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Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots

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Abstract

In the present investigation, methanolic extracts from shoots and roots of Tunisian *Nigella sativa* were assayed for their antioxidant and antimutagenic activities. The phenolic composition of the methanolic extracts was determined by RP–HPLC. The predominant phenolic compound was vanillic acid with a mean concentration of 143.21 and 89.94 mg per 100 g dry weight of shoots and roots, respectively. Shoots and roots showed comparable and strong superoxide scavenger activity; however, shoots exhibited higher DPPH radical scavenging, reducing and chelating activities than roots. Mutagenic and antimutagenic activities were determined by using the Ames test. Shoots and roots demonstrated important antimutagenic effects. Roots exhibited stronger activity than shoots with an inhibition percentage of 71.32%. ***To cite this article: S. Bourgou et al., C. R. Biologies 331 (2008).***

Résumé

Composition phénolique et activités biologiques des parties aériennes et des racines de la nigelle tunisienne. Dans le présent travail, les extraits méthanoliques obtenus à partir des parties aériennes et des racines de *Nigella sativa* ont été étudiés pour leurs activités antioxydante et antimutagène. L'analyse du contenu phénolique par RP–HPLC a révélé que l'acide vanillique est le composé majeur dans les parties aériennes et les racines, présentant des teneurs de 143,21 et 89,94 mg par 100 g de matière sèche. Les deux organes ont montré une capacité importante et comparable à éliminer l'ion superoxyde, mais les parties aériennes ont montré des pouvoirs antiradicalaire, réducteur et chélateur plus élevés que ceux des racines. Ces dernières ont présenté une meilleure activité antimutagène que les parties aériennes, exhibant un pourcentage d'inhibition de 71,32%. ***Pour citer cet article : S. Bourgou et al., C. R. Biologies 331 (2008).***

Keywords: *Nigella sativa* L; Shoots; Roots; Antiradical activity; Reducing power; Chelating power; Antimutagenic activity; Phenolic composition

Mots-clés : *Nigella sativa* L ; Partie aérienne ; Racines ; Activité antiradicalaire ; Pouvoir réducteur ; Pouvoir chélateur ; Activité antimutagène ; Composition phénolique

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1. Introduction

Polyphenols are naturally occurring secondary metabolites in all plant materials, and prominently ubiquitous in herbs, vegetables, fruits, and seeds [1]. The most accruing polyphenols are flavonoids, which are benzo-γ-pyrone derivatives consisting of phenolic and pyrane rings [2] that can be divided into six classes including flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanidins [3]. The other common polyphenols are phenolic acids. They are simple phenolic compounds of the non-flavonoid family and are synthesised through the shikimic acid pathway. Two main groups can be distinguished, both of which are hydroxyl derivatives of aromatic carboxylic acids: benzoic acids and cinnamic acids [4]. Phenolic compounds differ in structure according to the number and position of hydroxylations and methoylations of the aromatic ring [5].

Polyphenols are chemopreventers, protecting the body tissues against oxidative stress and modulating gene expression and inhibiting UV and carcinogen-induced tumorigenesis [6,7]. They also exhibit a wide range of biological activities including antimutagenic, antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic and vasodilatory actions [8–10]. In particular, they have been found to be strong antioxidants with free radical scavenging, inhibiting enzymatic systems responsible for free radical generation, metal chelation, and reducing properties [5].

Nigella sativa L. (*Ranunculaceae*), commonly known as black cumin, is an annual herbaceous plant used as a popular aromatic herb and culinary spice. Traditionally, it is used as a natural remedy for a number of illnesses that include asthma, cough, hypertension, bronchitis, diabetes, headache, eczema, fever, inflammations, and other diseases [11]. Several biological activities have been reported in *N. sativa* seeds, including antioxidant [12], anti-inflammatory [13] and antiulcer [14]. However, no studies have been conducted on the other plant parts: shoots and roots. In fact, no information is available on their biological capacities.

Thus, the aim of the present work was to identify and quantify phenolic acids and flavonoids composition of *N. sativa* shoots and roots by RP–HPLC and to evaluate the antioxidant capacity of methanol extracts from these plant parts using different systems, including DPPH and superoxide anion radical scavenging, metal chelating and reducing power, as well as to screen their mutagenic and antimutagenic activities.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT) was purchased from Sigma. Nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma. Folin–Ciocalteu reagent and sodium azide were purchased from Aldrich. Authentic standards of phenolic compounds were purchased from Sigma and Fluka. Stock solutions of these compounds were prepared in HPLC-grade methanol. These solutions were wrapped in aluminium foil and stored at 4 °C. All other chemicals used were of analytical grade.

2.2. Plant material

N. sativa shoots and roots were collected in May 2005 at the flowering stage from cultivated plants from the Menzel Temime region (northeastern Tunisia).

2.3. Preparation of methanolic extracts

The air-dried and finely ground shoots and roots (2.5 g) were extracted by stirring with 25 ml of absolute methanol at room temperature for 30 min. Extracts were kept for 24 h at +4 °C, and then filtered through Whatman filter paper. Extracts were evaporated under vacuum to dryness to give yields of 29.3 and 19% for shoots and roots respectively, and stored at +4 °C until analysis.

2.4. Determination of total polyphenol content

Total phenolics of *N. sativa* extracts were determined using the Folin–Ciocalteu (F-C) reagent, according to the method described by Dewanto et al. [15]. An aliquot of 0.125 ml of diluted extract was added to 0.5 ml of deionized water and 0.125 ml of the (F-C) reagent. After shaking, the mixture was incubated for 3 min at room temperature. Then, 1.52 ml of 7% Na_2CO_3 solution was added. The volume obtained was adjusted to 3 ml using distilled water, mixed vigorously, and held for 90 min at ambient temperature. The absorbance of the solution was then measured at 760 nm against a blank. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of dry weight through the calibration curve of gallic acid. The sample was analyzed in three replications.

2.5. Hydrolysis and identification of phenolic compounds using HPLC

Dried samples from shoots and roots were hydrolysed according to the method of Proestos et al. [16], slightly modified. Forty millilitres of methanol containing BHT (1 $g\,l^{-1}$) were added to 0.5 g of a dried *N. sativa* sample. Then 10 ml of 6 M HCl were added. The mixture was stirred carefully and then sonicated for 15 min and refluxed in a water bath at 90 °C for 2 h. The obtained mixture was injected to HPLC. The phenolic compounds' analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph (RP–HPLC) coupled with an UV-Vis multiwavelength detector. The separation was carried out on a 250 × 4.6-mm, 4-μm Hypersil ODS C18 reversed phase column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 $ml\,min^{-1}$. The gradient programme was as follows: 15% A/85% B 0–12 min, 40% A/60% B 12–14 min, 60% A/40% B 14–18 min, 80% A/20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min. The injection volume was 20 μl, and peaks were monitored at 280 nm. Samples were filtered through a 0.45-μm membrane filter before injection. Peaks were identified by congruent retention times compared with standards. Analyses were performed in triplicates.

2.6. DPPH assay

The electron donation ability of the obtained methanol extracts was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) according to the method of Hanato et al. [17]. Extracts (2 ml, 10–1000 $\mu g\,ml^{-1}$) were added to 0.5 ml of 0.2 mM DPPH methanolic solution. After an incubation period of 30 min at room temperature, the absorbance was determined against a blank at 517 nm. Percentage inhibition of free radical DPPH (PI%) was calculated as follow: $PI\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. Extract concentration providing 50% inhibition (IC_{50}) was calculated form the regression equation prepared from the concentration of the extracts and the inhibition percentage. BHT was used as a positive control. Samples were analyzed in triplicate.

2.7. Determination of anion superoxide-scavenging activity

The superoxide anion was generated in a PMS-NADH system by oxidation of NADH and assayed by the reduction of NBT. The method described by Duh et al. [18] was used to determine the superoxide anion radical scavenging activity. The reaction mixture, containing 0.2 ml of extract (10–1000 $\mu g\,ml^{-1}$) in distilled water, 0.2 ml PMS (60 μM), 0.2 ml of NADH (677 μM), and 0.2 ml NBT (144 μM), was incubated at room temperature for 5 min. Then the absorbance was read at 560 nm. All solutions were prepared in a phosphate buffer (0.1 M, pH 7.4). The scavenging activity was calculated as follows: $PI\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$, where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. The IC_{50} was calculated from the plot of the inhibition percentage against the extract concentration. BHT was used as a reference compound control. Samples were analyzed in triplicate.

2.8. Reducing power

The method of Oyaizu [19] was used to assess the reducing power of *N. sativa* shoot and root extracts. Methanol extracts (1 ml) were mixed with 2.5 ml of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$), and incubated in a water bath at 50 °C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added to the mixture that was centrifuged at 650 *g* for 10 min. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The intensity of the blue–green colour was measured at 700 nm. The EC_{50} value ($mg\,ml^{-1}$) is the extract concentration at which the absorbance was 0.5 for the reducing power and was obtained from the linear regression equation prepared from the concentrations of the extracts and the absorbance values. High absorbance indicates high reducing power. Ascorbic acid was used as a positive control.

2.9. Metal-chelating power

Extracts (0.1 ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml) [20]. The reaction was initiated by the addition of 5 mM ferrozine (0.1 ml) and 2.75 ml of distilled water. The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was then measured at 562 nm. The scavenging activity was calculated as follows: $PI\% = [(A_{blank} -$

$A_{sample})/A_{blank}] \times 100$, where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. IC_{50} was calculated from the plot of inhibition percentage against extract concentration. EDTA was used as a positive control. Samples were analyzed in triplicate.

2.10. Mutagenic and antimutagenic tests

The mutagenicity assay was performed with *Salmonella typhimurium* strain TA 1535 using the plate incorporation procedure described by Maron and Ames [21]. Fifteen microlitres of bacterial stock were incubated in 5 ml of oxoid nutrient for 16 h at 37 °C in an orbital shaker. Then 100 µl of the overnight culture of bacteria (approximate cell density: 10^8 cells ml^{-1}) and 100 µl of extracts dissolved in DMSO (0.05–0.5 mg ml^{-1}) were added to tubes containing 2 ml of top Agar (supplemented with 0.5 mM *l*-histidine and 0.5 mM *d*-biotine). The mixture was then poured on minimal agar plates previously prepared as described by Maron and Ames [21]. The plates were incubated at 37 °C for 48 h and the revertant bacterial colonies of each plate were counted. Negative control cultures gave numbers of revertants per plate that were within the normal limits found in the laboratory. An extract was considered mutagenic if the number of revertants per plate was at least doubled. The spontaneous revertants are known to be responsive to sodium azide [21]. This mutagen was chosen to study the antimutagenic activity, and 15 µl of the mutagenic agent (dissolved in DMSO) were added per tube of top agar containing the bacterial strain and the extract. The resulting mixture was poured on the minimal agar plate. The plates were incubated at 37 °C for 48 h and the revertant bacterial colonies of each plate were counted. The inhibition percentage of mutagenicity (PI%) was calculated according to the following formula: PI = [1 – (number of revertants on test plates/number of revertants on control plates)] × 100.

2.11. Statistical analysis

All data are reported as the mean ± SD of three measurements. Means of total phenolic content, IC_{50} on DPPH, superoxide and chelating powers and EC_{50} on reducing power were statistically compared using the STATI-CF program with the Student *t*-test at the $P < 0.05$ significance level. A one-way analysis of variance (ANOVA) and the Newman–Keuls multiple-range test were carried out to test any significant differences between shoot and root phenolic content used at $P < 0.05$. A two-way analysis of variance (ANOVA), with the organ (O) and dose (D) as factors, was achieved for mutagenic and antimutagenic data, using the STATI-CF statistical program. Means were compared using the Newman–Keuls test at the $P < 0.05$ level, when significant differences were found.

3. Results and discussion

3.1. Total phenolics' content

Based on the absorbance values of extract solutions reacted with the Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents, total phenolics are given in Table 1. Phenolic contents varied significantly between organs. Shoots exhibited an important amount of 10.04 mg of GAE/g DW, which was about 2.5 times higher than the total polyphenols of roots (4.01 mg of GAE/g DW). The levels of total polyphenols were superior to those reported in *N. sativa* seeds [22].

3.2. Identification and quantification of phenolic compounds by HPLC

The free forms of phenolic compounds are rarely present in plants. More often, they occur as esters, glycosides or bound to the cell wall. For this reason, acidic hydrolysis was used to release the aglycones in order to simplify the identification process [23]. Moreover, BHT, a powerful antioxidant, was added to prevent degradation of phenolics during hydrolysis [24]. RP-HPLC coupled with a UV-Vis multiwavelength detector was employed to separate and to quantify phenolic compounds. Fig. 1 shows the chromatograms of authentic standards and *N. sativa* shoots and roots. Fourteen phenolic compounds were successfully identified, including gallic acid, (–)-*p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, *p*-coumaric, ferulic acid, *trans*-2-hydroxycinnamic acid, *trans*-cinnamic acid, epicatechin, (+)-catechin, quercetin, apigenin, amentoflavone, and flavone. These compounds have been identified according to their retention time and the spectral characteristics of their peaks compared to those of standards, as well as by spiking the sample with standards. Results demonstrated that differences in *N. sativa* shoots and roots phenolic composition were significantly more quantitative than qualitative. With the exception of *p*-coumaric and ferulic acids detected only in roots, and amentoflavone present in shoots, *N. sativa* plant parts possess similar composition.

The amounts of the different identified phenolic acids and flavonoids are shown in Table 2. Vanillic

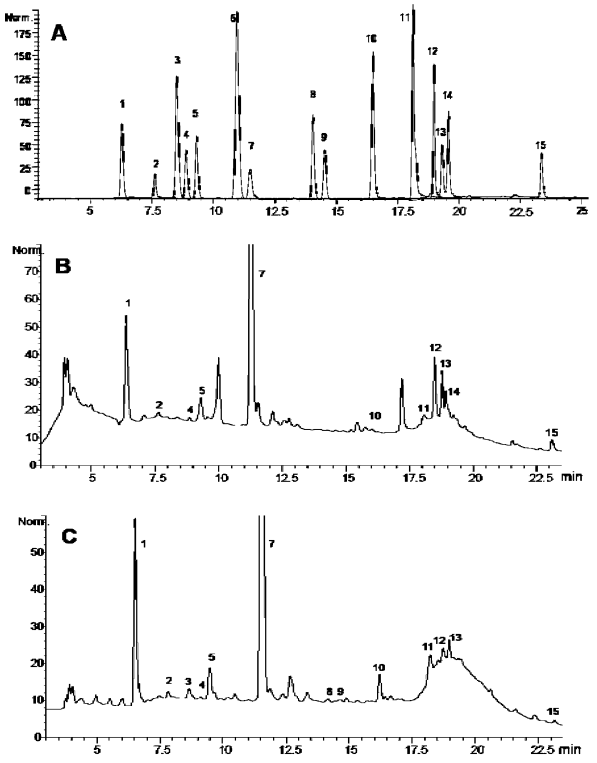

Fig. 1. RP-HPLC Chromatographic profiles of standard mixture (**A**), *Nigella sativa* shoots (**B**) and *Nigella sativa* roots (**C**) monitored at 280 nm. The peaks correspond to: **1**, gallic acid; **2**, (–)-epicatechin; **3**, *p*-hydroxybenzoic acid; **4**, chlorogenic acid; **5**, (+)-catechin; **6**, syringic acid; **7**, vanillic acid; **8**, *p*-coumaric acid; **9**, ferulic acid; **10**, *trans*-2-hydroxycinnamic acid; **11**, quercetine; **12**, *trans*-cinnamic acid; **13**, apigenine; **14**, amentoflavone; **15**, flavone.

acid was detected to be the major phenolic component in the two plant parts (shoots and roots), contributing about 66% to the total amount and showing the levels of 143.21 and 89.94 mg/100 g DW in shoots and roots, respectively. Gallic acid was also predominant, but slightly higher in roots (30.59 mg/100 g DW) than in shoots (27.86 mg/100 g DW). *trans*-Cinnamic acid, (+)-catechin and apigenin were also found with appreciable level in shoots. The amounts of the detected compounds are close to those reported in similar aromatic and medicinal herbs [25].

The levels of total phenolic compounds in *N. sativa* shoots and roots determined by HPLC were 2.15 and 1.35 mg/g DW for shoots and roots, respectively, and thus lesser than the ones obtained by the Folin–Ciocalteu method. This result is predictable due to the weak selectivity of the Folin–Ciocalteu reagent, as it reacts positively with different antioxidant compounds (phenolic and non-phenolic substances).

3.3. Antioxidant capacity

The stable DPPH radical is widely used to evaluate the free radical scavenging activity in many plant extracts [26]. The assessment of antioxidant activity showed that both *N. sativa* shoots and roots were able to scavenge this radical (Table 1). Shoots displayed a higher activity than roots (IC_{50} = 280 and 450 μg ml^{-1}, respectively). Although this scavenging effect was lower than that of BHT (Table 1), it was stronger than the antiradical activity reported in the seeds of the same specie from India [22], which showed an IC_{50} value of 1240 μg ml^{-1}.

Results demonstrated also that both *N. sativa* shoots and roots exhibited significant superoxide anion scavenging capacity (Table 1). As for DPPH, shoots were more effective O_2^- scavengers than roots. The IC_{50} values of *N. sativa* shoots and roots extracts on superoxide radical scavenging activity were 12 and 18 μg ml^{-1}, respectively. These results revealed that methanolic extracts of *N. sativa* organs were free radical scavengers, acting possibly as primary antioxidants.

The reducing capacity of a compound may serve as indicator of its potential antioxidant activity [27]. The presence of reducers (i.e. antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex to the ferrous form. *N. sativa* shoots exhibit a significant reducing power (Table 1); in fact EC_{50} (57 μg ml^{-1}) was comparable to that of the standard ascorbic acid (38 μg ml^{-1}). Roots showed lower reducing ability than roots, presenting an EC_{50} of 1.85 mg ml^{-1}. These results suggest that *N. sativa* shoots were electron donors reacting with free radicals to convert them into more stable products and to terminate radical chain reactions as described by [28].

Although iron is essential for oxygen transport, respiration, and enzymes activity, it is a reactive metal that catalyzes oxidative damage in living tissues and cells [29]. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Results indicate that methanol extracts of *N. sativa* shoots and roots interfered with the formation of ferrous and ferrozine complex (Table 1). However, this chelating power activity was weak, in fact, compared to the standard EDTA, IC_{50} were high (3.8 and 7.5 mg ml^{-1} for shoots and roots, respectively).

The strong antioxidant activity of *N. sativa* shoots and roots assessed by the different systems could be attributed to their high total polyphenolic contents; in fact, it has been found that polyphenols are one of the most effective antioxidative constituents in the

Table 1
Amounts of total phenolic compounds and antiradical (DPPH, superoxide anion), reducing and chelating activities of *Nigella sativa* shoots and roots and BHT, ascorbic acid and EDTA

Samples	Total phenolics (mg GAE/g DW)	IC_{50}(μg/ml) on DPPH	IC_{50}(μg/ml) on superoxide	EC_{50}(μg/ml) on reducing power	IC_{50}(μg/ml) on chelating power
Nigella. sativa shoots	10.04 ± 0.006	280 ± 0.02	16 ± 0.09	57	3800
Nigella. sativa roots	4.010 ± 0.05	450 ± 0.07	18 ± 0.05	1850	7500
BHT	–	16 ± 0.02	0.2	–	–
Ascorbic acid	–	–	–	38	
EDTA	–	–	–	–	30

Data are reported as means ± SD of three measurements. $P < 0.05$ compared to control (C; ANOVA followed by the Student *t*-test).

Table 2
Content (mg/100 g) of phenolic acids and flavonoids in *Nigella sativa* shoots and roots

Phenolic compounds	Content (mg/100 g dry sample)	
	Nigella. sativa shoots	*Nigella. sativa* roots
Phenolic acids		
Gallic acid	27.86 ± 0.97	30.59 ± 0.9
p-Dihydroxybenzoic acid	n.d.	1.73 ± 1.6
Chlorogenic acid	1.51 ± 0.64	0.36 ± 0.48
Syringic acid	n.d.	n.d.
Vanillic acid	143.21 ± 0.23	89.94 ± 0.92
p-Coumaric acid	n.d.	0.36 ± 0.72
Ferulic acid	n.d.	0.18 ± 0.2
trans-2-Hydroxycinnamic acid	1.25 ± 0.8	2.58 ± 0.8
trans-Cinnamic acid	15.47 ± 0.54	0.98 ± 0.28
Flavonoids		
(−)-Epicatechin	1.28 ± 0.61	0.64 ± 0.54
(+)-Catechin hydrated	7.26 ± 0.97	3.4 ± 0.74
Quercetine	2.56 ± 0.43	2.61 ± 0.69
Apigenin	6.83 ± 0.44	1.77 ± 0.61
Amentoflavone	2.91 ± 0.81	n.d.
Flavone	3.4 ± 0.33	0.54 ± 0.8
Total	215.373	135.756

Data are reported as means ± SD of three measurements. $P < 0.05$ compared to control (C; ANOVA followed by the Student *t*-test). n.d.: not detected.

plant [30]. Moreover, the high yield of the different phenolic compounds found in *N. sativa* plant parts (Table 2) might contribute to the potent antioxidant activity of the methanol extracts, since a positive correlation between phenolic composition and antioxidant activity was proved [31]. Thus, antioxidant property of shoots and roots could be attributed to the significant amount of benzoates, especially vanillic acid, present in our study with the high amounts of 143.21 ± 0.23 and 89.94 ± 0.92 μg/100 g DW, respectively in shoots and roots. Numerous studies indicated that this phenolic acid was a potent antioxidant agent, quenching radicals, singlet oxygen and hydrogen peroxide [32–34]. Moreover, the amount of vanillic acid was about 1.5 higher in shoots than in roots, which may explain the superiority of shoots extracts as antioxidants. Other minor phenolic compounds should not be neglected, since synergy of the different chemicals with each other should be taken into consideration for the biological activity. Likewise, the molecular structure and differences in number and position of the hydroxyl group on the aromatic ring influence the antioxidant activity [5]. The presence of the CH=CH–COOH group in the hydroxycinnamic acids found in *N. sativa* shoots and roots (*trans*-cinnamic, *trans*-2-hydroxy-cinnamic, *p*-coumaric, ferulic and chlorogenic acids) is considered to be key element for the antioxidative efficiency [35]. As well, catechin was found to be the most abundant flavonoid in our study for both shoots (7.26 ± 0.97 mg/100 g DW) and roots (3.4 ± 0.74 mg/100 g DW). This flavonol is a well-known antioxidant, due to the presence of the *o*-dihydroxy and *o*-hydroxyketo groups [3].

3.4. Mutagenic and antimutagenic activity

The results of the Ames test are reported in Table 3. Spontaneous revertants were 18 in number and none of the tested extracts, even at high concentrations, induced significant increase of the revertants' number in *Salmonella typhimurium* (TA1535) strains. The absence of mutagenicity within the extracts of *N. sativa* in the *Salmonella* tested strain indicated that DNA does not seem to be a relevant target.

Sodium azide damages DNA and thus induces mutagenecity. A dose of 1.5 μg per plate of this mutagen was chosen for the antimutagenicity studies, since this dose was not toxic and induced 1320 revertants in *S. thyphimurium* TA1535. The inhibitory effect of the methanol *N. sativa* extracts on the mutagenicity of sodium azide using the plate incorporation assay is illustrated in Table 3.

The results indicated that shoots and roots demonstrated significant inhibitory effect. The highest antimutagenic activity for *N. sativa* shoots (45.84% inhibition) and roots (71.32% inhibition) was obtained at a low con-

Table 3
Mutagenic (number of revertants/plate) and antimutagenic effects (inhibition percentage of mutagenicity) of *Nigella sativa* shoots and roots in *Salmonella typhimurium* TA1535

	Dose (µg/ml)	Nb revertants/plate	Nb revertants/plate in presence of sodium azide	% inhibition of mutagenicity
Nigella sativa shoots	50	22 ± 1.1	725.66 ± 4.5	45.84
	250	25.5 ± 2.3	961.66 ± 8.6	27.64
	500	28 ± 1.1	770.33 ± 1.7	42.4
Nigella sativa roots	50	19.33 ± 1.7	395.33 ± 0.09	71.32
	250	24.66 ± 2.8	920 ± 2.2	30.85
	500	24 ± 1.1	1093.66 ± 8	17.45
Spontenous revertants		18 ± 1.13	1320 ± 18.3	

Data are reported as means ± SD of three measurements. $P < 0.05$ compared to control (% inhibition per control = 0%; C; ANOVA followed by the student *t*-test).

centration (50 µg/plates), although higher doses failed to increase antimutagenicity (Table 3). This type of response has been already obtained in other antimutagenic studies [36].

The relevant antimutagenic activity of *N. sativa* extracts associated with the absence of genotoxicity suggests that these extracts contain interesting active compounds and our data on antioxidant activity of *N. sativa* shoots and roots imply that their antigenotoxic activity is mediated by their antioxidative property, as it is has already been reported [37,38]. In fact, many carcinogens and/or mutagens produce oxygen-free radicals for interaction with cellular macromolecules; thus the antioxidant and antiradical properties of antioxidants, especially polyphenols, give them the ability to inhibit DNA lesions generated by oxidative stress, and to prevent mutagenicity [37]. In our study, shoots and roots exhibited a high level of phenolic compounds (Table 2) and several of these phenolic acids and flavonoids, including gallic acid, catechin and apigenin, were reported as powerful antimutagenic and anticancer agents [9,39,40]. Moreover, Sawa [41], who studied the antiradical property of 17 authentic phenolics, demonstrated that vanillic acid, the major phenolic component in *N. sativa* shoots and roots, has a strong anti-tumour-promoter effect through its remarkable antiradical capacity.

Finally, the fact that roots of *N. sativa* have a stronger antimutagenic capacity than that of shoots, in spite of their lower total polyphenolic content, suggests that the activity may be due more to the combinatory effect of different compounds than to their quantity.

4. Conclusion

In conclusion, this is the first study focused on the biological activities of *N. sativa* shoots and roots. Methanol extracts of parts of plants showed significant antimutagenic activity and antioxidant capacity in different assays in vitro. Eight phenolic acids and six flavonoids were identified, and vanillic acid was the dominant phenolic compound in shoots and roots. The present study provides data for supporting the use of Tunisian *N. sativa* shoot and root extracts as natural antimutagenic and antioxidant agents, and confirms that these extracts represent a significant source of phenolic compounds. To understand their mechanism of action as bioactive components, further fractionation of methanolic extracts and isolation of phenolic compounds (especially vanillic acid) and determination of their biological activities in vitro and in vivo are needed.

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