Pak. J. Bot., 41(3): 1321-1330, 2009.

NUTRITIONAL PROFILE OF INDIGENOUS CULTIVAR OF BLACK CUMIN SEEDS AND ANTIOXIDANT POTENTIAL OF ITS FIXED AND ESSENTIAL OIL

MUHAMMAD TAUSEEF SULTAN[1*], MASOOD SADIQ BUTT[1], FAQIR MUHAMMAD ANJUM[1], AMER JAMIL[2], SAEED AKHTAR[3] AND MUHAMMAD NASIR[1]

[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[2]Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan
[3]University College of Agriculture, BZ University, Multan, Pakistan

Abstract

Medicinal plants gained momentous support in the recent era for their therapeutic potential. The core objective of the research study was to characterize the indigenous variety of black cumin (*Nigella sativa* L.), locally known as "Kalonji" and its fixed and essential oils. Compositional analysis revealed that it contains appreciable quantities of carbohydrates, proteins and fats. Moreover, potassium, calcium, phosphorous and magnesium were predominant minerals, whilst considerable quantities of sodium, iron, manganese, zinc and copper were also present. Characterization of fixed oil enumerated that polyunsaturated fatty acids were the dominating fraction i.e., 60.17±1.53% as compared to saturated and monounsaturated fatty acids i.e., 16.64±0.91 and 22.47±0.59%, respectively. Carotenoids and tocopherols were 450.66±16.21 mg/kg-oil, whereas thymoquinone contents were observed to be 201.31±13.17 mg/kg of seeds. In comparison, analysis of essential oil revealed that it contains functional ingredients like thymoquinone, dihydrothymoquinone, p-cymene, carvacrol, α-thujene, thymol, α-pinene, β-pinene and t-anethole as major constituents. Furthermore, *In vitro* antioxidant capacity indicated that fixed and essential oils inhibited lipid peroxidation by 25.62 and 92.56% and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity by 32.32 and 80.25%, respectively. The present findings showed that black cumin fixed and essential oils are rich source of phytochemicals and can be utilized against lifestyle disorders like hyperglycemia and hypercholesterolemia.

Introduction

The reliance of humans on plants to cure various pathologies is as old as their history and developments in the domain of nutrition during last few decades unveiled therapeutic potential of many culinary herbs (Hameed *et al*., 2008; Jabeen *et al*., 2008). Black cumin (*Nigella sativa* L.) locally known as "Kalonji" is good source of nutritionally essential components (Black *et al*., 2006; Atta-ur-Rahman *et al*., 1985a; Bukhari, 1985). Scientific investigations have depicted its composition i.e., moisture, oil, proteins, ash and total carbohydrates contents in the range of 3.8-7.0%, 22.0 to 40.35%, 20.85-31.2%, 3.7-4.7% and 24.9-40.0%, respectively (Takruri & Dameh, 1998; Atta, 2003).

The health enhancing potential of black cumin has been attributed to the active ingredients that are mainly concentrated in fixed and essential oil (Ramadan, 2007). Black cumin seed fixed oil contains appreciable quantities of unsaturated especially polyunsaturated fatty acids; constitute the bulk of oil ranging from 48-70%, while monounsaturated (18-29%) and saturated fatty acids (12-25%) are in lesser proportions (Nickavar *et al*., 2003; Cheikh-Rouhou *et al*., 2007). Besides better fatty acid profile, it also contains considerable quantities of tocopherols and allied bioactive compounds that

*Corresponding author: E-mail: tauseefsultan@hotmail.com; Phone# 0092-333-9949100

are important in attenuating the overall antioxidant capabilities of the body (Valko *et al*., 2007). Moreover, presence of phytosterols further strengthens its hypoglycemic and hypercholesterolemic perspectives (Cheikh-Rouhou *et al*., 2008; Ramadan & Mörsel, 2003; Atta, 2003). Likewise, pharmacological investigations explored the effectiveness of essential oil and its active ingredient i.e., thymoquinone against various maladies like oxidative stress, cancer, immune dysfunction and diabetic complications (Gali-Muhtasib *et al*., 2004, 2006; Hussein *et al*., 2005).

Like most herbs, the composition of black cumin varies with the geographic distribution, time of harvest and agronomic practices. This project was designed to characterize indigenous variety of black cumin seeds and thus nutritional profile of fixed and essential oils could possibly be used for their potential applications against lifestyle disorders.

Materials and Methods

Materials: Black cumin seeds of indigenous variety were obtained from Barani Agricultural Research Institute (BARI), Chakwal. Raw materials for cookies preparation were procured from local market while reagents (analytical and HPLC grade) and standards were purchased from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany).

Characterization of black cumin seed: Black cumin seeds were analyzed for various quality attributes including proximate analysis, mineral composition, polyphenols and alkaloids. The procedures followed are given below:

Proximate analysis: Black cumin seeds were analyzed for moisture, ash, crude protein, crude fat, crude fiber and nitrogen free extract using their respective methods i.e. Method No. 44-15A, Method No. 08-01, Method No. 46-30, Method No. 30-25, Method 32-10, respectively, described in AACC (Anon., 2000).

Mineral contents: The black cumin seeds were analyzed for mineral profile following the protocols described in AOAC (Anon., 2003). Concentrations of calcium (Method 968.08), magnesium (Method 968.08), zinc (Method 991.11), iron (Method 985.01) and phosphorous (Method 965.17) were determined by Atomic Absorption Spectrophotometer (Varian AA240, Australia) while sodium (Method 968.08) and potassium (Method 968.08) were measured through Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge).

Total polyphenols: Total polyphenols were determined using Folin-Ciocalteu method and values were expressed as gallic acid equivalent (Singleton *et al*., 1999).

Extraction and analysis of fixed oil: The oil from the black cumin seed was extracted through solvent extraction technique as described in AOCS (Anon., 1998); hexane used as solvent was recovered by Rotary Evaporator (Eyela, Japan). The extracted oil was stored in dark place at room temperature. The black cumin fixed oil was analyzed for physical & chemical characteristics and fatty acid profile using their respective methodologies. Physical characteristics include color, refractive index, flavor, specific gravity, specific extinction, smoke point and fire point. Chemical parameters include free fatty acids, peroxide value (POV), thiobarbituric acid (TBA) value, acid value, iodine value and saponification value. Fatty acid profile of black cumin fixed oil was determined by the method (Ce 1f-96) given in AOCS (Anon., 1998). Tocopherols of the oil sample were analyzed on HPLC (Model: Perkin Elmer Series 200, USA) following the

procedures of Katsanidis & Addis (1999). Thymoquinone in black cumin fixed oil sample was estimated using method described by Al-Saleh *et al*., (2006).

Extraction of essential oil: Essential oil was extracted following the method of Kantar *et al*., (2003). Essential oil was subjected to GC–MS analysis using a Gas Chromatograph (Singh *et al*., 2005).

Antioxidant potential of fixed and essential oil: Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was evaluated using the method described by Taga *et al*., (1984). The degradation rate of the extracts was calculated according to first order kinetics (Al-Saikhan *et al*., 1995). DPPH radical scavenging activity of black cumin fixed oil was measured according to the method of Brand-Williams *et al*., (1995).

Statistical analysis: Data obtained was analyzed statistically using statistical package i.e. Cohort V-6.1 (Co-Stat-2003). Sample for each analysis was run quadruplet and values expressed are means ± standard deviation.

Results

Black cumin seeds analyzed for different quality attributes contain 6.46±0.17, 22.80±0.60, 31.16±0.82, 6.03±0.16 and 4.20±0.11% of moisture, proteins, fat, fiber, ash contents, respectively, while nitrogen free extract was found to be 29.36±0.78%. Mineral composition indicated that potassium is dominant (808.00±6.61 mg/100g) followed by calcium (570±21.5 mg/100g), phosphorous (543±10.04 mg/100g) and magnesium (265±4.87 mg/100g), respectively. Moreover, considerable quantities of sodium, iron, manganese, zinc and copper were present in the indigenous variety of black cumin seeds. Total polyphenols contents were also determined and found to be 310.26±6.82 mg gallic acid/kg of oil while alkaloids 2.06±0.12% on dry weight basis (Table 1).

Black cumin fixed oil extracted through solvent extraction was tested for various physical & chemical characteristics, fatty acid profile and phytochemicals like thymoquinone, tocopherols and carotenoids (Table 2). The CieLab color values (*L**, *a**, *b**, chroma and hue angle) were found to be 32.62±0.27, 2.25±0.26, 56.26±0.89, 56.30±0.79 and 2.30±0.20, respectively. Means values for remaining physical parameters of fixed oil including specific gravity, refractive index, K_{232} and K_{270} values were 0.923±0.001, 1.473±0.001, 1.323±0.006 and 0.389±0.001, respectively. Likewise, means for chemical parameters like free fatty acid, POV, TBA, iodine & acid value, saponification value and unsaponifiable materials were 0.670±0.032%, 5.703±0.032 meq/kg, 0.060±0.003 mg malonaldehyde/kg-oil, 112.32±0.922 g/100g, 0.34±0.007 mg KOH/g, 172.56±1.594 and 1.80±0.089%, respectively.

Fatty acid profile of black cumin fixed oil is presented in Table 3. It is obvious from the results that linoleic, oleic and palmitic acids were the dominant fractions amounting 57.38±1.53, 19.65±0.61 and 12.07±0.87%, respectively. Myristoleic, stearic, eicosenoic and dihomolionolenic acids were present in quantities of 2.49±0.03, 2.35±0.04, 1.47±0.05 and 1.80±0.11% of the fixed oil, respectively. Similarly, linolenic acid was also present with percentage of 1.13±0.05% while myristic, arachidonic and behanic acids were in meager proportions i.e., 0.42±0.03, 0.33±0.06 and 0.19±0.03%, respectively. It is evident from the results that sum up of saturated and monounsaturated fatty acids were i.e., 16.64±0.91 and 22.47±0.59%, respectively while polyunsaturated fatty acids 60.17±1.53%.

Table 1. Proximate and mineral composition of black cumin seeds.

Proximate composition (%)	
Moisture	6.46 ± 0.17
Crude Protein	22.80 ± 0.60
Crude Fat	31.16 ± 0.82
Crude Fiber	6.03 ± 0.16
Ash	4.20 ± 0.11
NFE	29.36 ± 0.78
Mineral (mg/100gm)	
Potassium	808 ± 6.61
Calcium	570 ± 21.5
Phosphorus	543 ± 10.04
Magnesium	265 ± 4.87
Sodium	17.6 ± 2.21
Iron	9.70 ± 0.65
Manganese	8.53 ± 0.11
Zinc	6.23 ± 0.21
Copper	2.60 ± 0.03
Total polyphenols (mg gallic acid/kg of oil)	310.26 ± 6.82

Table 2. Physical and chemicals parameters of black cumin fixed oil.

Physical parameters	
CieLab color values	
*L**	32.62 ± 0.27
*a**	2.25 ± 0.14
*b**	56.26 ± 0.89
Chroma	56.30 ± 0.79
Hue angle	2.30 ± 0.20
Specific gravity (g/cm^3)	0.923 ± 0.001
Refractive index	1.473 ± 0.001
K_{232}	1.323 ± 0.006
K_{270}	0.389 ± 0.001
Chemical parameters	
Free fatty acid (%)	0.670 ± 0.032
POV (meq/kg)	5.703 ± 0.032
TBA value (mg malonaldehyde/kg-oil)	0.060 ± 0.003
Iodine value (g/100g)	112.32 ± 0.922
Acid value (mg KOH/g)	0.34 ± 0.007
Saponification value	172.56 ± 1.594
Unsaponifiable material (%)	1.80 ± 0.089

It is obvious from Table 4 that thymoquinone computed in the fixed oil was 201.31±13.17 mg/kg-oil. Different forms of tocopherols including α-, β-, γ- and δ- were 182.56±6.82, 18.56±0.13, 142.97±7.56 and 17.62±0.20 mg/kg-oil, respectively. From carotenoids estimation, it is revealed that black cumin fixed oil contains appreciable quantity of this trait i.e., 88.95±3.91 mg/kg-oil. Total tocopherols contents were 361.71±10.23 mg/kg-oil whilst carotenoids and tocopherols collectively were 450.66±16.21 mg/kg-oil.

Table 3. Fatty acid composition of black cumin fixed oil.

Fatty acids (%)	
Myristic acid (14:0)	0.42 ± 0.03
Myristoleic acid (14:1)	2.49 ± 0.03
Palmitic acid (16:0)	12.07 ± 0.87
Stearic acid (18:0)	2.35 ± 0.04
Oleic acid (18:1)	19.65 ± 0.61
Linoleic acid (18:2)	57.38 ± 1.53
Linolenic acid (18:3)	1.13 ± 0.05
Eicosenoic acid (20:1)	1.47 ± 0.05
Arachidonic acid (20:6)	0.33 ± 0.06
Behanic acid (22:0)	0.19 ± 0.03
Dihomolionolenic acid (22:2)	1.80 ± 0.11
Saturated fatty acids	16.64 ± 0.91
Monounsaturated fatty acids	22.47 ± 0.59
Polyunsaturated fatty acids	60.17 ± 1.53

Table 4. Functional components of black cumin fixed oil.

Parameters (mg/kg-oil)	
Thymoquinone	201.31 ± 13.17
Tocopherols	
α-Tocopherol	182.56 ± 6.82
β-Tocopherol	18.56 ± 0.13
γ- Tocopherol	142.97 ± 7.56
δ-Tocopherol	17.62 ± 0.20
Total tocopherols	361.71 ± 10.23
Carotenoids	88.95 ± 3.91
Tocopherols and carotenoids	450.66 ± 16.21

Table 5. Functional components of black cumin essential oil.

Parameters (%)	
Thymoquinone	23.25 ± 1.03
Dihydrothymoquinone	3.84 ± 0.12
ρ-Cymene	32.02 ± 1.01
Carvacrol	10.38 ± 0.30
α-Thujene	2.40 ± 0.06
Thymol	2.32 ± 0.26
α-Pinene	1.48 ± 0.02
β-Pinene	1.72 ± 0.05
t-Anethole	2.10 ± 0.42
Minor components	23.81 ± 0.92

Black cumin essential oil was evaluated for its active ingredients through GC-MS; indicated that it contains thymoquionone, dihydrothmoqionone, p-cymene, carvacrol, α-thujene, thymol, α-pinene, β-pinene and t-anethole as major constituents i.e., 23.25±1.03, 3.84±0.12, 32.02±1.01, 10.38±0.30, 2.40±0.06, 2.32±0.26, 1.48±0.02, 1.72±0.05 and 2.10±0.42%, respectively (Table 5).

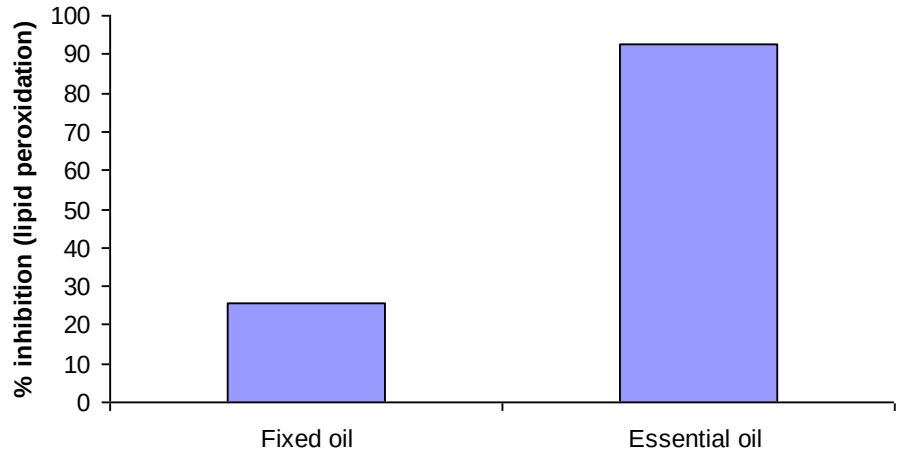

Fig. 1. Antioxidant activity of black cumin fixed and essential oils.

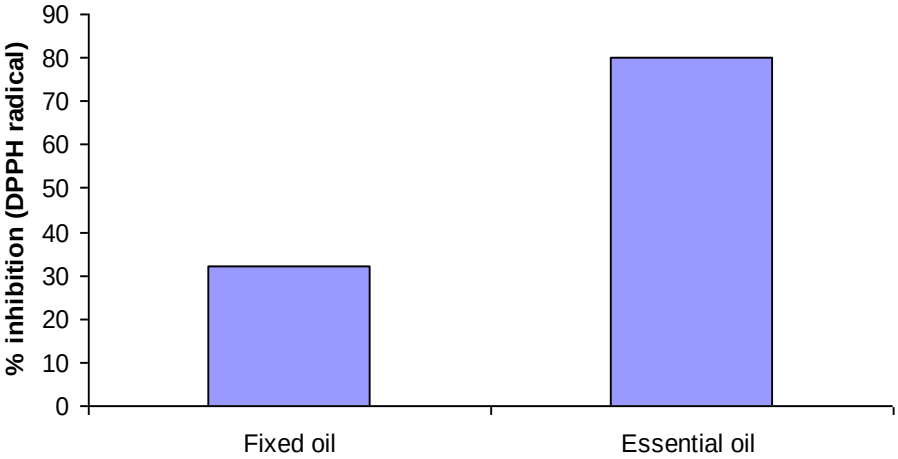

Fig. 2. DPPH radical scavenging activities of black cumin fixed and essential oils.

Antioxidant potential of fixed and essential oils was also assayed. Antioxidant activity based on coupled oxidation of β-carotene and linoleic acid was determined; black cumin fixed and essential oils inhibited lipid peroxidation by 25.62 and 92.56%, respectively (Fig. 1). Later, DPPH assay was also conducted that is another module to study the antioxidant potential of test materials; black cumin fixed and essential oils inhibited DPPH radical formation by 32.32 and 80.25%, respectively (Fig. 2).

Discussion

Black cumin seeds are important in herbal medicines commonly employed in eastern civilization (Ramadan, 2007). In Pakistan according to author knowledge, little efforts had been made to characterize the black cumin seed fixed and essential oils. Findings of

present research regarding characterization were in close conformity with the values described in the literature with slight differences. The environmental factors like climate and location might be a possible reason for these variations. Moreover, difference in genetic makeup could also be a contributing factor as indigenous variety was tested in the trial. Various scientists across the globe gave some evidences about the composition of black cumin seeds. Overall, moisture, fat, protein, ash and total carbohydrates contents were in the range of 3.8-7.0, 22.0-40.35, 20.85-31.2, 3.7-4.7 and 24.9-40.0% respectively (Abdel-Aal & Attia, 1993; El-Dhaw & Abdel-Manaem, 1996; Takruri & Dameh, 1998; Atta, 2003). In another research study, Cheikh-Rouhou *et al*., (2007) compared Tunisian and Iranian varieties for various quality characteristics. They observed that Tunisian variety contains 8.65, 28.48, 26.7, 4.86 and 40.0% of moisture, oil, proteins, ash and carbohydrates as compared to 4.08, 40.35, 22.6, 4.41, and 32.7% for respective traits in Iranian variety.

In the present study, it was observed that minerals content accounts for 4.20%. Some studies also supported the present results regarding mineral contents like Ashraf *et al*., (2006); Nickavar *et al*., (2003) and Atta (2003). However, findings of Cheikh-Rouhou *et al*., (2007) showed slight differences while comparing the composition of minerals but they also reported that potassium is dominant mineral in black cumin seed. Possible causes of these differences could be climatic variations and genotype. Likewise, findings of Takruri & Dameh (1998) also strengthened this instant exploration as they reported presence of iron, copper, sodium, potassium, calcium, zinc and phosphorous i.e., 105, 18.4, 496.0, 5257, 1859, 60.4 and 5265mg/kg, respectively in black cumin seeds of Indian origin.

Black cumin seeds contain appreciable quantities of fixed oil that needs further to be explored as a source of edible oil. The physical parameters like specific gravity, refractive index, K_{232} and K_{270} are important in quantitative estimation of oxidative stability of fat and oils. Present findings remained in the ranges described in literature (Ramadan & Mörsel, 2003; Atta, 2003; Cheikh-Rouhou *et al*., 2007). Chemical attribute like iodine value indicates the presence of unsaturated fatty acids and higher value is an indication of the presence of lower amount of saturated fats and *vice versa*. The iodine value usually ranged from 15.0 to 150g/100g in vegetable oils (Hsu & Chung, 2002). The present findings are in corroboration with the values reported earlier by Atta (2003) and Cheikh-Rouhou *et al*., (2007). Thymoquinone is more concentrated in black cumin essential oil ranging from 18-24% (Burits & Bucar, 2000) whereas extraction of fixed oil by means of n-hexane retains meager quantity as in case of present investigation. Some organic extracts of black cumin seed also reported to possess appreciable amounts of thymoquinone. In one such study, Singh *et al*., (2005) estimated thymoquinone contents of 11.8% in acetone extract seed. Moreover, thymoquinone belongs to class of compounds known as terpenoids; most members are volatile in nature and their heating losses ranged from 20-50% (Bendahou *et al*., 2008) that could also be a possible reason for lower quantity of thymoquinone in fixed oil. Consumption of different isomeric forms like α-, β-, γ- and δ-Tocopherols contribute substantially towards the health improvement. Presence of these important phytochemicals has also been highlighted in two earlier studies; initially conducted by Ramadan & Mörsel (2002) and later by Al-Saleh *et al*., (2006). According to Ramadan & Mörsel (2002), one gram of black cumin fixed oil contain 284, 40, 225, 48µg of α-, β-, γ- and δ-Tocopherols, respectively. They also reported the presence of β-carotene amounting 593µg/g oil. Similarly, Al-Saleh *et al*., (2006) reported the concentrations of 10.41 and 6.95mg/kg-seed of α- and γ-

Tocopherols, respectively. They observed the concentration of thymoquinone (3098.5±1519.66 mg/kg) in black cumin seeds.

Black cumin essential oil is also important owing to its rich phytochemistry. Some other studies conducted in different part of the world showed varying picture about the composition of black cumin essential oil. Burits & Bucar (2000) analyzed essential oil using GC-MS and characterized many components including thymoquinone; 27.8%–57.0%, ρ-cymene; 7.1%–15.5%, carvacrol; 5.8%–11.6%, t-anethole; 0.25%–2.3%, 4-terpineol; 2.0%–6.6% and longifoline; 1.0%–8.0% (Mozzafari *et al*., 2000; Nickavar *et al*., 2003). Afterwards, Ashraf *et al*., (2006) and Wajs *et al*., (2008) determined p-cymene as major component of black cumin seed essential oil. Among these research investigations, results reported by Nickavar *et al*., (2003) and Burits & Bucar (2000) are in agreement with the present findings. Overall, black cumin is naturally bestowed with antioxidant rich volatile oil (0.40-1.50%) that contains 18.4-24% thymoquinone and 46% monoterpenes such as p-cymene and α-pinene (Al-Jassir, 1992; El-Tahir *et al*., 1993; Singh *et al*., 2005; Ashraf *et al*., 2006). Several other scientists explored the antioxidant potential of black cumin oil and its various fractions containing active ingredients. Because of the ease and convenience, DPPH assay has widespread use in free radical scavenging assessment (Thaipong *et al*., 2006; Erkan *et al*., 2008; Scherer & Godoy, 2009). According to Burits & Bucar (2000), IC50 value (DPPH assay) for different test compounds like essential oil, thymoquinone and carvacrol were found to be 460.0, 211.0 and 28.8 mg/mL, respectively. In another investigation, black cumin seeds inhibited DPPH radical formation and mean IC50 (μM) was found to be 515 ± 20.1 while ABTS.+ (TEAC, mM Trolox) assay gave readings of 2.0 ± 0.7, 2.4±0.3 and 2.5±0.6 after 1, 4 and 6 minutes of reaction (Erkan *et al*., 2008).

Conclusion

Black cumin holds nutraceutical potential against various physiological threats owing to its rich phytochemistry especially due to the presence of thymoquinone, tocopherols, etc. Finally, fixed and essential oil supplementation in food products especially bakery items is feasible and can be employed to achieve the allied health claims.

Acknowledgement

Corresponding author is thankful to Higher Education Commission of Pakistan for providing funds for PhD project entitled “Characterization of black cumin fixed and essential oils and exploring their role as functional foods”.

References

Abdel-Aal, E.S.M. and R.S. Attia. 1993. Characterization of black cumin (*Nigella sativa*) seeds. 2-Proteins. *Alex. Sci. Exch*., 14: 483-496.

Abu-Jadayil, S., S.K.H. Tukan and H.R. Takruri. 1999. Bioavailability of iron from four different local food plants in Jordan. *Pl. Foods. Human. Nut*., 54: 285-294.

Al-Jassir, S.M. 1992. Chemical composition and microflora of black cumin (*Nigella sativa*) seeds growing in Saudi Arabia. *Food Chem*., 45: 239-242.

Al-Saleh, I., A.G. Billedo and I.I. El-Doush. 2006. Levels of selenium, DL-a-tocopherol, DL-g-tocopherol, all-trans-retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. *J. Food Comp. Anal*., 19: 167-175.

Anonymous. 1998. *Official Methods and Recommended Practices of American Oil Chemical Society*. 5th Ed. AOCS, Campaign, Illinois, USA.

Anonymous. 2000. *Approved Methods of the American Association of Cereal Chemists*, 10th Ed. AACC, St. Paul, MN, USA.

Anonymous. 2003. *Official Methods of Analysis*.13th Ed. Association of Official Analytical Chemists (AOAC), Washington, DC, USA.

Ashraf, M., Q. Ali and Z. Iqbal. 2006. Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. *J. Sci. Food Agric*., 86: 871-876.

Atta, M.B. 2003. Some characteristics of nigella (*Nigella sativa* L.) seed cultivated in Egypt and its lipid profile. *Food Chem*., 83: 63-68.

Atta-ur-Rahman, S. Malik, H. Cun-Hung and J. Clardy. 1985a. Isolation and structure determination of nigellicine, a novel alkaloid from seeds of *Nigella sativa*. *Tetrahedron. Lett*., 26: 2759-2562.

Bendahou, M., A. Muselli, M. Grignon-Dubois, M. Benyoucef, J.M. Desjobert, A.F. Bernardini and J. Costa. 2008. Antimicrobial activity and chemical composition of *Origanum glandulosum* Desf. Essential oil and extract obtained by microwave extraction: comparison with hydrodistillation. *Food Chem*., 106: 132-139.

Black, M., J.D. Bewley and P. Halmer. 2006. *The Encyclopedia of Seeds: Science, Technology and Uses*. CAB International. Cambridge, USA.

Brand-Williams, W., M.E. Cuvelier and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol*., 28: 25-30.

Bukhari, A.A. 1985. *Sahih-ul-Bukhari* (Arabic). Federal Ministry of Education, Govt. of Pakistan, Islamabad.

Burits, M. and F. Bucar. 2000. Antioxidant activity of *Nigella sativa* essential oil. *Phytother. Res*., 14: 323-328.

Cheikh-Rouhou, S., S. Besbes, B. Hentati, C. Blecker, C. Deroanne and H. Attia. 2007. *Nigella sativa* L.: Chemical composition and physicochemical characteristics of lipid fraction. *Food Chem*., 101: 673-681.

Cheikh-Rouhoua, S., S. Besbesa, G. Lognayb, C. Bleckerc, C. Deroannec and H. Attia. 2008. Sterol composition of black cumin (*Nigella sativa* L.) and Aleppo pine (*Pinus halepensis* Mill.) seed oils. *J. Food Comp. Anal*., 21: 162-168.

El-Dhaw, Z.Y. and N.M. Abdel-Munaem. 1996. Chemical and biological values of black cumin seeds. *J. Agric. Sci. Mansoura Univ*., 21: 4149-4159.

El-Tahir, K.E., M.M. Ashour and M.M. Al-Harbi. 1993. The cardiovascular effects of the volatile oil of black seed (*Nigella sativa*) in rats:elucidation of the mechanism(s) of action. *Gen. Pharmacol*., 24: 1123-1131.

Erkan, N., G. Ayranci and E. Ayranci. 2008. Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol, *Food Chem*., 110: 76-82.

Gali-Muhtasib, H., M. Diab-Assaf, C. Boltze, J. Al-Hmaira, R. Hartig, A. Roessner and R. Schneider-Stock. 2004. Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. *Int. J. Oncol*., 25: 857-866.

Hameed, I., G. Dastagir and F. Hussain. 2008. Nutritional and elemental analyses of some selected medicinal plants of the family polygonaceae. *Pak. J. Bot*., 40(6): 2493-2502.

Hussein, M.R., E.E. Abu-Dief, M.H. Abd El-Reheem and A. Abd-Elrahman. 2005. Ultrastructural evaluation of the radioprotective effects of melatonin against X-ray-induced skin damage in Albino rats. *Int. J. Exp. Pathol*., 86: 45-55.

Jabeen, F., M. Shahbaz and M. Ashraf. 2008. Discriminating some prospective cultivars of maize (*Zea mays* L.) for drought tolerance using gas exchange characteristics and proline contents as physiological markers. *Pak. J. Bot*., 40(6): 2329-2343.

Katsanidis, E. and P.B. Addis. 1999. Novel HPLC analysis of tocopherols, tocotrienols and cholesterol in tissue. *Free Rad. Biol. Med*., 27: 1137-1140.

Mozzafari, F.S., M. Ghorbanli, A. Babai and M. Farzami Scpehr. 2000. The effect of water stress on the seed oil of *Nigella sativa* L. *J. Essential. Oil Res*., 12: 36-38.

Nickavar, B., F. Mojab, K. Javidnia and M.A. Roodgar Amoli. 2003. Chemical composition of the fixed and volatile oils of *Nigella sativa* L. *Zeitschrift. Fur. Naturforschung*., 58: 629-631.

Ramadan, M.F. 2007. Nutritional value, functional properties and nutraceuticals applications of black cumin (*Nigella sativa* L.): an overview. *Int. J. Food Sci. Technol*., 42: 1208-1218.

Ramadan, M.F. and J.T. Mörsel. 2002. Direct isocratic normalphase assay of fat-soluble vitamins and beta-carotene in oilseeds. *Eur. Food Res. Technol*., 214: 521-527.

Ramadan, M.F. and J.T. Mörsel. 2003. Analysis of glycolipids from black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) oilseeds. *Food Chem*., 80: 197-204.

Scherer, R. and H.T. Godoy. 2009. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. *Food Chem*., 112: 654-658

Singh, N., M. Verma, D. Mehta and B.K. Mehta. 2005. Two new lipid constituents of *Nigella sativa* (Seeds). *Ind. J. Chem*., 44: 742-744.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol*., 299: 152-178.

Taga, M.S., E.E. Miller and D.E. Pratt. 1984. China seeds as source of natural lipid antioxidation. *J. Am. Oil Chem. Soc*., 61: 928-931.

Takruri, H.R.H. and M.A.F. Dameh. 1998. Study of nutritional value of black cumin seeds (*Nigella sativa* L.). *J. Sci. Food Agric*., 76: 404-410.

Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D.H. Bryne. 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Comp. Anal*., 19: 669-675.

Valko, M., C.J. Rhodes, J. Moncol, M. Izakovic and M. Mazur. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact*., 160: 1-40.

Wajs, A., R. Bonikowski and D. Kalemba. 2008. Composition of essential oil from seeds of *Nigella sativa* L., cultivated in Poland. *Flavour Fragr. J*., 23: 126-132.

(Received for publication 20 April, 2009)