms of health and medicine. The oil is thermally stable and has a melting point of -16°C. *A. ampeloprasum* oil is a rich source of tocotrienols (133.82 mg/100 g oil) possessing potent biological functions. The oil showed an appreciable antioxidant capacity owing to a significant content of tocotrienols, phenolic and flavonoid compounds. Overall, *A. ampeloprasum* seed oil can be used as a raw material for diverse applications.

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Conflict of Interest Statement
The authors declare that there are no potential conflicts of interest.
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