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Review on phytochemical evaluation and extraction of *Nigella sativa* (Kalongi) with pharmacological and traditional applications

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Abstract

Nigella sativa is an annual flowering plant and belonging to family Ranunculaceae. The plant is commonly grown and nurtured in India and Pakistan. In addition, Mediterranean countries have also been reported to cultivate the plant. The height of the herb is about 45 cm and length of the leaves is from 2.5 to 5.0 cm. The shape of the leaves is lanceolate and linear. The color of petals of the flower is pale blue and its seeds are externally black in color and internally white. The plant has been utilized conventionally as well as pharmacologically for treatment of various diseases, i.e. chest congestion, hypertension, obesity, piles, bacterial infections, fever, jaundice, fungal infections, cancers, inflammation, oxidative stress, paralysis, fatigue etc. Seeds are consumed in a variety of ways interchangeably i.e. as condiment and spice. *Nigella sativa* is enriched with various significant phytochemicals, including: glycoscides, carbohydrates, alkaloids, tannins, alkaloids, volatile oil, terpenoids, and flavonoids, steroids resinous and phenolic compounds. The other most important chemical constituents are thymol, carvone, thymoquinone, nigellicimine, nigellicine, dithymoquinone and thymohydroquinone. The medicinal herb contains relatively good amounts of copper (Cu), iron (Fe), potassium (K), phosphorous (P), calcium (Ca) and zinc (Zn). Natural products have been used as a major source of treatment and diet for the provision of essential nutrition and health for both animal and humans. All these aspects catch the attention of researchers to approach the utility, efficiency and potency of *Nigella sativa*.

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Introduction

Nigella sativa which is commonly known as Kalongi is an annual flowering plant. It is responsible for causing the stimulation of energy in the body and it alleviates fatigue and dispiritedness (Abbas *et al.,* 2012). It has been widely used in Unani Tibb system of medicine as a cure for many diseases. According to different researches in medicine it has been approved to be used as astringent, bitter stimulant, diuretic, emmenagogue, anthelminitic. Moreover, it has also been directed for treating piles, paralysis, intermittent fever, jaundice, dyspepsia and skin diseases Javed and Shahid, 2010). World Health Organization (WHO) has estimated that almost 80% of the people globally treat their ailments with medicinal plants. *Nigella sativa* as a whole plant could be utilized for the purpose of phytomedicine. Plants main activity and therapeutic profile is due to the presence and formation of secondary metabolites which are biosynthesized within the plant from primary metabolites. Information of occurrence of these metabolites within the *Nigella Sativa* has been obtained from various analytical and pharmacological procedures (Malika *et al.,* 2004). The characterization of these phytochemical constituents could be done as glycoscides, carbohydrates, alkaloids, tannins, alkaloids, volatile oil, terpenoids, and flavonoids, steroids resinous and phenolic compounds. The chemical nature of these constituents is entirely diverse due to which their pharmacological action also differs (Khan, 1999). These phyto constituents are formed through different metabolic pathways and this is the reason for their diversification and different pharmacological uses as well as other roles such as veterinary sciences, agriculture, forestry and human therapy. Besides these usages have been experimentally tested and corroborated scientifically. *Nigella sativa* is native herbaceous *plant*. It is grown is different countries and is named in a different languages depending on the region where it is grown e.g. kalajira (Bangali), shonaiz (Persian), kalonji (Urdu), habba-tusawda (Arabic) and black cumin (English). *Nigella sativa* is commonly grown and nurtured in India and Pakistan. In addition Mediterranean countries have also been reported to cultivate the plant well (Sofowora, 1993). Religiously black seeds have been in use by various communities. For instance Muslims has a strong acceptance of its use for curing every disease excluding death. Hence the seeds have a popular use as nutritional, folk, natural and medicinal plant. Its use has an ancient history which has been thoroughly verified by modern day scientific researches. For this reason and because of the presence of several active constituents *Nigella sativa* is utilized for the treatment and healing of different diseases (Shafi *et al.,* 2009). This study is aimed to provide comprehensive review about the plant *Nigella sativa* and its pharmacological activities.

Nigella sativa

Botanical description

In English *Nigella sativa* is also commonly known as Black caraway and Black seed. Though whole part of the plant could be utilized as medicine but the main part of the plant that constitutes main phyto-constituents is seeds. It is the member of family Ranunculaceae. It is found in southwest and South Asia. The height of the herb is about 45 cm and length of the leaves is from 2.5-5.0 cm. The shape of the leaves is lanceolate and linear. The color of petals of the flower is pale blue and its seeds are externally black in color and internally white also seeds are dicotyledonous and are angular. *Nigella Sativa* has a characteristic aromatic odor and bitter in taste (Chopra *et al.,* 1958; Atta-ur-Rahman *et al.,* 1995). The plant has divided but not thread like leaves with overall height of the plant is 20-30cm or is 7.9-11.8 inches tall. The petals are five to ten in number (Daba and Abdel Rahman, 1998; Saleem and Hossain, 2000). The arrangement of flower is delicate. The plant usually follows sexual reproduction and is required to cultivate from seeds. Seeds are developed indoor four- six weeks prior to the initiation of last average frost date. This is to avoid any sort of damage to young seedlings by extreme weather conditions. Then the seeds are sown and grown outdoor in mixture of clay. The seed cultivation requires specific sort of sandy or heavy clays and soil requires moist conditions for its appropriate cultivation and growth.

Furthermore, water and fertilizers play essential role in the proper growth of the plant. The fruit of the plant constitutes united follicles which are three to seven in number each comprising various seeds. The size of fruit is quite large and inflated and is in capsule form. The plant bears flowers and fruits from January to April. Generally, the appropriate time for the cultivation of plant is from November to April and the germination of seed occurs within 10-15 days. It could be grown asexually in vitro from the callus culture from roots, leaf and stem plants from special seedlings. Seeds besides being small aretrigonus, angular, tubercular, dicotyledonous, trigonus, and are regulose-tubercular (Rifat-uz-Zaman and Khan, 2004; Bamosa *et al.,* 2010). The seeds when cut into transverse section show a single epidermal layer comprising of oval cells which have dense walls and has an external covering of papillose cuticle consisting filling of dark brown contents. Epidermal layer is then followed by parenchymatous cells which are comprised of 2-4 dense walled layers elongated tangentially, followed by a reddish brown pigmented layer composed of thick walled, rectangular, elongated cells (Rifat-uz-Zaman and Khan, 2004). Seeds are consumed in a variety of ways interchangeably i.e. as condiment and spice (El-Dakhakhny, 1963; Agrawal *et al.,* 1979; Beckstrom and Duke, 1994; El-Dakhakhny *et al.,* 2000).

Ethnopharmacology of Nigella sativa

In Northern Africa, the plant has been used traditionally used for the treatment of skin diseases (eczema), upper respiratory tract diseases (bronchitis, cough, influenza) and for other ailments like fever, headache and rheumatic diseases. However, In Indian traditional medicine system black cumin seeds are utilized in variety of ways such as anti-paralytic, antipyretic, anti-flatulent, anti-carminative, bitter stimulating agent, diuretic, anthelmintic, as astringent, anti-hepatic, as emmenagogue, for treatment of jaundice, piles, dyslipidemia, hypertension and dermatological problems. Also, it has been used as tonic, analgesic, as liver tonic and for other GIT diseases. Moreover, Gulf region of Arabia has been using black seeds for the management of hypertension, paralysis, obesity, heart diseases and chest congestion as well as for the treatment of infection, inflammation, GIT diseases, rheumatoid ailments, diabetes, dysmenorrhea, chronic headache, migraine, dizziness, hemiplegia, back pain and for treating flatulence (Gilani *et al.,* 2004; Ahmad *et al.,* 2013). Additionally, it has also been utilized as spice and preservative. Besides there are few more applications which are included relieving toothache, preventing radiation damage and post- surgical adhesions along with lethargy (Randhawa *et al.,* 2002).

General collection and extraction methods for experiments

The dry seeds of *Nigella sativa* are grounded in a mortar and pestle to convert it into the powder form. Then, the powder was passed through the sieve to get rid of any unwanted particle or enities. Different solvents are used for the extraction of *Nigella sativa* through different methods such as, chloroform, methanol hydro-alcoholic solution (20% DM water in methanol). Powder is loaded in the extraction equipment which is run by solvent with (no. of cycles required). Collect the extract, filter it and evaporate it (El-Kadi and Kandil, 1987; El-Tahir *et al.,* 1993; El-Daly, 1998).

Extraction by speed extractor: The crushed powder is placed in the extractors together with purified sand. The solvent containing principle is in its purified form. This is then preceded by automatic extraction which is continued till three cycles are completed and the colour of the extract begins to change and becomes potent. The procedure remains sustained till potent dark colored extract is obtained. Same procedure is followed with four different polar and non- polar solvents. Then the resultant extract is kept in dried and clean container (Saleem and Hossain, 2000; El-Dakhakhny *et al.,* 2000).

Extraction by speed extractor: Similarly active constituents could be obtained through extraction by soxhlet extractor which is also a hot continuous extraction. The extraction is continued and repeated

with different solvents of varied polarity till the desired extract is obtained. For prevention of impurities dried and clean extractor is used (Beckstrom and Duke, 1994; El-Daly, 1998).

Extraction by aspirator: Furthermore extraction could be carried out with aspirator as well. Powdered form is obtained after drying and crushing the seeds. Solvents of diverse polarity are used and decanted. The solvent is then subjected to undisturbed overnight placement preceded by its collection and storage the next day. These resultant stored extracts are then concentrated and dried by using Rota evaporator equipment till concentrated oil or sticky solid is obtained. Then the division of hydro-alcoholic portion is done against chloroform and ethyl-acetate. The remainder portion which is organic in nature is collected and dried till all the oil droplets are collected. They are mixed with parent extract. Then, it is stored in cool and dry place. The left part is only dried to form solid (El Tahir *et al.,* 1993; Hailat *et al.,* 1995; El-Daly, 1998 ;).

Phytochemistry

There are various chemical constituents which are isolated from the black cumin. The main chemical constituents are thymol, carvone, thymoquinone, nigellicimine, nigellicine, dithymoquinone and thymohydroquinone as shown in figure 1.

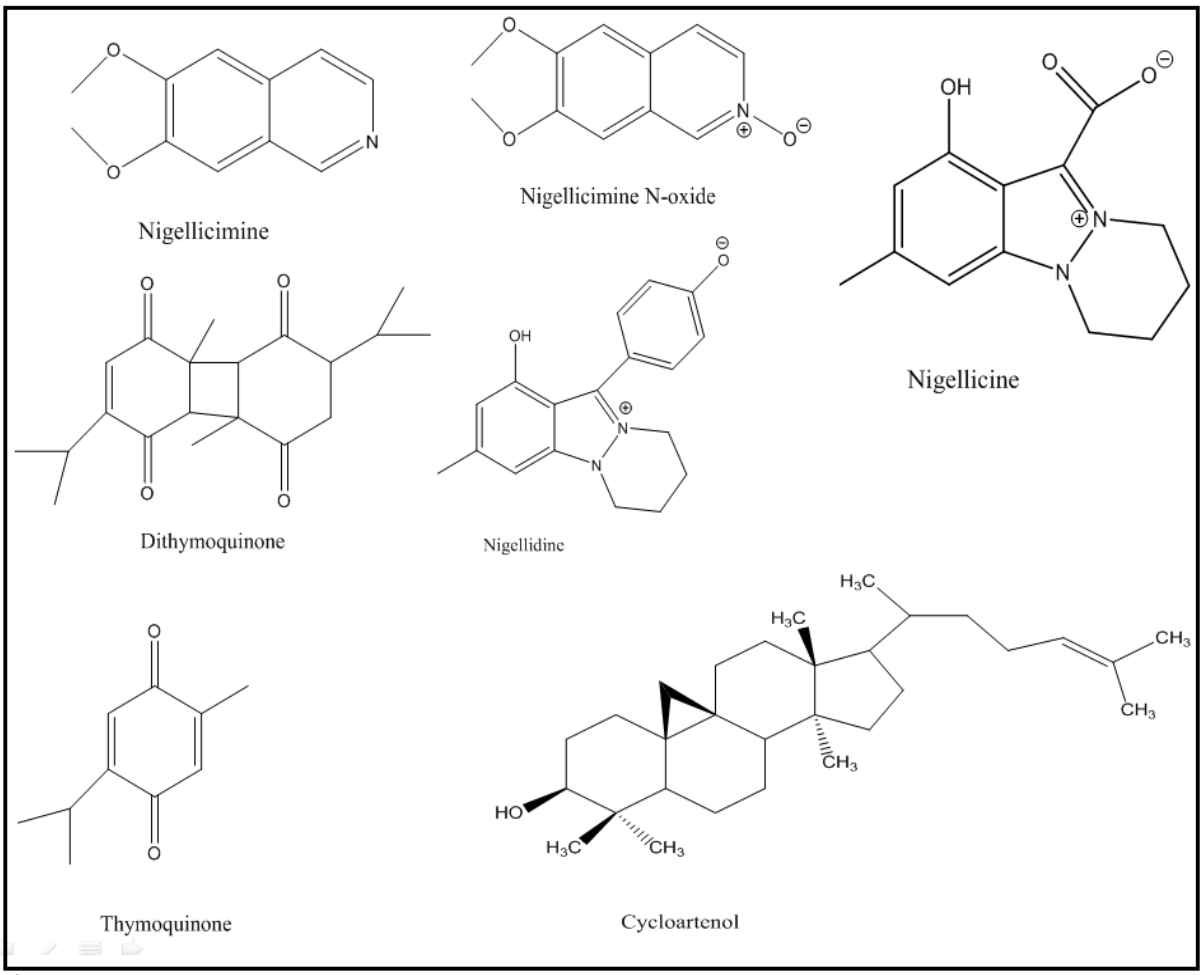

Fig. 1. Various important phytochemicals of *Nigella sativa*.

Extensive studies were done to evaluate the constitution of black cumin seeds. *Nigella sativa* seeds have the composition of saponins, essential oils, alkaloids, fixed oils, and proteins **(**Staphylakis and Gegiou, 1986; Khan, 1999; Goreja, 2003). The unsaturated fatty acids comprises of myristic acid, linoleic acid, palmitic acid, arachidonic acid, oleic acid, eicosadienoic acid, palmitoleic acid and stearic

acid. However fixed oils constitutes 32-40% apart from other constituents such as sterol glycosides, beta-sitosterol, sterolesters, cycloartenol and cycloeucalenol. (Tembhurne *et al.,* 2014). The saturated fatty acids are also present alongside volatile oils (0.4-0.45%) which are carvacrol, *p*-cymene, d-limonene, α and β-pinene, d-citronellol and nigellone which serves as the only constituent with the carbonyl content. Seeds of *Nigella sativa* have volatile oils which contains t-anethole, 4-terpineol and longifoline (Enomoto *et al.,* 2001; Ahmad *et al.,* 2013); Black seed also have few traces of pentacyclictriterpene (Hosseinzadeh *et al*., 2005). Also there are two different forms of alkaloids in the black cumin seed: Isoquinoline alkaloids that comprises of the constituents mentioned earlier as nigellicimine, nigelliciminen-oxide and Pyrazol alkaloids that includes: nigellidine and nigellicine. Besides this *Nigella sativa* is also composed of nutritional content such as proteins involving nearly nine essential amino acids, fats, mineral elements, carbohydrates and vitamins (Haq *et al.,* 1999). Sodium dodecyl sulfate poly acryl amide gel electrophoresis (SDS-PAGE) was used for the fractionation of the plant seed) which showed bands that ranged from 94 to 100kDa molecular mass. There are some more contents present in black seeds such as alpha hederine, citronellol and limonene as well as have relatively good amounts of Copper(Cu), Iron (Fe), Potassium (K) , Phosphorous (P), Calcium (Ca) and Zinc (Zn). In N. sativa, the therapeutic actions are generally due to the occurance of quinine which in turn has high content of thymoquinone (TQ). TQ is responsible for treating convulsions (Hosseinzadeh *et al.,* 2004; Parvardeh *et al.,* 2005, Hosseinzadeh *et al.,* 2005), reduces oxidative stress levels (Hosseinzadeh *et al.,* 2012), reduces inflammation (El Gazzar *et al.,* 2006), used as anti-carcinogenic (Gali-Muhtasib *et al.,* 2008), antifungal activity (Abdel Azeiz *et al.,* 2013) and antibacterial activity (Halawani, 2009).

Physicochemical analysis

Nigella sativa oil has been investigated for the free fatty acid extinction coefficients (K232andK270), relative density (Ph.Eur.8.0/2.2.5), iodine value (Ph.Eur.8.0/2.5.4) (%Oleicacid) (Ph.Eur.8.0/2.5.1), saponification value (Ph.Eur.8.0/2.5.6), un-saponification matter(Ph.Eur.8.0/2.5.7), peroxide value (Ph.Eur.8.0/2.5.5) refractive index (Ph.Eur.8.0/2.2.6), (Satyavati *et al.,* 1987; Aitzetmüller *et al.,* 1997; Cheikh-Rouhou *et al.,* 2007), iodine value and refractive index were determined according to AOCS recommended practices Ca5a-40,Cd8b-90,Ch5-91,Cc7-25,Cd1c-85; Free fatty acid content, peroxide value (PV), respectively (AOCS,1998). Free fatty acid content was expressed as percent to oleicacid, extinction coefficient (K 232andK 270) was expressed as the specific extinction of a 1%(w/v) solution of oil in cyclohexanein 1cm cellpathlength PV was stated as milli equivalent of active oxygen per kilogram of oil (MeqO2/kg oil), and, a CARY100 Variant UV spectrometer was used (Aitzetmüller *et al.,* 1997).

Oxidative stability of seed oils

Determination of oxidative stability of each sample was done as per the AOCS (American Oil Chemical Society) recommendations Cd12b-92 (AOCS,1998) such as the documentation of the induction period (IP,h) was done by a 743 Rancimat (Metrohm,Switzerland) apparatus using 3 go foil sample was done. Samples were poured in standard tubes of Rancimat and tested by placed in Rancimat standard tubes were investigated by warming at 110°C with an adjustment of an air flow of 20L/h (Cheikh-Rouhou *et al.,* 2007).The values have been determined and compared statistically (Aitzetmüller *et al.,* 1997; Cheikh-Rouhou *et al.,* 2007).

Nigella sativa pharmacological actions

Extensive *in vitro* and *in vivo* research has been done in order to confirm plant various pharmacological and toxicological effects.

Anti-cancer properties: The plant is a valuable source of thymoquinone which has significant potential to cure cancer. It inhibits the different signaling pathways that are involved in abnormal cell division and production hence proved to be useful for the cure

of different malignant tumors (Chaieb *et al.,* 2011; Kouidhi *et al.,* 2011).

Anti-obesity: Previous studies have confirmed its beneficial effect in reduction of the body weight. The plant has an ability to lessen the body mass index (BMI) especially in diabetic patients. This effect is may be due to the peroxidation of the lipids which then resulted into the overall decrease in the body weight (Qidwai *et al.,* 2009; Razavi and Hosseinzadeh, 2014).

Reproductive system: Many investigations have proven that the black seed oil could impede the involuntary actions of uterine smooth muscle and also effect the oxytocin induced contractions (Parhizkar *et al.,* 2016).

Bronchodilator effect: *Nigella sativa* has potential to inhibit the histamine H1 and can be used as the bronchodilator. Further, it also stimulates the inhibitory non-adrenergic, non-cholinergic nervous system (NANC) or inhibition of stimulatory NANC. The opening of potassium channels and inhibition of phosphodiesterase may be the alternative methods for the bronchodilator effect (VanAmsterdam *et al.,* 1989; Buckle *et al.*, 1993) and most significantly calcium blockage (Miyaharaetal. 1993), due to the calcium inhibitory effect it also relaxes the bronchial muscles (Boskabady *et al.,* 2010).

Antioxidant effect; the plant isolated phytochemicals by the thin layer chromatography have demonstrated the compounds radical scavenging abilities and shown the potent anti-oxidant activity (Burits and Bucar, 2000).

Anti-inflammatory and analgesic actions: By applying the three different nociceptive experiments on the rats and mice i.e. hot plate method, tail-pinched method and acetic acid-induced writhing test it was concluded that the plant has ability to reduce the pain stimulus. The findings have confirmed that the plant black seeds fixed oil has potential anti-nociceptive actions that are may be due to an opioid part in the oil. This portion is act as naloxoneis i.e. an inhibitory effect for the pain. Moreover, the plant oil has considerable CNS depressing activities (Al-Ghamdi MS, 2001).

Anti-carcinogenic and mutagenic activity: Various studies have been conducted to evaluate the anti-cancer effects of the plant. The crude methanol seed extract has showed a significant cytotoxicity activity on tumors i.e. Erlich ascites carcinoma, Dalton's ascites lymphoma and sarcoma. The researchers have verified the cytotoxic ability of the black seeds *in vivo* by deterring the growth of Erlich ascites carcinoma (Salem, 2005; Randhawa and Alghamdi, 2011).

Hepatic and nephrotoxicity protection: Around the globe, this herb is used to treat various liver related disorders in traditional medicine. Its hepatic protective role has been confirmed on rats. The findings of the study have verified that the plant phyto constituent named thymoquinone has an ability to reduce the hepatic toxicity which was induced by the tert-butyl hydro peroxide (TBHP). A study has illustrated the thymoquinone effect as kidney protection when administered cisplatin. Further, it also increases the antitumor activity of the medicine (Mahmoud *et al.,* 2002).

Respiratory actions: It has been investigated that the intravenous administration of the seeds volatile oils increase the intra tracheal pressure and respiratory rate and this effect is dose dependant (El-Tahir *et al.,* 1993a). However, TQ has little or no effect on rate of respiration but increased the intra tracheal pressure (Mathur *et al.,* 2011).

Antidiabetic action: Traditionally the plant has been used to treat the diabetes and this anti-diabetic activity of the herb was evaluated in rabbits. The findings of a study has described that after 4-6 hour of intra-peritoneal administration of the plant oil at dose 50mg/kg significantly decreased (about15%–23%) the fasting blood glucose concentration in both normal and hyper glycemic rabbits. Insulin concentration was not affected which highlighted that

the hypoglycemic effect was moderated by an unknown mechanism (Bamosa *et al.,* 2010).

Cardiovascular activities: The plant is proved to be quite beneficial to treat various cardiovascular disorders. Many studies were carried on the rats and results have demonstrated that the herb oil is effective to treat hypertension. It is also helpful in reducing the heart rate and could be possibly use to treat arrhythmia. The methanol soluble portion of seeds oil showed inhibitory effects on arachidonic acid induced platelet aggregation and blood coagulation. Several compounds having anticoagulation effect were isolated and some of them are more potent than aspirin (Houghton *et al.,* 1995).

Antiulcer action: An investigation on rats has demonstrated that the aqueous extract of the herb seeds was effective in reducing the ulcer index that was induced by aspirin. The treatment decreased peptic activity and production of the acid and it did not alter the mucin activity. Hence, these studies have proven the gastric protective effect of the plant (Hanafy and Hatem, 1991).

Anti-microbial and anti-parasitic properties: The oil and the extract have been investigated to have a broad spectrum of activity against microbes. For example, in *in vitro* studies have shown that the essential oils have marked antibacterial effects even in 1:100 dilutions against number of organisms, included: *Staphylococcus albus*, *Salmonella typhi*, *Escherichia coli*, *Shigella niger* and *Vibrio cholera*. A study has demonstrated the effectiveness in reducing the number of *Schistosoma mansoni* worms in the liver and total number of ova in both the liver and intestine pointed when volatile oil was administered orally to dose of 2.5 and 5.0mL/kg for two weeks (Hanafy and Hatem, 1991).

Nigella sativa toxicological effects

The plant seed extract and its isolated phtyoconstituents have demonstrated the minimum level of the toxicity i.e. the TQ value of LD_{50} was determined to be 2.4g/kg (range1.52–3.77). The acute administration of the high doses resulted into the liver, kidney and heart toxicity. Introduction of TQ in the drinking water of mice with concentrations of upto 0.03% for 3 months resulted in no sign of toxicity, except for a significant reduction in concentration of fasting plasma glucose. Oil of the plant is used topically to treat various skin infections and to reduce the inflammation. Despite the fact that it is quite useful, two case of contact dermatitis was reported after application of the pure seed oil. Furthermore, it also enhances the concentrations of the certain enzymes i.e. creatine phosphokinase, lactate dehydrogenase, alanine amino transferase and creatinine concentrations. In clinical trials which were conducted on recruited patients it was concluded that the plant has potential to reduce the appetite and overall decrease in the body weight (Ali and Blunden, 2003; Khader *et al.,* 2009).

Conclusion

Nigella sativa is enriched with valuable phytochemicals which are responsible for its various therapeutic activities. Detailed studies are required to ensure its safety and efficacy and possible use of phytochemicals as drug candidate.

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