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ANTIOXIDANT, ANTI-INFLAMMATORY, ANTICANCER AND ANTIBACTERIAL ACTIVITIES OF EXTRACTS FROM *NIGELLA SATIVA* (BLACK CUMIN) PLANT PARTS

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ABSTRACT

Nigella sativa is a one of the most commonly found aromatics in the Mediterranean kitchen. This plant is traditionally used for the treatment of several pathologies. In this study, the antioxidant, anti-inflammatory, anticancer and antibacterial activities of the shoots, roots and seeds methanol extracts from *N. sativa* were studied. The three organs exhibited strong antioxidant activity using the oxygen radical absorbance capacity method and a cell-based assay. Furthermore, the seeds hexane fraction (SHF) of the methanol extract showed significant anti-inflammatory activity, inhibiting nitric oxide release with an IC_{50} value of 6.20 μg/mL in lipopolysaccharide-stimulated RAW 264.7 macrophages. The SHF was found to be active against A-549 lung carcinoma cells and DLD-1 colon carcinoma, with IC_{50} values of 31.0 and 63.0 μg/mL, respectively, as well as against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) bacteria with IC_{50} values of 38.0 and 80.0 μg/mL, respectively. The shoots methanol extract exhibited moderate anti-inflammatory activity but no anticancer and antibacterial activities.

PRACTICAL APPLICATIONS

The results obtained suggest that *Nigella sativa* plant parts, including the shoots, the roots and the seeds, have the potential to be developed into dietary supplements as food preservative and for the improvement of human nutrition and health. In addition, the seeds could be useful for therapeutical purposes and could be developed as anticancer agent and as foodborne preservative as well as for the treatment of chronic inflammatory pathologies associated with overproduction of nitric oxide.

INTRODUCTION

Reactive oxygen species (ROS) include superoxide radical, hydrogen peroxide, peroxyl radicals ($ROO^{\cdot}$), hydroxyl radical, as well as reactive nitrogen species such as nitric oxide (NO) and peroxynitrite. ROS are mainly responsible for the initiation of oxidation reaction in foods; they change the functionalities of proteins, lipids and carbohydrates by forming oxidized dimmers and trimers (Frankle 2005). ROS make food products less acceptable or unacceptable to consumers and lower the overall nutritional, chemical and physical qualities of food during storage and marketing (Choe and Min 2006). In biological systems, oxidative stress affects major cellular components, including lipids, proteins and DNA, and is involved in such pathologies as cardiovascular diseases (atherosclerosis), inflammatory diseases (asthma, rheumatoid arthritis, allergies), neurodegenerative diseases (Parkinson and Alzheimer diseases) and aging (Kris-Etherton *et al*. 2004; Laguerre *et al*. 2007).

Several synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and tert-butylhydroquinone are used for the prevention of the oxidative reactions in foods, pharmaceuticals and cosmetics, and the management of oxidative stress-related diseases.

However, there has been growing concern over their safety because several studies documented the mutagenesis and carcinogenesis potential associated with some synthetic antioxidants (Pan *et al*. 2007). Therefore, the extraction and characterization of natural antioxidants are of considerable interest.

In the recent decades, the need of new anti-infective agents because of the emergence of multiple antibiotic resistances has led to the search of new sources of potential antimicrobials (Carson and Riley 2003). Among them, the plant kingdom offers a wide range of biodiversity of great value for the pharmaceutical industry.

Nigella sativa L., commonly known as black cumin, is an aromatic and medicinal plant which belongs to Ranunculacea family. It is used traditionally in several countries for culinary and medicinal purposes. The seeds are used as a spice in the flavoring of foods, especially bakery products and cheese, and in the preparation of a traditional sweet dish, composed of black cumin paste, which is sweetened with honey or syrup. The plant has been used traditionally as a remedy for a wide range of illnesses including asthma, cough, bronchitis, headache, rheumatism, dysentery, infections, fever, influenza and eczema (Salem 2005). Our previous investigations on the Tunisian *N. sativa* revealed that methanol extracts obtained from the different plant parts including the shoots, roots and the seeds expressed high antioxidant activity. In fact, they exhibited reducing and chelating powers as well as free radical scavenging ability by scavenging 2,2-diphenyl-1-picrylhydrazyl and superoxide anions (Bourgou *et al*. 2008a,b). Phytochemical investigation revealed that the different plant parts are a source of polyphenols. They were found to be especially rich on phenolic acids where vanillic acid represented the major compound (Bourgou *et al*. 2008a,b).

Bioactivities of several extracts with different polarities from *N. sativa* seeds have been investigated. Methanolic, chloroform and hexane extracts were active against cervical cancer by inducing apoptosis in HeLa cells (Shafi *et al*. 2009). In addition, the aqueous extract possessed anti-inflammatory and analgesic activities in animal models (Al-Ghamdi 2001). Moreover, Swamy and Tan (2000) found that the ethyl-acetate fraction of the ethanolic extract exhibited immunomodulatory and cytotoxic properties. However, except for our previous study indicating the antioxidant and antimutagenic capacities of the shoots and roots of *N. sativa* (Bourgou *et al*. 2008b), there are no other investigations on the biological potential of these plant parts as well as the seeds from the Tunisian genus.

In this study, we investigated the *in vitro* and *ex vivo* antioxidant activities, as well as the anti-inflammatory, anticancer and antibacterial activities of the methanol extracts obtained from the shoots and the roots and the polar (water) and apolar (hexane) fractions of the methanol extract of the seeds.

MATERIALS AND METHODS

Plant Material and Extraction Procedure

Shoots and roots of *N. sativa* were collected in May 2006, while the seeds were collected at maturity in July 2006 from cultivated plants from the region of Menzel Temime (Northeastern Tunisia).

The shoots, roots and seed powders (10 g) were extracted under reflux with absolute methanol for 1 h and then with 80% methanol for 3 h. Seed methanol extract was further partitioned with water and hexane. Afterward, the different extracts were concentrated under vacuum and finally freeze dried to yield shoots methanol extract (SME), roots methanol extract (RME), seeds water fraction (SWF), seeds hexane fraction (SHF).

Cell Culture

The human lung carcinoma A-549 (American Type Culture Collection [ATCC] #CCL-185), colon adenocarcinoma DLD-1 (ATCC #CCL-221), normal skin fibroblast (WS-1) and murine macrophage RAW 264.7 (ATCC#TIB-71) cell lines were obtained from the ATCC (Manassas, VA). Cell lines were grown in Minimum Essential Medium with Earle's salts. The medium was supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), a solution of vitamins (1×), sodium pyruvate (1×), nonessential amino acids (1×), penicillin (100 IU) and streptomycin (100 µg/mL) (Mediatech Cellgro®, Manassas, VA). Cells were cultured in a humidified atmosphere at 37C under 5% CO_2.

Evaluation of *In Vitro* and *Ex Vivo* Antioxidant Activity

$ORAC_{FL}$ Assay. The procedure was modified from the method described by Ou *et al*. (2001). Briefly, the oxygen radical absorbance capacity (ORAC) assay was carried out on a Fluoroskan Ascent Fl™ plate reader (Thermo-Labsystems, Helsinki, Finland). Trolox was used as a control standard. The experiment was conducted at 37.5C and pH 7.4, with a blank sample in parallel. The fluorimeter was programmed to record the fluorescence of fluorescein every 30 s after addition of 2,2′-azobis (2-amidinopropane) dihydrochloride. The final results were calculated by comparing the net areas under the fluorescein decay curves between the blank and the samples. ORAC values were expressed in micromoles of Trolox equivalents (TE) per milligram (µmol TE/mg).

Antioxidant Cell Assay Using 2′,7′-Dichlorofluorescin-Diacetate

Antioxidant activity was evaluated using the 2′,7′-dichlorofluorescin-diacetate (DCFH-DA) assay as described

by Girard-Lalancette *et al*. (2009), with some modifications. Briefly, WS-1 cells were plated in 96-well microplates at 10,000 cells per well and incubated for 24 h at 37C under 5% CO_2. The cells were washed with 150 µL Hank's balanced salt solution (HBSS) at pH 7.4 and incubated for 30 min with 100 µL HBSS (pH 7.4) containing 5 µM DCFH-DA. The cells were then washed again with 150 µL HBSS. To assess the antioxidant activity, the cells were incubated with increasing concentrations of the extracts, in the absence or the presence of 200 µM tert-butylhydroperoxide (*t*-BuOOH). Fluorescence was measured immediately after *t*-BuOOH administration and again 90 min later, using an automated 96-well Fluoroskan Ascent Fl™ plate reader (Fluoroskan Ascent Fl, Thermo-Labsystems) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

Evaluation of Anti-inflammatory Activity

To investigate the anti-inflammatory activity of *N. sativa* extracts, NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells was examined. Exponentially growing macrophages were plated in 24-well microplates (BD Falcon, Mississauga, ON, Canada) at a density of 2×10^5 cells per well in 400 µL of culture medium and were allowed to adhere for 24 h at 37C under 5% CO_2. Cells were then treated with increasing concentrations of extracts dissolved in dimethyl sulfoxide (DMSO). The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Cells were then stimulated with 100 µg/mL LPS and incubated at 37C under 5% CO_2. After 24 h, cell-free supernatants were collected and nitrite production was measured using the modified method of Green *et al*. (1990). Griess reagent (50 µL of 1% sulphanilamide and 50 µL of 0.1% N-1-naphtylethylenediamine dihydrochloride in 2.5% H_3PO_4) was added in equal volume (100 µL) to cell supernatant and incubated at room temperature for 30 min. N(G)-nitro-L-arginine methyl ester (L-NAME) was used as a positive control. The absorbance at 540 nm was then measured using an automated 96-well Varioskan Ascent plate reader (Thermo Electron, Vantaa, Finland) and nitrite was quantified by comparison with a $NaNO_2$ standard curve.

Evaluation of Anticancer Activity

Exponentially growing cells were plated at a density of 5×10^3 cells per well, in 96-well microplates (Costar, Corning Inc., Albany, NY) into 100 µL of culture medium and were allowed to adhere for 24 h at 37C under 5% CO_2 before treatment. Then 100 µL of increasing concentrations of extracts dissolved in DMSO were added. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. Then the cells were incubated for 48 h in the presence or absence of extracts. The cytotoxicity was assessed using the resazurin reduction test as described by O'Brien *et al*. (2000). Fluorescence was measured using an automated 96-well Fluoroskan Ascent Fl™ plate reader (Thermo-Labsystems) at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxic activity was expressed as the concentration of extract inhibiting cell growth by 50% (IC_{50}).

Evaluation of Antibacterial Activity

Antibacterial activity was assessed according to the microdilution method described by Banfi *et al*. (2003). Briefly, exponentially growing bacteria were plated in 96-well round bottom microplates (Costar, Corning Inc.) at a density of 25×10^3 gram-positive *Staphylococcus aureus* (ATCC 25923) or 5×10^3 gram-negative *Escherichia coli* (ATCC 25922) per well in 50-µL nutrient broth (Difco, Lawrence, KS). Then 100 µL of increasing concentrations of extract was added per well. The final concentration of solvent in the culture medium was maintained at 0.1% (v/v) to avoid solvent toxicity.

Antibacterial activity was assessed by adding 50 µL of 4% resazurin to each well; the microplates were then incubated at 37C. Fluorescence was measured after 6 h on an automated 96-well Fluoroskan Ascent FlTM plate reader (Thermo-Labsystems) using excitation and emission wavelengths of 530 and 590 nm, respectively. Antibacterial activity was expressed as the concentration of extract inhibiting bacterial growth by 50% (IC_{50}).

Data Analysis

Data were reported as mean values calculated from replicates (n P 3). Statistical treatments were performed using Sigma Stat software (Jandel Scientific, San Rafael, CA) at 5% significance error level.

RESULTS AND DISCUSSION

Evaluation of *In Vitro* and *Ex Vivo* Antioxidant Activity

The antioxidant potential of *N. sativa* plant parts was assessed using the ORAC assay. The ORAC index has been widely employed in the antioxidant capacity evaluation (Davalos *et al*. 2004). This methodology measures the protection afforded by an antioxidant to a target molecule that is being oxidized by $ROO^{\cdot}$. As it is shown in Table 1, the extracts exhibited important antioxidant activity and the shoots extract was found to be the most active with ORAC value of 4.07 µmol of TE/mg followed by the roots (ORAC value of 2.57 µmol of TE/mg). The activity of the polar fraction (water) of the seeds

TABLE 1. ANTIOXIDANT ACTIVITY OF *NIGELLA SATIVA* PLANT PART EXTRACTS

Extracts	ORAC value (μmol trolox/mg DW)
SME	4.07 ± 0.50
RME	2.57 ± 0.75
SWF	1.85 ± 0.28
SHF	0.23 ± 0.05
Quercetin	26.00 ± 2.00

Quercetin was used as standards. Each value represents the mean ± SD of three determinations.
ORAC, oxygen radical absorbance capacity; RME, roots methanol extract; SHF, seeds hexane fraction; SME, shoots methanol extract; SWF, seeds water fraction.

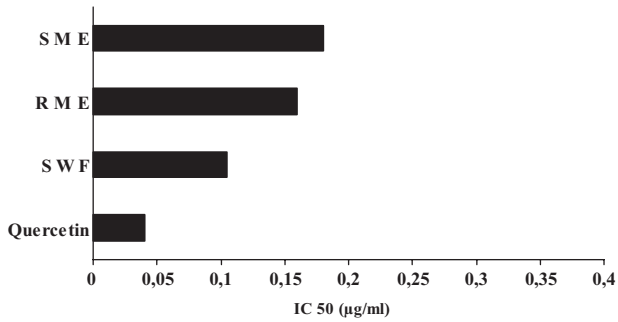

FIG. 1. ANTIOXIDANT EFFECT OF *NIGELLA SATIVA* PLANT PART EXTRACTS ON WS-1 CELL LINES TREATED WITH TERT-BUTYL HYDROPEROXIDE
Quercetin was used as standards. Each value represents the mean ± SD of three determinations.

methanol extract was high and eight times stronger than the apolar one (hexane fraction), which suggest that the water fraction was richer on antioxidant compounds and confirm earlier studies, where polar solvents are more suitable to extract antioxidants than apolar ones (Negi *et al*. 2005).

The rank range was as follow: SME > RME > SWF > SHF.

However,, ORAC values observed for *N. sativa* extracts are consistently close compared with those generally observed in extracts from plants known for their antioxidant properties such as bilberry, strawberry and oregano (Ou *et al*. 2001; Huang *et al*. 2002; Su *et al*. 2007). Thus, *N. sativa* plant parts extracts have been demonstrated to be for the first time efficient in scavenging $ROO^{\cdot}$ and to possess radical chain-breaking antioxidant activity by H-atom transfer (Ou *et al*. 2001). Peroxyl is formed by a direct reaction of triplet oxygen with alkyl radicals in fatty acid oxidation. This radical produces hydroperoxide (ROOH) by abstracting oxygen from other molecules. Oxidation of unsaturated fatty acids and their esters to ROOHs cause deterioration of foods (Frankle 2005). Moreover, $ROO^{\cdot}$ has been proposed to be mediators of the ROOH-dependent oxidations related to human diseases (Spiteller 2006).

The antioxidant activity of shoots, roots and seeds extracts was also assessed using a cell-based assay using DCFH-DA, a useful indicator of ROS. This test has been developed as a new sensitive test which allows detection of both the anti- and pro-oxidant properties (Girard-Lalancette *et al*. 2009). As the hexane fraction of the seeds methanol extract exhibited weak activity *in vitro*, the *ex-vivo* activity of the water fraction was assessed only. The results presented in Fig. 1 demonstrated that *N. sativa* plant parts extract strongly inhibited *t*-BuOOH-induced DCFH oxidation. SWF extract was the most effective, exhibiting an IC_{50} value of 0.11 μg/mL, followed by the roots and the shoots extracts (IC_{50} values of 0.16 and 0.18 μg/mL, respectively). This result indicates that *N. sativa* plant part extracts significantly inhibits ROS production and thus exhibits the ability to protect cells from oxidative stress. Moreover, compared with literature data, cellular antioxidant activity of *N. sativa* extracts is higher than that of medicinal plants including blackberry, peach, blueberry, strawberry and kiwi (Elisia *et al*. 2007; Girard-Lalancette *et al*. 2009). To the best of our knowledge, this is the first report on the cellular antioxidant property of *N. sativa* plant parts.

In vitro and *ex vivo* antioxidant activity of *N. sativa* plant part extracts is likely because of the action of known *N. sativa* plant parts phenolic compounds. In fact, anti-peroxyl radical properties of natural extracts are generally attributed to redox reactions with some bioproducts present in the extracts, notably phenolics (Ehlenfeldt and Prior 2001). Phenolic compounds may act as rapid donators of a hydrogen atom to $ROO^{\cdot}$, before the latter react with biological molecules or, fluorescein for ORAC assay. Their presence in *N. sativa* tissues (shoots, roots and seeds) at high levels was reported previously (Bourgou *et al*. 2008a,b). Besides, regarding the phenolic composition, *N. sativa* shoots, roots and seed are known to be rich in phenolic acids, mainly vanillic acid (Bourgou *et al*. 2008a,b), and this monohydroxyhenolic compound (vanillic acid) has been found to exhibit a high ORAC value (Kayano *et al*. 2004). Moreover, this compound has been reported to possess a high antioxidant activity at the cellular level; in fact, Kanski *et al*. (2002) demonstrated that several phenolic acids including vanillic ones protect efficiently cultured cells from oxidative stress.

Evaluation of Anti-inflammatory Activity

The anti-inflammatory activity of *N. sativa* plant parts extracts was evaluated by measuring their capacity to inhibit cellular NO generation. In fact, NO is an endogenous free radical species that is involved in a number of diverse physiological processes, including smooth muscle relaxation, platelet inhibition, neurotransmission and immune regulation. However, high levels have been closely correlated with the pathophysiology of a variety of diseases and inflamma-

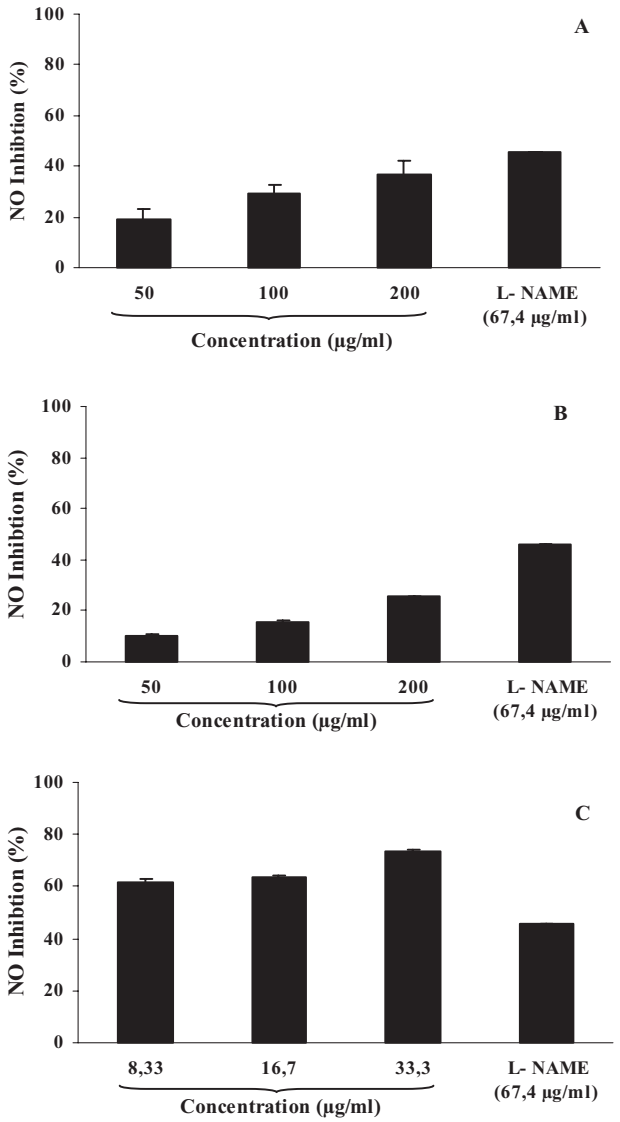

FIG. 2. EFFECTS OF *NIGELLA SATIVA* PLANT PART EXTRACTS, SHOOTS METHANOL EXTRACT (A), SEEDS WATER FRACTION (B) AND SEEDS HEXANE FRACTION (C) ON NITRIC OXIDE (NO) PRODUCTION IN LIPOPOLYSACCHARIDE-STIMULATED RAW-264.7 MACROPHAGES
Each value represents the mean ± SD of three determinations. L-NAME, N(G)-nitro-L-arginine methyl ester.

tions (Colasanti and Suzuki 2000). Therefore, the inhibition of NO production may be a useful strategy for the treatment of various inflammatory disorders (Choi *et al.* 2007). NO is synthesized from L-arginine by NO synthase (NOS). Upon stimulation by various cytokines and LPS, macrophages express the inducible NOS (iNOS), and produce large amounts of NO.

The results indicated that roots extract was inactive (data not shown), however; as it is shown in Fig. 2, shoots and seeds were able to inhibit NO production in a dose-dependant manner. The SHF exhibited particularly strong ability to inhibit LPS-induced NO secretion at concentrations ranging from 8 to 33 µg/mL, with 73.7% inhibition observed at 33.3 µg/mL and an IC_{50} value of 6.2 µg/mL. Moreover, this activity was higher than that of the positive control, L-NAME. In fact, the latter inhibited NO release by 45.7% at 67.4 µg/mL. This inhibition is not because of its cytotoxicity; in fact, doses up to 33.3 µg/mL produced no significant cytotoxic effect on RAW 264.7 cells, and the cells remain viable (data not shown). These results suggest that the SHF may contain potent anti-inflammatory constituents and are consistent with reports dealing with the inhibitory effect of apolar components of the seeds on many inflammatory mediators. Indeed, thymoquinone, which is a terpene ketone characteristic *N. sativa* seeds, has been reported as a strong anti-inflammatory compound. It inhibits the production of NO by macrophages by suppressing the expression of iNOS in rat macrophages (El-Mahmoudy *et al.* 2002). It is also a potent inhibitor of eicosanoid generation by inhibiting both cyclooxygenase and lipooxygenase, respectively (Houghton *et al.* 1995). Furthermore, Chakravarty (1993) reported the inhibition of histamine release from mast cells by the *N. sativa*-derived nigellone (polythymoquinone).

Furthermore, SWF and SME showed moderate anti-inflammatory activity inhibiting NO release by 36. 8 and 25.5% at 200 µg/mL. As SWF and SME also exhibited potent antioxidant activities (they showed high cellular antioxidant activity with respective IC_{50} values of 0.11 and 0.18 µg/mL), their anti-inflammatory activity might be linked, at least in part, to the presence of antioxidant compounds; phenolics. Indeed, several phenolic compounds have been reported to possess anti-inflammatory activity (Middleton 1998). In addition, phenolic compounds have been reported to be beneficial in the treatment of chronic inflammatory diseases associated with overproduction of NO (Jiang and Dusting 2003).

Evaluation of Cytotoxicity against Tumor Cell Lines

In this study, the anticancer activity of *N. sativa* plant parts extracts was evaluated against human lung carcinoma A-549 and colon adenocarcinoma DLD-1 cell lines. The results presented in Table 2 indicates that the SHF exhibited strong antiproliferative activity against the two cell lines, with IC_{50} values of 31.0 and 63.0 µg/mL for A-549 and DLD-1, respectively. The polar fraction of the seeds extract as well as the shoots and roots extracts is considered inactive as they showed IC_{50} values higher than 150 µg/mL. These results suggest that the anticancer activity of the seeds might be linked to the presence of active apolar compounds. From the characteristic *N. sativa* compounds, thymoquinone was

TABLE 2. CYTOTOXIC ACTIVITY OF *NIGELLA SATIVA* PLANT PART EXTRACTS AGAINST A-549 AND DLD-1 CELL LINES

	IC_{50} (μg/mL)	
Extracts	A-549	DLD-1
SME	>150	>150
RME	>150	>150
SWF	>150	>150
SHF	31.00 ± 3.00	63.00 ± 3.00
Etoposide	2.00 ± 0.58	15.89 ± 2.89

Etoposide was used as positive control. Each value represents the mean ± SD of three determinations.
RME, roots methanol extract; SHF, seeds hexane fraction; SME, shoots methanol extract; SWF, seeds water fraction.

reported to exhibit a potent anticancer potential against four human cancer cell lines including A-549 (Rooney and Ryan 2005).

Furthermore, in concordance with ours results, Mbarek *et al*. (2007) studied the anti-tumor property of the ethanol extract of *N. sativa* seeds and demonstrated that the ethyl acetate fraction was active against P815, Vero and ICO1 cancer cell lines. These authors suggest that the activity might be because of the presence of terpenes. Moreover, Kumara and Huat (2001) studying the *in vivo* anticancer ethanolic extract of *N. sativa* seeds, isolated a triterpene saponin, α-hederin, which shows dose-dependent tumor inhibition when given intraperitoneally for 7 days at 5 and 10 mg/kg to mice with formed tumors.

Evaluation of Antibacterial Activity

The antimicrobial activity of *N. sativa* plant parts was investigated against *S. aureus* and *E. coli*. The results presented in Table 3 indicate that the SHF possess considerable inhibitory effects against both strains with IC_{50} values of 38.0 μg/mL for *S. aureus* and 80.0 μg/mL for *E. coli*, whereas the polar fraction (SWF) of the seeds as well as the shoots and roots extracts remained inactive against the bacteria. Thus, SHF seems to contain active apolar antibacterial compounds. The activity may be linked at least to the action of the characteristic *N. sativa* quinone; thymoquinone. In fact, Inouye *et al*. (2006) studied the activity of various quinones using vapor and solution contact assays, and reported that thymoquinone is a potent antifungal and antibacterial agent.

TABLE 3. ANTIBACTERIAL ACTIVITIES OF *NIGELLA SATIVA* PLANT PART EXTRACTS

	IC_{50} (μg/mL)	
Extracts	*Staphylococcus aureus*	*Escherichia coli*
SME	NA	NA
RME	NA	NA
SWF	NA	NA
SHF	38.00 ± 3	80.00 ± 2
Chloramphenicol	2.26 ± 1	0.29 ± 0.1

Chloramphenicol was used as positive control. Each value represents the mean ± SD of three determinations.
NA, not active; RME, roots methanol extract; SHF, seeds hexane fraction; SME, shoots methanol extract; SWF, seeds water fraction.

However, the results indicate that SHF activity was more potent against $Gram^+$ bacteria than $Gram^-$. Singh *et al*. (2005) tested the antibacterial activity of acetone extract obtained from *N. sativa* seeds and found a greater activity against $Gram^+$ bacteria than $Gram^-$. In general, the antimicrobial activity of the medicinal plant extracts tested was more pronounced against $Gram^+$ than against $Gram^-$ bacteria. (Chandrasekaran and Venkatesalu 2004; Mothana and Lindequist 2005). The difference in sensitivity between $Gram^+$ and $Gram^-$ bacteria might be attributed to the differences in morphological constitutions between these microorganisms. $Gram^-$ bacteria have an outer phospholipidic membrane, almost impermeable to lipophilic compounds (Nikaido and Vaara 1985). The absence of this barrier in $Gram^+$ bacteria allows the direct contact of the hydrophobic constituents with the phospholipids bilayer of the cell membrane, where they bring about their effect, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Cowan 1999).

CONCLUSION

In conclusion, this study shows that Tunisian *N. sativa* plant parts extracts possess broad activities. The shoots, roots and seeds methanol extracts exhibited powerful antioxidant activity assessed *in vitro* and *ex vivo*, which suggest that they can be used as easily accessible source of natural antioxidants and as a possible food supplement to combat free radical-mediated organoleptic deterioration. Furthermore, the potent anti-inflammatory, anticancer as well as the antibacterial activities of the SHF suggest that it can also be a potential source of cytotoxic, anti-inflammatory and foodborne preservative compounds. Studies for the isolation of these components are now in progress.

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