Physicochemical and phytochemical standardization with HPTLC fingerprinting of *Nigella sativa* L. seeds

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Abstract: This study was designed to perform the physicochemical and phytochemical standardization with HPTLC fingerprinting of *Nigella sativa* seeds in order to establish the standard pharmacognostical parameters of this miracle herb. Different parameters like extractive values; total ash value, acid insoluble ash value and water soluble ash value, moisture content, loss on drying, pH values of *Nigella sativa* seeds were performed. Preliminary phytochemical screening was done to detect different phytoconstituents by using the Harborne's phytochemical methods. Quantification of phenolic and flavonoid contents, determination of pesticides residues, aflatoxin and heavy metals were also carried out. HPTLC fingerprinting of methanolic extract was performed using CAMAG-HPTLC system connected with win CAT software. Preliminary phytochemical screening of the extracts in different solvent revealed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, lipids, sterols and tannins. Total flavonoid and phenolic contents in methanolic extract was found to be 1.4 mg/gm and 9.8 mg/gm extract respectively. Concentrations of heavy metals were found within acceptable limits. Pesticides residues and aflatoxins were not detected. The physicochemical and phytochemical standards along with HPTLC fingerprint profile established as an outcome of this research may be utilized as substantial data for identification, purification and standardization of *Nigella sativa* seeds.

Keywords: *Nigella sativa*; miracle herb; ranunculaceae; Habat-ul-Sauda; HPTLC fingerprint; black seeds.

INTRODUCTION

Nigella sativa is considered as a miracle herb due to its wonderful power of healing. It exhibits wide spectrum of pharmacological and therapeutic potential as revealed in many researches. It has been used for curing various diseases for many centuries in different indigenous systems of medicine as well as folk medicines (Goreja, 2003). *Nigella sativa* is commonly known as karayal in Hindi, kalonji in Urdu, black cumin, black caraway or small fennel in English, Habat-ul-Sauda or "Habbat-ul-Barakah (seed of blessing)" in Arabic, siyah daneh in Persian and kalajira, kalajaji, mugrela, upakuncika in Sanskrit etc. *Nigella sativa* is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, Saudi Arabia**,** Turkey, Syria, Pakistan and India (Sharma *et al.,* 2005). *Nigella sativa* (a member of family Ranunculaceae) is an annual flowering plant with finely divided leaves and 20-90 cm in height. The delicate flowers have 5-10 petals. The fruits are big and exaggerated capsules are made-up of 3-7 united follicles having various seeds**.** The seeds are small dicotyledonous, trigonus, angular, 2-3.5×1-2 mm, apparent black and internally white, somewhat aromatic odor and bitter in taste (Warrier *et al.,* 2004). According to one of the Prophetic hadith, it is considered among Muslims as one of the enormous forms of remedial medicine. Holy Prophet Mohammad (Peace be upon Him) once told his companions that black seed is the remedy for all diseases except death (Al-Bukhari, 1976). The *Nigella sativa* seeds have been widely used for the treatment of different diseases and ailments. Seeds exhibits a wide spectrum of biological and pharmacological activities which include antihypertensive, antidiabetic, diuretics, anticancer, immunomodulator, analgesic, antioxidant, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepato-protective, pulmonary-protective, nephro-protective, gastro-protective, antioxytocic and anticonvulsant properties etc. Due to its miraculous power of healing, *N. sativa* has got the place among the top ranked evidence based herbal medicines (Abel-Salam, 2012; Abdel-Sater, 2009; Assayed, 2010; Abdel-Zaher *et al.,* 2011; Boskabady *et al.,* 2010). Impurities, substitution and adulterations are very common troubles among drugs of herbal origin. The modern techniques are being used today to ensure quality control of medicinal plant products as per WHO guidelines. The adulteration was a

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major problem due to unavailability of sophisticated scientific research, approved methods pertaining to legitimacy of herbal drug in the past days. But in present scenario of scientific research, adulteration can be controlled by means of modern scientific methods.

Keeping in view the importance, the seeds of *Nigella sativa* were standardized as per standard methods available in WHO and Pharmacopoeial guidelines for herbal drugs. Therefore, present work was designed to perform the physicochemical and phytochemical investigations of *Nigella sativa* seeds. HPTLC fingerprinting of different extracts of the seeds were also developed in order to authenticate the genuineness of the drug. The output of this study may be used as a standard reference for identification, authentication, purification and to differentiate the seeds of this plant from its adulterants.

MATERIALS AND METHODS

Collection and authentication of drug

The *Nigella sativa seeds* Linn were purchased from a local supplier (Subhash Chawla and Sons) in Delhi and Identified and authenticated by Dr. Mohd Mujeeb, Assistant Professor, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi. A voucher specimen No.NS/FP-JNU was submitted in the herbarium of Jodhpur National University-Jodhpur, Rajasthan, India, for future reference. The black seeds were thoroughly rinsed with plenty of water to get rid of the adherent impurities and shade dried. The clean seeds were used for the preparation of extracts.

Preparation of extracts

The seeds of *Nigella sativa* were dried below 60ºC in an oven, powdered and passed through sieve no 14. The dried powdered seeds of *Nigella sativa* (500gm) were subjected to extraction with different solvents (petroleum ether, *n*-butanol, chloroform, acetone and methanol) for 6 h in a Soxhlet apparatus on water bath. The extracts obtained were filtered and evaporated to dryness by Rota evaporator (Buchi, Rotavapor-R-210 Switzerland) and stored at low temperature for further studies.

Physicochemical standardization

The extracts of the plants were standardized according to WHO guidelines and other Pharmacopoeial procedures. Physicochemical standardization which includes extractive values in different solvents, total ash value, acid insoluble ash value and water soluble ash value, moisture content, loss on drying, pH values (1% and 10% solutions), aflatoxins, heavy metals and pesticides residues in different extracts were analyzed as per the standard methods (Anonymous, 1998; Khandelwal 2007, WHO, 2011).

Preliminary phytochemical analysis

The seed extracts in different solvents were subjected to preliminary phytochemical investigations to determine the different phytoconstituents like carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, lipids, steroids and tannins using different standard methods (WHO, 2011). Total phenols and flavonoids contents in the seed extracts were also determined by UV spectroscopy according to the standard official procedure (McDonald, 2001; Chang, 2002).

Determination of total phenolic contents by UV spectrophotometer

The phenolic contents of methanolic extract were measured by Folin Ciocalteu method with the help of UV spectrophotometer. Standard calibration curve was plotted by using gallic acid as standard phenolic compound. The Folin Ciocalteu reagent was diluted (1: 10) with distilled water and freshly used. 10mg of standard Gallic acid was accurately weighed and dissolved in 100mL distilled water in a volumetric flask (100µg/mL of stock solution) and then pipette out 0.5 to 2.5mL of aliquots into 25mL volumetric flasks from the above prepared stock solution. Then 10 mL of distilled water and 1.5mL of Folin Ciocalteu reagent, diluted as per the label details to each of the above volumetric flasks were added. After 5 min, 4mL of 0.7M sodium carbonate was added, and then distilled water was added to make the volume up to 25 mL. After 30 min, absorbance at 765nm was recorded and calibration curve of absorbance v/s concentration was plotted (fig. 1). A dilute methanolic seed extract (0.5mL of 1:10g mL-1) was mixed with Folin Ciocalteu reagent (5mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4mL, 1M). The final mixtures were maintained to stand for 15 min and the total phenols were measured by colorimetric method at 765 nm. Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass). The total phenolic contents of the methanolic extract were calculated by using standard calibration curve (McDonald, 2001).

Determination of total flavonoid contents by UV spectrophotometer

The total flavonoids were determined by aluminum chloride colorimetric method using quercetin as a standard flavonoids compound. UV spectrophotometer was used for measurement of absorption of different concentrations of standard. Aluminium chloride (10%) and 1M sodium acetate solutions were prepared. 10gm of anhydrous aluminium chloride was dissolved in 100mL of distilled water to prepare a 10% aluminium chloride solution and 82.0 gm of sodium acetate was dissolved in 1000 mL distilled water to prepare a 1M sodium acetate solution. Seed extracts (0.5mL of 1:10g/mL) were separately mixed with 1.5mL of methanol, 0.1mL of 10%

aluminium chloride, 0.1mL of 1M sodium acetate and 2.8mL of distilled water. This mixture was maintained at room temperature for 30 min; the absorbance of the mixture was measured at 415nm with a double beam UV/Visible spectrophotometer. The calibration curve was also prepared by preparing quercetin solutions at concentrations 10, 20, 40, 50 to 100 µg/mL in methanol. A standard calibration curve of absorbance vs concentration was plotted (fig. 2). The total flavonoid contents in the sample extract were calculated by using standard calibration curve (Chang, 2002).

Development of chromatographic HPTLC fingerprint profile of methanolic extract of Nigella sativa

The methanolic extract of *Nigella sativa* was subjected to HPTLC fingerprint profile to check the presence of different phytopharmaceuticals. Numbers of peaks, peak areas, Rf values were recorded. Different combination of solvent systems were tried to get good separation and sharp peaks. The adequate resolution was obtained in the chloroform: methanol (4:1) solvent system and it was found to be the best for separation of compounds present in methanolic extract of *Nigella sativa.* HPTLC Fingerprinting was carried out by using CAMAG HPTLC system (Switzerland) with a Linomat 5 sample applicator. The whole investigation process was carried out in air-conditioned room maintained at 22ºC and 55% humidity. TLC was also done on pre-coated silica gel HPTLC aluminium plates $60F_{254}$ (20 cm×20 cm, 0.2mm thickness, 5-6 µm particle size, E-Merck, Germany)**.** Five µL of extract was spotted as bands of 6 mm width by using the auto sampler fitted with a 100 µL Hamilton syringe. The plates were established using Chloroform: Methanol (4:1) as a solvent system. The solvent system was transferred in CAMAG twin-trough plate development chamber which was lined with filter paper and pre-saturated with 30 ml mobile phase. The resulted plates were dried in air and scanned. A spectrodensitometer (Scanner 3, CAMAG) equipped with 'win CATS' planar chromatography manager (version 1.3.0) software was used for the densitometry measurements, spectra recording and data processing. Absorption/remission was the measurement mode at a scan speed of 20 mm/s. Chromatograms were recorded at 254 and 366 nm. The R_f value of each compound which were separated on plate and data of peak area of each band were recorded.

Determination of heavy metal residues

Residues of heavy metals (Cd, Pb, As and Hg) in the extracts were determined according to the American Organization of Analytical Chemists (AOAC) official method of analysis (Anonymous, 2006). The detailed working parameters of atomic absorption spectrophotometer for the analysis of heavy metals are shown in the table 1.

Protocol for digestion of samples

The sample of *Nigella sativa* (1gm) was carefully weighed into a clean beaker, 15ml of the digesting acid mixture comprising of HNO_3 and HCl in ratio of 1:3 was added to the sample in the beaker. The beaker was heated on a hot plate in the fume cupboard until the observed brown fumes has disappeared. The beaker was carefully removed and allowed to cool. The solution was filtered with Whatman filter paper no 42 and rinsed with 10 ml of digesting acid mixture to make up to 25 ml and stored in a sample bottle for analysis. The analysis of the digested samples was carried out using atomic absorption spectrophotometer for lead and cadmium. The hydride generator was attached to AAS for arsenic estimation. The cold vapour technique was used for estimation of mercury. The calibration plot method was used for the analysis. The digested samples were analyzed in triplicates. Microsoft Excel software was used to get standard plot and regression equation for determination of concentration of heavy metal in samples.

Determination of pesticide residues

Pesticides (organochlorides, organophosphates and pyrethroids) residues in the extracts were determined by GC-MS according to AOAC guidelines (Anonymous, 2006).

Aflatoxin analysis

Aflatoxins were analysed in the different plant extracts by HPLC method as described by AOAC method 980.20-ITEM-I (Scott, 1990).

RESULTS

Physicochemical standardization

Physicochemical parameters include extractive values, total ash value, acid insoluble ash value and water soluble ash value, moisture content, loss on drying, pH values (1% and 10% solutions) were determined to check the purity of the drug. The results of physicochemical parameters are summarized in table 2.

Preliminary phytochemical analysis

The seeds extracts in different solvents were qualitatively examined for the presence of major phytochemicals (carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, lipids, steroids and tannins). The screening of the preliminary phytochemicals showed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, lipids, steroids and tannins. The methanolic extracts showed the presence of all above constituents except lipid contents. The results of phytochemicals screening are represented in table 3.

Total phenolic contents

The total phenolic contents of methanolic extract were measured by UV spectrophotometric method. The total content of phenolic compounds was found to be 9.8367±

0.023 mg/gm in methanolic extract of *Nigella sativa* seeds. The presented values are Mean ±S.D. of triplicate determinations. The total phenolic content was expressed as gallic acid equivalent in mg/gm of the extract. The standard calibration curve was used to calculate the total phenolic contents in the methanolic extract of the seeds (fig. 1).

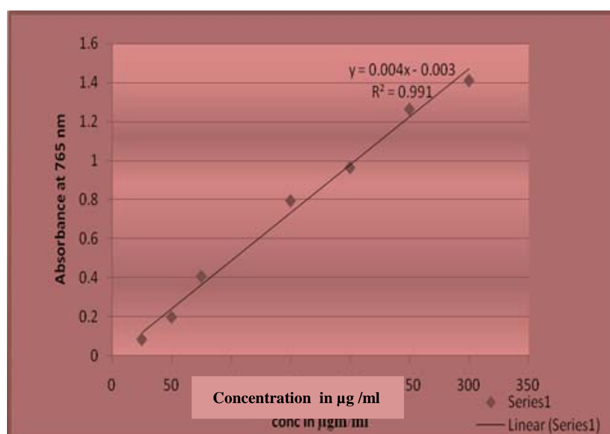

Fig. 1: Standard calibration curve for determination of total phenolic contents

Total flavonoids contents

The flavonoids content was determined by UV spectrophotometric method. The total content of flavonoids was found to be 1.400±0.029 mg/gm in methanolic extract of *Nigella sativa* seeds. The presented values are Mean ±S.D. of triplicate determinations.The total flavonoids contents in the sample extract were calculated by using standard calibration curve (fig. 2).

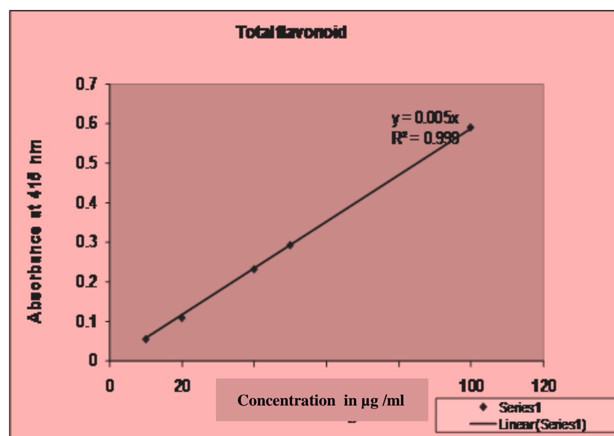

Fig. 2: Standard calibration curve for determination of total flavonoids content

HPTLC fingerprint profile of methanolic extract of Nigella sativa

HPLTC is an important analytical tool in the separation, detection and estimation of different classes of natural products. HPTLC fingerprinting of methanolic extract of the *Nigella sativa* seeds was performed using CAMAG-HPTLC system connected with win CAT software.

HPTLC fingerprinting of methanolic extract of the *Nigella sativa* had been carried out by using chloroform: methanol (4:1) solvent system for separation of as many as phytochemicals. Results of this experiment revealed the presence of several constituents in the extracts.

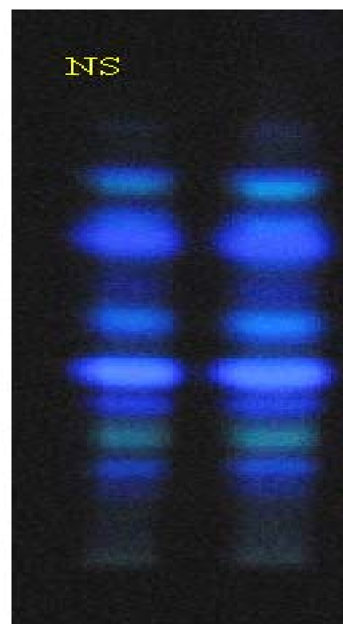

Fig. 3: HPTLC photograph of methanolic extract of *Nigella sativa* at 366 nm

HPTLC chromatogram of methanolic extract of *Nigella sativa* showed 11 peaks at different R_f values and peak area at 366 nm in chloroform: methanol (4:1) solvent system (figs. 3-4, table 4) where as 8, peaks were present in HPTLC chromatogram at 254 nm of methanolic extract of *Nigella sativa* (figs. 5-6, table 4). The number of constituents (No. of peaks) in the extract and their retention factor (R_f) are summarized in table 4 and chromatographic profile had been shown by figs. 3-6.

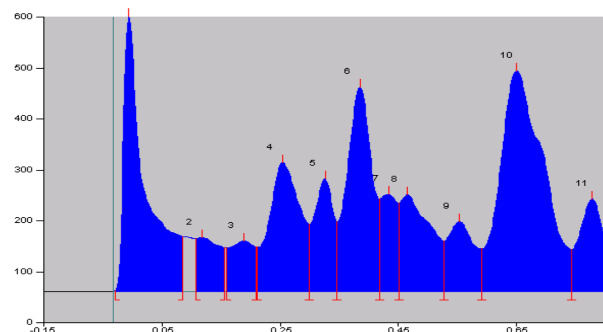

Fig. 4: Chromatogram of the methanolic extract of *Nigella sativa* at 366 nm

Determination of heavy metal residues

The determination of heavy metals (e.g. Cadmium, Lead, Arsenic and Mercury) analysis was carried out in the extracts of seeds of the *Nigella sativa* on atomic absorption spectrophotometer. All necessary safety measures were adopted to avoid any possible contamination of the sample as per the AOAC guidelines. Cadmium was found to be 0.12±0.02 mg/kg. It was below the acceptable limit of 0.3 mg/kg as prescribed by WHO in all the samples. Lead was found to be 0.15±0.06 mg/kg which was far below the acceptable limit of 10 mg/kg as prescribed by WHO in all the samples. Arsenic and Mercury were detected in all samples. Both metals were found to be within acceptable limits of 0.5 ppm and 1.0 ppm, respectively (table 5).

Fig. 5: HPTLC photograph of methanolic extract of *Nigella sativa* at 254 nm.

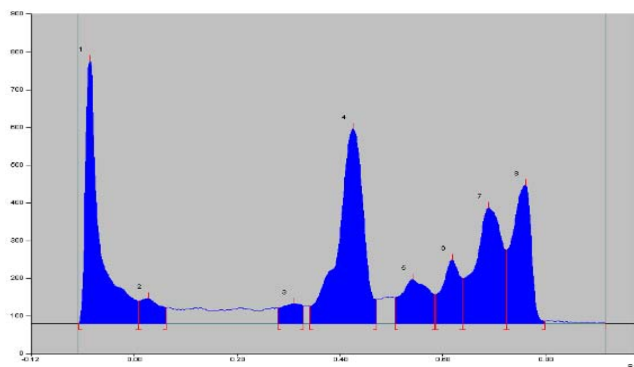

Fig. 6: Chromatogram of the methanolic extract of *Nigella sativa* at 254 nm.

Determination of pesticide residues

Pesticides (organochlorides, organophosphates and pyrethroids) residues in the extracts were determined by GC-MS according to AOAC guidelines (Anonymous, 2006).

Total 40 pesticides were tested in all the samples, none of the pesticides was found in the *Nigella sativa* extract.

Aflatoxin analysis

Aflatoxins were analysed in the different plant extracts by HPLC method as described by AOAC method 980.20-ITEM-I (Scott, 1990). Different aflatoxins like B1, B2, G1 and G2 were determined in the black seeds extract. No aflatoxin was detected in the sample of the extract.

DISCUSSION

Standardization is an important modern tool of analysis used to ensure the quality of herbal drugs. There are various physicochemical parameters used for the quality evaluation of the herbal drugs. The extractive values were used to find out the amount of active principles. The higher percentage yield of *Nigella sativa* extract was found to be 16.67% in petroleum ether (table 2). This indicated that seeds of *Nigella sativa* contain higher concentration of fatty constituents. The ash values were used to detect the presence of any foreign matters e.g. sand and soil, water soluble salts adhering to the surface of the drugs. There is always a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthy materials. The water-soluble ash was used for the measurement of inorganic elements. The total ash values of a plant drugs are not always trustworthy due to the possibility of presence of non-physiological substances. So, the authentication of acid insoluble ash was also done which showed lowest content of acid insoluble ash in the extract of the seeds (table 2). Loss on Drying (LOD) and Moisture Content determines the amount of moisture as well as volatile components present in a drug. Higher moisture content in the drug sample may causes hydrolysis of active ingredients of the drug and decreases its quality and efficacy. The final dryness of the drug and rate of moisture removal are equally important and it was observed that the moisture content in the seeds of *Nigella sativa* was found to be 0.51% (table 2) which showed that the drug was well dried and stored. Loss on drying (LOD) of the black seeds was found to be 0.51±0.032% and expressed in table 2. The pH values of the *Nigella sativa* extracts (1% and 10% solutions) was also investigated using digital pH meter as per the standard method. The pH of the drug (1% and 10% solutions) was found to be 5.55±0.56 and 7.51±0.32 respectively (table 2). The pH of the extracts reveals the concentration of acidic and basic

compounds. The plants considered as biosynthetic laboratory for a multitude of compounds. The compounds that are responsible for therapeutic effect are usually the secondary metabolites. The results of preliminary phytochemical screening showed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, sterols and tannins, in the methanolic extract of seeds (table 3). Tannins act by binding to proline rich proteins and interfere with the protein synthesis. Therefore, this preliminary phytochemical screening may be useful in the detection and further quantitative analysis of such compounds. The phenolic and flavonoid compounds act as antioxidants. These compounds are also reported to have anticancer, antimicrobial, anti-inflammatory and anti-allergic activities etc. Phenolic compounds are most widely occurring groups of phytochemicals and derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants. These compounds are secondary metabolites which have vital role in reproduction and growth, gives protection against harmful predators and pathogens (Eleazu *et al.,* 2012). Therefore, quantitative analysis of these compounds is very important to check the quality of drug. The total quantitative analysis of phenolic and flavonoids content of black seeds were performed by UV spectroscopic method. The total phenolic and flavonoid contents in the methanolic extract of *Nigella sativa* seeds were found to be 9.8367±0.023 mg/ gm and 1.400±0.029 mg/gm respectively. These findings revealed that the *Nigella sativa* extract contain considerable amount of phenolic and flavonoid compounds. HPLTC is an important analytical tool in the separation, detection and estimation of different classes of natural products and chromatographic fingerprint is a rational approach for more strong and efficacious quality control of the natural products (Mauji *et al.,* 2011). HPTLC fingerprinting of methanolic extract of the *Nigella sativa* seeds was performed. Results revealed the presence of several constituents in the extracts. HPTLC chromatogram of methanolic extract of *Nigella sativa* showed 11 peaks at different R_f values and peak area at 366 nm in chloroform: methanol (4:1) solvent system (figs. 3-4, table 4) where as 8, peaks were present in HPTLC chromatogram at 254 nm of methanolic extract of *Nigella sativa* (figs. 5-6, table 4). The number of constituents (No. of peaks) in the extract and their retention factor (R_f) are summarized in table 4 and chromatographic profile had been shown by figs. 3-6.

Table 1: Parameters of Atomic Absorption Spectrophotometer (AAS) for analysis of heavy metals in *Nigella sativa*

Parameters	Heavy Metals			
	Hg	As	Pb	Cd
Instrument	AAS	AAS	AAS	AAS
Model	AA-6300	AA-6300	AA-6300	AA-6300
Manufacture	Shimadzu	Shimadzu	Shimadzu	Shimadzu
Lamp	Mercury EDL	Arsenic EDL	Lead EDL	Cadmium EDL
Wavelength (λmax)	253.6 nm	193.7 nm	283.3nm	228.8 nm
Flame Type	off	off	Air-Acetylene	Air-Acetylene
Fuel gas	5.5 L/min -Argon	5.5 L/min -Argon	2.5 L/min -Acetylene	2.5 L/min -Acetylene
Support gas	15.0 L/min -Air	15.0 L/min -Air	15.0 L/min -Air	15.0 L/min -Air
Technique (AAS)	Cold vapour	Hydride generation	Flame	Flame

Table 2: Results of physicochemical analysis of *Nigella sativa* Linn. seeds

S. No.	Physicochemical parameters	Average values % Mean ± SE
A.	Extractive values	
1.	Petroleum ether	16.67±2.23%
2.	Chloroform	9.9.2±1.32%
3.	*n*-butanol,	7.12±0.67%
4.	Methanol	11.87±1.54%
B.	Ash Values	
1.	Total Ash Value	5.5±0.24%
2.	Acid Insoluble Ash Value	0.41±0.02%
3.	Water Soluble Ash Value	3.07±0.46%
C.	Loss on Drying (LOD)	3.55±0.43%
D.	Moisture contents	0.51±0.032%
E.	pH of the drug (1% solution)	5.55±0.56
F.	pH of the drug (10 % solution)	7.51±0.32

Table 3: Results of Phytochemicals screening of different extracts of the *Nigella sativa* Linn. seeds

Constituents	Seed Extracts			
	Petroleum ether	Chloroform	*n*- Butanol	Methanol
Alkaloids	-	+	+	+
Carbohydrates	-	-	-	+
Phenolic Compounds	-	-	+	+
Flavonoids	-	+	+	+
Proteins and Amino- Acids	-	-	+	+
Saponins	-	+	+	+
Lipids / Fats	+	-	-	-
Tannins	-	-	-	+
Sterols	-	+	-	+

Table 4: HPTLC finger printing profile of *Nigella sativa* seeds

HPTLC finger printing of methanolic extracts at 366 nm			
Solvent systems	No. of peak	Rf values	Percentage peak area
Chloroform: Methanol (4:1)	11	0.04, 0.09, 0.13, 0.21, 0.41, 0.47, 0.53, 0.56, 0.58, 0.65, 0.68	12.95, 3.12, 3.57, 6.37, 6.12, 14.07, 5.44, 9.22, 2.84, 18.87, 6.56
HPTLC finger printing of methanolic extract at 254 nm			
Chloroform: Methanol (4:1)	8	0.08, 0.09, 0.36, 0.42, 0.59, 0.67, 0.72, 0.81	21.54, 2.93, 2.37, 27.62, 7.00, 6.4, 17.44, 14.71

In conclusion, the fingerprint images obtained from HPTLC analysis of *Nigella sativa* seeds Linn can provide standard fingerprints which can be used as a reference for the identification, authentication, purification, quality control and to differentiate the seeds of this miracle drug from its adulterants in order to ensure its therapeutic efficacy. Heavy metals are toxic inorganic materials even at very low concentrations. Heavy metals enter the biological cycle of plants and are stored in various parts of plants. Moreover, these could also be dangerous for the health of humans and animals after an excess dietary intake of contaminated plants (Rai *et al.,* 2011). Cadmium, mercury, arsenic and lead are the most common toxic metals usually present in various herbal drugs (Haider *et al.,* 2004). Lead is reported to cause, anemia, miscarriage, kidney damage, lower sperm count, some neurological disorders and hepatotoxicity in higher concentration (Mudipalli*,* 2007). Acute or chronic cadmium exposure leads to breast and pulmonary cancers, hemorrhagic trauma, respiratory agony, anemia and cardiovascular diseases (Huff *et al.,* 2007). Arsenic exposure resulted in dermatological disorders, neurotoxicity, hypertension and peripheral arteriosclerosis (Lee *et al.,* 2003). Determination of heavy metals like Cadmium, Lead, Arsenic and Mercury was carried out in the extracts of seeds of the *Nigella sativa* on Atomic Absorption Spectrophotometer. All necessary steps were taken under consideration during whole study to avoid any possible contamination of the sample as per the AOAC guidelines (Anonymous, 2006; Scott, 1990). Concentration of lead, arsenic, mercury and cadmium in *Nigella sativa* was found to be under the limit. Pesticides are the toxic substances; the drugs should be free from these substances. Determination of pesticide residue was carried out in extract by standard methods as described in AOAC guidelines (Anonymous, 2006; Scott, 1990). Total 40 pesticides were tested in the samples, none of the pesticides was found in samples of the extract.

Fungi produced secondary metabolites called mycotoxins (Leong *et al.,* 2011). Mycotoxins producing fungi may lead to contamination of herbal preparations during different stages of production and processing, especially in favorable conditions like humidity and temperature. *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides* are the most common mycotoxins producing fungi. Aflatoxins B1, B2, G1 and G2 are produced by Aspergillus species which are reported to cause liver cancer in human (Chu, 1991). Aflatoxins B1, B2, G1 and G2 were determined in the black seeds extracts. No aflatoxin was detected in the black seed extract.

Table 5: Determination of heavy metal residues (mg/kg)

S. No.	Test parameter	*Nigella sativa*
1	Cadmium (Cd)	0.12±0.02
2	Lead (Pb)	0.15±0.06
3	Arsenic (As)	0.26±0.08
4	Mercury (Hg)	0.20±0.06

CONCLUSION

This is concluded that the outcome of this study may be utilized as a diagnostic tool for the standardization of this miraculous drug and will be helpful in characterization of

its. Thus, the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality control of medicinal plant.

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