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Seasonal variation of antioxidant activity of *Nigella* spp. in Ukraine

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This study aimed to evaluate the antioxidant potential of ethanol extracts of *Nigella* L. species from the Kherson Oblast of Ukraine. The extracts of seeds and above-ground parts of *N. damascena* L., *N. hispanica* L., *N. orientalis* L., and *N. sativa* L. cv. Diana were used to determine the total phenolic content (TPC), total flavonoid content (TFC), total phenolic acid content (TPAC), free radical scavenging activity (FRSA), and molybdenum reducing power (MRP). The plant raw material taken for the experiment in sprouting, budding, flowering, and ripening. The TPC of seed extracts was from 8.11 to 35.12 mg GAE.g^{-1}, TFC from 3.21 to 12.65 mg QE.g^{-1}, TPAC from 2.1 to 8.21 mg CAE.g^{-1}, FRSA from 3.56 to 7.32 mg TE.g^{-1}, and MRP from 23.47 to 68.34 mg TE.g^{-1}. The study of TPC investigated *Nigella* spp. during vegetation showed accumulation of them from 33.65 to 54.11 mg GAE.g^{-1} in the sprouting, from 54.89 to 65.76 mg GAE.g^{-1} in the budding, from 43.18 to 88.43 mg GAE.g^{-1} in the flowering, and from 49.21 to 71.45 mg GAE.g^{-1} in the ripening depending on species. The TFC was from 17.87 to 27.18 mg QE.g^{-1} at the sprouting, from 31.87 to 43.54 mg QE.g^{-1} in the budding, from 29.11 to 57.34 mg QE.g^{-1} in the flowering, and from 23.98 to 50.32 mg QE.g^{-1} in the ripening, depending on species. The TPAC was 12.11–17.32 mg CAE.g^{-1} in the sprouting, 11.17–18.43 mg CAE.g^{-1} in the budding, 10.09–28.45 mg CAE.g^{-1} in the flowering, and 17.86–22.43 mg CAE.g^{-1} in the ripening. FRSA was in the sprouting 4.56–7.77 mg TE.g^{-1}, in the budding 6.38–8.11 mg TE.g^{-1}, in the flowering 7.12–9.67 mg TE.g^{-1}, and in the ripening 5.12–9.54 tmg TE.g^{-1} depending on species. The MRP in the sprouting was 27.14–65.29 mg TE.g^{-1}, in the budding 45.48–77.5 mg TE.g^{-1}, in the flowering 77.89–94.32 mg TE.g^{-1}, and in the ripening 96.11–110.87 mg TE.g^{-1}. A strong correlation found between MRP and TPC, TFC, TPAC (r = 0.824–0.965) in the seed extracts. The results obtained in this study can be used for further biochemical and farmaceutical research.

Keywords: Black cumin, total polyphenol content, total flavonoid content, total phenolic acid content, correlation

Introduction

Species of *Nigella* L. genus belong to Ranunculaceae Juss. and well-known in many countries in the world as medicinal and culinary plants. The center of species origin and diversity is the Western-Irano-Turanian region (Zohary, 1983). The species quantity of this genus is approximately 20. The most known species are *N. damascena* L. (also named lady-in-a-mist or

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ragged lady) and *N. sativa* L. (also named black cumin or black seeds) which are used for ornamental and medicinal purposes and have a high commercial interest (Salehi et al., 2021). From a therapeutic point of view, *N. sativa* showed the possibility of treating respiratory, gastrointestinal, and rheumatic disorders and is used as a spice in foodstuffs in many countries (Tiji et al., 2021).

Most research on *Nigella* spp. raw concerning of seed or essential oil seed (Bessedik, 2016; Busari et al., 2020) composition and biological activities (Boudiaf et al., 2010). In most cases, it relates to *N. sativa* raw. *N. sativa* seed extracts are used in the cosmeceutical branch as soaps, creams, oils, shampoos, and capsules (Eid et al., 2017).

The biochemical composition of *N. sativa* seeds from Yemen, Iran, and Malaysia, according to Haron et al. (2014), was the following: crude fat from 20.63 to 28.71%, crude protein from 11.35 to 14.04%, total moisture from 5.37 to 7.93%, total ash from 4.15 to 4.51%, total carbohydrates from 48.69 to 57.18%, calcium 2,242 $mg.kg^{-1}$, potassium 6,393 $mg.kg^{-1}$, magnesium 2,234 $mg.kg^{-1}$, saturated fatty acids 1.42%, polyunsaturated fatty acids 65.13%. The nutrient content of seeds of *N. damascena* and *N. sativa* from Algeria, according to Benazzouz-Smail et al. (2023), was the following: 3.41 and 4.71% of moisture, 34.3 and 42.3% of crude fat, 25.10 and 21.60% of crude protein, 32.47 and 27.23% of total carbohydrates, 5.62 and 6.90% of reducing sugars, 4.72 and 4.16% of ash, 0.56 and 0.33% of sodium, respectively.

There exist numerous reports about the biological activities of *N. sativa* plant parts. As reported Bourgou et al. (2008), the shoot and root methanol extracts of *N. sativa* demonstrated significant antimutagenic activity. The investigation of biological activities of *N. sativa* seeds found the antioxidant (Ashraf et al., 2011; Alenzi et al., 2013; Akinwumi et al., 2020; Adam and Shuiab, 2022), antiviral, antimicrobial, anti-inflammatory (Eid et al., 2017), anti-toxic, anticancer, immunomodulatory (Ciesielska-Figlon et al., 2023) and antidiabetic (Houcher et al., 2007; Alenzi et al., 2013) activities of its extracts. The seed extracts of *N. sativa* were effective against *Salmonella typhi*, *Bacillus cereus*, *Klebsiella pneumonia*, *Escherichia coli*, etc. (Hassan et al., 2016).

The existence of different seed components can be used in cryobiological investigations (Awan et al., 2018). Also, the antioxidant, antiproliferative, and antiangiogenic activities of *N. sativa* pulp were found (Tan, 2018).

The essential oil composition is the most studied topic of these plants study. The main components of essential oil of *N. sativa* seeds are thymoquinone (57.9%), *p*-cymene (28.9%), alpha-phellandrene (6.3%), alpha-terpineol (2.7%), limonene (1.3%), etc. (Ibrahim et al., 2022). The thymoquinone also demonstrated an important antioxidant (Alenzi et al., 2013; Chung et al., 2023), cytotoxic, antifungal, and anticancer activities (Elsharkawy et al., 2021).

Also, these species are promising honey plants. The *Nigella honey* contains 14.3% of moisture, 73% of total soluble solid, 0.99% of protein, 0.18% of ash, 84.4% of total carbohydrates, 350.4 Kcal of energy (per 100 g), 558 ppm of sodium, 1063 ppm of potassium, 75.4 ppm of calcium, 58.8 ppm of magnesium, 344.2 ppm of iron, 5.6 ppm of lead, 95.5 mg of gallic acid equivalent of total polyphenols (per 50 ml), 2.66 mg of catechin equivalent of total flavonoid content (per 50 ml), and 0.69 mg of ascorbic acid (per 100 ml) (Linkon et al., 2015).

This study aimed to evaluate the antioxidant activity of the above-ground part and seeds of *Nigella* spp. from South Ukraine as a potential source of antioxidants depending on the stage of growth.

Material and methodology

Plant material

The species of *Nigella* L. from the South of Ukraine (Kherson Oblast) were used in this study: *N. damascena* L., *N. hispanica* L., *N. orientalis* L., and *N. sativa* L. cv. Diana (Figure 1). The seeds were planted and above-ground parts (herb) were taken for analysis at the sprouting, budding, flowering, and ripening stages during 2020–2021 from the experimental collection of the Institute of Climate Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine (Kherson region, v. Plodove). Also, the seeds of the investigated species were analyzed.

Biochemical analyses

All biochemical analyses were conducted at the Slovak University of Agriculture in Nitra (Slovak Republic).

Chemicals

All chemicals used were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) and CentralChem (Slovakia).

Figure 1 Plants of *Nigella* spp. in the flowering
1 – *Nigella* hispanica L.; 2 – *N. orientalis* L.; 3 – *N. damascena* L.; 4 – *N. sativa* L. cv. Diana is in the flowering stage

Preparations of extracts

An amount of 0.25 g of each sample was extracted with 20 mL of 80% ethanol for 2 h in a laboratory shaker GFL 3005 (GFL, Burgwedel, Germany). Then, the samples were centrifuged at 4,605 RCF (Rotofix 32 A, Hettich, Germany) for 10 min and the supernatant was used for measurement of FRSA (antiradical activity) using DPPH, MRAP (antioxidant activity) using phosphomolybdenum method and measurement of other antioxidant properties (detection of total polyphenol, total flavonoid, and phenolic acid content).

Total polyphenol content of extracts

The total polyphenol content (TPC) was measured by the method of Singleton and Rossi (1965) using the Folin-Ciocalteu reagent. A quantity of 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured with the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25–300 $mg.L^{-1}$; R^2 = 0.998) was used as the standard. The results were expressed as $mg.g^{-1}$ DW gallic acid equivalent.

Total phenolic acid content

The content of phenolic acids (TPAC) was determined using the procedure described by Árvay et al. (2017). 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v), and 0.5 ml of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid 1–200 $mg.L^{-1}$ (R^2 = 0.999) was used as a standard. The results were expressed in $mg.g^{-1}$ caffeic acid equivalents (CAE).

Total flavonoid content of extracts

The total flavonoid content (TFC) was determined by the modified method described by Shafii et al. (2017). An aliquot of 0.5 mL of the sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1 M potassium acetate, and 4.3 mL of distilled water. After 30 min in darkness, the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (1–400 $mg.L^{-1}$; R^2 = 0.997) was used as the standard. The results were expressed in $mg.g^{-1}$ DW quercetin equivalent.

Free radical scavenging activity

Free radical scavenging activity (FRSA) of samples (antiradical activity) was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchéz-Moreno et al., 1998). An amount of 0.4 mL of sample was mixed with 3.6 mL of DPPH solution (0.025 g DPPH

in 100 mL ethanol). The absorbance of the reaction mixture was determined with the spectrophotometer Jenway (6,405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg.L^{-1}; R^2 = 0.989) was used as the standard and the results were expressed in mg.g^{-1} DM Trolox equivalents.

Molybdenum-reducing power of extracts

The molybdenum-reducing power (MRP) of samples was determined by the method of Prieto et al. (1999) with slight modifications. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M), and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then cooled to room temperature. The absorbance at 700 nm was detected with the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1,000 mg.L^{-1}; R^2 = 0.998) was used as the standard and the results were expressed in mg.g^{-1} DM Trolox equivalent.

Statistical analysis

The results are expressed as mean values of three replications ± standard deviation (SD); Data were analyzed with the ANOVA test and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

Results and discussion

Polyphenol compounds are plant-produced second metabolites with numerous biological activities and health benefits (Rana et al., 2022). The total content of polyphenol compounds depends on numerous factors such as period of growth, ecological conditions of growth (Rini et al., 2023), species and genotypes, plant raw, extracts, etc. (Vergun et al., 2023).

The study of *Nigella* spp. antioxidant activity and antioxidant compounds as a rule related to *N. sativa* seeds as the most popular raw among other species (Neha et al., 2014; Pop et al., 2020; Muzolf-Panek and Gliszczyńska-Świgło, 2022).

This study showed that the TPC of seed extracts of investigated plants was from 8.11 to 35.12 mg GAE.g^{-1}, TFC from 3.21 to 12.65 mg QE.g^{-1}, TPAC from 2.1 to 8.21 mg CAE.g^{-1}, FRSA from 3.56 to 7.32 mg TE.g^{-1}, and MRP from 23.47 to 68.34 mg TE.g^{-1} (Figure 2). It should be noted that minimal values were found for seed extracts of *N. sativa* cv. Diana and maximal for *N. hispanica* except for FRSA. In this case maximal value found for *N. orientalis*.

It was found 31.15 mg GAE.g^{-1} of TPC and 16.34 mg GAE.g^{-1} of the total flavonoid content of seed extracts obtained by Soxhlet extraction (Goga et al., 2012). Kaushik and Barmanray (2012) determined in ethanol extracts of *N. sativa* seeds 138.59 mg GAE.100 g^{-1} of TPC. The study of different seed extracts of *N. sativa* demonstrated the most TPC (81.31 mg GAE.mg^{-1})

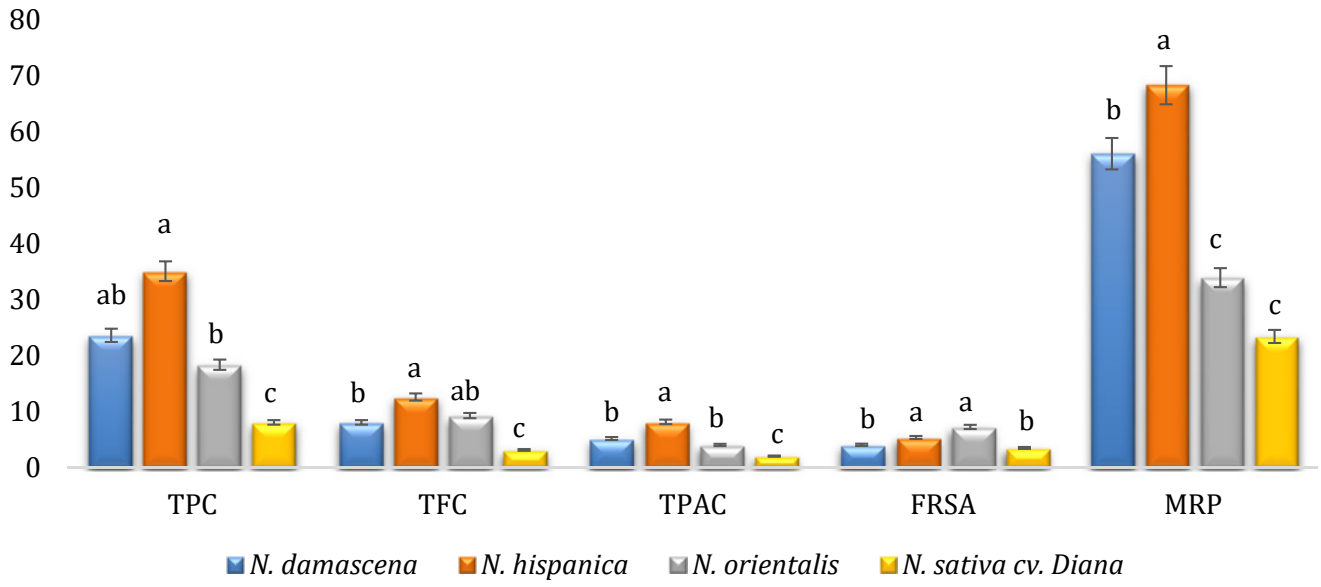

Figure 2 The antioxidant parameters of *Nigella* spp. seed extracts
TPC – total phenolic compounds, mg of gallic acid equivalent per gram; TFC – total flavonoid content, mg of quercetin equivalent per gram; TPAC – total phenolic acid compounds, mg of caffeic acid equivalent per gram; FRSA – free radical scavenging activity, mg of Trolox equivalent per gram; MRP – molybdenum reducing power, mg of Trolox equivalent per gram. Means in each column followed by different letters are significantly different ($p < 0.05$)

and TFC (5.20 mg QE.mg^{-1}) in the chloroform extracts (Meziti et al., 2012). Mazandarani (2015) determined 121.3 mg GAE.g^{-1} of TPC and 194.04 mg QE.g^{-1} of TFC in seed extracts of *N. sativa*. Guergouri et al. (2017) determined 16.67 µg GAE.g^{-1} of TPC and 3.83 µg QE.g^{-1} of TFC in the seed oil of *N. sativa*. Sadik et al. (2017) detected 19.9 mg GAE.g^{-1} DW of TPC in seed extracts of this species. The study of different extracts of *N. sativa* seeds showed that TPC in water, methanol, and ethanol extracts was 51.63, 31.16, and 5.47 µg GAE.g^{-1}, respectively. The chloroform and dichloromethane extracts demonstrated the absence of TPC (Dalli et al., 2021). The TFC in the same study was 10.11, 18.4, and 39.82 µg QE.g^{-1} in water, methanol, and ethanol extracts, respectively. It should be noted that chloroform and dichloromethane extracts showed 14.11 and 16.4 µg QE.g^{-1} of TFC, respectively (Dalli et al., 2021). The study of Alrashidi et al. (2022) demonstrated that TPC of oil extracts from seeds *N. sativa* was 116.39 mg GAE.g^{-1} in methanol extracts, 106.94 mg GAE.g^{-1} in ethanol extracts, and 238.80 mg GAE.g^{-1} in mixed extracts (methanol-water (1 : 1)).

Benazzous-Smail et al. (2023) determined in *N. sativa* and *N. damascena* seed extracts 6.28 and 18.41 mg GAE.g^{-1} of TPC, respectively, and 0.59 and 1.38 mg QE.g^{-1} of TFC, respectively.

Flavonoids are biochemical substances widely presented in plant raw and demonstrated antimicrobial, antioxidant, anticancer, and anti-inflammatory activities (Tungmunnithum et al., 2018).

The study of TPC of investigated *Nigella* spp. herb extracts during vegetation showed accumulation of them from 33.65 to 54.11 mg GAE.g^{-1} in the sprouting, from 54.89 to 65.76 mg GAE.g^{-1} in the budding, from 43.18 to 88.43 mg GAE.g^{-1} in the flowering, and from 49.21 to 71.45 mg GAE.g^{-1} in the ripening depending on species (Figure 3). At all, the TPC accumulation was found in a range of 33.65–88.43 mg GAE.g^{-1} depending on species and stage of growth.

As reported Bourgou et al. (2008), the TPC of shoots and root extracts of *N. sativa* was 10.04 and 4.01 mg GAE.g^{-1}, respectively. *Consolida regalis* Gray (Ranunculaceae) ethanol extracts of leaves, stems, and flowers demonstrated 12.41, 5.15, and 16.29 mg GAE.g^{-1} of TPC, respectively (Ucar, 2018). Cuce (2023) determined 19.17, 18.59, and 21.24 mg GAE.g^{-1} of the TPC in flower, root, and leaf extracts of *Adonis paryadrica* (Boiss.) Kandemir & Autaç (Ranunculaceae), respectively.

We determined TFC from 17.87 to 27.18 mg QE.g^{-1} at the sprouting, from 31.87 to 43.54 mg QE.g^{-1} in the budding, from 29.11 to 57.34 mg QE.g^{-1} in the flowering, and from 23.98 to 50.32 mg QE.g^{-1} in the ripening, depending on species (Figure 4). At all TFC of investigated plants was from 17.87 to 57.34 mg QE.g^{-1} depending on species and period of growth.

The study of different solvents of *N. sativa* seeds demonstrated 413.33 mg QE.100 g^{-1} of TFC (Kaushik and Barmanray, 2012). In the flower, root, and leaf extracts of *Adonis paryadrica* detected 32.42, 0.54, and 54.97 mg RE.g^{-1} (rutin equivalent) of TFC, respectively (Cuce, 2023).

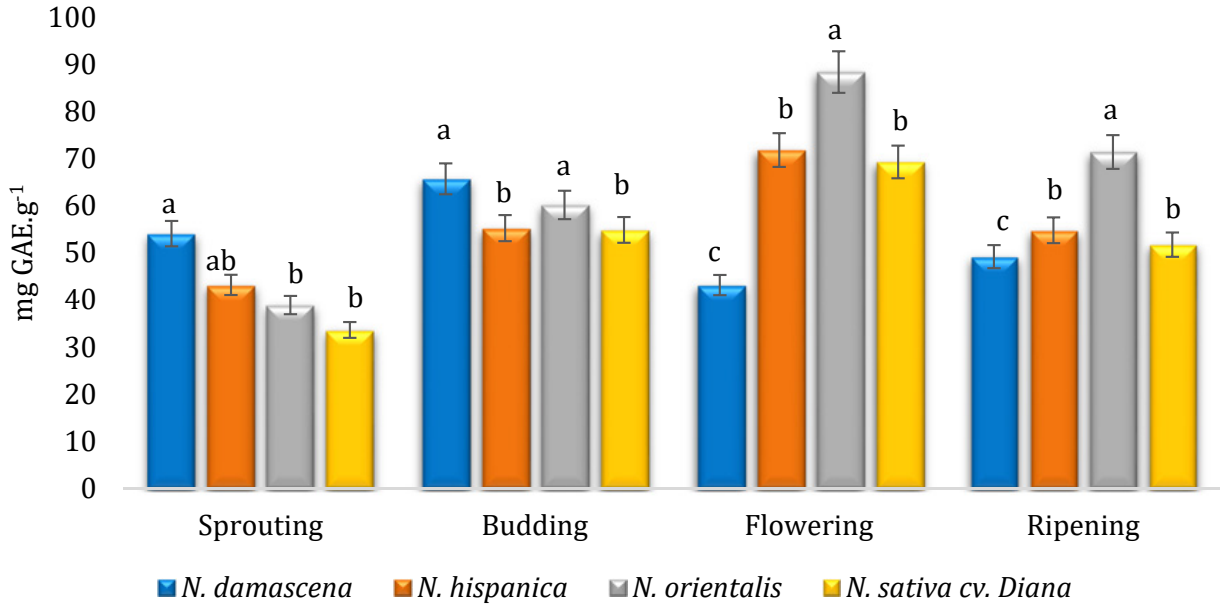

Figure 3 The seasonal variation of the total polyphenol content of *Nigella* spp. herb extracts
GAE – gallic acid equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

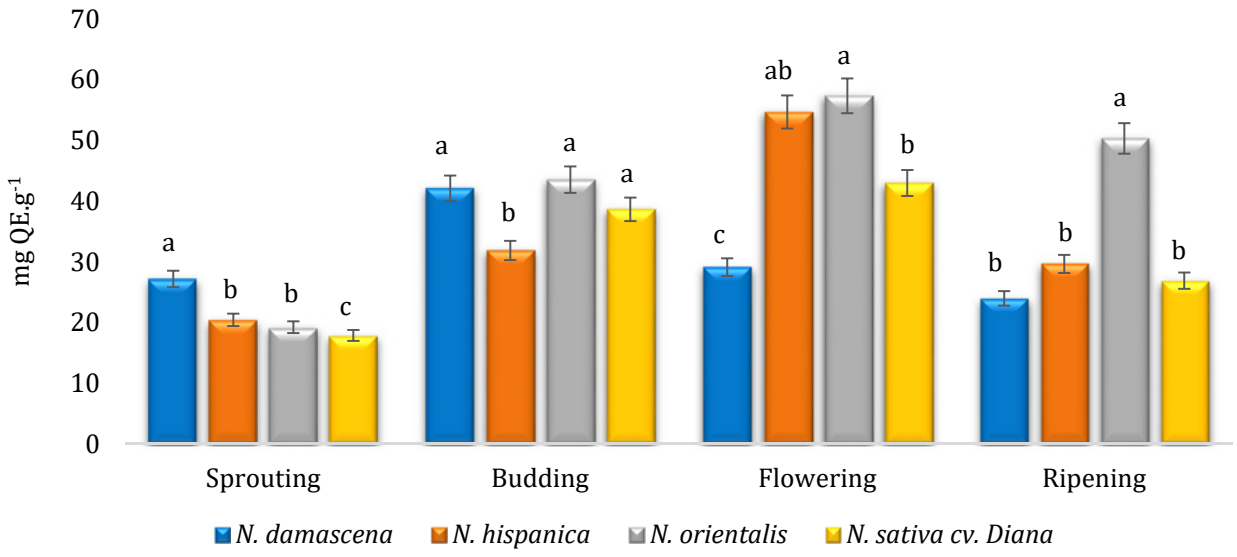

Figure 4 The seasonal variation of the total flavonoid content of *Nigella* spp. herb extracts
QE – quercetin equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

The TPAC of investigated extracts was from 10.09 to 28.45 mg CAE.g^{-1} depending on species and stage of growth (Figure 5). This parameter was 12.11–17.32 mg CAE.g^{-1} in the sprouting, 11.17–18.43 mg CAE.g^{-1} in the budding, 10.09–28.45 mg CAE.g^{-1} in the flowering, and 17.86–22.43 mg CAE.g^{-1} in the ripening. The minimal values of TPAC were found in the sprouting and budding stage for *N. sativa* cv. Diana plants, in the flowering stage for *N. damascena*, and the ripening stage for *N. orientalis*. The maximum values of TPAC in the sprouting were determined for *N. damascena*, in the budding and ripening for *N. hispanica*, and the flowering period for *N. orientalis*.

The gallic, hydroxybenzoic, syringic, vanillic, caffeic, coumaric, and cinnamic acids were identified in extracts of *N. damascena* and *N. sativa* (Benazzous-Smail et al., 2023).

The most widely used method of antioxidant activity determination is the DPPH method which changes the extract coloring from purpure to yellow or green depending on raw. Kaushik and Barmanray (2012) studied different solvents and found high DPPH scavenging ability in methanol extracts (58.08%) and low in water extracts (20.81%) of *N. sativa* seeds. The study of *N. sativa* seed extracts from antioxidant potential by the DPPH method showed an increase in the

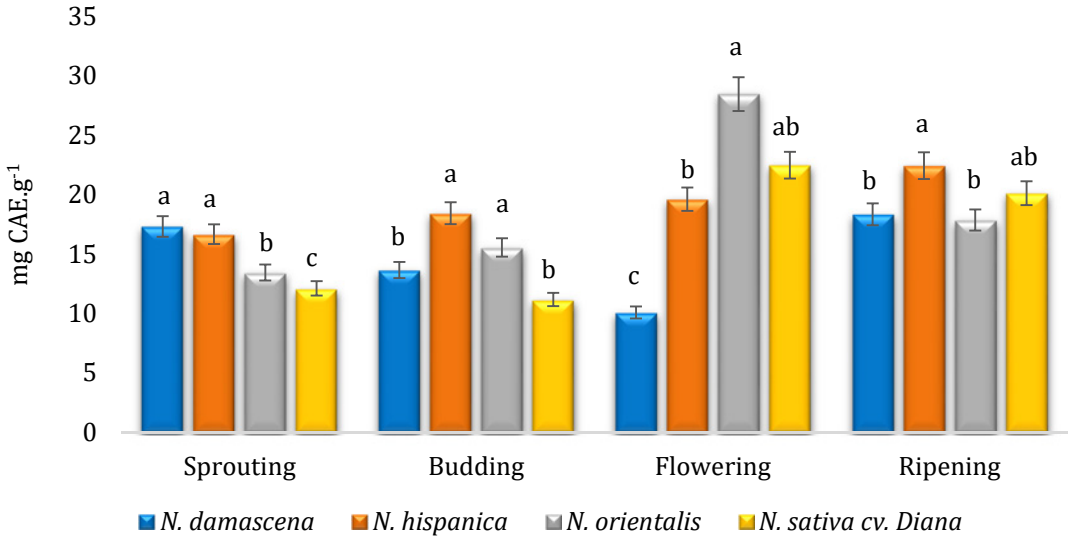

Figure 5 The seasonal variation of the total phenolic acid content of *Nigella* spp. herb extracts
CAE – caffeic acid equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

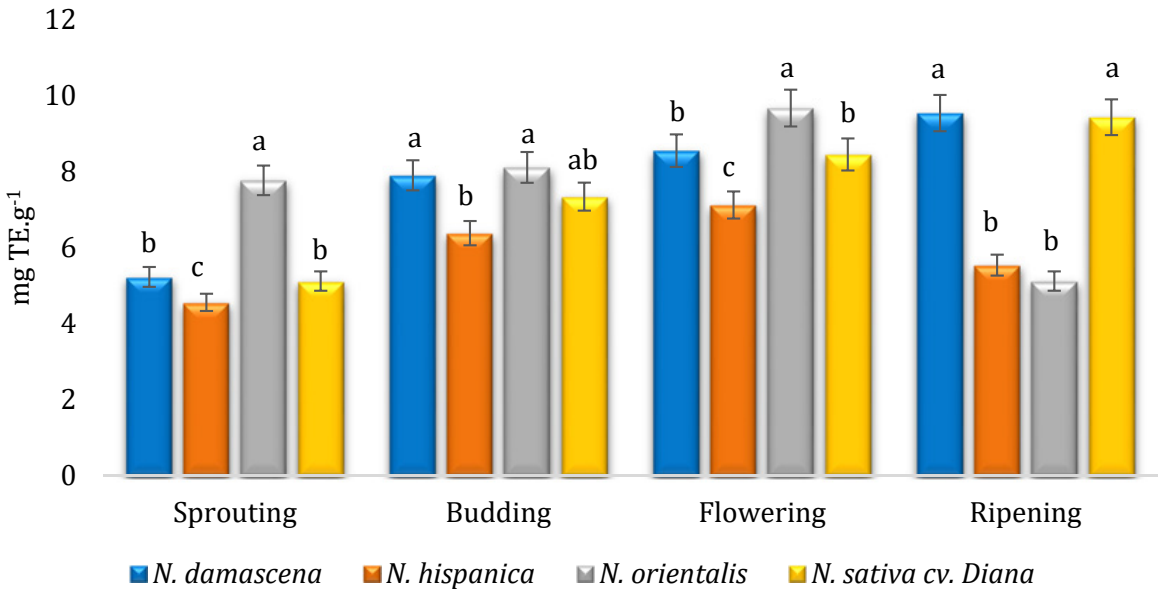

Figure 6 The seasonal variation of antioxidant activity by DPPH method of *Nigella* spp. herb extracts
TE – Trolox equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

radical scavenging ability depending on concentration (Hassan et al., 2016). Mammad et al. (2017) found that methanol seed extracts of *N. sativa* had moderate antioxidant activity by the DPPH method.

FRSA of ethanol extracts of *Nigella* species showed that in the sprouting it was 4.56–7.77 mg TE.g^{-1}, in the budding it was 6.38–8.11 mg TE.g^{-1}, in the flowering it was 7.12–9.67 mg TE.g^{-1}, and in the ripening, it was 5.12–9.54 mg TE.g^{-1} depending on species (Figure 6). It should be noted that highest values of this parameter were determined for *N. orientalis* in the sprouting, budding, and flowering, and for *N. damascena* cv. Diana in the ripening.

The study by Malik et al. (2017) demonstrated *in vitro* antioxidant activity by the DPPH method of different Ranunculaceae (root extracts) from 0.016 to 0.251 g TE.g^{-1} DW (or from 16 to 251 mg TE.g^{-1} DW). Among these species are representatives of Aconimum L., Anemone L., and Pulsatilla L. genera. The study concerning *Adonis paryadrica* flower, root, and leaf

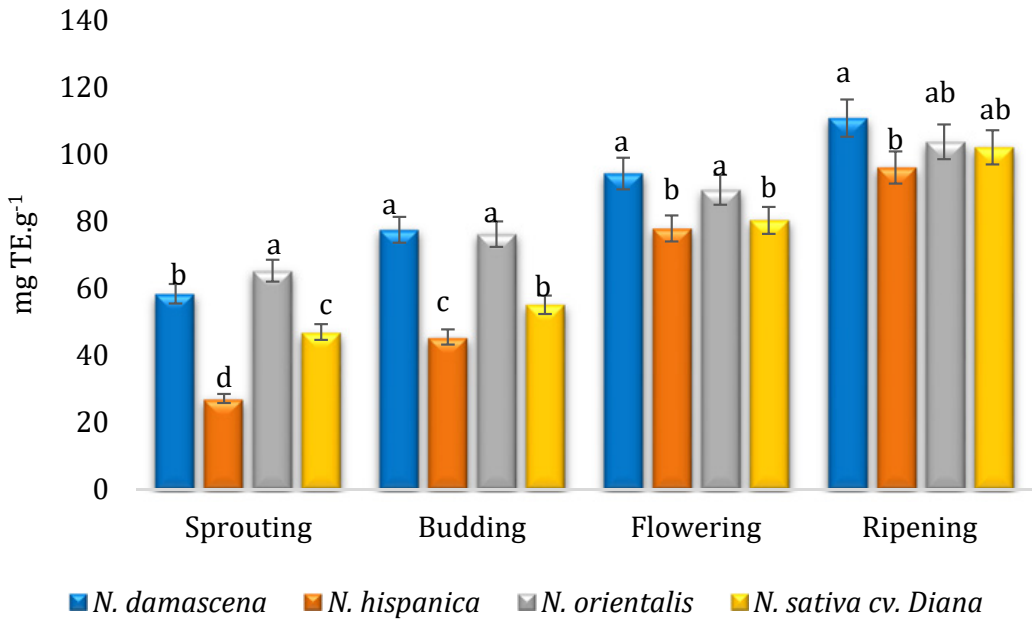

Figure 7 The seasonal variation of reducing power of *Nigella* spp. herb extracts by phosphomolybdenum method
TE – Trolox equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

extracts showed that antioxidant activity by the DPPH method was 30.94, 12.58, and 38.26 μmol TE.g^{-1}, respectively (Cuce, 2023).

The MRP of investigated plant extracts depended on the stage of growth and species. In the sprouting, it was 27.14–65.29 mg TE.g^{-1}, in the budding it was 45.48–77.5 mg TE.g^{-1}, in the flowering 77.89–94.32 mg TE.g^{-1}, and in the ripening it was 96.11–110.87 mg TE.g^{-1} (Figure 7).

The study of relations between antioxidant activity by various methods and numerous groups of polyphenol compounds in plant extracts demonstrated a strong correlation between them (Kędzierska-Matysek et al., 2021; Szabo et al., 2021). A strong positive correlation can observed between different polyphenol compounds and antioxidant activity considering also conditions such as different times and temperatures of conducting (Lim et al., 2019).

The correlation analysis of obtained data concerning antioxidant parameters of investigated species showed that between two methods of antioxidant activity of herb extracts was a moderate correlation (r = 0.568) (Table 1). However, both FRSA and MRP had a weak correlation with TPC (r = 0.298 and r = 0.373, respectively), TFC (r = 0.263 and r = 0.336, respectively), and TPAC (r = 0.228 and r = 0.340, respectively).

Also, between accumulated compounds and the molybdenum-reducing power of seed extracts existed a strong correlation (r = 0.824–0.965). However, between two assays of antioxidant activity was a very weak correlation (r = 0.051). Between FRSA and TFC of seed extracts, a moderate correlation (r = 0.597) was found whereas with TPC (r = 0.285) and TPAC (r = 0.251) a correlation was weak.

According to Dalli et al. (2021), a strong correlation was found between the total flavonoid compounds and antioxidant activity by the DPPH method in seed extracts (r = 0.986). In our study, the TFC of seed extracts demonstrated a moderate correlation with FRSA (r = 0.597). Khiya et al. (2021) in the study of *Salvia officinalis* extracts found a strong correlation between TPC and FRSA by the DPPH method (r = 0.771) and between TPC and MRP (r = 0.932). In our study, between TPC and MRP of seed extracts was found a trong correlation, and in the rest extracts observed weak or moderate correlation.

Conclusions

The obtained data demonstrate the high antioxidant potential of different extracts of *N. damascena*, *N. hispanica*, *N. orientalis*, and *N. sativa* cv. Diana from the Kherson Oblast (Ukraine) during four stages of growth. The higher values of TPC, TFC, TPAC, and MRP were determined in seed extracts of *N. hispanica*, and FRSA in extracts of *N. orientalis*. The lowest values of all parameters demonstrated the study of seed extracts of *N. sativa* cv. Diana. The seasonal variation of TPC was the following: in the sprouting and budding maximal values found in extracts *N. damascena*, in the flowering and ripening in extracts of *N. orientalis*. The extracts of *N. damascena* showed high values of TFC in the sprouting and *N. orientalis* in the budding, flowering, and ripening. The maximal values of TPAC

Table 1 Pearson's coefficients of correlation of antioxidant parameters of *Nigella* species

Parameters	TPC	TFC	TPAC	FRSA	MRP
Herb					
TPC	1.000	0.941**	0.685*	0.298	0.373
TFC	0.941**	1.000	0.461	0.263	0.336
TPAC	0.685*	0.461	1.000	0.228	0.340
FRSA	0.298	0.263	0.228	1.000	0.568*
MRP	0.373	0.336	0.340	0.568*	1.000
Seeds					
TPC	1.000	0.939**	0.997**	0.285	0.965**
TFC	0.939**	1.000	0.925**	0.597*	0.824**
TPAC	0.997**	0.925**	1.000	0.251	0.960**
FRSA	0.285	0.597*	0.251	1.000	0.051
MRP	0.965**	0.824**	0.960**	0.051	1.000

TPC – total phenolic content; TFC – total flavonoid content; TPAC – total phenolic acid content; FRSA – free radical scavenging activity; MRP – molybdenum reducing power; ** – correlation is significant at the level of 0.01; * – correlation is significant at the level of 0.05

were found in sprouting in *N. damascena* extracts, in the budding and ripening in *N. hispanica* extracts, and in the flowering in *N. orientalis* extracts. FRSA and MRP were maximal in the sprouting in *N. orientalis* extracts, in the ripening in *N. damascena* extracts. The maximal values of FRSA in the budding and flowering were determined in *N. orientalis* extracts, and MRP in the same period in *N. damascena* extracts. This study can be helpful for the identification of more needed periods of growth of *Nigella* spp. for obtained plant raw with high antioxidant activity. Also, these results can be useful for further structural biochemical investigations.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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References

Adam, G.O., & Shuiab, Y.A. (2022). Antioxidant and anti-tyrosinase potentials of extracts of *Nigella sativa* and *Senna alexandrina* from Sudan. In *Journal of Applied Veterenary Sciences*, 7(2), 41–45. http://dx.doi.org/10.21608/javs.2022.122398.1127

Akinwumi, K.A., Jubril, A.J., Olaniyan, O.O., & Umar, U.U. (2020). Ethanol extracts of *Nigella sativa* has antioxidant and ameliorative effect against nickel chloride-induced hepato-renal injury in rats. In *Clinical Phytoscience*, 6(64), 1–12. https://doi.org/10.1186/s40816-020-00205-9

Alenzi, F.Q., Altamini, M.A.A., Kujan, O., Tarakji, B., Tamimi, W., Bagader, O., Shangiti, A.A., Alanazi, A.B.T., Alenezy, A.K., Al-Swailmi, F., Alenizi, D., Salem, M.L., & Wyse, R.K.H. (2013). Antioxidant properties of *Nigella sativa*. In *Molecular and Genetic Medicine*, 7(3), 1–5. http://dx.doi.org/10.4172/1747-0862.1000077

Alrashidi, M., Derawi, D., Salimon, J., & Yusoff, M.F. (2022). The effects of different extraction solvents on the yield and antioxidant properties of *Nigella sativa* oil from Saudi Arabia. In *Journal of Taibah University for Science*, 16(1), 330–336. https://doi.org/10.1080/16583655.2022.2057673

Árvay, J., Hauptvogl, M., Ivanisova, E., Tidil'ová, I., Hrstková, M., Bajčan, D., and Lazor, P. (2017). Methylxanthines and catechines in different teas (*Camellia sinensis* L. Kuntze) – influence on antioxidant properties. In *Potravinarstvo Slovak Journal of Food Science*, 11(1), 510–516. http://dx.doi.org/10.5219/796

Ashraf, S.S., Rao, M.V., Kaneez, F.S., Qadri, S., Al-Marzouqi, A.H., Chandranath, I.S., & Adem, A. (2011). *Nigella sativa* extract as a potent antioxidant for petrochemical induced oxidative stress. In *Journal of Chromatographic Science*, 49, 321–326.

Awan, M.A., Akhter, S., Husna, A.U., Ansari, M.S., Rakha, B.A., Azam, A., & Qadeer, S. (2018). Antioxidant activity of *Nigella sativa* seeds aqueous extracts and its use for cryopreservation of buffalo spermatozoa. In *Andrologia*, e13020. https://doi.org/10.1111/and.13020

Benazzouz-Smail, L., Achat, S., Brahmi, F., Bachir-Bey, M., Arab, R., Loreno, J.M., Benbouriche, A., A., Boudiab, K., Hauchard, D., Boulekbache, L., & Madani, K. (2023). Biological properties, phenolic profile, and botanical aspect of *Nigella sativa* L. and *Nigella damascena* L. seeds: a comparative study. In *Molecules*, 28, 571. https://doi.org/10.3390/molecules28020571

Bessedik, A. (2016). Toxicity and antioxidant activity of the essential oil of *Nigella sativa*. In *Der Pharmacia Lettre*, 8(15), 245–249.

Boudiaf, K., Houcher, Z., Sobhi, W., & Benboubetra, M. (2010). Evaluation of antioxidant and anti-xanthine oxidoreductase activities of *Nigella sativa* Linn seeds' extracts. In *Journal of Applied Biological Sciences*, 4(1), 7–16.

Bourgou, S., Ksouri, R., Bellila, A., Skandrani, I., Falleh, H., & Marzouk, B. (2008). Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. In *Comptes Rendus Biologies*, 331, 48–55. https://doi.org/10.1016/j.crvi.2007.11.001

Busari, A.A., Adejare, A.A., Kelani, S.O., Imam, K.O., Awesu, A.A., & Adefila-Sanni, I. (2020). Comparative pre- and post-treatment effects of *Nigella sativa* oil on lipid profile and antioxidant enzymes in a rat model of *diabetes mellitus*. In *Nigerian Journal of Medicine*, 29, 415–421. https://doi.org/10.4103/NM_42_20

Chung, K.X., Wei, Y.L.P., & Akowuah, G.A. (2023). Different extraction methods for thymoquinone from *Nigella sativa* L. seeds and antioxidant activity. In *Indian Journal of Natural Products and Resources*, 14(1), 75–80. https://doi.org/10.56042/ijnpr.v14il.1134

Ciesielska-Figlon, K., Wojciechowicz, K., Wardowska, A., & Lisowska, K.A. (2023). The immunomodulatory effect of *Nigella sativa*. In *Antioxidants*, 12, 1340. https://doi.org/10.3390/antiox12071340

Cuce, M. (2023). Antioxidant and antimicrobial activities of methanol extracts from *Adonis paryadrica* (Ranunculaceae) – a critically endangered endemic species growing in the Turkish flora. In *International Journal of Secondary Metabolite*, 10(1), 147–157. https://doi.org/10.21448/ijsm.1271974

Dalli, M., Azizi, S., Kandzi, F., & Gseyra, N. (2021). Evaluation of the *in vitro* antioxidant activity of different extracts of *Nigella sativa* L. seeds, and the quantification of their bioactive compounds. In *Materials Today: Proceedings*, 45, 7259–7263. https://doi.org/10.1016/j.matpr.2020/12/743

Eid, A.M., Elmarzugi, N.A., Ayyash, L.M.A., Sawafta, M.N., & Daana, H.I. (2017). A review on the cosmeceutical and external applications on *Nigella sativa*. In *Journal of Tropical Medicine*, 2017, 7092514. https://doi.org/10.1155/2017/7092514

Elsharkawy, E.R., Abdallah, E.A., & Markb, A.A. (2021). Potential cytotoxic, antifungal, and antioxidant activity of ditymoquinone and tymoquinone. In *Journal of Hunan University* (*Natural Sciences*), 48(9), 90–99.

Goga, A., Hasić, S., Bećirovićć, Š., & Ćavar, S. (2012). Phenolic compounds and antioxidant activity of extracts of *Nigella sativa* L. In *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, 39, 15–19.

Guergouri, F.Z., Sabhi, W., & Benbaubetra, M. (2017). Antioxidant activity of Algerian *Nigella sativa* total oil and its unsaponifiable fraction. In *The Journal of Phytopharmacology*, 6(4), 234–238.

Haron, H., Grace-Lynn, C., & Shahar, S. (2014). Comparison of physicochemical analysis and antioxidant activities of *Nigella sativa* seeds and oils from Yemen, Iran, and Malaysia. In *Sains Malaysiana*, 43(4), 535–542.

Hassan, W., Noreen, H., Khalil, S.U., Hussain, A., Rehman, S., Sajjad, S., Rehman, A.U., & Rocha, J.B.T. (2016). Ethanolic extract of *Nigella sativa* protects Fe(II) induced lipid peroxidation in rat's brain, kidney and liver homogenates. In *Pakistan Journal of Pharmacological Sciences*, 29(1), 231–237.

Houcher, Z., Boudiaf, K., Benboubetra, M., & Houcher, B. (2007). Effects of methanolic extract and commercial oil of *Nigella sativa* L. on blood glucose and antioxidant capacity in alloxan-induced diabetic rats. In *Pteridines*, 18(1), 8–18.

Ibrahim, M.A., Mohammed, B.A., & El-Kamali, H.H. (2022). GC/MS profiling and antioxidant activity of *Nigella sativa* essential oil from Sudan. In *World Journal of Pharmaceutical and Life Sciences*, 8(12), 152–156.

Kaushik, N., & Barmanray, A. (2012). Evaluation of flavonoids content, phenolic compounds and antioxidant properties of *Nigella sativa* seed extracts using different solvents. In *International Journal of Food and Nutritional Sciences*, 11(3), 2964–2973.

Kędzierska-Matysek, M., Stryjecka, M., Teter, A., Skałecki, P., Domaradzki, P., & Florek, M. (2021). Relationships between the content of phenolic compounds and the antioxidant activity of Polish honey varieties as a tool for botanical discrimination. In *Molecules*, 26, 1810. https://doi.org/10.3390/molecules26061810

Khiya, Z., Oualcadi, Y., Gamar, A., Barrekhis, F., Zair, T., & Hilali, F.E. (2021). Correlation of total polyphenolic content with antioxidant activity of hydromethanolic extract and their fractions of the *Salvia officinalis* leaves from different regions of Morocco. In *Journal of Chemistry*, 2021, 8585313. https://doi.org/10.1155/2121/8585313

Lim, Y.P., Pang, S.F., Yusoff, M.M., & Gimbun, J. (2019). Correlation between the antioxidant, total flavonoid and total phenolic content of *Phaleria macrocarpa* fruit extracts. In *International Journal of Recent Technology and Engineering*, 8(1), 38–42.

Linkon, K.M.M.R., Prodhan, U.K., Hakim, M.A., & Alim, M.A. (2015). Study on the physicochemical and antioxidant properties of *Nigella honey*. In *International Journal of Nutrition and Food Sciences*, 4(2), 137–140. https://doi.org/10.11648/j.ijnfs.20150402.13

Malik, J., Tauchen, J., Landa, P., Kutil, Z., Marsik, P., Klaucek, P., Havlik, J., & Kokoska, L. (2017). *In vitro* antiinflammatory and antioxidant potential of root extracts from Ranunculaceae species. In *South African Journal of Botany*, 109, 128–137. http://dx.doi.org/10.1016/j.sajb.2016.12.008

Mammad, Z., Mammad, K., Aqel, T., Kribii, A., & Ounine, K. (2017). Antibacterial and antioxidant activity of *Nigella sativa*. In *International Journal of Innovation and Scientific Research*, 31(1), 167–172.

Mazandarani, M. (2015). Aut ecology, total phenol, and total flavonoid content, antioxidant activity, and ethnopharmacological survey of *Nigella sativa* Linn. in traditional medicine of Golestan province, North of Iran. In *Crescent Journal of Medical and Biological Science*, 2(3), 95–99.

Meziti, A., Meziti, H., Boudiaf, K., Mustapha, B., & Bouriche, H. (2012). Polyphenolic profile and antioxidant activities of *Nigella sativa* seed extracts *in vitro* and *in vivo*. In *International Journal of Biotechnology and Bioengineering*, 6(4), 109–117.

Muzolf-Panek, M., & Gliszczyńska-Świgło, A. (2022). Extraction optimization of the antioxidants from *Nigella sativa* seeds using response surface methodology. In *Journal of Food Measurement and Characterization*, 16, 4741–4753. https://doi.org/10.1007/s11694-022-01575-z

Neha, R., Vali, V., Gunaseelan, J., & Perinbam, K. (2014). Antioxidant and antiproliferative activity of the methanol extract from *Nigella sativa* seeds. In *Asian Journal of Biological Sciences,* 7(3), 122–130. https://doi.org/10.3923/ajbs.2014.122.130

Pop, R.M., Bocsan, I.C., Buzoianu, A.D., Chedea, V.S., Sacaci, S.A., Pecoraro, M., & Papolo, A. (2020). Evaluation of the antioxidant activity of *Nigella sativa* L. and *Allium ursinum* extracts in a cellular model of doxorubicin-induced cardiotoxicity. In *Molecules*, 25, 5259. https://10.3390/molecules25225259

Prieto, P., Pineda, M., & Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. In *Analytical Biochemistry*, 269(2), 337–341. https://doi.org/10.1006/abio.1999.4019

Rana, A., Samtiya, M., Dhewa, T., Mishra, V., Aluko, R.E. (2022). Health benefits of polyphenols: a concise review. In *Journal of Food Biochemistry*, 46(10), e14264. https://doi.org/10.1111/jfbc.14264

Rini, S., Shweta, V., Vijender, S., & Sakshi, B. (2023). Impact of geographical location on the polyphenolic content of *Nigella sativa* seed extract and the relative anti-inflammatory and antioxidant potential thereof: a comparative study. In *Indian Journal of Natural Products and Resources*, 14(1), 89–96. https://doi.org/10.56042/ijnpr.v14i1.1132

Sadik, I., Allah, A.A., Abdulhameed, M., Abdelkader, M., Sayed, N., Nour, A., & Tom, A.A. (2017). Antioxidant activity and *in vitro* potential inhibition of *Nigella sativa* and *Saussurea lappa* against LDL oxidation among Sundance. In *E3 Journal of Medical Research*, 6(3), 22–26. http://dx.doi.org/10.18685/EJMR(6)3 EJMR-17-013

Salehi, B., Quispe, C., Imran, M., Ul-Haq, I., Živković, J., Abu-Reidan, I.M., Sen, S., Taheri, Y., Acharya, K., Azadi, H., Contreras, M.M., Segura-Carretero, A., Mnayer, D., Sethi, G., Martorell., M., Razis, A.F.A., Sunusi, U., Kamal, R.M., Suleria, H.A.R., & Sharifi-Rad, J. (2021). *Nigella* plants – traditional uses, bioactive phytoconstituents, preclinical and clinical studies. In *Frontiers in Pharmacology*, 12, 625386. https://doi.org/10.3389/fphar.2021625386

Sánchéz-Moreno, C., Larrauri, A., & Saura-Calixto, F. 1998. A procedure to measure the antioxidant efficiency of polyphenols. In *Journal of the Science of Food and Agriculture*, 76, 2, 270–276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

Shafii, Z. A., Basri, M., Malek, E. A., & Ismail, M. 2017. Phytochemical and antioxidant properties of *Manilkara zapota* (L.) P royal fruit extracts and its formulations for cosmeceutical application. In *Asian Journal of Plant Science and Research*, 7(3), 29–41.

Singleton, V. L., & Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. In *American Journal of Enology and Viticulture*, 16(3), p. 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Szabo, E., Marosvölgyi, T., Kőrösi, L., Schmidt, J., Csepregi, K., Márk, L., Bóna, Á. (2021). Correlations between total antioxidant capacity, polyphenol and fatty acid content of native grape seed and pomace of four different grape varieties in Hungary. In *Antioxidants* (Basel), 10(7), 1101. https://doi.org/10.3390/antiox10071101

Tan, G. (2018). Antioxidative, antiproliferative and antiangiogenic activities of *Nigella sativa* pulp, a waste material remaining from oil production. In *Haceteppe Journal Biology and Chemistry*, 46(4), 629–642. https://doi.org/10.15671/HJBC.2018.268

Tiji, S., Benayad, O., Berrabah, M., Mounsi, I.E., & Mimouni, M. (2021). Phytochemical profile and antioxidant activity of *Nigella sativa* L. growing in Marocco. In *The Scientific World Journal*, 2021, 6623609. https://doi.org/10.1155/2021/6623609

Tungmunnithum, D., Thongboonyou, A., Phalboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolics compounds from medicinal plants for pharmaceutical and medicinal aspects: an overview. In *Medicines*, 5, 93. https://doi.org/10.3390/medicines5030093

Ucar, E. (2018). *In vitro* antioxidants and antimicrobial activities and various enzyme inhibitory activities of ethanol extracts from different organs of *Consoloda regalis*. In *Fresenius Environmental Bulletin*, 27(9), 5950–5957.

Vergun, O., Shymanska, O., Rakhmetov, D., Fishchenko, V., & Ivanišová, E. (2023). A comparative study of *Silphium* spp. antioxidant activity. In *Agrobiodiversity for Improving Nutrition, Health and Life Quality*, 7(1), 48-60. https://doi.org/10.15414/ainhlq.2023.0006

Zohari, M. (1983). The genus *Nigella* (Ranunculaceae) – a taxonomic revision. In *Plant Systematics and Evolution*, 142, 71–105. https://doi.org/10.1007/bf00989605