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Phenolic profile and investigation of biological activities of *Allium scorodoprasum* L. subsp. *rotundum*

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ABSTRACT

In this study, phenolic profile of *Allium scorodoprasum* L. subsp. *rotundum* and its bioactivity in multiway approach including antioxidant, antimicrobial, antidiabetic, cytotoxic and antiinflamatory activities were investigated. The extraction of phenolic and flavonoid compounds from *Allium scorodoprasum* L. subsp. *rotundum* was optimized as a function of ethanol percentage, extraction temperature and extraction time by employing a response surface method. The optimal extraction conditions were found as 79 °C extraction temperature, 28% ethanol and 118 min duration of the extraction. Quercetin was determined as the major phenolic compound of the extract obtained under the optimal conditions and followed by vanillic, caffeic, 2,4 hydroxybenzoic and ellagic acid. In addition to high antioxidant capacity, the obtained extract showed antimicrobial activity, against different bacteria and molds. The concentrations of the extract obtained at the optimal conditions that inhibited 50% of *Aspergillus oryzae* and pancreatic α-amylases and α-glycosidase activities were calculated as 11.56, 14.35 and 19.35 mg extract/mL, respectively. The antiinflammatory effect of the obtained extract was also examined, and it was more inhibitory against xanthine oxidase activity than against lipoxygenase activity. The cytotoxicity of the optimized extract was observed against breast and bone cancer cells, and it showed significant cytotoxic activity especially on bone cancer cells. As a result, *Allium scorodoprasum* L. subsp. *rotundum* could serve as a promising source of natural bioactive compounds for application in food and non-food products.

1. Introduction

A. scorodoprasum L. subsp. *rotundum* also known as wild garlic or leek, belongs to the *Alliaceae* family that is one of the world's oldest cultivated plants with 900 species, grows in East Anatolia and Northern Anatolia of Turkey (Emir et al., 2020a; Tasci et al., 2019; Tubives, 2015). They have some volatile sulfur compounds that give characteristic flavors to them as well as dietary fiber, sugars, flavonoids and essential oils (Benkeblia & Lanzotti, 2007a, 2007b; Guillamón et al., 2021; Majewski, 2014a, 2014b; Tasci et al., 2019). Their distinct flavor is due to the chemical transformation of volatile organosulfur compounds formed by the division of the odorless flavor precursor S-Ally-L-cysteine sulfoxide by alliinase and lachrymatory factor synthase (Teshika et al., 2019). Besides flavoring foods, *Allium* species have traditionally been used for various medical purposes since ancient times (Mitic et al., 2014).

In raw or cooked form *A. scorodoprasum* L. subsp. *rotundum* leaves and bulbs can be used for flavoring and increasing the nutritive value or shelf life of different types of foods including soups, meals, cheese and salads (Tasci and Koca, 2015). Like other species of the genus *Allium (Allium sativum, Allium cepa, Allium schoenoprasum)* (Tasci et al., 2019), it has some therapeutic properties. It has been claimed to have antimicrobial, antioxidant, diuretic, antihypertensive, antiobesity, hepatoprotective (liver protector) and antitumor activities (Kurnia et al., 2021; Tasci and Koca, 2015).

Researches on nutraceutical preparations used in natural food supplements have increased in recent years, and the bioactive properties of plant species containing "alliin" have been studied in detail. Ferulic, gallic, protocatechuic acids, quercetin and kaempferol, which are found in the phenolic composition of these species, have been identified to have strong antioxidant and antimutagenic properties (Guillamón et al., 2021; Singh et al., 2009). Up to now, numerous studies have been done on the bioactive components of *Allium* species (Lee et al., 2007; Woo et al., 2007; Kim et al., 2011; Köseahmetoğlu, 2012; Colina-Coca et al., 2013; Keles et al., 2014; Guillamón et al., 2021; Rocchetti et al., 2022). It

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has been emphasized that they have compounds with high antibacterial activity against different microorganisms including antibiotic resistant, Gr (+) and Gr (−) bacteria (El-Saber et al., 2020). Organosulfur compounds of the *Allium* genus have antioxidant, antiinflamatory, regulated redox, proenergetic, antiproliferative and cytotoxic activity (Kurnia et al., 2021; Liu et al., 2015; Souza et al., 2011). Studies have shown that the *Allium* plant's bioactive constituents exhibit immunomodulatory, antiobesity, antihepatotoxicity, neuroprotection, cardioprotective, digestive system protective, and renal protective properties (Shang et al., 2019; González et al., 2021). Flavonoids of *Allium* extracts have been widely used in in-vivo studies, and especially those of garlic and onion have been supported by several clinical studies (neuroprotective effects, cognitive activities, antialzheimer and antiparkinson) (Farooqui & Farooqui, 2018; Marefati et al., 2021).

Phenolic and flavonoid compounds of *A. scorodoprasum* L. were analyzed in different studies (Mitic et al., 2014; Sokmen et al., 1999; Štajner et al., 2006). It was recorded that its extract contained high levels of phenolic substances and had a high antioxidant capacity (Demir et al., 2013). Besides the antioxidant and antimicrobial activities of it, cytotoxic (colon cancer) activities of the *A. scorodoprasum* L. extract were determined, and different phenolic compounds were identified (Izol et al., 2021). In a study examining the anticancer/cytotoxic effects of some plants, specific to Sivas region, which are thought to have antineoplastic properties; the IC_{50} inhibition of the *A. scorodoprasum* L. was reported to be 102 μg/mL and 108 μg/mL for MDA-468 and MDA-231 cancer cells (breast cancer) (Turan et al., 2010). Also, Mollica et al. (2018) reported *A. scorodoprasum* L. extract had antioxidant, α-amylase and α-glucosidase inhibitory capacities.

This study aimed to extract and identify the phenolic compounds from *A. scorodoprasum* L. *subsp. rotundum* and characterize its biological activities. Although the biological activities of other species of *Allium* have been investigated in several studies, up to the now limited number of research has been conducted on *A. scorodoprasum* L. *subsp. rotundum* which is mainly on the extraction and identification of phenolic compounds, antioxidant and antimicrobial capacities and a few cytotoxic studies against some cancer cells. According to our literature survey, we have not come across the optimizations of the phenolic and flavonoid compounds extraction from *A. scorodoprasum* L. *subsp. rotundum* and in addition to antioxidant and antimicrobial activities, investigation of its bioactivity in multiway approach, including antidiabetic, cytotoxic and particularly antiinflamatory activities. For this purpose, in this study the extraction conditions were optimized in terms of ethanol percentage (%), extraction time (min) and temperature (°C) to obtain the high amount of Total Phenolic (TP) and Total Flavonoid (TF) compounds from *A. scorodoprasum* L. *subsp. rotundum.* The phenolic compositions together with antimicrobial, antioxidant, antidiabetic, antiinflammatory and cytotoxic activities of the extract obtained under the optimal conditions were determined.

2. Materials and methods

2.1. Materials

Allium scorodoprasum L. *subsp. rotundum* were obtained from Zara region of Sivas province, Turkey, ground and stored until analyzed (+4 °C). Mueller-Hinton Broth (MHB), Mueller-Hinton Agar (MHA), Sabouraud Glucose Agar, Chapman Agar and Eosin Methylene Blue (EMB) were obtained from Merck (Merck KGaA, Germany). The microorganisms [*E. faecalis* (ATCC29212), *E. coli* (ATCC25922), *S. aureus* (ATCC29213), *A. flavus* (ATCC9170) and *A. niger* (ATCC6275)] were provided from cultural collection of Cumhuriyet University Research Hospital. The ampicillin disc was obtained from Oxoid™ (ThermoFisher Scientific, USA) and HPLC column was Brownlee Analytical C18 (4.6 × 250mm, 5 μm, Perkin Elmer USA). The phenolic compounds (gallic, caffeic, ferulic, 2,4′-hydroxybenzoic, vanillic, chlorogenic, ellagic and rosmarinic acids, quercetin, catechin, epicatechin, kaempferol, naringenin), lipoxygenase (LOX), xanthine oxidase (XO), α-amylase from *Aspergillus oryzae* and α-amylase from porcine pancreatic, α-glucosidase from *Saccharomyces cerevisiae* and cancer cell lines [(MCF-7; breast cancer (86012803), (MG-63; bone cancer (86051601)] were purchased from Sigma-Aldrich (USA). Microplate reader was obtained BioTek, Epoch, USA. Acarbose (Glucobay®) from Bayer AG, allopurinol (Urikoliz©) from Sandoz™, methotrexate from Koçak Farma™ were taken. Other chemicals were supplied by Merck (MerckKGaA, Darmstadt, Germany) or Sigma-Aldrich (USA).

2.2. Method

2.2.1. Plant extract preparation

Ground plant material was mixed with the solvent at a ratio of 10 mL solvent/1 g and extracted at different temperatures, times and ethanol percentages to optimize the extraction conditions. At the end of the extraction period, the samples were centrifuged (5000 rpm; 10 min), and filtered (Cai et al., 2004). After that the TP and TF compounds of the extracts were analyzed as described in section 2.2.4.

2.2.2. Optimization of the extraction using response surface method

Box-Behnken response surface design, allowing fewer number experiments (17 sets of the experiments) than other designs, with three factors (x_1; ethanol percentage, x_2; temperature and x_3; time) and three levels (Table 1), was used in this study to examine and optimize the effect of the factors on the amount of the extracted TP and TF. Table 1 presents the experimental range and values of the independent variables tested for *A. scorodoprasum* L. extractions. The optimal point was estimated by a quadratic model that was explained by the following equation.

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad \text{Eq 1}$$

Where x_1, x_2 and x_3 are the independent variables studied, Y is the response variable (TP and TF concentration), b_0 is the interception coefficient; b_1, b_2 and b_3 are the linear terms, b_{11}, b_{22} and b_{33} are the quadratic terms, and b_{12}, b_{13} and b_{23} are the coefficients for the interaction.

A statistics program (The Design-Expert 7.0.0 software State-Ease Inc. Minneapolis, MN, USA) was used to analyze the interaction between dependent and independent variables. Fisher's and Student's t-test were employed to get both models (TP and TF concentrations) equations and their regression coefficients. The optimal ethanol percentage (x_1), extraction temperature (x_2) and extraction time (x_3) were assigned based on the desirability function approach. The optimal conditions were verified by performing the extraction in triplicate under the optimal experimental conditions.

2.2.3. Large scale extraction under the optimal experimental conditions

Plant material, 20 g, was mixed with 200 mL of optimal ethanol percentage and its phenolic compounds were extracted at optimal temperature and time found in Section 2.2.2. At the end of the extraction period, the sample was centrifuged (5000 rpm; 10 min) and filtered. To obtain the sufficient amount of extract, this process was repeated several

Table 1
Experimental range and levels of independent variables used for *A. scorodoprasum* L. subsp. *rotundum* extraction.

Independent Variables	Symbol	Range and level		
		−1	0	+1
Ethanol (%)	X_1	25	50	75
Temperature (°C)	X_2	40	60	80
Time (min)	X_3	10	70	130

times and all the extracts were combined, evaporated and stored at −18 °C until used (Maisuthisakul et al., 2007).

2.2.4. Total phenolic and flavonoid contents

The total phenolic (TP) content of the extracts was analyzed according to Singleton and Rossi (1965). Folin-Ciocalteu reagent, 2 N 100 μL was mixed with extract/standard gallic acid solutions (100 μL extract/100 μL of standard), 2.3 mL of purified water and 1 mL of aqueous sodium carbonate solution (7%). The mixture was kept at 25 °C for 2 h and then its absorbance at 750 nm was measured. The determination of the total amount of the flavonoids of the *A. scorodoprasum* L. extract was conducted according to the method provided by Zhishen et al. (1999). The extract was reacted with the order of 5% $NaNO_2$, 10% $AlCl_3$ and NaOH (1 M). The absorbance of the mixture was read at 510 nm. The TP and TF of the extract were explained as mg "Gallic Acid Equivalent" (GAE) and "Quercetin Equivalent" (QE) per gram of *A. scorodoprasum* L. used for the extraction.

2.2.5. Antioxidant capacity

The antioxidant capacity of the *A. scorodoprasum* L. extract was determined with three different methods. DPPH (2,2 Diphenyl-1-picrihydrazyl) method was performed according to the method provided by Brand-Williams et al. (1995) by reacting DPPH working solution (1.95 mL, 100 μM) with 50 μL plant extract or 50 μL standard Trolox solutions at 25 °C for 10 min and their absorbances were determined at 517 nm. *FRAP* (Ferric Reducing/Antioxidant Power) method was performed according to the method defined by Benzie and Strain (1996) by mixing 100 μL extract/100 μL Trolox standard solutions with 2.9 mL of FRAP working solution (sodium acetate buffer, $FeCl_3$, TPTZ; 10/1/1) and measuring the absorbances at 593 nm. TEAC (Trolox Equivalent Antioxidant Capacity) method was performed according to Re et al. (1999). After mixing 2.9 mL ABTS'+ working solution with 100 μL extract/100 μL Trolox standard solutions, the absorbances of the mixtures were read at 734 nm. All results were expressed as μmol "Trolox Equivalent" (TE) after constructing of TE standard curve.

2.2.6. Antimicrobial activity

Disk diffusion method; The method defined by Ebrahimabadi et al. (2010) was conducted with the slight modifications for the determination of the antimicrobial activities of the extract. Bacteria and molds (18–24 h) colonies were grown in the solid medium from fresh cultures. Microorganisms were prepared in the physiological saline solution to the concentration of 10^8 cfu/mL by comparing with 0.5 McFarland turbidity tube. They (100 μL) were inoculated onto petri plates containing MHA. The sterile empty discs (6 mm) were impregnated with the *A. scorodoprasum* L. extract and placed on the petri plates. After that, petri plates were incubated for 24 h (37 °C bacteria; 30 °C molds) and the inhibition zones diameters were measured. Standard antibiotic discs (ampicillin) and water were used as the controls.

Minimum Inhibitory Concentrations (MIC); To determine the MICs values, macrobroth method was applied (Oskay et al., 2007). Five different microorganism cultures, prepared as described above, 25 μL (1×10^8 cfu/mL), were added to MHB (3 mL) and mixed with the declining concentration of the extract (30–0.46 mg/mL). They were incubated for 24 h (37 °C bacteria; 30 °C molds). MIC values were recorded according to the lowest concentration that observed no microbial growth and for positive control, ampicillin was used.

2.2.7. Identification of phenolic compounds

The phenolic composition of the *A. scorodoprasum* L. extract, obtained at the optimal conditions, was quantitatively analyzed by HPLC system with DAD detector that had C18 column. For the gradient elution of the phenolic compounds, acidified water (pH 2.5, adjusted with orthophosphoric acid) (solvent A) and acetonitrile (solvent B), were used (Ávila et al., 2019). The phenolic compounds were removed from the column by decreasing the ratio of the Solvent A from 100% to 57% at 25 °C (60 min) with the flow rate of 0.8 mL/min. Then, concentrations of phenolic compounds were computed using average peak-areas by comparison with the standards.

2.2.8. Antidiabetic activity

Inhibititory activities the extract and acarbose (used as control) against α-amylase and α-glucosidase enzymes were determined by Worthington (1993) with slight modifications. The various concentrations of the plant extract [from 0 to 10 mg extract (or acarbose)/mL] were prepared and their antidiabetic activities were measured by determining their ability to inhibit two α-amylase from pancreatic and microbial (*A. niger*) origin. Preliminary experiments were designed to select appropriate concentrations of enzymes (pancreatic α-amylase 36 units/mL and microbial α-amylase 29 units/mL). The enzyme solutions, 0.5 mL, were mixed with 0.5 mL of extract (0–10 mg/mL) and allowed to preincubate at 25 °C (10 min). A solution of 0.5 mL of 1% potato starch prepared in sodium phosphate buffer (pH 6.9, 20 mM) was added to the enzyme/extract mixture and incubated (25 °C, 30 min). The amount of reducing sugar formed at the end of the incubation was determined by the using glucose standard with dinitrosalicylic acid method (Miller, 1959), spectrophotometrically at 560 nm. After selecting the appropriate concentration of enzyme (based on the preliminary experiment), 0.5 mL of α-glucosidase at a concentration of 0.40 U/mL was mixed with 0.5 mL of the phosphate buffer (pH 6.9, 20 mM) and 0.5 mL of different concentrations of extract (0–10 mg extract/mL) and the resulting mixtures were preincubated at 25 °C for 5 min. After the preincubation, 0.5 mL of p-nitrophenyl-α-D-glucapiranoside solution (5 mM) was added to the samples and incubated (37 °C, 25 min). The reaction was stopped with 2 mL Na_2CO_3 (0.1 M) and the absorbance was determined at 405 nm. IC_{50} was defined as the concentration of the plant extract required to inhibit 50% of the activity of α-amylase and α-glucosidase.

2.2.9. Antiinflamatory activity

15-Lipoxygenase (LOX) inhibitory activity; 15-LOX solution with 8460 units/mL activity was get ready in phosphate buffer (0.1 M, pH 8). The solution of linoleic acid used as a substrate was prepared at a concentration of 1980 μmol/L. The LOX inhibitory assay was applied according to Alaba et al. (2014). A total of 50 μL LOX was mixed with 2740 μL phosphate buffer and 10 μL of the different concentrations of the extract (0–8 mg/mL plant extracts). Following the preincubation at 30 °C for 5 min, linoleic acid was added (200 μL) to the mixtures and then their absorbances were monitored at 234 nm for 5 min with 30 s intervals. Quercetin solution (3.74 M) was used as a positive control.

Xanthine oxidase (XO) inhibitory activity; The XO inhibitory assay was applied according to the method provided by Nessa et al. (2010) with slight modifications. Different concentrations of the plant extract, 1 mL, (0–10 mg/mL) wertr mixed with 0.1 mL of XO (0.1 units/mL) and 1.9 mL of phosphate buffer (pH 7.5, 0.1 M). After 5 min of preincubation, 1 mL of xanthine (1.52 mg/mL) was added to the samples and they were incubated (25 °C, 10 min). The reaction was terminated with 1 mL of HCl (1 M) and uric acid formation was measured at 295 nm. For positive control, allopurinol was used. IC_{50} was defined as the concentration of the plant extract required to inhibit 50% activity of LOX and XO.

2.2.10. Cytotoxic activity

In vitro cytotoxicity against MCF-7 and MG-63 cells were determined by modifying the method provided by Betancur-Galvis et al. (2002). The plant extract (0.2 g) was dissolved in DMSO (1 mL) and filtered through a 0.22 μm filter. The cell lines were grown in DMEM and RPMI1640 (Content L-glutamine; 2 mg/mL $NaHCO_3$) medium at 37 °C containing 5% CO_2 and the developed cells, 100 μL, was transferred to the plate (1×10^4 cell/well). At the end of the incubation (24 h), 100 μL of fresh medium was added to the cells. Then, 20 μL of the different concentrations of the extract (8.33–266.62 μg/mL) was added to them and incubated (37 °C, 24 h). At the end of the incubation, 10 μL MTT (3-(4,

5-dimethyl thiazolyl-2-yl)-2,5-diphenyltetrazolium bromide) was added to the samples on the plate and incubated for further at 37 °C (2 h). After the addition of 100 μL SDS (Sodium Dodecyl Sulfate) (1%) to the samples on the plate, they were agitated at 100 rpm for 10 min and their resulting colors were measured with a microplate reader at 540 nm. For positive controls, methotrexate and doxorubicin were used and the cytotoxicity of the plant extract was defined as its concentration (IC_{50}) which inhibited 50% of MCF-7 and MG-63 cells.

2.2.11. Statistical analysis

All analyses were conducted at least in triplicate and the results were given with mean and standard deviation. Microsoft Excel 2010 (Microsoft, Redmond, WA, USA) program and Design-Expert 7.0.0 statistical package were used for the analysis of data.

3. Results and discussion

3.1. Extraction of TP and TF compounds

Fig. 1 presents the amount of TP and TF of the extracts, after 17 different extraction conditions. The highest TP (23.20 mg GAE/g) and TF (2.60 mg QE/g) amount of *A. scorodoprasum* L. extracts were observed at an experiment of 17 (50%, 80 °C, 130 min) (Fig. 1) and their lowest amount was at the 4th experiment (50%, 60 °C, 70 min) as 11.21 mg GAE/g and 1.12 mg QE/g, respectively. When the TP and TF results of the extracts *A. scorodoprasum* L. were examined, both indicated a decrease with the increase in ethanol percentage, but the increment in the temperature and time boosted the amount of extracted TP and TF compounds. In all conditions tested, it was determined that the content of water soluble dry matter was found in different ratios independently from TP and TF contents. This shows that besides the TP and TF compounds, different compounds have been extracted depending on the extraction conditions.

A past study of TP and TF compounds in methanol extracts of the *A. scorodoprasum* L. (seed and bulb) demonstrated that the bulb was rich in TP and TF (Mitic et al., 2014). The other study about the bulb, flower and stem of *A. scorodoprasum* L. showed that the richest part in terms of flavonoid compounds was its stem (14.95 mg Rutin Equivalents/g plant extract) (Mollica et al., 2018). The study conducted on the root, stem and flower parts of *Allium* species stated that the total phenolic ratio was higher in the above parts (Emir et al., 2020b). The highest phenolic and flavonoid content of *A. paniculatum* was determined as 21.88 mg GAE/g extract, 2.89 mgQE/g (Emir et al., 2020a). If the amount of TP and TF compounds extracted in our research are considered based on extracted WSDM of *A. scorodoprasum* L., the TP and TF contents of the extract of our study coincide to the literature studies.

Since the structures of compounds with antioxidant capacity are very different from each other, measurement with a single method is not sufficient (MacDonald et al., 2006). Therefore, the antioxidant capacities of the extracts were evaluated with three methods, namely: DPPH, TEAC and FRAP methods. The highest antioxidant capacity of the *A. scorodoprasum* L. extracts determined with three methods (DPPH, TEAC, FRAP) was found in the experiment of 17 (50%, 80 °C, 130 min) as 1.63 μmol TE/g, 2.89 μmol TE/g, 2.72 μmol TE/g, respectively. Like the antioxidant capacity results, the highest antibacterial and antifungal activity of *A. scorodoprasum* L. extracts against the selected microorganisms were determined in the 17th experiment (50%; ethanol percentage, 80 °C; temperature, 130 min; extraction time) and all extracts obtained at 17 different conditions showed higher antimicrobial and antifungal activities against Gr (+) bacteria and *A. niger,* respectively than the others. The increase in antioxidant and antimicrobial capacities of the extracts was found to be in parallel with the increase in TP and TF amounts (Fig. 1).

3.2. Statistical modelling

Variables were coded according following equations (Eq. (2) and Eq. (3)), where Y_1 and Y_2 represent TP and TF compounds, respectively, as the function of X_1 [ethanol (%)], X_2 [temperature (°C)] and X_3 [time (min)].

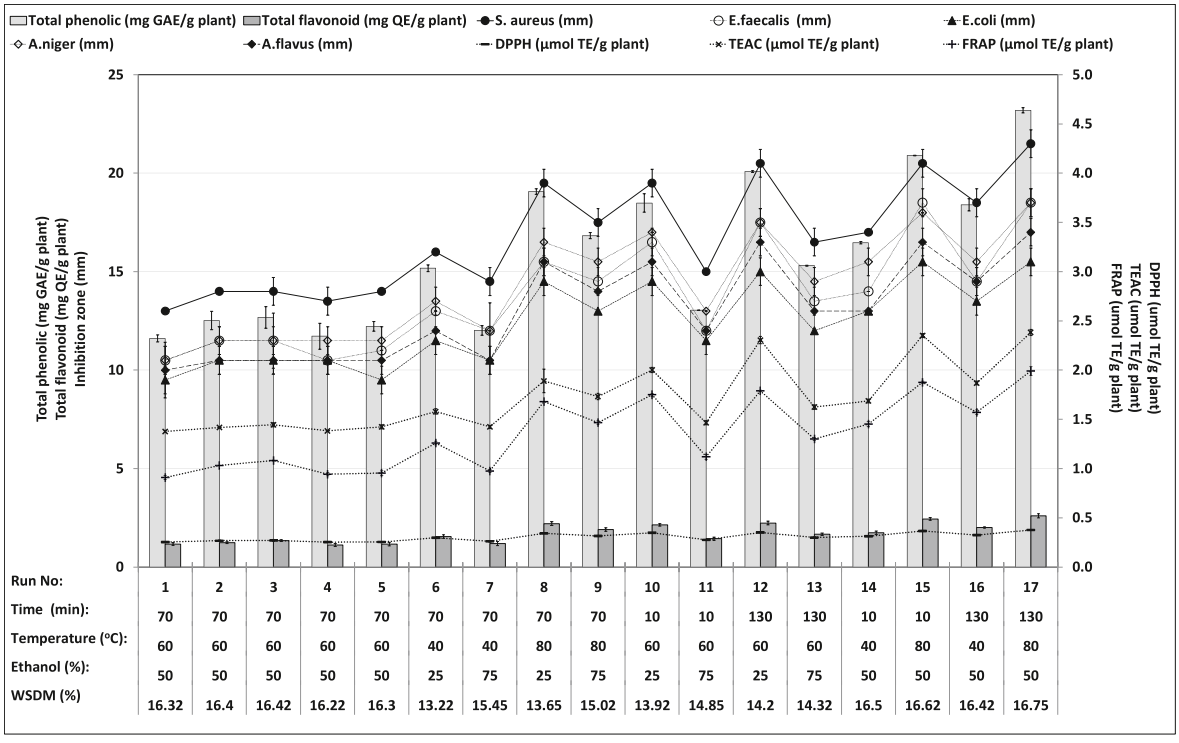

Fig. 1. Total phenolic, flavonoid compounds, antioxidant and antimicrobial activities of A. scorodoprasum L. subsp. rotundum extracts obtained at different conditions.

$$y_1 = 12.15 - 1.95x_1 + 2.24x_2 + 0.84x_3 + 0.31x_1^2 + 3.31x_2^2 + 2.97x_3^2 + 0.23x_1x_2 + 0.14x_1x_3 + 0.08x_2x_3 \quad \text{Eq 2}$$

$$y_2 = 1.21 - 0.24x_1 + 0.33x_2 + 0.08x_3 + 0.09x_1^2 + 0.42x_2^2 + 0.40x_3^2 + 0.02x_1x_2 + 0.03x_1x_3 - 0.02x_2x_3 \quad \text{Eq 3}$$

The evaluations of TP and TF amounts of the *A. scorodoprasum* L. extracts with ANOVA is presented in Table 2. The p values (<0.05) and F-values (42.58 and 37.32) indicate the significance of both models. Both of them did not present lack of fit and the same regression coefficients, 0.98, were observed (0.98) (Table 2). Table 2 shows predicted R^2 (Pred R^2) values that are in reasonable agreement with the adjusted determination (Adj R^2) values, showing satisfactory adjustment between the determined and estimated data, and Adj R^2 values (0.96 and 0.95) also confirm the validy of the models. It was seen that the experiments were precise and reliable due to low values of the coefficients of variation of both models (4.68 and 6.14%). Adequate precisions (signal to noise ratio) of both the models (20.61 and 18.20) were higher than 4 confirming the adequacy of the model precision.

Fig. 2 presents the diagnostic plots to be used to evaluate the sufficiency of the models. Fig. 2A and D shows the sufficient agreement between the predicted and determined values. Normal distribution was observed in the normal % probability plots of residuals for both responses without deviation of the variance (Fig. 2B and E). All the data points were observed within the limits (±3) in the internally studentized residuals plots (Fig. 2C and F).

Fig. 3 presents the response surface and contour plots showing the effect of concentration, temperature and time on the extraction of TP and TF substances. The maximum TP (19.74 mg GAE/g) and TF (2.27 mg QE/g) compounds were obtained at 80 °C and 25% ethanol percentage when extraction time was 70 min (Fig. 3A and B). Fig. 3C and D shows the effects of the ethanol percentage and time on the TP and TF compound extraction at 60 °C. The maximum TP and TF content were observed in the 25% ethanol percentage for 120 min of the extraction time as 18 mg GAE/g and 1.98 mg QE/g, respectively. The highest TP (21.52 mg GAE/g) and TF (2.41 mg QE/g) contents at 50% ethanol percentage were found at the end of the 120 min extraction time at 80 °C.

All the independent variables showed significant linear ($p < 0.05$) effects on both responses, and their interactions showed non-significant effects on TP and TF content. Unlike ethanol percentages, the quadratic coefficients of temperature and time presented positive significant effects on both of them. The plant tissue becomes soft at high temperatures and long extraction times, resulting in weakening of the cell membrane and thus phenolics are released more easily into the solvent (Sulaiman et al., 2017). As seen from Figs. 1 and 3, decreasing the ethanol percentage, increasing extraction time and temperature favor the extraction of TP and TF compounds from the plant.

3.3. Optimization

The extraction of TP and TF compounds from *A. scorodoprasum* L. with 28% of ethanol percentage at 79 °C for 118 min were suggested by the Design-Expert program as the optimal conditions, and the extracted TP and TF compounds were predicted as 22.47 mg GAE/g, 2.56 mg QE/g at these conditions. Triplicate extractions of TP and TF compounds from the *A. scorodoprasum* L. under the suggested conditions were performed to confirm the optimal extraction conditions. The TP and TF contents of the extract were determined as 25.60 mg GAE/g and 2.21 mg QE/g, respectively (Table 3) that were in agreement approximately with the estimated values.

3.4. Phenolic composition of A. scorodoprasum L. extract

Medicinal plants have rich phenolic compounds that can show different bioactive properties. It has been reported that the amount and composition of phenolic compounds of plants could be used to predict their biological activities (Mollica et al., 2018). The phenolic compounds of the *A. scorodoprasum* L. extract obtained under the optimal conditions were analyzed by the HPLC (Fig. 4) and the results are shown in Table 4. The number of phenolic compounds identified by HPLC analysis was 13. Among them, the highest amount of the phenolic compound was quercetin. The other important phenolics determined in the extract were ellagic, caffeic, 2,4 hydroxybenzoic and vanillic acids.

The flower extracts of *A. scorodoprasum* L. were found to be rich in rosmarinic acid (Mollica et al., 2018). In another study, high levels of malic acid (0.787 mg/g) and quercetin (0.127 mg/g) were determined in the ethanol extract of *A. scorodoprasum* L. subsp. *rotundum* using the LC/MS/MS system (Izol et al., 2021). In different studies about *Allium* species, rosmarinic acid (Radovanović et al., 2015), p-coumaric acid (Emir et al., 2020a), hydroxybenzoic acid, epigallocatechin gallate and genistein (Emir et al., 2020b) were identified. In this study, in addition to high levels of quercetin (1.32 mg/g), vanillic 0.74 mg/g), caffeic (0.67 mg/g) and ellagic acids (0.57 mg/g), hydroxybenzoic (0.59 mg/g extract) and rosmarinic acids (0.19 mg/g extract) were determined in the plant extract obtained under optimal conditions (Table 4). The optimal extraction conditions (28%, 79 °C, 118 min) found in this study were successfully carried out to provide maximum yields of phenolic compounds from *A. scorodoprasum* L.

Table 2
Analysis of variance for the extracted total phenolic compounds and flavonoids.

Source	Sum of squares		Degree of freedom		Average of squares		F-value		p-value	
	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2	Y_1	Y_2
Model	211.19	3.74	9	9	23.47	0.42	42.58	37.32	<0.0001	<0.0001
X_1	30.47	0.46	1	1	30.47	0.46	55.30	41.21	0.0001	0.0004
X_2	40.18	0.89	1	1	40.18	0.89	72.92	79.77	<0.0001	<0.0001
X_3	8.18	0.07	1	1	8.18	0.07	14.84	6.42	0.0063	0.0390
X_1 X_2	0.21	0.00	1	1	0.21	0.00	0.38	0.08	0.5587	0.7803
X_1 X_3	0.11	0.00	1	1	0.11	0.00	0.20	0.36	0.6685	0.5650
X_2 X_3	0.03	0.00	1	1	0.03	0.00	0.06	0.24	0.8085	0.6391
X_1^2	0.40	0.03	1	1	0.40	0.03	0.72	2.94	0.4231	0.1299
X_2^2	46.26	0.74	1	1	46.26	0.74	83.96	66.39	<0.0001	<0.0001
X_3^2	76.85	1.38	1	1	76.85	1.38	139.48	123.55	<0.0001	<0.0001
Residual	3.86	0.08	7	7	0.55	0.01				
Lack of fit	2.97	0.05	3	3	0.99	0.02	4.44	2.19	0.0920	0.2315
Pure error	0.89	0.03	4	4	0.22	0.01				
Total	215.04	3.82	16	16						

Significant ($p < 0.05$)
Y_1: R^2: 0.98. Adj R^2: 0.96. Pred R^2: 0.77. C.V. %: 4.68 Adeq Precision: 20.61
Y_2: R^2: 0.98. Adj R^2: 0.95. Pred R^2: 0.79. C.V. %: 6.14 Adeq Precision: 18.20

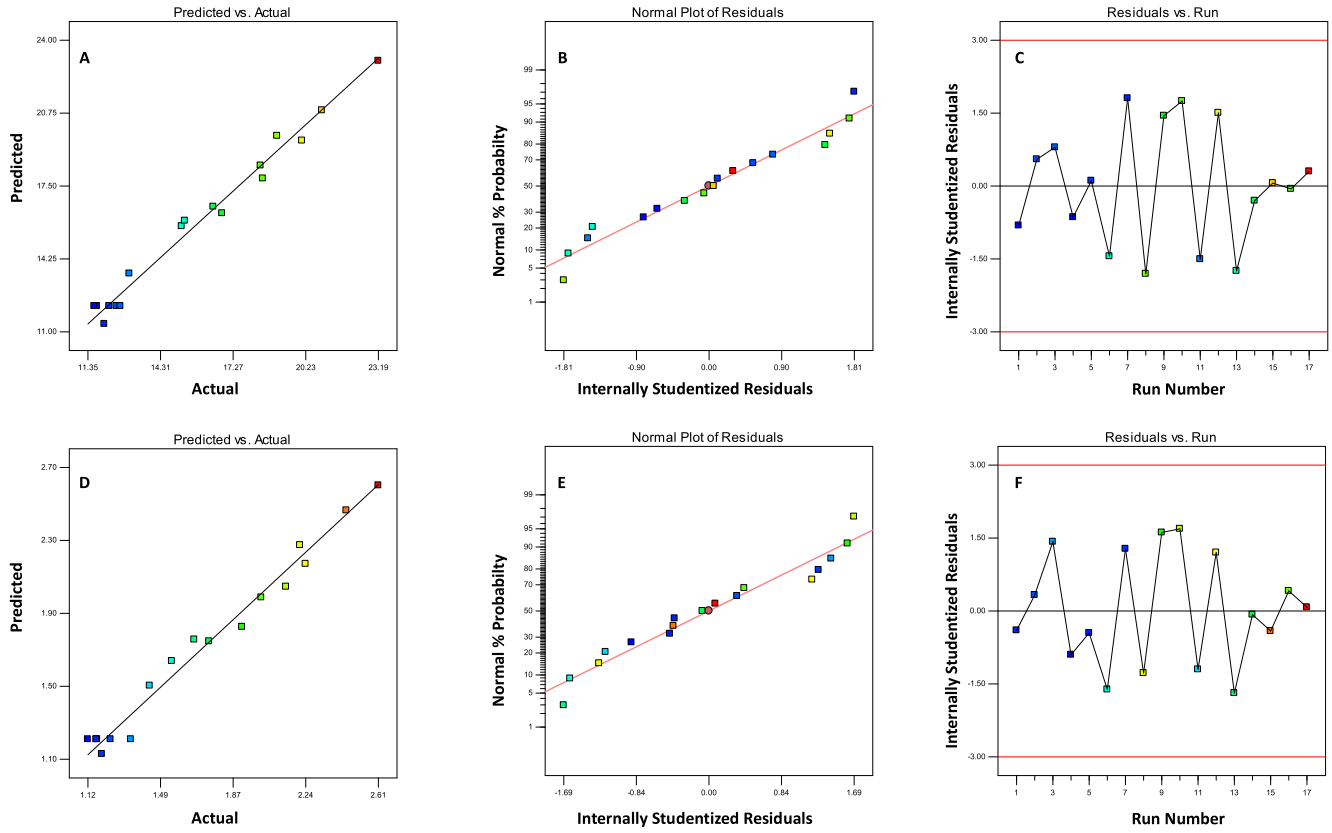

Fig. 2. Diagnostic plots for the model adequacy for the total phenolic and flavonoid compounds.

3.5. Antioxidant and antimicrobial activities

The antioxidant and antimicrobial activities of the concentrated extract, obtained under optimal conditions, were presented in Tables 5 and 6. Table 5 shows the inhibition zone diameters of the concentrated extract, obtained under optimal conditions, for different microorganisms that are important in terms of food technology (*S. aureus*; 20.00 mm, *E. faecalis;* 17.50 mm, *E. coli*; 14.00 mm, *A. niger*; 18.50 mm, *A. flavus*; 14.5 mm). MIC results showed that only *E. coli* presented a high resistance (7.5 mg/mL) against *A. scorodoprasum* L. extract. Among the selected bacteria and mold, *A. scorodoprasum* L. extract indicated the highest antibacterial activity against *S. aureus* and the highest antifungal activity against *A. niger*.

Stopping the development of microorganisms and preventing a secondary infection are the expected properties of plant-based antimicrobial agents (Farag et al., 2015; Sinan et al., 2021). A lot of research is being done for the treatment of infectious diseases with natural resources instead of antibiotics (Reiter et al., 2020). In the literature, it is stated that numerous medicinal plants have antibacterial and antifungal activities as a result of a series of reactions against microorganisms. Phenolic compounds are major compounds in the defense system of plants that stop the development of pathogenic effects of many microorganisms (Karuppiah et al., 2012; Mollica et al., 2021). The different *Allium* species have been shown to have antimicrobial activity depending on their phenolic content (Farhat et al., 2021; Kyung, 2012; Shang et al., 2019). Izol et al. (2021) determined the antimicrobial activity of *A. scorodoprasum* L. subsp. *rotundum* root extract against two Gr (−) (*P. aeruginosa, E. coli,)*, two Gr (+) (*S. pyogenes*, *S. aureus*) bacteria and one yeast (*C. albicans*) and it was reported that its root extract showed the highest minimum inhibition against *S. aureus* (MIC; 150 μg/mL). The studies about allicin and its analogs (of plant origin) presented that they had antimicrobial activity against *Campyrobactor jejuni, Listeria monocytogenes, E. coli O157:H7, S. aureus* (Reiter et al., 2020; Sheppard & Long, 2016; Yin & Cheng, 2003). In a study examining the antibacterial and antifungal properties of *A. sativum* extract, the highest antibacterial effect was reported against *S. aureus*, the highest antifungal effect was determined against *Candida ablicans* and MIC (10^5 cfu/mL) values were reported as 100 μg/mL and 150 μg/mL, respectively (Meriga et al., 2012). The results of this study comply with the previous studies on *Allium* species.

As seen in Table 6, antioxidant capacity was investigated by three methods (FRAP, TEAC and DPPH) and calculated as Trolox equivalent and FRAP, TEAC and DPPH values were measured 2.89, 2.72 and 1.63 μmol TE/g extract, respectively. The studies on *A. scorodoprasum* L. extracts reported their strong radical scavenging activities (Demir et al., 2013; Mitic et al., 2014; Mollica et al., 2018). Some *Allium* species, namely, *A. cepa* (Jaiswal et al., 2017) and *A. sativum* (Kallel et al., 2014) were reported to have antioxidant properties. It was found that the antioxidant capacities of the different species of the *Allium* species measured by DPPH were determined as 41.56 μmol TE/g extracts (Abdel-Hady et al., 2018), 89.3 μmol TE/g extracts (Koleva et al., 2002) and 95.6 μmol TE/g extracts (Pourmorad et al., 2006). The differences in antioxidant activities between this study and previous studies can arise from the use of different antioxidant measurement methods, different species of the same family and different extraction conditions.

3.6. Antidiabetic activity

Diabetes mellitus is a condition that develops from high blood sugar (hyperglycemia) due to incomplete or a certain level of insulin deficiency. Today, in the treatment of diabetes, insulin and orally taken antidiabetic drugs are used (Haller et al., 2019). Nowadays, the increase in the prevalence of diabetes mellitus (type 1 and type 2) and the unfavorable effects related to commercially available oral antidiabetic

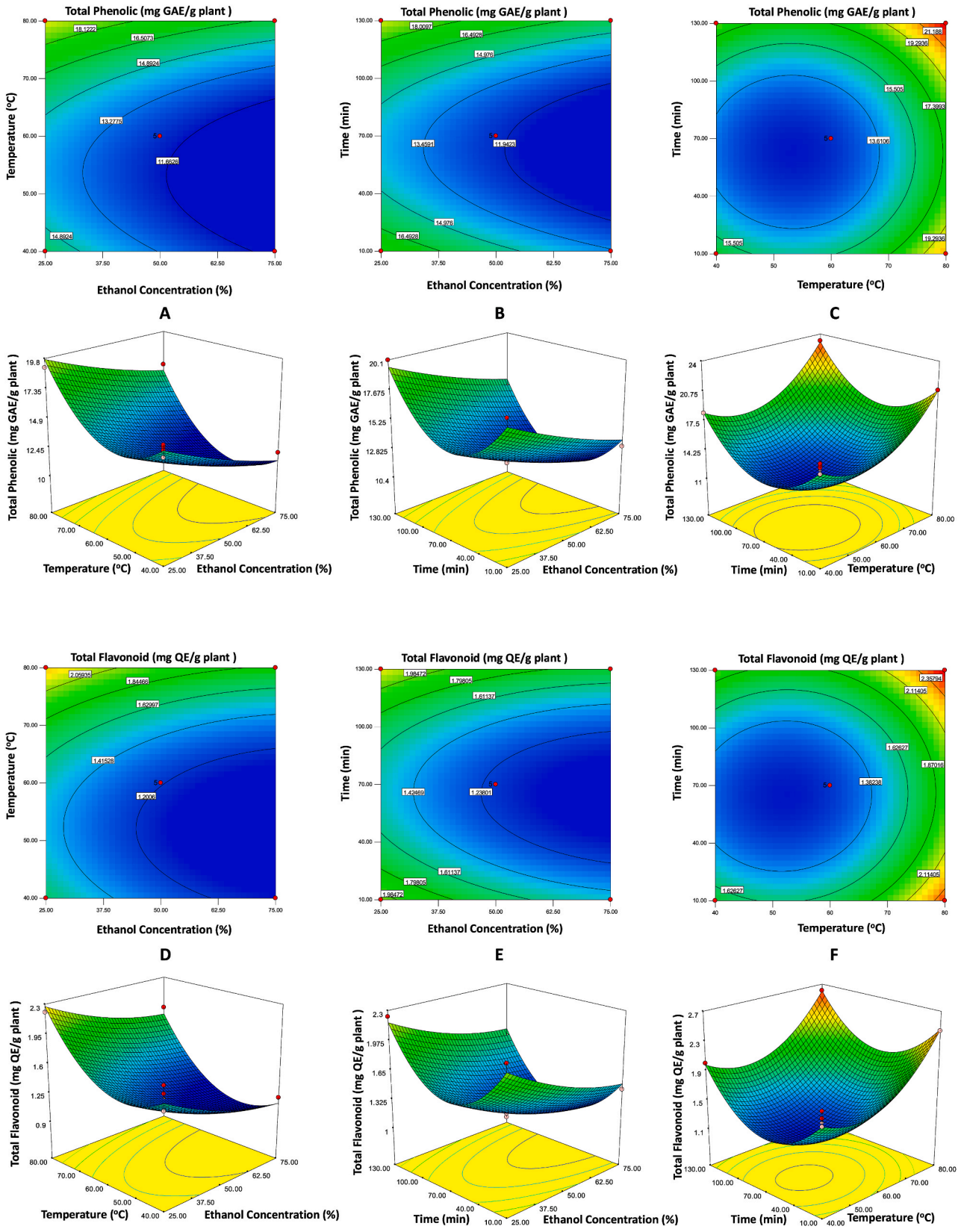

Fig. 3. Response surface graphs showing the effect of temperature, time and ethanol concentration on total phenolic content and flavonoid of the extracts. A: Effect of temperature and ethanol concentration on total phenolic content in 70 min, B: Effect of ethanol concentration and time on total phenolic content at 60 °C, C: Effect of temperature and time on the total phenolic content in a 50% ethanol concentration, D: Effect of temperature and ethanol concentration on total flavonoid content in 70 min, E: Effect of ethanol concentration and time on total flavonoid content at 60 °C, F: Effect of temperature and time on the total flavonoid content in a 50% ethanol concentration.

Table 3
The analysis results of *A. scorodoprasum* L. subsp. *rotundum* extract obtained under optimum conditions (estimated and actual).

Optimum conditions	Ethanol (%)	28
	Temperature (°C)	79
	Time (min)	118
Estimated	Total Phenolic (mg GAE/g)	22.47
	Total Flavonoid (mg QE/g)	2.56
Actual	Total Phenolic (mg GAE/g)	25.60 ± 0.48
	Total Flavonoid (mg QE/g)	2.21 ± 0.04
	WSDM (%)	16.22 ± 0.04

Values are expressed as means ± SD. WSDM: Water Soluble Dry Matter.

drugs have brought along many problems. Such reasons have led the researchers to investigate new therapeutical and phytopharmacological methods focused on controlling postprandial glucose concentrations. Utilizing plant-based resources is one of the strategies developed for this purpose. The use of carbohydrate digestive enzyme inhibitor agents from natural-based compounds can be a feasible strategy to inhibit dietary carbohydrate absorption and prevent drug damage with fewer adverse effects than synthetic drugs (Etxeberria et al., 2012). In this study, the antidiabetic properties of *A. scorodoprasum* L. extract were determined against α-amylase and α-glucosidase. The amounts of the extract inhibiting the 50% activity of α-amylase from pancreatic and *A. oryzae* and α-glucosidase were 11.56, 14.35 and 19.35 mg extract/mL, respectively (Table 6). IC_{50} values of acarbose for three enzymes were found to be 2.16, 2.54 and 3.00 mg/mL, respectively (Table 6).

Mollica et al. (2018) reported that the stem extract of *A. scorodoprasum* L. showed inhibitory action against α-amylase (0.55 mmol acarbose equivalent/g extract). The antidiabetic effects of different *Allium* species were investigated in-vivo/in-vitro by many researchers (Eidi et al., 2006; Kothari et al., 2020). In one of the studies, *A. sativum, A. akaka, A. cepa* and *A. porrum* showed IC_{50} values of 16.36 mg/mL, 17.95 mg/mL, 16.74 mg/mL and 15.73 mg/mL, respectively. Kim et al. (2011) determined the inhibitory activity of the ethanol extract of *A. cepa* peel as $IC_{50} > 3$ mg/mL against the pancreatic α-amylase. The same study reported that the highest phenolic compound present in *A. cepa* peel was quercetin, which showed an IC_{50} value of 0.60 mg plant extract/mL. It was also reported to be effective in α-glucosidase inhibition. The antidiabetic activity of flavonoid derivatives obtained from the peel of *Allium* species was investigated and the association of the inhibition ability of the α-glucosidase with the antioxidant capacity of the plant was reported (Vu et al., 2020). Kothari et al. (2020) reported that kaempferol, another important *Allium* flavonol, which displayed promising antidiabetic potential (Kothari et al., 2020). This study presented that *A. scorodoprasum* L. had quercetin (1.32 mg/g) and kaempferol (0.34 mg/g) (Table 4) that could contribute significantly to antidiabetic activity together with the other phenolic compounds. When all the findings are interpreted together with the results of this study, phytochemicals of *Allium scorodoprasum* L. subsp. *rotundum* can be used as an antihyperglycemic agent to control postprandial glucose levels.

Table 4
Phenolic compounds composition of concentrated *A. scorodoprasum* L. subsp. *rotundum* extract obtained under optimum conditions.

No	Phenolic compound	Amount
1	Gallic acid (mg/g extract)	0.10 ± 0.00
2	Caffeic acid (mg/g extract)	0.67 ± 0.01
3	Ferulic acid (mg/g extract)	0.50 ± 0.01
4	Catechin (mg/g extract)	0.30 ± 0.00
5	2.4 hydroxybenzoic acid (mg/g extract)	0.59 ± 0.01
6	Chlorogenic acid (mg/g extract)	0.46 ± 0.01
7	Ellagic acid (mg/g extract)	0.57 ± 0.01
8	Vanillic acid (mg/g extract)	0.74 ± 0.01
9	Quercetin (mg/g extract)	1.32 ± 0.02
10	Epicatechin (mg/g extract)	0.19 ± 0.00
11	Kaempferol (mg/g extract)	0.34 ± 0.00
12	Rosmarinic acid (mg/g extract)	0.19 ± 0.00
13	Naringenin (mg/g extract)	0.36 ± 0.00
Total Phenolic (mg GAE/g extract)		76.96 ± 0.01
Total Flavonoid (mg QE/g extract)		12.20 ± 0.01

Values are expressed as means ± SD.

3.7. Antiinflamatory activity

Researches have indicated that phenolics could inhibit LOX and XO activity and act as antiinflammatory factors (Gawlik-Dziki et al., 2011). According to our knowledge based on the literature review, this is the first study about the antiinflamation properties of *A. scorodoprasum* L. by evaluating the inhibition of LOX and XO enzymes. The IC_{50} values of the extract, quercetin and allopurinol are presented in Table 6. The extract concentration inhibiting the 50% activity LOX and XO were found as 9.75 and 9.71 mg extract/mL, respectively while the quercetin and allopurinol IC_{50} values for LOX and XO were 1.22 and 2.69 mg/mL, respectively.

The inhibitory activities of various medicinal plant extracts against LOX and XO were investigated in several studies (Alaba et al., 2014; Jacob, 2015; Nessa et al., 2010; Prakash et al., 2011). In a study on the

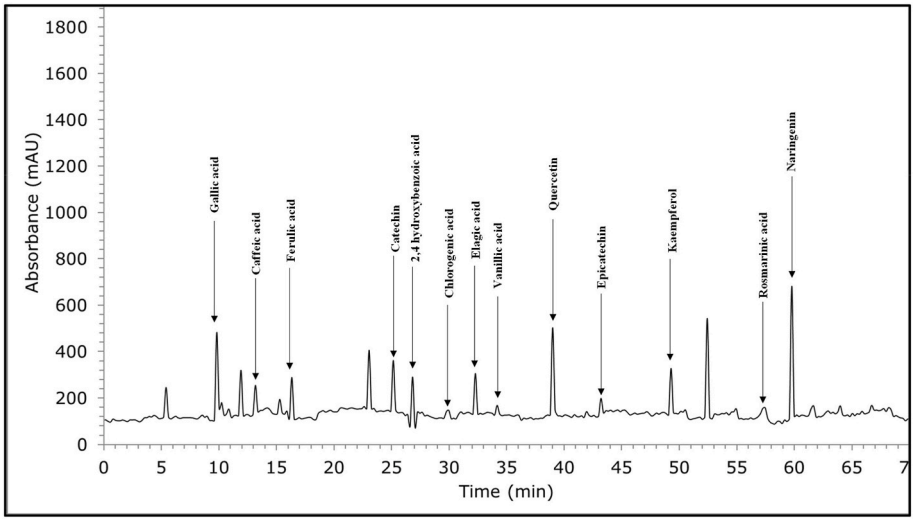

Fig. 4. HPLC chromatogram of A. scorodoprasum L. subsp. rotundum extract.

Table 5
Antimicrobial activities of concentrated *A. scorodoprasum* L. subsp. *rotundum* extract obtained under optimum conditions.

		Antimicrobial Activities
S. aureus	Extract: Inhibition zone (mm)	20.00 ± 0.00
	Standard Ampicillin: Inhibition zone (mm)	26.00
	Extract: MIC (mg extract/mL)	3.75 ± 0.00
	Extract: MBC (mg extract/mL)	3.75-^{NI}
	Standard Ampicillin: MIC (mg/mL)	0.48
E. faecalis	Extract: Inhibition zone (mm)	17.50 ± 0.71
	Standard Ampicillin: Inhibition zone (mm)	24.00
	Extract: MIC (mg extract/mL)	3.75 ± 0.00
	Extract: MBC (mg extract/mL)	3.75-^{NI}
	Standard Ampicillin: MIC (mg/mL)	0.48
E. coli	Extract: Inhibition zone (mm)	14.00 ± 0.00
	Standard Ampicillin: Inhibition zone (mm)	23.50
	Extract: MIC (mg extract/mL)	7.50 ± 0.00
	Extract: MBC (mg extract/mL)	7.50-^{NI}
	Standard Ampicillin: MIC (mg/mL)	0.96
A. niger	Extract: Inhibition zone (mm)	18.50 ± 0.71
	Standard Ampicillin: Inhibition zone (mm)	25.50
	Extract: MIC (mg extract/mL)	3.75 ± 0.00
	Extract: MBC (mg extract/mL)	3.75-^{NI}
	Standard Ampicillin: MIC (mg/mL)	0.96
A. flavus	Extract: Inhibition zone (mm)	14.50 ± 0.00
	Standard Ampicillin: Inhibition zone (mm)	25.00
	Extract: MIC (mg extract/mL)	3.75 ± 0.00
	Extract: MBC (mg extract/mL)	3.75-^{NI}
	Standard Ampicillin: MIC (mg/mL)	0.96

NI: No Inhibition. Values are expressed as means ± SD.

xanthine oxidase, the IC_{50} of onion solid waste was determined as 12.5–32.5 mg/mL (Nile et al., 2017). In another study, antiinflammation effects of *Allium* species with different plant combinations were also evaluated. Results showed the combined plant extracts (*Allium hookeri* and *Curcuma longa*) synergistically inhibited inflammation via the NF-κB/COX-2/iNOS pathway (Lee et al., 2020). Quercetin and its derivatives are used as antiinflammatory agents (Murota & Terao, 2003) that can help to prevent certain diseases, such as cancer, atherosclerosis, and chronic inflammation (Gawlik-Dziki et al., 2011). The ability of quercetin isolated from different *Allium* species to inhibit the xanthine oxidase enzyme was investigated, and its antigout activity was determined. *A. flavum* was reported to have both high antioxidant capacity and antiinflammatory activity in human platelets in vitro studies, and it was concluded that it could be anti-gout agents based on its high antioxidant capacities (Simin et al., 2020). The association of antiinflammatory activity with antioxidant capacity can be considered as a result of the reduction of free radicals. The metal chelating ability of phenolics could be responsible of antiinflammatory activity by inactivating the enzymes that cause the inflammation (Jacob, 2015). As a result, *A. scorodoprasum* L. extract had inhibitory activity against the enzymes responsible for inflammation and could be used as an anti-inflammatory agent. Also, the high quercetin concentration of the extract was thought to contribute to these results.

3.8. Cytotoxic activity

The first of the most important problems in cancer chemotherapy is the toxicity of medicines. Although there are several drugs on the market to treat different types of cancer, no medication has been claimed to be completely effective and safe. Researchers have reported that the use of plants and plant-derived compounds are effective and safe in the treatment of different cancers (Shukla et al., 2018). Biological activities of plants are known for their positive effects on health and can also treat a variety of diseases, including cancer, without causing toxicity in the metabolism since they are powerful immunomodulator, and they had antioxidant properties that protect cells from oxidative damage (Kothari et al., 2020; Patil et al., 2013).

Cytotoxicity is a common method used in the determination of anticancer activity; generally, it is based on the evaluation of the toxic effect of biological and chemical substances on the cell (Szymanowska et al., 2018). The amounts of the *A. scorodoprasum* L. extract inhibiting the 50% activity of MCF-7 and MG-63 cells were recorded as 82.78 and 76.53 μg/mL, respectively (Table 6). The IC_{50} values of doxorubicin and methotrexate for these cell lines were found as 45.81, 44.49, 47.23 and 44.30 μg/mL, respectively. It has been reported in the literature that *Allium* species have antioxidant and cytotoxic effects on different cancer cells (prostate cancer, breast cancer, cervical cancer) (Bhandari et al., 2017). In this study, the high antioxidant capacity of the concentrated *A. scorodoprasum* L. extract was considered the reason for its cytotoxic activity against MG-63 and MCF-7 cell lines.

A previous study reported that the root extracts of *A. scorodoprasum* subsp. L. *rotundum* showed cytotoxic activity against colon cancer cells (IC_{50} = 84.9) (Izol et al., 2021). The cytotoxic activity of different *Allium* species (*A. flavum and A. carinatum*) on MRC-5 cancer cells was examined and reported to have close cytotoxicity compared to doxorubicin (Aleksandar et al., 2019). Radovanović et al. (2015) demonstrated effective anticancer activity in human melanoma FemX and human colon carcinoma LS174 cell lines in stem and leaf extracts of *Allium porrum*. Abdel-Hady et al. (2018) detected multiple phenolic and flavonoid substances in *Allium kurrat* extract that had anticancer activity against colon cancer (Caco-2) and liver cancer (HepG2). Previously, it has been reported the cytotoxic effect of different phenolic compounds (rosmarinic acid, quercetin, rutin, apigenine quercetin, kaempferol, genisteine, apigenine, myricetine and coumaric acid) (Radovanović et al., 2015; Taheri et al., 2020). In this study; phenolic compounds were determined in different ratios (Table 4) and quercetin (1.32 mg/g), kaempferol (0.34 mg/g) and rosmarinic acid (0.19 mg/g) were determined in the extract and considered to be effective cytotoxic agents

Table 6
Biological activities of concentrated *A. scorodoprasum* L. subsp. *rotundum* extract obtained under optimum conditions.

		Biological Activities
Antioxidant Activities (μmol TE/g extract)	DPPH (μmol TE/g extract)	1.63 ± 0.01
	FRAP((μmol TE/g extract)	2.89 ± 0.01
	TEAC((μmol TE/g extract)	2.72 ± 0.01
Antidiabetic Activities (mg extract or standard/mL)	Extract: α−Amylase inhibitory *(A. oryzae)*($IC_{50\%}$)	11.56 ± 0.48
	Extract:α−Amylase inhibitory (pancreatic)($IC_{50\%}$)	14.35 ± 0.73
	Extract:α−Glycosidase inhibitory ($IC_{50\%}$)	19.35 ± 1.09
	Standard Acarbose: α−Amylase inhibitory *(A. oryzae)*($IC_{50\%}$)	2.16 ± 0.03
	Standard Acarbose: α−Amylase inhibitory (pancreatic)($IC_{50\%}$)	2.54 ± 0.04
	Standard Acarbose: α−Glycosidase inhibitory ($IC_{50\%}$)	3.00 ± 0.03
Antiinflamatory Activities (mg extract or standard/mL)	Extract: Lipoxygenase inhibitory ($IC_{50\%}$)	9.75 ± 0.04
	Extract: Xanthine Oxidase inhibitory ($IC_{50\%}$)	9.71 ± 0.21
	Standard Quercetin: Lipoxygenase inhibitory ($IC_{50\%}$)	1.22 ± 0.03
	Standard Allopurinol: Xanthine Oxidase inhibitory ($IC_{50\%}$)	2.69 ± 0.08
Cytotoxic Activities (μg extract or standard/mL)	Extract: MCF-7($IC_{50\%}$)	82.78 ± 0.13
	Extract: MG-63 ($IC_{50\%}$)	76.53 ± 0.17
	Standard Doxorubicin: MCF-7 ($IC_{50\%}$)	45.81 ± 0.44
	Standard Doxorubicin: MG-63 ($IC_{50\%}$)	44.49 ± 0.47
	Standard Methotrexate: MCF-7 ($IC_{50\%}$)	47.23 ± 0.44
	Standard Methotrexate: MG-63 ($IC_{50\%}$)	44.30 ± 0.58

Values are expressed as means ± SD.

against both cancer cell lines (Table 4). When the findings were compared with the literature, the cytotoxic properties of the *A. scorodoprasum* L. extract were found to be similar to the literature since they contained different amounts of the aforementioned compounds.

4. Conclusion

In this study, the extraction conditions to obtain the phenolic and flavonoid compounds from the *A. scorodoprasum* L. were optimized and the optimal conditions were determined as 79 °C, 28% ethanol percentage, 118 min of the extraction time. The extract had found to be high in antioxidant and antimicrobial activities. These show the potential of *A. scorodoprasum* L. extracts to prevent the growth of the pathogenic microorganism and the formation of the free radicals that have harmful effects on the different stages of life. Besides antioxidant and antimicrobial activity, *A. scorodoprasum* L. showed antidiabetic, antiinflammatory and anticancer activity. Due to its bioactive properties, the use of its extract in food and nonfood applications can serve as a natural plant-based additive that the consumer could demand.

CRediT authorship contribution statement

Tuğba Demir: Methodology, Conceptualization, Investigation, Formal analysis, Writing – original draft. **Özlem Akpınar:** Methodology, Writing – review & editing, Supervision. **Haki Kara:** Methodology, Writing – review & editing. **Hüseyin Güngör:** Methodology.

Declaration of competing interest

The authors declare no conflict of interest.

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