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BLACK SEEDS (*NIGELLA SATIVA*)- A SOURCE OF IRON AND ANTIOXIDANTS

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ABSTRACT

Iron is determined in the seeds of *Nigella sativa* spectrophotometrically and iron-opt complexation is selected. Calibration curve method is employed for this study. From this curve molar absorptivity (ε) was calculated to determine the concentration of metal in different samples, molar absorptivity (ε) was found to be $10,000M^{-1}$. cm^{-1} at 515nm. Average values of concentrations for the five ´amples were found to be 110.4239ppm, 139.7700ppm, 138.0647ppm, 141.6273ppm and 139.2660ppm.respectively. Fe(III) solution was treated with extract of *N. sativa* as well as two other active biological reductants, hydroquinone and hydroxyl ammonium chloride. It has been found that *N. sativa* is stronger reducing agent than hydroxyl ammonium chloride and weaker then hydroquinone.

Key-words: Kalonji, Fe^{2+}/Fe^{3+}, antioxidant

INTRODUCTION

Black seed is also included in the list of natural drugs of Al-Tibb al-Nabawi, and, according to tradition, "Hold onto the use of the black seed for it has a remedy for every illness except death" (Ibn Al Qayyim). Naturally occurring alternative medicine could help to solve many of the common as well as peculiar medicinal problems facing the people (Vohora and Dandiya, 1992; Takri and Dameh, 1998). There are many herbs used for the treatment of different diseases, one of them is *Nigella sativa* (Miller, 1998; Ali, 1992). Chemical composition]. It is a herbaceous plant belongs to the family Ranuculaceae, its seeds are commonly known as Kalonji, Black cumin, black seed and black caraway (Sweet sunnah, 2003). Our goal is to determine the role of seeds of *N. sativa* for human health. In order to understand the use of these seeds and their mode of action, it is important to know about their constituents, amazingly its chemical composition is very rich and diverse. These seeds have over one hundred different chemical constituents having active ingredient thymoquinone (TQ). Others are dithymoquinone, thymohydroquinone, nigellone, ascorbic acid (Vitamin C), tocopherol (Vitamin E), linoleic acid, lipase, oleic acid, carvacrol, t-anethole and 4-terpineol etc. Most of these show antioxidant ability (Al-Yahya, 1981; Bose *et al*., 1981; Jukneviciene *et al*., 1977; Khan, 1999; Abdel-Fateh and Matsumoto, 2000; Burits and Bucar, 2000). Seeds of *N. sativa* were used in the indigenous sytem of medicine (Salomi and Nair, 1992). It has many pharmacological actions such as a anti-phlegmatic, stimulant, carminative, diuretic, expectorant, anti-fertility, stomach and liver tonic (Bratter and Schramel, 1980).

The role of elements in health and disease is now an established fact (Siddiqi and Kan, 1990). Trace elements are crucial to virtually all biochemical and physiological processes in plant, animal and human beings (Nergiz and Otle, 1992). Of these, iron, zinc, cobalt, manganese, nickel, copper, chromium and molybdenum are now thought to be essential for animal life (Cheblowski and Coleman, 1976).

The black seed is also a source of Ca, K, Fe, Zn, Mg, Se and Na, required only in small amounts by the body (Khan, 1999). The main action of these elements is to act as essential cofactors in various enzymatic functions. Iron has the longest and best-described history among all the metals (Cowan, 1997). An in vivo study of bioavailability of iron from four different plants i.e. black cumin seeds, milk thistle seeds, sesame seeds and thyme leaves shows that iron was better utilized from black cumin seeds as indicated by liver storage of iron. On the other hand, thyme had the highest iron absorption but lowest utilization (Jadayil *et al*., 1999).

The bioavailability of iron from *N. sativa* was investigated and it was concluded that iron from *N. sativa* was better utilized (Siong *et al*., 1989). In the present work iron is determined in the seeds of *N. sativa* spectrophotometrically. For this purpose most reliable and convenient method “Iron-opt complexation is selected (Jeffery *et al*., 1989). Calibration curve method is employed for this study (Sawyer *et al*., 1984).

MATERIALS AND METHODS

The samples of *N. sativa* were purchased from the local market. They were cleaned and dried at 40 ± 5^0C. Accurately weighed amount of sample (about 5gms) was soaked in 100ml of 1M HCl for 24hours, filtered with Whatman filter paper #542. The filtrate was then made upto 250ml with distilled water.

Iron was estimated by Fe-Opt method that is accurate and convenient. Iron was standardized first, 0.0101gm ammonium iron(II)sulphate $Fe(NH_4)_2(SO_4)_2.5H_2O$ was dissolved in 100ml deionized water, few drop of concentrated sulphuric acid was added, diluted to 250ml and mixed thoroughly($1x10^{-4}$M). 0.25% solution of the 1,10 phenanthroline monohydrate (Opt) in ethanol, 1M sodium acetate and 1% hydroquinone in deionized distilled water were prepared. Different volumes of standard solutions were transferred to 25ml volumetric flasks sodium acetate solution was added to bring the pH to 3.5± 1.0 checked by pH meter model Orion 720 with a resolution of ±0.1. 2ml 1% äydroquinone solution for reduction of iron into ferrous and 2.5ml of 1:10 orthophenanthroline for complexation was added to each flask. The volume was then made upto 25ml. Mixed well, and allowed to stand for 1 hour for the completion of complexation. The absorbances were recorded at 515nm on Shimadzu model-160 (UV-VIS) Spectrophotometer.

The same procedure was used to analyze the iron content in the extract of the sample of *N. sativa*. Reduction of $Fe(NO_3)_3.9H_2O$ was studied with different reductants, hydroquinone, hydroxyl ammonium chloride and extract of the sample of *N. sativa*. For this purpose different volumes of 0.0001M solution of Fe(III) was transferred to 25ml volumetric flasks then same procedure was applied for reduction using different reducing agents. For first set 2ml Hydroquinone (1%), for the second set 2ml hydroxyl ammoium chloride (10%) and for the third set 5ml extract of the *N. sativa* was used.

RESULTS AND DISSCUSSION

Iron in *N. sativa* has been determined by atomic absorption Spectrophotometric and colorimetric method (Siong et al., 1989). Reported concentration of iron in seeds is 140ppm (Duke, 1992). Iron is required to the body in trace amount but this little quantity is very important and essential. Iron is present in the body in both ferrous as well as ferric forms but absorbable form is the ferrous (Iffat *et al*., 2004; Iffat *et al*., 2005). Iron present in *N. sativa* in both forms but it has also contained reducing agents, which help to provide the iron to the body in the bioavailable form(ferrous) and also reduce iron which is already present in the body.

Iron was detected in the sample spectrophotometrically, by choosing most reliable method " Iron(II) Opt complexation". For this purpose reducing agent was added to the accurately measured volume of sample and then orthophenanthroline was added as a chelating agent. So that the iron-Opt complex was prepared in pH 4.0 buffer. Before taking the absorbance, calibration curve with known concentration of Fe-Opt solutions was plotted. From this curve molar absorptivity (ε) was calculated to determine the concentration of metal in different samples, molar absorptivity (ε) was found to be $10{,}000 M^{-1}. cm^{-1}$ at 515nm (**Fig. 1**).

Five samples of *N. sativa* were also treated with Opt in the same manner taking absorbance at 515nm for different volumes of sample. Concentrations were calculated using molar absorptivity value (ε) $10{,}000\ M^{-1}. cm^{-1}$. Average values of concentrations for all five samples were found to be 110.423ppm, 139.770ppm, 138.064ppm, 141.627ppm and 139.266ppm in five different samples respectively (Tables 1-5). Mean of these five values 133.829ppm was comparable with the reported value of iron in seeds. *N. sativa* acts as active antioxidant, to determine and to confirm this activity, Fe(III) solution was treated with extract of *N. sativa* as well as two other active biological reductants, hydroquinone and hydroxyl ammonium chloride. Order of reducing strength of *N. sativa* can be shown in following manner:

Hydroquinone > Extract of *N. sativa* > Hydroxyl Ammonium Chloride.

It shows that *N. sativa* is stronger than hydroxyl ammonium chloride and weaker then hydroquinone (Table 6, **Fig. 2**). It means that *N. sativa* is a source of ferrous (the bioavailable iron) as well as an antioxidant.

Table 1. Calculations of Fe-Opt complex for determination of Fe(II) in sample 1.
Sodium acetate=2.5M; 1,10-Orthophenanthroline (Opt)= 0.25% = 0.0126M (2.5ml in each)
Sample extract = 5g /100ml in 1 M HCl diluted to 250ml with distilled water. ε = $10{,}000\ M^{-1}\ cm^{-1}$ λ max =515 nm

S[#]	sample/ 25ml	Abs.	C =A/ε [M]	mmoles	gms/5g	gm/gm	ug/g	Overall factor
1	2.5	0.052	0.0000052	0.0130	0.0007198	0.00014396	143.962	143.962
2	5.0	0.094	0.0000094	0.0118	0.0006506	0.00013012	130.120	130.120
3	7.5	0.121	0.0000121	0.0101	0.0005583	0.00011166	111.663	111.663
4	10.0	0.147	0.0000147	0.0092	0.0005087	0.00010174	101.742	101.742
5	12.5	0.175	0.0000175	0.0088	0.0004845	9.6898E-05	96.898	96.898
6	15.0	0.204	0.0000204	0.0085	0.0004706	9.4129E-05	94.129	94.129
7	17.0	0.232	0.0000232	0.0085	0.0004723	9.4455E-05	94.455	94.455

Mean value = 110.424

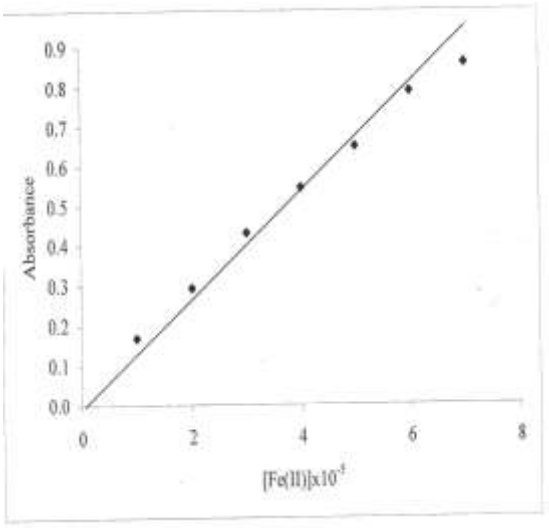

Fig.1. Calibration curve for Fe(II)-opt complex.

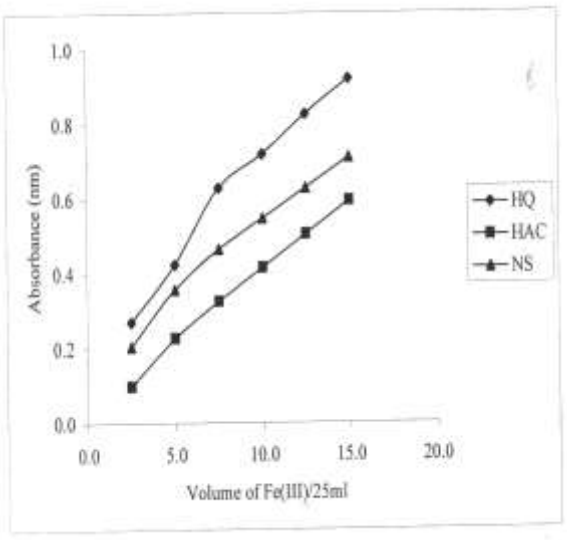

Fig.2. Plot for reducing effect of *Nigella sativa* and other reductants on Fe (III).

Table 2. Calculations of Fe-Opt complex for determination of Fe(II) in sample 2.
Sodium acetate=2.5M; 1,10-Orthophenanthroline (Opt)= 0.25% = 0.0126M (2.5ml in each)
Sample extract = 5g /100ml in 1 M HCl diluted to 250ml with distilled water. ε = 10,000 M^{-1} cm^{-1} λ max =515 nm

S[#]	sample/ 25ml	Abs.	C =A/ε [M]	mmoles	gms/5g	gm/gm	ug/g	Overall factor
1	2.5	0.051	0.0000051	0.0128	0.000706	0.0001412	141.194	141.194
2	5.0	0.101	0.0000101	0.0126	0.000699	0.0001398	139.809	139.809
3	7.5	0.149	0.0000149	0.0124	0.0006875	0.0001375	137.502	137.502
4	10.0	0.201	0.0000201	0.0126	0.0006956	0.0001391	139.117	139.117
5	12.5	0.251	0.0000251	0.0126	0.0006949	0.0001390	138.979	138.979
6	15.0	0.301	0.0000301	0.0125	0.0006944	0.0001389	138.886	138.886
7	17.0	0.351	0.0000351	0.0129	0.0007145	0.0001429	142.903	142.903

Mean value = 139.770

Table 3. Calculations of Fe-Opt complex for determination of Fe(II) in sample 3.
Sodium acetate=2.5M; 1,10-Orthophenanthroline (Opt)= 0.25% = 0.0126M (2.5ml in each)
Sample extract = 5g /100ml in 1 M HCl diluted to 250ml with distilled water. ε = 10,000 M^{-1} cm^{-1} λ max =515 nm

S[#]	sample/ 25ml	Abs.	C =A/ε [M]	mmoles	gms/5g	gm/gm	ug/g	Overall factor
1	2.5	0.048	0.0000048	0.0120	0.0006644	0.0001329	132.888	132.888
2	5.0	0.111	0.0000111	0.0139	0.0007683	0.0001537	153.652	153.652
3	7.5	0.145	0.0000145	0.0121	0.0006691	0.0001338	133.811	133.811
4	10.0	0.211	0.0000211	0.0132	0.0007302	0.000146	146.038	146.038
5	12.5	0.244	0.0000244	0.0122	0.0006755	0.0001351	135.103	135.1028
6	15.0	0.291	0.0000291	0.0121	0.0006714	0.0001343	134.272	134.272
7	17.0	0.321	0.0000321	0.0118	0.0006534	0.0001307	130.689	130.689

Mean value = 138.065

Table 4. Calculations of Fe-Opt complex for determination of Fe(II) in sample 4.
Sodium acetate=2.5M; 1,10-Orthophenanthroline (Opt)= 0.25% = 0.0126M (2.5ml in each)
Sample extract = 5g /100ml in 1 M HCl diluted to 250ml with distilled water. ε = 10,000 M^{-1} cm^{-1} λ max =515 nm

S[#]	sample/ 25ml	Abs.	C =A/ε [M]	mmoles	gms/5g	gm/gm	ug/g	Overall factor
1	2.5	0.049	0.0000049	0.0123	0.0006783	0.0001357	135.657	135.657
2	5.0	0.111	0.0000111	0.0139	0.0007683	0.0001537	153.652	153.652
3	7.5	0.149	0.0000149	0.0124	0.0006875	0.0001375	137.502	137.502
4	10.0	0.215	0.0000215	0.0134	0.000744	0.0001488	148.807	148.807
5	12.5	0.241	0.0000241	0.0121	0.0006672	0.0001334	133.442	133.442
6	15.0	0.311	0.0000311	0.0130	0.0007175	0.0001435	143.501	143.501
7	17.0	0.341	0.0000341	0.0125	0.0006942	0.0001388	138.832	138.832

Mean value = 141.627

Table 5. Calculations of Fe-Opt complex for determination of Fe(II) in sample 5.
Sodium acetate=2.5M; 1,10-Orthophenanthroline (Opt)= 0.25% = 0.0126M (2.5ml in each)
Sample extract = 5g /100ml in 1 M HCl diluted to 250ml with distilled water. ε = 10,000 M^{-1} cm^{-1} λ max =515 nm

S[#]	sample/ 25ml	Abs.	C =A/ε [M]	mmoles	gms/5g	gm/gm	ug/g	Overall factor
1	2.5	0.049	0.0000049	0.0123	0.0006783	0.0001357	135.657	135.657
2	5.0	0.101	0.0000101	0.0126	0.000699	0.0001398	139.809	139.809
3	7.5	0.161	0.0000161	0.0134	0.0007429	0.0001486	148.576	148.576
4	10.0	0.201	0.0000201	0.0126	0.0006956	0.0001391	139.117	139.117
5	12.5	0.241	0.0000241	0.0121	0.0006672	0.0001334	133.442	133.442
6	15.0	0.311	0.0000311	0.0130	0.0007175	0.0001435	143.501	143.501
7	17.0	0.331	0.0000331	0.0122	0.0006738	0.0001348	134.761	134.761

Mean value = 139.266

Table 6. Data for reducing effect of *Nigella sativa* and other reductants on Fe(III).
Concentration of Fe(III) = 0.0001 M, Orthophenanthroline = 0.25% = 0.0126M (2ml in each)
Hydroxy Ammonium Chloride = 10% (2ml), Sample extract = 2ml; Hydroquinone = 1% (2ml)
Sample extract = 5g /100ml in 1 M HCl diluted to 250ml with distilled water, λ max =515 nm

S[#]	Fe(III) soln./ 25ml	Hydroquinone	Hydroxyl Ammonium Chloride	Sample extract
1	2.50	0.271	0.101	0.205
2	5.00	0.424	0.211	0.357
3	7.50	0.629	0.325	0.465
4	10.00	0.719	0.415	0.547
5	12.50	0.825	0.505	0.629
6	15.00	0.92	0.595	0.711

CONCLUSION

Iron is required to the body in trace amount and if it is in excess it create toxicity. From the results above we can conclude that the Kalonji is a source of iron as well as antioxidants. Which help to provide iron according to the

body requirement that is actually the ferrous form. Its constant use would be helpful to control the anaemic problems. Kalonji is a safe and effective herb.

REFERENCES

Abdel-Fateh, M., A. K. Matsumoto and H. Watanabe (2000). Antinoceceptive effects of *Nigella sativa* oil and its major component, thymoqinone in mice. *European J. Pharmacology*, 400: 89-97.

Ali, J. M. S. (1992). Chemical composition andmicroflora of black cmin (*Nigella sativa* L.) seeds growingin Saudi Arabia. *Food Chemistry*, 45: 239-242.

Al-Yahya, M.A. (1981). Phytochemical studies of the plants used in traditional medicine of Saudi Arabia. *Fitoterapia*, 57: 179-82.

Bose, B., O. Ghosh and R.P. Singh (1981). Study on the chemical constituents of seeds of *Nigella sativa* (Kalajeira)-a preliminary note. *J. Inst. Chem*., 53: 273-277.

Bratter, P. and P. Schramel (1980). Trace Elements, In: *Analytical Chemistry in Medicine and Biology*. pp. 125-126, (Walter D Ggruyter, Berlin Ed.)

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

Cheblowski, J. I. And J. E. Coleman (1976). Zinc and its role in enzymes, In: *Metal ions in biological systems*. pp. 198-200, (H. Sigel, Dekker eds.)

Cowan, J. A. (1997). Fundamentals of inorganic biochemistry, In: *Inorganic biochemistry an introdction*. pp. 2-3, Willey-VCH, Inc. USA.

Duke, A. (1992) Chemicals in *Nigella sativa* L. (Renunculaceae), In: *White lotus aromatics newsletters*. pp. 5-6, http://www.ars-grain.gov/cgi-bin/duke/farmacy2.pl

Ibn Al Qayyim Al Jawziyyah: "Al Tibb al Nabavi".

Iffat, A. T., Z. T. Maqsood, K. Ali and S. Nisar (2004). Interaction of tannic Acid with higher oxidation state of iron. *J. Chem. Soc. Pak*., 26: 151-156.

Iffat, A. T., Z. T. Maqsood and N. Fatima (2005). Study of complex formation of Fe(III) with tannic acid. *J. Chem. Soc. Pak*., 27: 174-177.

Jadayil, S. Abu., S. K. H. Tukan and H. R. Takruri (1999). Bioavailability of iron from four different local food plants in Jordan. *Plant Foods Hum. Nutr*, 54: 285-294.

Jeffery, G. H., J. Bassett, J. Mendham and R. C. Denny (1989). Colorimet ¨ and spectophotometry, In: *Vogel's Textbook of Quantitative Chemical Analysis*. pp. 691-692, 5th ed. John Wiley and Sons N. Y.

Jukneviciene, G., S. Dogyte and N. Stankeviciene (1977). Biological properties and essential oils of some spice plants grown at the Kaunas Botanical Garden. Ser. C., 9: 9-16.

Khan, M. A. (1999). Chemical composition and medicinal properties of *Nigella sativa* Linn. *Inflammopharmacology*, 7:15-35.

Miller, L. G. (1998). Herbal Medication, Neutraceuticals and Diabetes, In: *A Clinical's Guide*. pp 115-133, Bighamton, N.Y.Haworth Press, Inc.

Nergiz, C. and S. Otle (1992). Chemical composition of *Nigella sativa* L. Seeds. *Food Chemistry*. 48: 4-6.

Salomi, N. J. and S. L. Nair (1992). Anti-tumour principles from *Nigella sativa* seeds. *Cancer Letters*, 63: 41-46.

Sawyer, D. T., W. R. Heineman and J. M. Beebe (1984). Ultraviolet visible absorption spectroscopy, In: *Chemistry Experiments for Instrumental Methods*. pp. 206-207, John Wiley & Sons N.Y.

Shahito, S.R., T. G. Kazi, G. H. Kazi and M.A. Jakhrani (2002). Determination of mineral constituents in medicinally important plants *Nigella sativa*, *Myristica fragrants* Houtt. and *Allium sativum* Linn. using atomic absorption spectrophotometry. *J. Chem. Soc. Pak*., 24: 134-138.

Siddiqi, T. O. and H. A. Kan (1990). Robable role of trace elements of some medicinal plants in cardiovascular diseases. *Acta Manilana*, 38: 19-24.

Siong, T.E., K. S. Choo and S. M. Shahid (1989). Determination of iron in foods by atomic absorption spectrophotometric and colorimetric methods. *Pertanika*, 12: 313-322.

Sweet Sunnah, (2003). A cure for every disease except death (Sahi Bukhari), In: *Black Seeds*. pp 1-7, www.sweetsunnah.com , Email: info@sweetsnnah.com.

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

Vohora, S. B., and P. C. Dandiya (1992). Herbal analgesic drugs. *Fitoterapia*, 63: 195-207.

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