1- Introduction

1.1- Introduction approach

Nigella (Nigella sativa L.) is an annual herbaceous plant belonging to the *Ranunculaceae* family (Atta, 2003). The genus *Nigella* contains more than 116 species, the most popular of which is *Nigella sativa L.* All *Nigella* species are therophytes: annuals that complete their life cycle in a short favorable period and survive harsh periods as seed (Ansari and Satish, 2013). *Nigella sativa*, perhaps the most well-known member of the genus, grows 8-35 inches (20-90 cm) in height and has finely divided somewhat threadlike leaves. This species has pale-blue to pale-purple flowers that bloom in the spring and produce seed capsules (fruit) that contain numerous seeds (Heiss *el at*., 2012). The seeds are angular, of generally small size (1-5 mg), dark grey or black color.

The genus likely originated in parts of the eastern Mediterranean, northeastern Africa, and south western Asian regions (Vavilo and Dorofeew, 1992). *Nigella sativa* is found growing wild in regions of northern Africa, Turkey, Syria, Iraq, and Iran. The species is also cultivated on a commercial scale in northern Africa (Egypt, Tunisia, Sudan), eastern Africa (Ethiopia), western Asia (Iraq, Israel, Jordan, Lebanon, Syria, Turkey, Yemen), and southern Asia (India, Iran, Pakistan)

The majority of the global commercial supply of *N. sativa* seed is obtained from cultivation in Egypt, Turkey, and India. In India, it is mainly grown in the far northern states of Punjab, Himachal Pradesh, Bihar, Madhya and Assam. Also cultivated extensively in Iran, *N. sativa* is traditionally farmed by communities situated in the provinces of Fars, Khorasan, and Qazvin. Most of the certified organic *N. sativa* seed in the global market originates from farms in Egypt's El-fayoum agricultural area in the Nile Valley, although there is some organic production in Turkey and India. It should be noted that in Turkey many farmers plant the seeds of *N. damascena* in the same fields with *N. sativa* (Engel and Brinckmann, 2017).

Nigella is mentioned in ancient Greek, Roman and *Hebrew texts*, under the name 'black cumin', as both a condiment and a component of herbal medicine. The earliest reference to *Nigella* is found in the Old Testament book of Isaiah. In a treatise on the early origins of Indian and Chinese drugs, Al-Biruni (973–1048) refers to the black seeds of *Nigella* as a

kind of grain known as *alwanak* in the *Sigzi* dialect of Kazakhstan; who cites its use during the tenth and eleventh centuries. Moreover Ibn Sina (980–1037), a contemporary of Hippocrates, listed the medicinal benefits of *Nigella* in the book *The Canon of Medicine*. *Nigella* has been used since antiquity by Asian herbalists and pharmacists and was used for culinary purposes by the Romans. It is best known thanks to the saying of the prophet Muhammad, that black cumin is a remedy for every illness except death. *Nigella* seeds were found in the tomb of pharaoh Tutankhamun in ancient Egypt, suggesting that they play a critical role in ancient embalming practices. Dioscorides, a *Greek* physician of the first century AD, reported that black cumin seeds taken orally can treat headaches, nasal catarrh, toothache and intestinal worms, as well as acting as a diuretic and increasing production of breast milk.

The name *Nigella* derives from the Latin *nigellus* or *niger*, meaning black. Common names applied to the genus *Nigella* are devil-in-a-bush or love-in-a-mist. As well as being commonly called black cumin, it is also known by a variety of popular names in different countries. It is called small fennel (or black cumin) in English; *cheveux de venus, nigelle, cumin noir* or *poivrette* in French; *nigella* in Italian; *Schwarz kümmel* in German; *neguilla* or *pasinara* in Spanish; *kolongi* in Turkish; *habba tu sawda* in Arabic; *shonaiz* in Persian; *jinten hitan* in Indonesian and Malay; and *kala zira*, *kalongi*, *krishanjirka*, *mangrail* and many other vernacular names in India. Some of the popular names of *nigella* are very similar to those given to other spices of the Apiaceae family, *viz. Siah Zira* (Black Cumin– *Carum carvi* L.), *Kala Zira* (Black Cumin – *Bunicum persicum* Bioss. Fedtsch syn. *Carum bulbocastanum* Koch.). Botanically and structurally, the *nigella* seed is entirely different from the seed spices mentioned above and belongs to a different family. In order to avoid confusion, it is therefore advisable to refer to this spice as *nigella* (Engel and Brinckmann 2017).

N. sativa used as a condiment in bread and other dishes. They are also used in the preparation of a traditional sweet dish, composed of black cumin paste, which is sweetened with honey or syrup, and in flavoring of foods, especially bakery products and cheese. *Nigella sativa* seed oil is considered as one among newer sources of edible oils, for its important role in human nutrition and health (Rajkapoor *et al.,* 2002).

1.2- Classification

The genus *Nigella* contains more than 116 species, the most popular of which is *Nigella sativa L.* As per the conventional classification of spices, it is classified as a mild spice; in addition, in terms of the plant organs used, *Nigella* is classified as a seed spice because it is mostly the dried seeds that are used as a spice. With regard to pollination behavior, *N. sativa L.* is a cross-pollinated crop and has a somatic chromosome number of 2n = 12 (Jha and Roy, 1979). The flowers are self-fertile, but cross-pollination occurs through insects. It belongs to the buttercup family (Ranunculaceae) and to the order Ranunculales. The other closely related species, *N. damascena L.* and *N. arvensis L.* are mostly used as ornamental plants and in medicines (Malhotra 2012).

1.3- Chemical structure

A qualitative examination of *N. sativa L.* seeds revealed the presence of sterols, triterpenes, tannins, flavonoids, cardiac glycosides, alkaloids, saponins, volatile oils, coumarins, volatile bases, glucosinolates and anthraquinones (Al-yahya, 1986). It has been shown that *N. sativa* seeds contain > 30% of a fixed oil and 0.40–0.45 % w/w of volatile oil. The volatile oil is a pale yellow liquid with characteristics aromatic odor and taste which is readily soluble in organic solvents such as ether, chloroform and ethanol but only sparingly soluble in water (Khan 1999).

Qualitative analysis of *N. sativa* seed oil using the capillary gas chromatography–mass spectrometry (GC/MS) technique has allowed the 67 different compounds to be identified; when these compounds were classified into various functional groups, the following results were obtained: monoterpenes (46 %); carbonyl compounds (25 %); phenols (1.7 %); alcohols (0.9 %) and esters (16 %) (Aboutable *et al.,* 1986). In the volatile oil of *N. sativa*, Adamu *et al.,* (2010) analyzed 2-methyl-5 (1-methyl ethyl)-bicyclo[3.1.0]hex-2-ene as the major constituent (62.28 %) of the volatile oil of *N. sativa,* while α-pinene was a minor constituent (2.28 %). El-Tahir *et al*., (1993) showed that the volatile oil contained 18.4–24 % thymoquinone and a total of 46 % of various monoterpenes such as *p*-cymene and pinene. The presence of thymoquinone, dithymoquinone, thymohydroquinone, thymol, carvacrol, oxy-coumarin, 6-methoxy-

coumarin and 7-hydroxy-coumarin, α-hedrin and steryl-glucoside as well as large amounts of flavinoids, tannins, essential fatty acids, essential amino acids, ascorbic acid, iron and calcium has been reported (Omar *et al*., 1999). According to Weiss (2002), the seeds contain 0.5 % volatile oil, of which the seven main constituents and their approximate proportions are *p*-cymene (31 %), thymoquinone (25 %), ethyl linoleate (9 %), α-pinene (9 %), ethyl hexadecanoate (3 %), ethyl oleate (3 %) and β-pinene (2 %). Sharma *et al*., (2009) found that in the essential oil (mean 0.5 %, max. 1.5 %), thymoquinone was identified as the main component (up to 50 %) alongside *p*-cymene (40 %), pinene (up to 15 %), dithymoquinone and thymohydroquinone. Other terpene derivatives were found only in trace amounts: carvacrol, carvone, limonene, 4-terpineol, citronellol. Furthermore, the essential oil contains significant (10 %) amounts of fatty acid ethyl esters. On storage, thymoquinone yields dithymoquinonene and higher oligo condensation products, which provide the spice with its aromatic flavour. Nickavara *et al*., (2003) investigated the chemical composition of the volatile oil of *N. sativa* seeds grown in Iran using GC and GC/MS methods and identified 32 compounds (86.7 %) in the volatile oils. The major compounds were *trans*-anethole (38.3 %), *p*-cymene (14.8 %), limonene (4.3 %), and carvone (4.0 %). The results are given in Table 1.1 (Nickavara *et al*., 2003).

Variations in the volatile composition have been observed in the two species, *N. damascene* and *N. sativa*: the former contained sesquiterpenes, including a large proportion of β-elemene (27.7 % extract, 54.7 % oil) and methyl 3-methoxy-*N*-methyl anthranilate (30.7 % extract, 12.7 % oil), which account for the characteristic aroma of this species; in the latter, however, only monoterpenes, including *p*-cymene (49 % extract, 47.4 % oil) and thymoquinone (20.6 % extract, 20.8 % oil), were detected (Rchid *et al*., 2004). Moretti *et al*. (2004) reported that the main components of *N. sativa* were *p*-cymene (33.8 %) and thymol (26.8 %), with only a small amount of thymoquinone (3.8 %) whereas *N. damascene* oil contained almost 100 % sesqui-terpenes, 73.2 % of which was made up by β-elemene.

Traces of two different types of alkaloids have been found in nigella seeds: isochinoline alkaloids, represented by nigellimin and nigellimin-N-oxide, and pyrazol alkaloids, including nigellidin and nigellicin. Rajkapoor *et al*., (2002) reported the

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alkaloids present in the seeds to be nigellicin, nigellidin, quanazoline, tannin, steroid α-spinasterol, campsterol, cholesterol, stigmas 7-en-3-β-ol, stigmasterol and flavonoids of trigillin quercetin-3-glucoside. Four dolabellane-type diterpene alkaloids have been isolated from the seeds of *Nigella sativa* (Morikawa *et al*., 2004 a, b). The active principles, nigellone and nigellidine, are reported to contain an indazol nucleus (Rahman *et al*., 1995). Three flavonoid glycosides and triterpene saponins were also identified from *N. sativa,* together with four phospholipid classes: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatitdylinositol (Ramadan and Mörsel, 2002).

Table (1.1) Chemical composition of the volatile constituent:

Compound	Percentage
n-Nonane	1.7
3-Methyl nonane	0.3
1,3,5-Trimethyl benzene	0.5
n-Decane	0.4
1-Methyl-3-propyl benzene	0.5
1-Ethyl-2,3-dimethyl benzene	0.2
n-Tetradecane	0.2
n-Hexadecane	0.2
Total non-terpenoid hydrocarbons	4.0
Thujene-α	2.4
Pinene-α	1.2
Sabinene	1.4
Pinene-β	1.3
Myrcene	0.4
Phellandrene-α	0.6
p-Cymene	14.8
Limonene	4.3
Terpinene-γ	0.5
Total monoterpenoid hydrocarbons	26.9
Fenchone	1.1
Dihydrocarvone	0.3
Carvone	4.0
Thymoquinone	0.6
Total monoterpenoid ketones	6.0
Terpinen-4-ol	0.7
p-Cymene-8-ol	0.4
Carvacrol	1.6
Total monoterpenoid alcohols	2.7
Longipinene-α	0.3
Longifolene	0.7
Total sesquiterpenoid hydrocarbons	1.0
Estragole	1.9
Anisaldehyde	1.7
trans-Anethole	38.3
Myristicin	1.4
Dill apiole	1.8
Apiole	1.0
Total phenyl propanoid compounds	46.1
Total compounds	86.7

Source: Nickavara *et al.* (2003).

1.4- *Nigella Sativa* Benefits

In Islamic culture, the Black seed is regarded as a remedy for all diseases.
Modern medicine places more focus on identification of the principal responsible for therapeutic activities.
The compound (called 'Nigellone ('extracted from *Nigella sativa* oil was injected into test animals, and a prominent bronchodilatory effect was observed, justifying its use in asthma and some other respiratory disorders.
The regular consumption of Black seed increased antibody production, significantly boosting the immune system.
The active principle of Black seed oil as thymoquinone is responsible for many of the plant's therapeutic uses.
The *Nigella sativa* enhanced the ability of macrophages to absorb 'polymorphonuclear leukocyte parasites'. This enhanced absorption served to increase the immune response against foreign bodies.
 Nigella sativa supplements are widely available in health food stores as well as some pharmacies. They often come in capsules of grounded Black seeds, soft gels of the oil or the oil in its free flowing state and are commonly sold under the name (Black seed).
Nigella extract taken by mouth daily in combination with Vitamin E, β-carotene and biotin allergy rhinitis symptom improve.

Nigella is comparable to that elicited by omeprazole, a well-known drug for the treatment of gastric hypersecretion and similar disorders.

Nigella sativa extract was also able to reduce the frequency of loose tooling in individuals exhibiting such symptoms.
A study performed comparing the kidney protection effect of Vitamin C and *N. Sativa* oil against gentamycin induced toxicity on the kidney of rabbits. The results proved that the organ protective effect of Vitamin C was enhanced with the administration of Black seed oil.

The Black seed is capable of protecting the testicles from the effects of drugs such as methotrexate and the cardioprotective effect was additionally observed for Black seed extract.

The constituents of the volatile oil, major thymoquinone, are thought to be responsible for *Nigella's* anticonvulsant effect.

The black seed powder can improve blood sugar levels in people with diabetes. Black seed might also improve levels of cholesterol in people with diabetes. Doses of 2 grams daily seem to be needed for any benefit. The black seed by mouth might reduce blood pressure by a small amount. The black seed oil increases the number of sperm and how quickly they move in men with infertility. A gel containing black seed oil to the breasts during the menstrual cycle reduces pain in women with breast pain.

There is also scientific literature that states that consumption of black seeds offer several beneficial properties for the liver. The Turkish study revealed the consumption of black seeds can offer protection against the occurrence of liver fibrosis. Although the study was performed with rabbits as study subjects, the result of this study are encouraging to continue clinical trials on the human population.

Scientific studies have also revealed that the consumption of black seeds can be a beneficial treatment for hypertension. A study performed by the Department of Internal Medicine and Cardiology and Medicinal Plant Research Center at Shahrekord University of Medical Sciences in Iran revealed that consumption of 100 mg to 200 mg of black seeds twice a day for an 8 week period produced a significant reduction in diastolic blood pressure. The study also revealed a significant reduction in low density lipoproteins, or LDL cholesterol, after the 8 weeks of treatment with black seeds.

Cosmetic benefits of *Nigella Sativa*: *Nigella* possesses beauty enhancing capabilities when the oil is administered topically or ingested.

Taking Black seed oil in capsule form leads to the straightening of the hair and fingernails, alongside increasing the luster.

People suffering from skin conditions such as psoriasis or eczema also benefit from applying the oil externally.

The oil can be used alone or mixed with cream. Many people also use homemade oil with *Nigella* oil and other carrier oils such as shea butter or Coconut Oil. This mixture can also be applied to burns or skin infections. The oil is additionally effective in relieving joint pain, moisturizing the skin and preventing the appearance of wrinkles on the skin.

It is important to note that supplementation with *Nigella* does not require extensive processing.

The seed oil or the crushed powder can be consumed since the active ingredients are present in the seed and are absorbed by the body upon consumption. 1 g of whole crushed Black seed taken twice daily reduces the low-density lipoprotein (or bad high cholesterol) and blood fats in people.

Black seed extract relieves pain and discomfort in people with a sore throat and swollen tonsils

Culinary uses of *Nigella Sativa*: Black seed has long been used as spices or condiments, and this method of use is also largely beneficial. The unique and pleasant taste and aroma of *Nigella* is added to the food or drink, and the medicinal benefits of the herb are still derived. No matter the form in which Black seed is used as a condiment, you can be sure you are getting a pleasing aroma and taste, alongside numerous therapeutic benefits.

1.5-Side effect of *Nigella sativa*

1.5.1 Skin

Topical application of *Nigella sativa* may induce a side effect called contact dermatitis. After handling or applying *N. sativa*, you may develop red, irritated patches of skin across the site of treatment. The skin may also become dry or begin to flake or itch. Seek prompt medical care if you experience a severe skin reaction, such as oozing or painful blisters, after using this supplement.

1.5.2- Kidney or Liver Damage

Oil extracted from *Nigella sativa* seeds may cause kidney or liver damage when used in high doses. These severe side effects of treatment were observed in rats given 2 grams per kilogram or more of NSO, according to MSKCC. Additional human research studies are necessary to further evaluate the effect *N. sativa* may have on the kidneys or liver;

however, if you experience symptoms of kidney or liver damage, such as back or abdominal pain, urination changes, jaundice, fatigue or itchy skin, seek prompt care from your medical provider.

1.5.3- Contraindications

The safety and efficacy of *Nigella sativa* have not been fully evaluated in pregnant women. Consequently, expectant mothers should not take *N. sativa* unless otherwise instructed by a medical professional, warns Dr. Michael Tierra with the American Association for the Advancement of Science.

1.5.4- Medication Interactions

Talk with your doctor about any medication you are currently taking before you begin treatment with *Nigella sativa.* This supplement is an antioxidant and may protect cells against damage. The protective effect of *Nigella sativa* may interfere with the action of certain types of cancer therapies, such as radiation therapy or chemotherapy, which destroy cells that grow rapidly. Additionally, concomitant use of *Nigella sativa* and antihypertensive drugs may cause a sudden drop in your blood pressure levels, MSKCC warns. Low blood pressure, also called hypotension, may result in symptoms of dizziness, headache or fatigue.

1.5.5- Hypotension

The Memorial Sloan-Kettering Cancer Center cautions that black seed can lower blood pressure to the point of hypotension, especially if you're currently taking a diuretic or antihypertensive medication. The drop in blood pressure impedes the transport of oxygen to the brain, heart and other organs. This can result in fatigue, nausea, blurred vision, shallow breathing, dizziness, lightheadedness and loss of consciousness. If blood pressure dips too low, it may be life-threatening.

1.5.6- Pregnancy

Women who are pregnant shouldn't take black seed for any purpose, according to the Memorial Sloan-Kettering Cancer Center. This is largely due to the fact that the herb may affect the smooth muscle contractions of the uterus. Further assessment is necessary to determine exactly how this can affect pregnancy, so it's best to avoid this substance when expecting a child.

1.5.7- Adverse Interactions

Black seed is also known to adversely interact with both chemotherapy medications and radiation, according to the Memorial Sloan-Kettering Cancer Center. Since it may act like an antioxidant in the body, this herbal supplement can decrease the efficacy of standard cancer treatments.

1.5.8- Other Allergic Reactions

As with almost any herbal supplement, there's the potential for an allergic reaction from ingestion. Unlike contact dermatitis, this allergic reaction isn't isolated to the skin. It may cause swelling of the lips, tongue, throat or face as well as difficulty breathing, a tingling sensation in the mouth, hives, dizziness, nausea, vomiting, diarrhea and abdominal cramping.

1.5.8.1- Children

Black seed oil is possibly safe for children when taken by mouth short-term and in recommended amounts.

1.5.8.2- Bleeding disorder

Black seed might lower blood sugar levels in some people. Watch for signs of low blood sugar (hypoglycemia) and monitor your blood sugar carefully if you have diabetes and use black seed.

Low blood pressure: Black seed might lower blood pressure. In theory, taking black seed might make blood pressure become too low in people with low blood pressure.

1.5.8.3- Surgery

Black Black seed might slow blood clotting and increase the risk of bleeding. In theory, black seed might make bleeding disorders worse.

1.5.8.4- Diabetes

Black seed might slow blood clotting, reduce blood sugar, and increase sleepiness in some people. In theory, black seed might increase the risk for bleeding and interfere with blood sugar control and anesthesia during and after surgical procedures. Stop using black seed at least two weeks before a scheduled surgery (Wade 2015).

1.6- How to use

- Eat black seeds plain.

- Eat a teaspoon of black seed mixed with honey.

- Boil black seed with water.

- Heat black seed and warm milk until it just begins to boil.

- Grind black seed and swallow it with water or milk.

- Sprinkle on bread and pastries.

- Burn black seed with bukhoor (incense) for a pleasant scent (amazing).

1.7- Functional properties

The nutritional constituents of *Nigella* seed from Europe and Ethiopia are given in Table (1.2) Proximate analysis of *N. sativa* seeds showed that the moisture content ranged from 5.52–8.50 %, crude protein from 20–26.7 %, ash from 3.77– 4.86 %, total carbohydrates from 23.5–33.2 % and ether extractable lipids from 34.49–38.72 % (Takruri and Dameh, 1993; Cheikh *et al*., 2007). Chemical analysis showed that nigella seed is a significant source of essential fatty acids, proteins, carbohydrates and other vitamins and minerals (Takruri and Dameh, 1993; Cheikh *et al*., 2007). *N. sativa* seeds include nutritional components such as carbohydrates (glucose, xylose, rhamnose and arabinose), vitamins (thiamine, riboflavin, pyridoxine, niacin and folic acid) (Khan, 1999), mineral elements and proteins. *N. sativa* seeds are also a source of calcium, iron, potassium and alkaloids (nigellidine, nigellimine and nigellicine) (Khan *et al*., 2003).

Table (1.2): Nutritional constituents of *Nigella* seed

Constituents	Ethiopian seed	European seed
Moisture (g)	6.6	4
Protein (g)	13.8	22
Fat (g)	32.2	41
Carbohydrate (g)	–	17
Fibre (g)	16.4	8
Ash (g)	7.5	4.5
N (g)	2.2	–
Na (g)	–	0.5
K (g)	–	0.5
Ca (g)	0.5	0.2
P (g)	0.6	0.5
Fe (mg)	17	10
Thiamine (mg)	0.62	1.5
Niacin (mg)	9.5	6
Pyridoxine (mg)	–	0.7
Tocopherol (mg)	–	34

Sources: Takruri and Damah, (1993)

The amino acids present in dormant seeds are cystine, lysine, aspartic acid, glutamic acid, alanine and tryptophan.

Nigella seed, its extracts and oils are known to have several pharmacological properties: some traditional medicinal preparations that contain *N. sativa* are listed below. The seeds are employed as a corrective of purgatives and other medicines and are believed to possess diuretic, anthelmintic and emmenagogue proper- ties, useful in indigestion, loss of appetite, fever, diarrhoea, dropsy, puerperal diseases, etc.

1.8- Uses of *Nigella sativa* in folk medicine

- Two drachm of lightly roasted *Nigella* seeds, with the addition of an equal quantity of treacle for the relief of intermittent fever.
- 10–20 grains of *Nigella* seed for the treatment of dysmenorrhoea; in large doses it may induce abortion.
- A confection made of *Nigella* seeds, cumin seeds, black pepper, raisins, tamarind pulp, pomegranate juice and sonchal salt with treacle and honey as a cure for loss of appetite and distaste for food.

- *Nigella* seeds with the addition of long- pepper, sonchal salt for the relief of pain, experienced after childbirth

- 10 g of each of the following: *Nigella* seeds, cumin seeds, anise seeds, *ajowain* seeds, carum seeds, *Anethum sowa, fenugreek,* coriander, ginger, long pepper, long pepper root, plumbago root, habusha (an aromatic substance), dried pulp of *Ziziphus jujuba*, root of *Aplotaxis auriculate* and Kamala powder, combined with 1000 g treacle, one seer (about 1 l) of milk and 40 g butter, boiled together. The resulting confection is known as *pancha jiraka paka*, and should be used in doses of about one drachm every morning for the relief of illness related to childbirth, including fever, loss of appetite and disordered secretions after delivery.

- Crushed seeds in vinegar to ease symptoms of skin disorders such as ring worm, eczema and baldness.

- Tea made from powdered *Nigella* seeds, fenugreek, garden cress, *Commiphora* spp. and dried leaves of *Cleome* spp., *Abrosia maritina L.* and *Centaurium pulchellum* Druce (used mainly in Egypt) as a cure for diabetes 500 mg Egyptian *Nigella* oil capsules as an antihistamine (Malhotra, 2012).

1.9 Important functional properties reported for *N. sativa* as

1.9.1- Antifungal activity

The essential oil of *N. sativa* has shown excellent activity against a number of fungi (Agarwal *et al.,*1979 ;Aboul ela *et al.,* 1996). he inhibition of aflatoxin formation by a number of medicinal plants including *N. sativa* at different concentrations has been studied. In one of these studies, the powdered seed and essential oil effectively inhibited the growth and aflatoxin production of a toxigenic strain of Aspergillus flavus (El-Shayeb and Mabrouk 1984, EI-Sayed *et al.,* 1997). The essential oil has also been reported to be effective against Col -letotrichum capsici, P*ythium vexans and Sclerotinia trifolium* ; while the seeds are ineffective against A. flavus, A .niger ,Geotrichum candidum and Penicillium roque- fortii (Rathee *et al.,*1982). Moreover, *N. sativa* oil could be valuable in the protection of plants against the fungus Candida *olivacum* ,a known parasite affecting the growth of economic crops such as rice, wheat and cotton (Aboul ela *et al.,* 1996).

1.9.2- Antibacterial activity

Thymoquinone, primarily present in seeds and oils, exhibits strong antimicrobial properties and is believed to be the active principle responsible for the antimicrobial profile of *N. sativa* oil el-Alfy et al., 1975; O'Mahony et al., 2005). However, another study has indicated the complementary involvement of other fatty acid compounds in the antimicrobial properties of the oil (Bourrel et al., 1993). Several studies have shown that *Nigella* seed, extracts and oils have an antibacterial action that inhibits the growth of both gram-positive and gram-negative micro- organisms except certain strains of Pseudomonas pyocyanea [P. aeruginosa] Ferdous *et al*., 1992). Water extract and hexane extract, however, have only weak activity against Streptococcus (Naqvi *et al.,* 1991). The antibacterial activity of the essential oil of *N. sativa* has also been further examined in a number of studies (Agarwal *et al.,* 1979; Rathee *et al.,* 1982). During testing against 21 pathogenic bacteria, it was found that the antibacterial activity of the seeds was predominantly related to the volatile oil fraction and the activity was much higher for all of the gram-positive strains tested. It has also been suggested that the volatile oil would be a good substitute for common antibiotics (Rathee *et al.,* 1982).

The crude extracts of N. sativa were reported to have a promising effect on multi- drug resistant organisms, including gram-positive and gram-negative bacteria ;*Nigella* oil was more effective against the former (Staphylococcus aureus, S. epidermis) than against the latter (Staphylococcus, Streptococcus pyogenes) (Alhaj *et al*., 2008). It has been reported to be effective against clinical isolates of methicillin-resistant S. aureus (Hannan *et al.,* 2008) and Helicobacter pylori (O'Mahony *et al.,* 2005). The biological activity of the oil, after fractionation, was ascribed to its phenolic content. A solution of the phenolic portion of the oil in propylene glycol had an enhanced antibacterial activity and was found to be non-toxic to humans with no adverse effects on blood pressure, heart or respiration (Toppozada *et al.,*1965).

Nigella extract, in combination with the commercial diagnostic antibiotics streptomycin and gentamycin, has been shown to exert a synergic antibacterial action .In an in vitro study, the volatile oil has shown antibacterial action against 37 enteric organisms; promising results have been observed on strains S. dysenteriae 1 ,S. flexneri, S. sonnei and S. boydii) Ferdous *et al.,* 1992). Moreover, the aqueous methanolic extract of *N.*

sativa has been screened against S. mutans and reported to show good antibacterial activity by preventing the adhesion of viable cells of S. mutans to smooth surfaces. The plant extract thus proved valuable in the prevention of dental caries and plaques) Namba *et al.,* 1985). In a collective study, some 32 plant species seed in Saudi folk medicine including *N. sativa* exhibited antidiarrheal activity (Shah *et al.*, 1988). Nigella sativa oil 0.1 % w/w) has also proven very useful in food preservation, as a potent inhibitor of food spoilage and hazardous bacteria (El-sayed *et al.*, 1997).

1.9.3- Nephroprotective activies

The nephroprotective effect of vitamin C and *Nigella sativa* oil was observed against gentamicin associated nephrotoxicity in rabbits. Serum creatinine, blood urea nitrogen, and antioxidant activity were measured as indicators of nephrotoxicity for all the groups of rabbits. It was revealed that vitamin C and *N. sativa* oil both had nephroprotective effect as they lowered the values of serum creatinine, blood urea nitrogen, and antioxidant activity as compared to GM control group values. When these two antioxidants were given as combination, they proved to have synergistic nephro protective effect.

1.9.4- Neuropharmacolgical activities

The aqueous and methanol extracts of defatted *N. sativa* seeds were shown to possess a potent central nervous system and analgesic activities, especially depressant action in the case of the methanolic extract. The neuroprotective effects of both the extracts of *N. sativa* in cerebral ischemia were observed. The neuroprotective effects could be due to its antioxidant, free radical scavenging, and anti-inflammatory properties (dubey *et al.,* 2016).

1.9.5-Antiparasitic activity

The volatile oil of *N. sativa* has been reported to exhibit fairly good antiparasitic activity, and particularly anthelmintic activity against earthworms (*Pheritima post-huma*), tapeworms (*Taenia solium*), hookworms (*Bunostomum trigonocephalum*) and nodular worms (*Oesophagostomum colombionum*), which was found to be comparable with that of the chemical agent piperizine phosphate (Agarwal *et al.*, 1979). Akhtar and Riffat (1991) reported the use of *N. sativa* seeds as a treatment against worms for children. The glycosides of *N. sativa* have considerable anti- cestodal potential in

animals (Akhtar and Riffat, 1991), with doses of 150 and 200 mg/kg showing activity comparable to that of levamisole hydrochloride and oxychozanide Nilzan TM   after 10 and 15 days. A lower dose of   100 mg/kg of the glycosides after 15 days was almost as effective as Nilzan (Akhtar and Aslam, 1997). Oil from *N. sativa* could therefore prove extremely useful as a natural antiparasitic treatment for children.

1.9.6- Anti-inflammatory activity

N. sativa seeds and oil are effective anti-inflammatory substances. The anti-inflammatory activities of the thymoquinone present in *N. sativa* are attributed to its antioxidant effects. It has been claimed that nigellone, the non-toxic carbonyl polymer of thymoquinone (Mahfouz and E1-Dakhakhny, 1966), thymoquinone (E1-Dakhakhny, 2000) and thymohydroquinone (E1-Alfy *et al*., 1975) are the active principles responsible for the anti-inflammatory properties of *N. sativa*. Nigellone in low concentrations proved to be an effective inhibitor of histamine release induced by antigens and calcium ionophores. The mechanism of action is thought to involve the inhibition of the protein kinase C and a decrease in the intracellular calcium concentration.

The fixed oil prepared from *N. sativa* has been tested as a possible inhibitor of eicosanoid generation and membrane lipid peroxidation using thymoquinone, a potent anti-inflammatory agent, as a reference parameter. The crude fixed oil acted as an inhibitor of the cyclooxygenase and 5-1ipoxygenase pathways of arachidonic acid metabolism in rat peritoneal leucocytes stimulated with a calcium ionophore, but the activity was greater than would be anticipated from the thymoquinone content of the oil. This has led to the conclusion that the anti-inflammatory actions of the expressed fixed oil are not solely related to the thymoquinone content; the unusual C 20:2 fatty acids that are known to be present in the seed oil may also contribute (Houghton *et al.*, 1995). The anti-inflammatory effects of the volatile oil and thymoquinone were observed in the significant reduction of both carrageenan- induced oedema formation and cotton seed pellet granuloma weight. On the basis of these results, it has been concluded that the volatile oil has an anti-inflammatory action that involves the inhibition of eicosonoids and lipid peroxidation (Muta- bagani and El-Mahdy, 1997). The soluble fraction of *N. sativa* seeds also had a stimulatory effect on the lymphocyte response to pooled allogenic cells, and also contributed to an increase in the production of interleukins (Haq *et al.*, 1995).

1.9.7-Antioxidant activities

N. sativa seeds and extracts have been reported to exhibit antioxidant properties, as they offer protection against damage caused by oxidation. Nagwa *et al.*, (2006) and Adamu *et al.*, (2010) have all found that *Nigella* oils may be used as an antioxidant, while Musa *et al.*, (2004) found that the ethanol extract can also generate antioxidants and was able to prolong the lifespan of mice. Recently Ibraheem *et al.*, (2010) reported that *N. sativa* has calcium antagonist and antioxidant properties, both of which play a major role in the management of diseases. The highest percentage of hydrogen peroxide inhibition in hepatic microsomes of mice treated with lindane was shown by *N. sativa* seed extract (Awney *et al.*, 1997). Nour and Mourad (2010) also found that *Nigella* oil had an antioxidant effect on mono- sodium glutamate-induced oxidative stress in the brain of rats, and *Nigella* seed extract has also prevented oxidative deterioration.

The chemotherapeutic toxicity caused by cisplatin has been shown to be greatly reduced by using cisplatin in combination with plant extracts of *Crocus sativus* stigmas (50 mg/kg) and *N. sativa* seed (50 mg/kg) (El-Daly, 1998). Similarly, the oil has been shown to be useful as a protective agent against the side-effects of methotrexate chemotherapy (Labib *et al.*, 2009). On the basis of these results, it has been suggested that a nutritional supplement of nigella seed extract may offer better protection to the human body against oxidative damage than supplementation with synthetic antioxidants. In fact, the antioxidant activity of *N. sativa* extract was found to be comparable with that of t-butylhydroquinon (Atta and Imaizumi, 1998).

1.9.8- Immunological activities

N. sativa seed extract had an inhibitory effect on the human immunodeficiency virus protease; however, the active principle(s) responsible for the activity were not identified. A number of traditional medicines including the seeds of *N. sativa* have been examined *in vitro* for their HIV protease-inhibiting properties (Ma *et al.*, 1994). Study has also been carried out on the effect of the volatile oil on T-cells. From the effect of the volatile oil on Jurkat T-cell leukemia polypeptides, a possible post-translational modification of P24 protein has been suggested as a biological action (Hailat *et al.*, 1995). In mice, cisplatin-induced falls in haemoglobin levels and leucocyte count have been reduced by treatment

with an extract of *N. sativa* seed. It has also been found that the plant extract modulates the immune system by increasing the number and activity of immune competent cells in humans (Medenica *et al.*, 1993). *Nigella* oil has played a significant role in altering the liver damage induced by *Schistosoma mansoni* infection in mice, and can help to improve the immunological system of the host. The immunomodulatory properties of *N. sativa* seed and thymoquinone support its traditional use for treatment in rheumatism and related inflammatory disorders. It may also be used as an immunopotentiating agent, and has strong immunomodulatory properties and interferon- like activity. *N. sativa* seed extract modulates the neurotransmitter amino acid release in cultured neurons in *vitro* (Malhotra, 2012).

1.9.9- Anticarcinogenic activity

An organic fraction obtained from *N. sativa* seed was tested on cancer cells and was found to have anti-tumour properties (Salomi *et al.*, 1992). An alcoholic extract of *N. sativa* was screened for anticancer properties and was found to be active against Ehrlich ascites carcinoma in mice. The extract (160 mg/kg body wt) produced a significant increase of lifespan (Abdel-Salam *et al.*, 1998). *N. sativa* extract (100 mg/kg body wt) inhibited skin carcinogenesis in mice by delaying the onset of papilloma formation. The extract (100 mg/kg body wt) also restricted the incidence of tumour to 33.3 % in chemically-induced soft tissue sarcomas in albino mice (Salomi *et al.*, 1992). *Nigella* extract inhibits cancer and endo-thelial cell progression and decreases the production of the angiogenic protein fibroblastic growth factor made by tumor cells. A monodesmosidic triterpene saponin, α-hederin, has also been isolated from the extract of *Nigella* seeds and has been shown to exert antitumoural activity (Muthu Kumara and Huat, 2001).

1.9.10- Antidiabetic activities

Several studies have reported that *N. sativa* oil and extracts have an antidiabetic effect. *N. sativa* and thymoquinone proved clinically useful for the treatment of diabetes and for protecting beta cells against oxidative stress. The oil has been reported to have significant effects in diabetic and dyslipidemic patients. The blood sugar lowering effect of the plant extract has been investigated in animal experiments, and the results have indicated that the plant extract could act as a useful therapeutic agent in the treatment of non-insulin

dependent diabetes mellitus. Crude *Nigella* appeared as effective as *n*-hexane extract for alleviating streptozotocin-induced diabetes mellitus. In a comparative study, the hypoglycaemic effect of the volatile oils of *N. sativa* and *Allium sativum* showed significant elevation of the serum insulin levels relative to glipizide. This synergic effect was higher for *A. sativum* than for *N. sativa. Nigella sativa* and four other medicinal plants of Egyptian origin have also been examined for their hypoglycaemic effect and an herbal formulation of the five plants was more effective in reducing blood glucose levels.

For thousands of year, the seeds, oils and extracts of *N. sativa* have been used as an anticancer agent by Unani, Ayurveda and the Chinese system of medicine that have originated from the Arab, Ind-Bangla and China, respectively. The modern scientific research with the investigation of anticancer activity of *N. sativa* is a comparatively recent affair (for the last 2~3 decades). There are not so many research works done in this field and very few review articles exist in this area. We have searched the scientific databases like Pubmed, Web of Science and Google scholar and summarized the current scientific information about the anticancer activities of *N. sativa* with mechanisms of action (Malhotra, 2012).

1.9.11- Anthelmintic properties

N. sativa was found to have an anthelmintic activity against tapeworm comparable to that of piperazine.

Analgesic qualities: The essential oil produced significant analgesic activity using chemical and thermal noxious stimuli methods such as acetic acid-induced writhing, hot plate and tail flick tests. The results show that *N. sativa* could reduce the presence of the nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy and mucosal pallor during the first 2 weeks (day 15). The anti-allergic effects of *N. sativa* components could be attributed to allergic rhinitis. Moreover, *N. sativa* should be considered for treating allergic rhinitis when the effects of other anti-allergic drugs need to be avoided (Khanna *et al.,* 1993).

1.9.12- Anticancer activity

1.9.12.1- Role of *N. sativa* as an anticancer agent

Many active ingredients have been found in the seeds of *N. sativa*. The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. The quantification of four

pharmacologically important components: thymoquinone (TQ), dithymoquinone, thymohydroquinone, and thymol, in the oil of *N. sativa* seed by HPLC. Much of the biological activities of the seeds have been shown to be due to thymoquinone, the major component of the essential oil, which is also present in the fixed oil. TQ is considered as potent anti-oxidant (Badary et al., 2003), anti-carcinogenic and anti-mutagenic agent (structure of thymoquinone is shown in Figure (1.1). Moreover, TQ is a relatively safe compound, particularly when given orally to experimental animals. Alpha (α)-hederin, a pentacyclic triterpene saponin (structure: Figure 1.2) isolated from the seeds of *N. sativa*, was also reported to have potent *in vivo* antitumor activity.
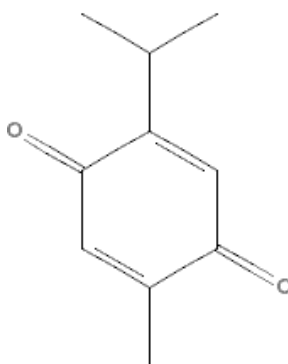

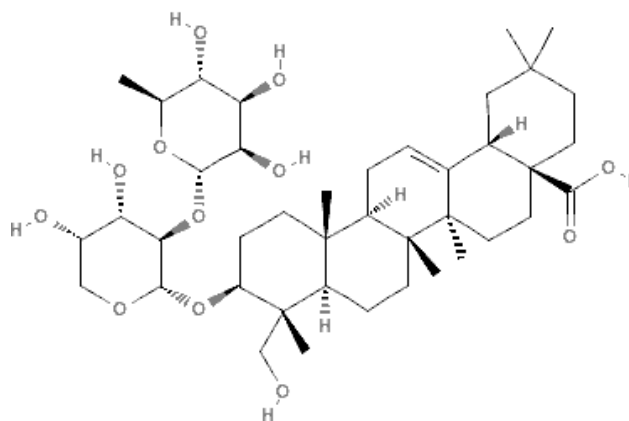Figure (1.1) Chemical structure of Thymoquinone

Figure (1.2): Chemical structure of anti-tumor agents isolated from *N. sativa* Alpha-hederin
(Khan et al., 2011)

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N. sativa seeds or oils or its active ingredients like TQ are effective against different cancers:

1.9.12.2- Breast Cancer

Aqueous and alcohol extracts of *N. sativa* were found to be effective *in vitro* in inactivating MCF-7 breast cancer cells.

1.9.12.3-Colon Cancer

The TQ suggest has anti-neoplastic and pro-apoptotic against colon cancer cell line HCT116 (Salim and Fukushima, 2003). The volatile oil of *N. sativa* has the ability to inhibit colon carcinogenesis (Salomi *et al.,* 1991).

1.9.13.4- Hepatic Cancer

The cytotoxic activity of *N. sativa* seed was tested on the human hepatoma HepG2 cell line and 88% inhibitory effect on HepG2 was found after 24-hr incubation with different concentrations (0-50 mg/ml) of the *N. sativa* extract.

1.9.12.5- Lung Cancer

The antitumor activity of α-hederin from *N. sativa* against LL/2 (Lewis Lung carcinoma) is mentioned in BDF1 mice (Thaberew *et al*., 2005). Also the supplementation of diet with honey and *N. sativa* has a protective effect against MNU (methylnitrosourea)-induced oxidative stress, inflammatory response and carcinogenesis in lung, skin and colon was showed by (Mobrouk *et al.,* 2002). However, α-hederin and TQ reported that

the two principal bioactive constituents of *N. sativa* enhance neither cytotoxicity nor apoptosis in A549 (lung carcinoma), HEp-2 (larynx epidermoid carcinoma) cells (Rooney and Ryan 2005).

1.9.12.6- Skin cancer

Topical application of *N. sativa* extract inhibited two-stage initiation/promotion [dimethylbenz[a]anthracene (DMBA)/croton oil] skin carcinogenesis in mice. Again, intraperitoneal administration of *N. sativa* (100 mg/kg body wt) 30 days after subcutaneous administration of MCA (20-methylcholanthrene) restricted soft tissue sarcomas to 33.3% compared with 100% in MCA-treated controls (Salomi *et al.,* 1991).

In addition to these cancer inhibiting properties, components of *N. sativa* have cancer protective roles (Ibrahim *et al.,* 2008).

Some studies have been for anther types of cancer such as Cervical Cancer, Prostate Cancer, Renal Cancer, Pancreatic Cancer and Fibrosarcoma.

1.9.13- Hepatoprotective activities

Thymoquinone, found in *N. sativa* as a major constituent of its volatile oil, has been found to be an efficient cytoprotective agent against chemically-induced hepatic toxicity in animal experiments (El-dakhakhny *et al.,* 2000, Bashandy, 1996).Using the well-known hepatoprotective agent silybin as areferance, the hepatoprotective properties of thymoquinone have been further examined in isolated rat hepatocytes. Pre- incubation of the hepatocytes with thymoquinone or silybin (1 mM) yielded isolated hepatocytes that offered protection against tertbutyl hydroperoxide induced injury (Daba and Abdelrahman,1998). *N. sativa* seeds are effective in treating patients suffering from liver cirrhosis and hepatocellular damage.

N. sativa oil (0.27 g/100 g body wt/day) was administered to adult and senile rats in order to determine its possible effect on the ageing process by measuring parameters thought associated with ageing, such as functional and structural changes in the liver and kidney of the animals. The treated senile animals showed a decrease in serum cholesterol, total lipids, γ-glutamyl transferase, urea, uric acid and nuclear DNA content. The results have suggested that nigella seed oil may be able to slow down the ageing process in senile

rats (Bashandy, 1996). *N. sativa* treatment has also been shown to protect the rat liver against hepatic ischemia-reperfusion injury.

1.9.14- Miscellaneous activities

1.9.14.1- Antispasmodic

In the early experimental work on *N. sativa,* which began in the 1980s, the alcoholic seed extract was fractionated by column chromatography on alumina to yield two organic fractions that showed hypotensive activities when tested on dogs. Fractionation of the alcohol–water extracts of the seeds afforded an organic fraction that displayed antispasmodic activity when tested on isolated rabbit intestine (Zawahry, 1963). The volatile oil was found to have some anti-oxytoxic potential based on *in vitro* experiments carried out on the uterine smooth muscle of rats and guinea pigs using isolated uterine horns (Aqel and Shaheen, 1996). However, the effects were concentration dependent and reversible by tissue washing. The volatile oil of *N. sativa* has been tested *in vitro* on the vascular smooth muscle, and it inhibited the norepinephrine-induced contractions of rabbit aortic rings in a solution containing Ca e+ ions; the activity was dose dependent and reversible (Aqel, 1992). In other studies the volatile oil functioned as a direct tracheal smooth muscle relaxant and a Ca 2+antagonist (Aqel, 1992), in which contractions were induced by histamine and acetylcholine, respectively.

1.9.14.2- Anti-hypertensive

In animal experiments, the volatile oil showed potent centrally acting antihypertensive properties that were partly attributed to the presence of thymoquinone in the oil (E1-Tahir *et al.*, 1993). Experiments evaluating the effects of *Nigella* volatile oil (30–120 zl/kg) on the arterial blood pressure and heart rate of urethane-anesthetized guinea pigs have indicated that the oil has cardiovascular-depressant effects which were speculated to be thymoquinone- related (El-Tahir and Ageel, 1994).

1.9.14.3- Analgesic

N. sativa oil has been shown to display CNS–depressant and potent analgesic properties in laboratory animal experiments. The analgesic activity of the oil has been attributed to the speculative presence of an opioid principle in the oil (Khanna *et al.*, 1993).

1.9.14.4- Growth-regulating

The effect of the seed oil on growth regulation in *Dysdercus similis* (F) has been examined. Petroleum ether fractions of the seeds at concentra- tions of 10.3–62.5 ppm were tested for growth–regulating [juvenile hormone (JH)] activity against larvae of *D. similis* (F). The *Nigella sativa* fraction showed high JH activity and this was considered to be due the fatty acid content in the seed extract (Kumar and Thakur, 1989).

1.9.14.5- Bronchodilatory

The bronchodilatory effects of the plant extract have long been known and studied (Mahfouz and El-Dakhakhny, 1966). Animal experiments have indicated that the volatile oil may act as a centrally acting stimulant of the respiratory system, provided the thymoquinone content in the oil can be removed (El-Tahir *et al.*, 1993). Nigellone is thought to be a promising substance for the prevention and control of bronchial asthma and other allergic conditions. The role *N. sativa* oil in the management of wheeze associated lower respiratory tract illness in children has also been investigated (Ahmad *et al.*, 2009).

1.9.14.6- Gastroprotective

N. sativa seeds in the diet have a favourable effect on the lipid profile by lowering the triglyceride, total cholesterol and LDL cholesterol and increasing the HDL cholesterol in albino rats (Buriro and Tayyab, 2007). The gastro- protective effects of *N. sativa* oil on the formulation of stress gastritis have been reported in hypothyroidal rats (Abdel-Sater, 2009). Gastric antiulcer effects have also been reported by Rajkapoor *et al.*, (2002).

1.10- health effects

N. sativa and gluthione have an antiperoxidative effect and are also beneficial in protecting against ionizing radiation-related tissue injury .The radioprotective properties of the seed oil of *N. sativa* were discussed ;its use showed significant improvement in DNA ,RNA, super oxide dismatase and glutathurane profiles and thereby enhanced longevity in animals. The expressed oil of *N. sativa* was the subject of earlier studies on radioprotection and has been shown to normalize enzymatic changes in the liver tissue that occur as a result of exposure to ionizing radiation in rabbits .

Ethanolic *nigella* extract helped in reducing the number of calcium oxalate deposites on ethylene glycol-induced kidney calculi.

The use of *N. sativa* oil to treat and heal chemically-induced wounds in rabbit skin was found to be effective.

The alcoholic extract of *N. sativa* ,administered orally on a daily basis, clearly improved the reproductive performance of male rats and brought about increased spermatogenesis in male albino rats .

Thymoquinone, the principal active component of *nigella* seeds has been shown to suppress epileptic seizures in rats.

Nigella powder can be used as vinegar and applied on spots caused by vitiligo, followed by exposure to sunlight. A decoction of seeds mixed with sesame oil is used externally in various skin eruptions. *Nigella* has also proved useful in the treatment of dermatitisb (Malhotra, 2012).

1.11- Toxicity

Nigella seed yields a volatile oil containing melanthin, nigelline, damascene and tannin. Melanthin is toxic in large doses and nigelline causes paralysis, so this spice must be used in moderation. The traditional use of *Nigella* seeds for the treatment of dyslipidaemia, hyperglycaemia and related abnormities; however, the same study indicates that the plant can be relatively toxic. Acute and chronic toxicity caused by *Nigella sativa* fixed oil. The methanol extract from the related species *N. damascene* seeds showed a high oestrogenic activity. Among the purified phenolic compounds tested, the phenolic ester 1-0-(2,4-dihydroxy) benzolglycelrol showed the strongest oestrogenic activity due to the presence of flavonoid compounds. As a result, *N. damascene* should not be used as a substitute for *N. sativa.* The seed powder did not produce any toxic effect at very high doses (28 mg/kg orally) and oral thymoquinone was also found to be safe at LD50 of 2.4 g/kg (Malhotra, 2012). *Nigella* is a safe and effective herb that can be used by almost anyone under the advice of an herbal practitioner. No health hazards or side-effects are associated with the proper administration of designated therapeutic dosages, although dermatitis has been reported in some cases (Sharma *et al.*, 2009).

1.12- Quality issues

1.12.1- Specification for whole seed

The quality of *Nigella* seed mainly depends on its appearance. The seeds should be matt-black, triangular, 1.5–3 mm long, uniform in size, shape and texture and have an oily, white interior. The odor of *Nigella* seeds when crushed should resemble strawberry. Some authors have mentioned that its smell is similar to that of oregano or carrots.

The Indian Agmark grade specifications for *Nigella* seeds as laid down in the Prevention of Food Adulteration Indian Act are given below:

Seed moisture: not more than 11 % by weight

Total ash: not more than 6 % by weight

Ash insoluble in acid: not more than 1 % by weight

Organic extraneous matters: not more than 3 % by weight

Inorganic extraneous matters: not more than 2 % by weight

Volatile oil: not less than 1 % (v/w)

Ether extracts (crude oil):   not less than 35 % (v/w)

Alcoholic acidity as: not more than 7 % (v/w) oleic acid

ASTA, ESA and ISO have not laid down specifications for *nigella* seed. This may be because there is less demand for the spice in European and American countries. India exports *nigella* to neighbouring countries, the Middle East and the Gulf states, though, to satisfy a demand from the many expatriate Asian workers.

Nigella powder is produced by grinding dried, cleaned and sterilized seed. After sieving through the required mesh size, the powder should be packed in airtight containers. The freeze-grinding technique can be used to avoid loss of flavor during heat grinding. The powder is creamy white in color with an aroma like strawberry. The specification for whole *nigella* seed should be strictly followed in powder production in addition to any seed powder quality specifications. The quality standards as laid down under the Prevention of Food Adulteration (PFA) Act and Rules for *Nigella* powder are given below (Anon, 1998):

Powder means the ground product of dried seeds of *N. sativa* L.

Moisture: not more than 12 % by weight

Total ash: not more than 7 % by weight

Ash soluble in dilute HCL: not more than 1.5 % by weight

Volatile oil: not less than 0.5 % (v/w)

It should be free from added coloring matter.

1.12.2- Volatile oil and fixed oils

The volatile oil content of *Nigella* seed averages 0.5–1.4 % and it contains primarily the glucosides melanthin and melathingenin, bitter substances, and a crystalline active principle, nigellone. The aroma of *Nigella* oil is warm, spicy and fatty and the flavor is strawberry-like with a burning sensation. The volatile oil of *Nigella* is yellowish brown with an unpleasant flavor. The physiological properties of *Nigella* oil are given below (Pruthi, 2001):

Specific gravity at 15°C: 0.875–0.886

Refractive index at 20°C: 1.4836–1.4844

Optical rotation at 20°C: +1.43° to + 2.86°

Acid value: up to 1.9

Ester value: 1.0–21.6

Ester value (after acetylation): 15–73

Solubility: 2-4.5 or more volumes alcohol

Fixed oils are also extracted from *Nigella* seeds. The fatty oil obtained from seeds is used for edible purposes. Extraction with benzene and subsequent steam distillation of the extract to remove volatile oil gave about 31 % of reddish brown, semi-drying oil with the following characteristics:

Specific gravity at 25°C: 0.9152

Refractive index at 21°C: 1.4662

Acid value: 42.83

Saponification value: 199.6

Iodine value: 117.6

RM value: 3.9 %

Unsaponifiable matter: 0.03 %

It shall be free from any other oils or fats, completely free from pig products or their derivatives or any other animal fats

It shall have the taste and odor distinguishing the product, and shall be free from strange taste and rancid odor.

No additives shall be allowed to the Virgin *Nigella Sativa* Seed Oil.

It shall be clear and free from any turbidity, or the raw materials from which it is extracted, and from materials used in its purification.

It shall be free from mineral oils.

Ratio of humidity and volatile matters at 105°C shall not exceed 0.2% (m/m).

Mineral matters ratio shall not exceed the following (mg/kg):

	Iron	Copper	Lead	Arsenic
Virgin Oil	5	0.4	0.1	0.1
Refined Oil	1.5	0.1	0.1	0.1

Nigella oleoresins can be extracted from the seeds but have little commercial value. Therefore, there are no ISO, ASTA or ESA specifications for *Nigella* oleoresins.

Packaging:

Without prejudice to the content of GSO standard mentioned in item (2.12), oil shall be packaged in appropriate containers, made of safe materials which do not affect its characteristics. Containers shall be adequate, dry, clean, free of any strange odor, not used before and have tight covers.

Storage:

Oil containers shall be stored in well ventilated stores, away from direct sunlight and sources of heat and contamination (GSO).

Shelf Life: 2 years max

Shelf life of this product is influenced by many conditions of which temperature, exposure to light / air and general good storage are the major factors. Material stored in

adverse conditions may deteriorate much faster. Our suggested "Re-test" date shown on this certificate reflects a minimum period in which we would expect product to remain in good usable conditions if stored as recommended. Thereafter its continued shelf life may be very much longer and we advise re-test at the indicated date and then every 3-6 months up to a suggested commercial expiry date as shown below. The expiry date is subjective and should be controlled by QC/QA. Typical indicators of re-test failure would be changes in organoleptic properties (clarity / color / sediment / haze / off odor etc) such changes may be gradual and slight and the commercial expiry date is intended to reflect a viable maximum proposal subject to earlier re-test approvals.

1.1.13- Adulteration

Nigella seed is available both whole and in ground form. The whole seed is subject to adulteration by onion seeds, because of their similarity. Onion seeds lose viability after one year and such unused seeds are used to adulterate batches of *Nigella* seed. Another form of adulteration is when the exhausted seed or spent seed after oil extraction is mixed in whole seed or ground form with unprocessed *Nigella* seeds. Essential oil extracted from seeds has also been found to be adulterated with chaff oil. The range of essential oil is 0.5–1.4 %. It should contain melangin as the major component, and levels of this compound should not go below 30 %. A high ratio of eicosadienoic acid to eicosamonoenoic acid combined with a high level of CO_2 fatty acids, is characteristic of *nigella* seed oils and could be used to identify genuine oil (Weiss, 2002). The adulterants can be detected through chromatographical techniques

1.14- Methods characterization of oil

1.14.1- Introduction

Methods for characterizing edible lipids, fats, and oils can be separated into two categories: those developed to analyze bulk oils and fats, and those focusing on analysis of foodstuffs and their lipid extracts. In evaluating foodstuffs, it is usually necessary to extract the lipids prior to analysis. In these cases, if sufficient quantities of lipids are available, methods developed for bulk fats and oils can be utilized.

Numerous methods exist for the characterization of lipids, fats, and oils. Traditional analytical methods for bulk fats and oils, many involving "wet chemistry.", methods of measuring lipid oxidation and methods for the analysis of lipid fractions, including fatty acids, triacylglycerols, and cholesterol.

The term lipids refer to a wide range of compounds soluble in organic solvents but only sparingly soluble in water. In contrast to lipids, the terms fats and oils often refer to bulk products of commerce, crude or refined, that have already been extracted from animal products or oilseeds and other plants grown for their lipid content. The term fat signifies extracted lipids that are solid at room temperature, and oil refers to those that are liquid. However, the three terms, lipid, fat, and oil, often are used interchangeably.

1.14.2- Methods for bulk oils and fats

Numerous methods exist to measure the characteristics of fats and oils. Some methods (e.g., titer test) have limited use for edible oils (in contrast to soaps and industrial oils). Other methods may require special apparatuses not commonly available or may have been antiquated by modern instrumental procedures [e.g., volatile acid methods (Reichert–Meissl, Polenske, and Kirschner values) have been replaced largely by determination of fatty acid composition using GC]. Methods to determined impurities, including moisture, unsaponifiable material in refined vegetable oil and insoluble impurities.

1.14.2.1- Sample Preparation

Ensure that samples are visually clear and free of sediment. When required (e.g., iodine value), dry the samples prior to testing (AOAC). Because exposure to heat, light, or air promotes lipid oxidation, avoiding these conditions during sample storage will retard rancidity.

1.14.2.2- Refractive Index

1.14.2.2.1- Principle

The refractive index) RI (of an oil is defined as the ratio of the speed of light in air (technically, a vacuum) to the speed of light in the oil .When a ray of light shines obliquely on an interface separating two materials, such as air and oil ,the light ray is refracted in a manner defined by Snell's law ,as shown in Equation [1]

$$\theta_1 n_1 = \theta_2 n_2 \qquad\qquad [1]$$

Where:

θ_1 = angle of the incident light

n_1 = refractive index of material 1; θ_2 = angle of the refracted

light n_2 = refractive index of material 2

From Equation [1], if the angles of incidence and refraction and the refractive index (n) of one of the two materials are known, the refractive index of the other material can be determined. In practice, the θ_1 and n_1 are constant, so n_2 is determined by measuring θ_2.

Because the frequency of light affects its refraction (violet light is refracted more than red light), white light can be dispersed or split after refraction through two materials of different refractive indexes (explaining the color separation of diamonds and rain- bows). Refractometers often use monochromatic light (or nearly monochromatic light from the sodium doublet D line, that has 589.0 and 589.6 nm wavelengths or light emitting diodes to provide 589.3 nm) to avoid errors from variable refraction of the different wave- lengths of visible light.

1.14.2.2.2- Applications

RI is related to the amount of saturation in a lipid ;the RI decreases linearly as iodine value (a measure of total unsaturation) decreases. RI also is used as a measure of purity and as a means of identification, since each substance has a characteristic RI .However , RI is influenced by such factors as FFA content, oxidation, and heating of the fat or oil. A relatively saturated lipid such as coconut oil has a different RI (n = 1.448 – 1.450) compared with a relatively unsaturated lipid such as menhaden oil (n = 1.472).

1.14.2.3- Color

Two methods for measuring the color of fats and oils are the Lovibond method and the spectrophotometric method.

In the Lovibond method, oil is placed in a standard- sized glass cell and visually compared with red, yellow, blue, and neutral color standards. Results are expressed in terms of the numbers associated with the color standards. Automated colorimeters are

available at 550, 620, and 670 nm. The photometric color index is calculated as shown in Equation [2]

Photometric color index = 1.29 (A_{460}) + 69.7 (A_{550}) +41.2 (A_{620}) − 56.4 (A_{670}) [2]

1.14.2.3.1-Applications

The color of fats and oils is most commonly evaluated using the Lovibond method. Oils and fats from different sources vary in color. But if refined oil is darker than expected, it is probably indicative of improper refinement or abuse. Though specifically developed for testing the color of cottonseed, soybean ,and peanut oils, the spectrophotometric method is probably applicable to other fats and oil (Nielsen, 2010).

1.14.2.4-Viscosity

A Viscosity is a measure of resistance between two immediate fluid layers in any fluid. If a fluid is having more viscosity or a more viscous fluid, it will experience more resistance during flow. For example, if you put the water and oil in the same pipe and impart the same velocity by means of pump, water will flow faster than oil because of lesser viscosity than oil. You can call it as 'thin' if the viscosity of that fluid is low and 'thick' if the viscosity of that fluid is high.

At room temperature viscosity of water is 1 m Pa. Sec and for vegetable oil is 57 m Pa. Sec. The above values are dynamics viscosity values. The kinematics' viscosity value can be found by dividing dynamic viscosity by density of each fluid. Thus,

     Kinematics' Viscosity= dynamic viscosity / density   [3]

Effect of temperature on viscosity of liquid increases as the temperature decreases while for gas, it works in reverse way. The viscosity of gas increase as the temperature increases.

Thus, we need to also consider temperature effects, while selecting oil for any particular applications. We all know that density is a property which is independent to temperature and some other physical quantities, but viscosity do have dependence to these properties. It should be emphasized that the viscosity as well as density comes from the same cause of fluid, i.e., its thickness, but these two are independent. Viscosity changes drastically, when there is a change in temperature.

The viscosity of oil is one of the main factors which govern oil absorption and drainage. The higher is the oil viscosity, the slower is the oil drainage. Oil viscosity depends on oil type as well as frying temperature and oil quality. Viscosity of vegetable oils measured from room temperature up to maximum of 130 ℃ using a glass capillary viscometer. Correlations were developed for viscosity with temperatures using empirical equations as well as relating fatty acid composition to the viscosity change. These studies give valuable information on the effect of temperature on oil degradation but fail to develop models which can be used as predictors of oil viscosity, especially at the frying temperatures. Viscometer and Rheometer are the most commonly used equipment's to measure oil viscosity at high temperatures.

1.14.2.5- Density

Density is an important factor which influences oil absorption as it affects the drainage rate after frying and also the mass transfer rate during the cooling stage of frying . Density has been experimentally shown to be linearly dependent on temperature However, there is no mathematical equation to predict the effect of temperature on density, especially at high temperatures at which frying is conducted. Frying occurs at temperatures around 180° C; however the densities of commonly used oils are known till around 110- 140° C Hence to accurately determine transport rates, density need to be determined and modeled at the frying temperatures. A liquid pycnometer is the most commonly used methods to determine density of a liquid at temperatures above room temperature. Both the methods involve use of glass apparatus (morar, 2013).

1.14.2.6- Specific gravity (SG)

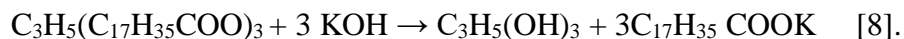Is the ratio of the density of a substance to the density of a reference substance equivalently, it is the ratio of the mass of a substance to the mass of a reference substance for the same volume.

1.14.2.7- Iodine Value

1.14.2.7.1- Principle

The iodine value (or iodine number) is a measure of degree of unsaturation, which is the number of carbon–carbon double bonds in relation to the amount of fat or oil. Iodine

value is defined as the grams of iodine absorbed per 100 g of sample. The higher the amount of unsaturation, the more iodine is absorbed and the higher the iodine value.

A common practice is to determine iodine value from the fatty acid composition. The calculated iodine value is not meant to be a rapid method, but instead gives two results (iodine value of triacylglycerols and FFAs) from one analysis (fatty acid composition).

$$ICl\ (excess) + R - CH = CH - R \rightarrow R - CHI - CHCl - R + ICL(remaining)\ [4]$$

$$ICl + 2KI \rightarrow KCl + KI + I_2 \qquad\qquad [5]$$

$$I_2 + starch + 2Na_2S_2O_3 \rightarrow 2NaI + starch + Na_2S_4O_6\ \ [6]$$

$$\text{(Blue)} \qquad\qquad \text{(Colorless)}$$

Calculated iodine value is obtained from fatty acid composition using Equation [7] for triacylglyc- erols. A similar equation allows calculation of the iodine value of FFAs.

Iodine value (triglycerides) = (% hexadecenoic acid $\times 0.950$) + (% octadecenoic acid $\times 0.860$) + (% octadecadienoic acid $\times 1.732$) + (% octadecatrienoic acid $\times 2.616$) + (% eicosenoic acid $\times 0.785$) + (% docosenoic acid $\times 0.723$)   [7]

1.14.2.7.2- Applications

Iodine value is used to characterize oils, follow the hydrogenation process in refining, and as an indication of lipid oxidation, since there is a decline in unsaturation during oxidation. The calculated value tends to be low for materials with a low iodine value and for oils with greater than 0.5% unsaponifiable.

1.14.2.8- Saponification Value

1.14.2.8.1- Principle

The saponification value (or saponification number) is defined as the amount of alkali necessary to saponify a given quantity of fat or oil. It is expressed as the milligrams of KOH required to saponify 1 g of the sample. The saponification value is an index of the mean molecular weight of the triacylglycerols in the sample. The mean molecular weight of the triacylglycerols may be divided by 3 to give an approximate mean molecular

weight for the fatty acids present; the smaller the saponification value, the longer the average fatty acid chain length.

$$C_3H_5(C_{17}H_{35}COO)_3 + 3\ KOH \rightarrow C_3H_5(OH)_3 + 3C_{17}H_{35}\ COOK \quad [8].$$

Stearin   Glycerol   Potassium stearate

The fractional molecular weight of each fatty acid in the sample must be determined first by multiplying the fatty acid percentage (divided by 100) by its molecular weight. The mean molecular weight is the sum of the fractional weights of all the fatty acids in the sample.

Saponification value = mg KOH per g of sample

$$= \frac{3 \times 56.1 \times 1000}{(\text{mean molecular weight} \times 3) + 92.09 - (3 \times 18)} \ldots\ldots[8]$$

where:

3 = the number of fatty acids per triacylglycerol

56.1 = M W of KOH (gmol)

1000 = conversion of units (mg/g)

92.09 = MW of glycerol (g/mol) 18 = MW of water (g/mol)

1.14.2.8.2- Applications

The calculated saponification value is not applicable to fats and oils containing high amounts of unsaponifiable material, FFAs (>0.1%), or mono- and diacylglycerols (>0.1%).

1.14.2.9- Unsaponifiable matter

Can be defined as the material present in oils and fats which after saponification of the oil or fat by caustic alkali and extraction by a suitable solvent remains non- volatile on drying at 80˚C.

The unsaponifiable matter includes hydrocarbons, higher alcohols and srerols (e.g. cholestrol, phytosterol). Most oils and fats of normal purity contain less than 2% of unsaponifiable matter.

1.14.2.10- Free Fatty Acids and Acid Value

1.14.2.10.1- Principle

Measures of fat acidity normally reflect the amount of fatty acids hydrolyzed from triacylglycerols.

FFA is the percentage by weight of a specified fatty acid (e.g., percent oleic acid). Acid value (AV) is defined as the mg of KOH necessary to neutralize the free acids present in 1 g of fat or oil. The AV is often used as a quality indicator in frying oils, where a limit of 2 mg KOHg oil is sometimes used. In addition to FFAs, acid phosphates and amino acids also can con- tribute to acidity. In samples containing no acids other than fatty acids, FFA and acid value may be converted from one to the other using a conversion factor Equation [9]. Acid value conversion factors for lauric and palmitic are 2.81 and 2.19, respectively.

$$\% \text{ FFA (as oleic)} = 1.99 \text{ acid value} \qquad [9]$$

Sometimes the acidity of edible oils and fats is expressed as milliliters of NaOH (of specified normality) required neutralizing the fatty acids in 100 g of fat or oil.

1.1.14.2.10.2- Applications

In crude fat, FFA or acid value estimates the amount of oil that will be lost during refining steps designed to remove fatty acids. In refined fats, a high acidity level means a poorly refined fat or fat breakdown after storage or use. However, if a fat seems to have a high amount of FFAs, it may be attributable to acidic additives (e.g., citric acid added as a metal chelator) since any acid will participate in the reaction. If the fatty acids liberated are volatile, FFA or acid value may be a measure of hydrolytic rancidity.

1.1.14.2.11- Ester value

The ester value is the number of mg of potassium hydroxide (KOH) required saponifying the esters in 1 g of a sample.

1.1.14.2.12- Peroxide Value

1.1.14.2.12.1- Principle

Peroxide value is defined as the milliequivalents (mEq) of peroxide per kilogram of sample. It is a redox titrimetric determination. The assumption is made that the compounds reacting under the conditions of the test are peroxides or similar products of lipid oxidation.

1.1.14.2.12.2- Applications

Peroxide value measures a transient product of oxidation, (i.e., after forming, peroxides and hydroperoxides break down to form other products). A low value may represent either the beginning of oxidation or advanced oxidation, which can be distinguished by measuring peroxide value over time or by using a procedure that measures secondary products of oxidation. For determination in foodstuffs, a disadvantage of this method is the 5 g fat or oil sample size required; it is difficult to obtain sufficient quantities from foods low in fat. This method is empirical and any modifications may change results. Despite its drawbacks, peroxide value is one of the most common tests of lipid oxidation. High-quality, freshly deodorized fats and oils will have a peroxide value of zero. Peroxide values >20 correspond to very poor quality fats and oils, which normally would have significant off flavors. For soy- bean oil, peroxide values of 1–5, 5–10, and >10 correspond to low, medium, and high levels of oxidation, respectively.

1.1.14.2.13- Fatty Acid Composition and Fatty Acid Methyl Esters

The fatty acid composition, or fatty acid profile, of a food product is determined by quantifying the kind and amount of fatty acids that are present, usually by extracting the lipids and analyzing them using capillary GC.

1.1.14.2.13.1- Principle

To increase volatility before GC analysis, triacylglycerols are typically esterified to form FAMEs. Acyl lipids are readily transesterified using base such as sodium hydroxide and methanol.

Determination of the fatty acid composition of a product permits the calculation of the following categories of fats that pertain to health issues and food labeling: percent saturated fatty acids, percent unsaturated fatty acids, percent monounsaturated fatty acids, percent polyunsaturated fatty acids and percent *trans* isomer fatty acids. Calculation of fatty acids as a percentage is referred to as normalization, that is, the areas of all of the FAMEs are summed and the percent area of each fatty acid is calculated relative to the total area. This is a reasonable procedure because with flame ionization detectors (FID), the weight of fatty acids in a mixture closely parallels the area on the chromatogram. However, this is not absolutely correct. Theoretical correction factors are needed to correct for the FID response, which is different depending on the level of unsaturation in FAMEs. A chromatogram showing separation of FAMEs of varying length and unsaturation is shown in The separation of FAMEs on this SP2560 column is typical of what is seen when using a highly polar (biscyanopropyl polysiloxane) column.

The separation of FAMEs on GC columns depends on the polarity of the liquid phase. On nonpolar liquid phases [such as 100% dimethyl polysiloxane (DB-1, HP-1, CPSil5CB) or 95% dimethyl, 5% diphenyl polysiloxane (DB5, HP5, CPSil8 CB)], FAMEs are separated largely based on their boiling points. This results in the elution order $18:3n\ 3 > 18:3n$

$6 > 18:1n\ 9 > 18:0 > 20:0$. On phases of medium polarity [such as 50% cyanopropylphenyl polysiloxane (DB225, HP225, CPSil43CB)], the order of elution is changed because of the interaction of the pi electrons of the double bonds with the liquid phase. The order of elution on these columns would be $18:0 > 18:1n\ 9 > 18:2n\ 6 > 18:3n\ 3 > 20:0$ (first eluted to last). When the polarity of the liquid is increased further with 100% biscyanopropyl polysiloxane columns (SP2560, CPSil88), the greater interaction of the double bonds with the very polar liquid phase results in an elution pattern $18:0 > 18:1n$

$9 > 18:2n\ 6 > 20:0 > 18:3n\ 3$. As the liquid phase polarity increases, the effect of double bonds on retention time increases. Additionally, *trans* fatty acids interact less effectively with the liquid phase than *cis* acids for steric reasons, so *trans* acids will elute before the corresponding *cis* acid; see Fig. 8-5 where $18:1\Delta9$ *trans* (elaidate)

elutes before 18 : 1Δ9 (oleic acid) and 18 : 2Δ9 *trans*, Δ12 *trans* (linoelaidate) elutes before linoleic acid (18 : 2n 6, 18 : 2Δ9 *cis* Δ12 *cis*) on this highly polar 100% biscyanopropyl polysiloxane column. It also can be seen that gamma linolenic (18:3n 6) elutes before linolenic acid (18: 3n 3). Because the double bonds are closer to the methyl side of the FAMEs in 18: 3n 3, the double bonds can more effectively interact with the liquid phase, resulting in greater retention by the column.

The complexity of the fatty acids found in various foods will affect the details of the GC analysis that can be used. Analysis of FAMEs of a vegetable oil is quite simple and can easily be accomplished in less than 20 min using a column with a medium polarity liquid phase. The fatty acids present in most vegetable oils range from C14 to C24. Coconut and palm kernel oils also contain shorter chain fatty acids such as C8–C12. Dairy fats contain butyric acid (C4) and other short chain fatty acids whereas peanut oil contains C26 at around 0.4–0.5% of the total FAMEs. Marine lipids contain a much wider range of fatty acids and require care in the separation and identification of FAMEs, many of which have no commercially available standards.

Trans fatty acids in foods originate from three main sources: biohydrogenation in ruminants, incomplete hydrogenation in the conversion of liquid oil to plastic fats, and high-temperature exposure during deodorization. The *trans* fatty acids formed from these three processes are quite different and require careful attention to achieve accurate analysis.

Separation of *trans* FAMEs is facilitated by selec- tion of the most polar column phases available. Currently, Sulepco SP2560 and Chrompak CPSil88 are most often used for analysis of *trans* fatty acids. These columns have liquid phases based on 100% biscyanopropyl polysiloxane. Even with optimized temperature programming and column selection, resolution of *trans* isomers from partially hydrogenated vegetable oil mixtures is incomplete and facilitated by use of a Fourier transform infrared (FTIR) detector or mass spectrometer (Nielsen, 2010)

Antioxidants

Food antioxidants are compounds that increase the resistance of fats to oxidation and consequent deterioration or rancidity. Only specifically approved antioxidants are able to be added to lard, tallow, and other foods susceptible to rancidity. In the United States,

authorization of antioxidants for use in meat products is a responsibility of the Food Safety Inspection Service of the United States Department of Agriculture (USDA). Antioxidants used in dairy products, salad dressings and oils are regulated by the Food and Color Additives Division of the U. S. Food and Drug Administration (FDA). Antioxidant inclusion is restricted to specific limits and must be declared on product labels. Some of the approved antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and tocopherols.

These primary antioxidants are often used in combination with citric or phosphoric acid. Use of one or more of the primary antioxidants in combination with one of the acids is common because combinations are much more effective than single antioxidants. Many food grade combinations are possible. It is important to note that antioxidants cannot be expected to stop rancidity. Their effectiveness lies only in slowing down the rate of oxidation and varies with the antioxidant combination used and with the food product to be protected.

Natural antioxidants, such as those contained in some spices, such as rosemary, sage, and marjoram, have met acceptance for the retardation of rancidity in meat products. These and other natural antioxidants not only retard the warmed over flavor in precooked meat products, but provide an agreeable aromatic aroma and flavor. Some spice extracts, particularly rosemary, are prepared primarily for their antioxidant activity and do not include strong flavor components.

Natural antioxidants from fruit products, including but not limited to pear and plum extracts, have also been shown to effectively reduce oxidative rancidity in ground meat products while providing additional sources of nutrients and flavor.

Many studies showed that *N. sativa* oil or TQ has antioxidant activity and increases the activities of antioxidant enzymes such as superoxide dismutase, catalase glutathione peroxidase. And antioxidant enzymes are clearly related to cancer- mostly their increased activities are beneficial against different types of cancer. Administration of *N. sativa* oil or TQ can lower the toxicity of other anti-cancer drugs (for example, cyclophosphamide) by an up-regulation of antioxidant mechanisms, indicating a

potential clinical application for these agents to minimize the toxic effects of treatment with anticancer drugs.

1.1.15- literature review

There is significant genetic variation and quantifiable differences in chemical and nutrient composition among *N. sativa* seed chemotypes of Egyptian, Iranian, Syrian, and Turkish origin (Ottai, 2012). Such differences may also exist in chemotypes found within a single country. In India, for example, samples of *N. sativa* seed obtained from 10 different states showed significant variation in chemical constituents and morphology (Shariq *et al.*, 2015).

The primary constituents in *N. sativa* seed are fatty oils (30-42%), mainly glycerol esters of linoleic, oleic, and palmitic acids, and aliphatic hydrocarbons, arachidonic acid (0.01-0.4%), γ-linolenic acid (0.1-1%), and tocopherols (about 170 mg/kg). The seed also contains essential oil (0.4-2.5%), which is composed mainly of monoterpenes, including p-cymene, thymoquinone, a-pinene, and carvacrol. The chemical nature of the constituent's nigellone and nigellin remains unclear. *Nigellone*, described as a component of the essential oil, is possibly a polymer of thymoquinone, while nigellin has been described as an alkaloid. There are also traces of isoquinoline alkaloids (nigellicine and nigellimin-N-oxide), an indazole-type alkaloid (nigellidine-4-*O*-sulfite), and dolabellane-type diterpene alkaloids (nigellamines A1 to A5, B1, B2, and C) (Blaschek*, et al.,* 2016). Analysis of essential oil composition can distinguish *N. sativa* and *N. damascena*. The essential oil of *N. damascena*, which is used in perfumery, contains approximately 8-10% of damascenine, a blue-fluorescing alkaloid. *Nigella sativa* essential oil does not contain this compound and therefore shows no fluorescence.

In vivo and in vitro studies have shown *nigella* seed powder and oil to have antibacterial, antifungal, antihistaminic, antihypertensive, anti-inflammatory, antinociceptive, antioxytocic, antiparasitic, antiviral, diuretic, hematological, hepatoprotective, hypoglycemic, immunopotentiating, wound-healing, and respiratory-stimulant properties (Shariq 2015 and Gali-Muhtasib *et al.,* 2006).

At least 38 clinical studies have investigated *N. sativa* seed and seed oil for their efficacy for various conditions, including respiratory, diabetic, hepatic, metabolic, mental, and dyspeptic disorders, male infertility, and others.

One (2008), RDBPC study, published in 2008, evaluated the effects of a boiled aqueous extract of *nigella* seed (NS; 50 mg/mL; no additional information provided) on subjects with respiratory symptoms including chest tightness, breathlessness, cough, and wheezing as a result of chemical inhalation. The participants (N = 40) were an average of 48.2 ± 11.91 years old and the exposure had occurred 18-20 years previously. Each participant drank either the *NS* extract (0.375 mL/kg of body weight) or a placebo solution daily for two months. Participants received medical exams wherein respiratory symptoms were measured at baseline, 30 days, and the end of the intervention. There were no significant differences between groups at baseline. Significant improvements in all symptoms were seen in the *NS* group at visits two (day 30) and three (day 60) compared to baseline, except for morning wheeze and cough at day 30. All symptoms were significantly improved by the end of the study compared to day 30 in the *NS* group. Additionally, all symptoms were significantly reduced in the NS group at day 30 and the end of the study compared to placebo (Bashabady and Farhadi, 2008).

Six additional studies have investigated the effects of *Nigella* on respiratory issues. Three of these studies addressed allergic rhinitis: One study found no significant improvement with 0.6-0.8 mg/kg NSO three times daily over six weeks (Alsamarai and Alabaidi, 2012); one found significant improvement in symptoms over one month with 2 g NS once daily (Isik *et al.,* 2010); and one claimed improvement in symptoms with NSO but did not specify the dosage (Nikakhlagh, 2011). Another study on asthma, which tested 1 and 2 g/day of NS, found that both doses resulted in significant improvements in markers at six and 12 weeks compared to baseline (Salim *et al.,* 2017). One study on lower respiratory tract illness in children reported significant improvement compared to baseline in pulmonary index scores and some improvement in peak expiratory flow rate with daily administration of NSO (0.1 mL/kg of body weight) over 14 days(Ahmed *et al.,* 2010). Finally, one study that investigated the effect of a NSO nasal spray (22 mg cold-pressed NSO/25 mL spray) on nasal symptoms in elderly patients for two weeks

found that nasal dryness, obstruction, and crusting improved significantly with NSO treatment compared to a saline solution (Oysu *et al.,* 2014).

At least two studies have assessed the effects of *Nigella* on patients with rheumatoid arthritis (RA). In one RDBPC study from 2014, patients with RA (N = 42) were randomly assigned to receive either 500 mg NSO (produced by Barij Essence Pharmaceutical Co.; Kashan, Iran; soft gel capsules containing 500 mg of cold-pressed NSO) or placebo (paraffin) twice daily for eight weeks. Blood samples were taken at baseline and the end of the study to measure markers of inflammation (serum tumor necrosis factor alpha [TNF$^-$] and interleukin 10 [IL-10]) and oxidative stress (serum malondialdehyde [MDA] and nitric oxide [NO]). The NSO group experienced a significant increase in serum IL-10, and a significant decrease in serum MDA and NO, compared to placebo. No significant changes in other biomarkers were observed between or within groups. Nevertheless, these results suggest that NSO could be a valuable adjunct therapy in RA, as it improves certain markers of inflammation and oxidative stress in patients (Hadi et al., 2016).

Another placebo-controlled study, published in 2012, investigated the effects of *Nigella* in female patients with RA. After taking starch-filled placebo capsules twice daily for one month, subjects (N = 40) took 500 mg of cold-pressed NSO twice daily for an additional month. Investigators reported significant improvements in the patients' Disease Activity Scores (a clinician-rated measure of joint swelling and tenderness) compared to ratings taken both before and after the one-month placebo period (Gheita and Knawy, 2012).

At least two studies have investigated the impact of *Nigella* on mental and cognitive health. In one 2013 RDBPC clinical trial, 40 healthy elderly volunteers were randomly assigned to take 500 mg crushed and encapsulated *NS* (no additional information provided) or placebo twice daily for nine weeks. Compared to baseline, the test group experienced significant differences in scores on various measures of memory, attention, and cognition, including logical memory tests I and II, a digit span memory assessment (total score), and the Rey-Osterrieth complex figure test (30-minute delayed recall and percent score). There were also significant differences compared to baseline in the time taken to complete a letter cancellation test and trail-making tests A and B. The authors

suggested that additional studies should be undertaken with large populations of patients with Alzheimer's disease over a longer period of time to determine if NS can enhance memory, attention, and cognition in that population (Binsayeed *et al.,* 2013).

Another RDBPC study explored the effectiveness of *Nigella* in treating mood, *anxiety*, and cognition. Young men (N = 48; 14-17 years old) were randomly assigned to take 500 mg crushed and encapsulated NS (no additional information provided) or placebo daily for four weeks. Compared to baseline, there was a statistically significant improvement in scores on the Bond-Lader visual analog scale (a measure of mood) for the NS group, but no statistically significant difference between the NS group and placebo group. There was also a statistically significant decrease in State-Trait Anxiety Inventory scores in the treatment group over four weeks compared to placebo. Both immediate free recall and delayed recall improved significantly over four weeks in the treatment group only. The authors postulated that cognition may have improved due to the improvements in anxiety and mood, and suggested that further long-term studies are warranted (Binsayeed *et al.,* 2014).

A 2016 RDBPC study examined the effect of *Nigella* on Hashimoto's thyroiditis, an immunological condition that impacts the thyroid gland. Patients with Hashimoto's (N = 40) were randomly assigned to take 2 g of ground NS (prepared by Goldaru Pharmaceutical Co.; Isfahan, Iran; no additional information provided) daily or placebo. The NS group experienced significantly reduced body weight, body mass index (BMI), and hip and waist circumference over eight weeks compared to the placebo group. Additionally, serum concentrations of thyroid stimulating hormone (TSH) and anti-thyroid peroxidase (anti-TPO) antibodies decreased in the NS group over eight weeks, while serum triiodothyronine (T3) increased. While there was no change in the concentration of nesfatin-1 (a neuropeptide involved in the regulation of hunger and fat storage) during the study, the authors noted that changes in anthropometric variables (weight, BMI, and hip and waist circumference) and thyroid hormones (TSH, anti-TPO, and T3) are often significant predictors of changes in nesfatin-1 concentrations (Frhangi *et al.,* 2016).

In a 2014 randomized, double-blind, controlled trial, NSO (verified seeds, dried, ground, and extracted with 96% ethanol, which was later evaporated) was compared to fish oil for the treatment of vitiligo, a condition characterized by a loss of skin pigmentation. Patients with vitiligo (N = 52) were randomly assigned to apply NSO or fish oil on lesions twice daily for six months. By the end of the study, Vitiligo Area Scoring Index scores had improved significantly in the NSO group compared to the fish oil group. There were no significant changes in VASI scores between groups in the first three months; not until the fourth month of the study did significant improvement appear in the NSO group (Gorbanibirgani *et al.,* 2014).

A 2016 randomized, triple-blind, placebo-controlled trial investigated the effectiveness of NSO in treating cyclic mastalgia. Female patients diagnosed with cyclic mastalgia (N = 156; 25-45 years old) were randomly assigned to apply twice daily 2 g of NSO gel (a gel base combined with cold-pressed NSO; Barij Essence Pharmaceutical Co.; Kashan, Iran), 20 mg of topical diclofenac, or a placebo gel for two menstrual cycles. There were no significant differences in patient characteristics or baseline pain scores between groups. Pain scores in active treatment groups also did not differ significantly at cycles one and two. However, both active treatment groups experienced a significant decrease in pain scores by the end of the study compared to baseline and placebo. The authors posited that thymoquinone, unsaturated fatty acids, and carvacrol may play a part in NSO's effectiveness for treating cyclic mastalgia. They also stated that the main shortcoming of the study was the lack of follow-up, which might have been helpful since recurrence of cyclic mastalgia is common (Huseini *et al.,* 2016).

Two outcomes from a single RDBPC study were published in 2015 and 2016 on NSO and cardiovascular risk, and NSO and inflammation, respectively. Obese women (N = 84; 25-50 years old) took 3 g per day of cold-pressed NSO (Dana Co.; Tabriz, Iran) or placebo and followed a low-calorie diet for eight weeks. In the cardiovascular-risk arm of the study, the women in the NSO group experienced significant decreases compared to baseline in weight, waist circumference, and levels of triglycerides and very-low-density lipoprotein (VLDL). The authors suggested that NSO supplementation combined with a low-calorie diet may reduce cardiometabolic risk factors in obese women, but they noted that more studies are needed to assess the efficacy of NSO as a complementary therapy

(Mahdavi *et al.*,2014) high-sensitivity C-reactive protein compared to placebo. No significant changes were seen in IL-6 levels. Based on these findings, the authors concluded that NSO supplementation and calorie-restriction may modulate systemic inflammatory biomarkers in obese women but that more studies are needed to clarify the findings.In the second arm of the study, NSO significantly decreased serum levels of TNF$^-$ (Mahdavi *et al.,* 2015).

A 2015 study assessed the effects of the previously mentioned NSO preparation on oxidative stress in obese women (N = 50; 25-50 years old). Investigators reported significant weight loss in the NSO group compared to the placebo group after eight weeks. Additionally, significant changes in superoxide dismutase (SOD, a natural antioxidant enzyme) occurred in the NSO group compared to the placebo group (Namazi *et al.,* 2015).

Two studies of varying quality have been conducted on *Nigella* for functional dyspepsia. In one RDBPC study, 70 patients diagnosed with functional dyspepsia took either a traditional formula consisting of 5 mL cold-pressed NSO, mineral oil, and honey (Barij Essence Pharmaceutical Co.; Kashan, Iran) or placebo daily for eight weeks. In both groups, significant decreases in dyspepsia severity scores as measured by the Hong Kong index of dyspepsia severity were seen in the second, fourth, and eighth weeks, but mean scores and the rate of *Helicobacter pylori* infection for the NSO group were significantly lower than in the placebo group at the end of the study.

A 2015 meta-analysis and systematic review assessed 17 randomized, controlled trials that examined the effects of *N. sativa* on plasma lipid concentrations. The authors suggested that there was a significant association between NS supplementation and reduction in total cholesterol and triglyceride levels. No significant effects were seen on high-density lipoprotein (HDL) cholesterol levels. NSO performed better than NS powder in lowering total serum cholesterol and low-density lipoprotein (LDL) cholesterol, but only NS powder was found to increase HDL cholesterol. The authors recommended that further randomized, controlled trials are needed to explore *nigella's* benefits for cardiovascular health (Sahebkar *et al.,* 2016).

Many studies assessed approximate analysis of *Nigella* seeds showed that crude protein (20.8%), moisture content (7.0%) and total carbohydrates (33.7%) while, ash content (3.7%) and extractable lipid (>30%) of the seeds were lower than those previously reported (Atta 2003). Cheick *et al,.(2007)* determined physicochemical properties of two *Nigella* seed varieties, having a Tunisian and Iranian origin and results suggested that *Nigella* seed oil could deserve further consideration and investigation as a potential new multi-purpose product for industrial, cosmetic and pharmaceutical uses. Salem *et al.,* (2017). Abbas studies the physiochemical studied and chemical composition of the extracted fixed oil and volatile oil of *Nigella sativa* L. seeds grown in variation regions.

Anther studies carried to *Nigella sativa* oil to determine physicochemical properties Abdel-Aal and Attia (1993), Argon (2016), Abdel-Aal and Attia (1993), Gad *et al.* (1963), Ustun *et al.* (1990).

The percentage of thymoquinone reported by Solati (2014).

A (2008) anther study showed the compression between antibacterial activity for *Nigella sativa L.* seed essential oils obtained by hydrodistillation (HD), dry steam distillation (SD), steam distillation of crude oils obtained by solvent extraction (SE-SD), and supercritical fluid extraction (SFE-SD) were tested for their antibacterial activities, using the broth microdilution method and subsequently analyzed by gas chromatography and gas chromatography– mass spectrometry. The results showed that the essential oils tested differed markedly in their chemical compositions and antimicrobial activities. The oils obtained by HD and SD were dominated by p-cymene, whereas the major constituent identified in both volatile fractions obtained by SD of extracted oils was thymoquinone (ranging between 0.36 and 0.38 g/ml, whereas in oils obtained by HD and SD, it constituted only 0.03 and 0.05 g/ml, respectively). Both oils distilled directly from seeds showed lower antimicrobial activity (HD and SD), than those obtained by SE-SD and SFE-SD. All oil samples were significantly more active against gram-positive than against gram- negative bacteria. Thymoquinone exhibited potent growth-inhibiting activity against gram-positive bacteria (Kokoska *et al.,* 2008).

A (2005) suggested the apoptotic mechanisms behind the anti-proliferative effect of TQ (from *N. sativa*) on myeloblastic leukemia HL-60 cells. They reported that TQ induces apoptosis, disrupts mitochondrial membrane potential and triggers the activation of caspases 8, 9 and 3 in HL-60 cells. The apoptosis induced by TQ was inhibited by a general caspase inhibitor, z-VAD-FMK; a caspase-3-specific inhibitor, z-DEVD-FMK; as well as a caspase-8-specific inhibitor, z-IETD-FMK. Moreover, the caspase-8 inhibitor blocked the TQ-induced activation of caspase-3, PARP cleavage and the release of cytochrome c from mitochondria into the cytoplasm. In addition, TQ treatment of HL-60 cells caused a marked increase in Bax/Bcl2 ratios due to upregulation of Bax and down regulation of Bcl2 proteins. Their results indicated that TQ-induced apoptosis is associated with the activation of caspases 8, 9 and 3; with caspase-8 acting as an upstream activator and activated caspase-8 initiates the release of cytochrome c during TQ-induced apoptosis. TQ action was also found as pro-apoptotic against colon cancer cell (Elmahdy *et al.,* 2005).

A (2010) investigated the radio-protective potential of *N. sativa* crude oil against hemopoietic adverse effects of gamma irradiation. He found that irradiation resulted in significant reduction in hemolysin antibodies titers and delayed type hypersensitivity reaction of irradiated rats, in addition to significant leukopenia and significant decrease in plasma total protein and globulin concentration and depletion of lymphoid follicles of spleen and thymus gland. Furthermore, there was a significant increase in malondialdehyde concentration with a significant decrease in plasma activities. But oral administration of *N. sativa* oil before irradiation considerably normalized all the above- mentioned criteria; and produced significant regeneration in spleen and thymus lymphoid follicles. Thus *N. sativa* oil is recognized as a promising natural radio protective agent against immunosuppressive and oxidative effects of ionizing radiation (Assayed 2010).

2- Materials and Methods

2.1- Materials

The seeds of *Nigella sativa* and its oil extracted by mechanical pressing were purchased from Omdurman market in Khartoum (Sudan) during December 2017. The plant material was authenticated by the taxonomist Yahya Suliman Mohamed at herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), National center for Research, Khartoum, Sudan, and voucher herbarium samples were deposited there for further reference .

2.2- Methods

2.2.1- Proximate analysis

Determination of moisture, protein, fibre, and ash were carried out in triplicate, while the oil content was in duplicate. All proximate results were expressed as a percentage of the weight of samples analyzed.

2.2.1.1- Moisture content

The moisture content was determined by moisture analyzer- MAC 50/1/WH at 110ºC for 15 minutes.

2.2.1.2- Protein content

The nitrogen content was estimated according to Pearson (1970). 2 g of sample was weighted and transferred to a Kjeldahl digestion flask. (1 g) of catalyst mixture (90% anhydrous sodium sulphate and 10% copper sulphate) were added followed by 3 ml of

conc. sulphuric acid. The flask was heated gently, cooled and digested into distilling flask with 15 ml of NaOH 40% solution. (50 ml) of boric acid solution and 3 drop of methyl red were placed in receiving flask. The distillation apparatus was connected up with delivery tube dipping below the boric acid solution. The tap was closed and the ammonia distilled into the boric acid solution. The distillate solution was titrated against 0.1 N HCL. The percentage of nitrogen was calculated:

1 ml 0.1 N = 0.00014 g N. and then crude protein was calculated using approximate factor: N × 6.25.

2.2.1.3- Crude fibre content

The crude fibre content was determined according to Pearson (1970) by extracting the sample with hexane in sohxlet apparatus. (5 g) of the extracted sample was dried in air and transferred to 500 ml conical flask. (200 ml) of 0.225 N sulphuric acid was added and brought to boiling point. The content was filtered using Buchner funnel while it was hot, washed with (200 ml) NaOH 0.313 N and boiled gently to boiling point for 30 minutes. Allowed to stand for 1 minute and then filtered through ash less dry weighted filter paper and washed with HCL 1%, hot water until free from acid and alcohol .Then dried at 105 ^{0}C to constant weight. The paper and content was ignited at 550 ^{0}C for 1 hour. The weight of the ash was subtracted from the increase of weight in the paper and the difference is reported as fiber.

-2.2.1.4Ash content

Determining the total ash was performed according to Pearson (1970), by weighted out (5 g) of the sample into platinum dish which had previously been ignited and cooled before weighed. Then the dish and content were ignited, first gently on fire and then on furnace at 550 ^{0}C. The dish and content were transferred to desiccators for 10 minutes .then the ash content was calculated as a percentage.

Ash content % $= \dfrac{\text{weight of ash (g)}}{\text{Weight of sample (g)}}$ 100%

2.2.1.5- Oil content

5g of *Nigella sativa* powder was weighted, the sample were placed into sealed of Soxhlet, then hexane was added to it, heated under reflux to six an hours, after that; the solvent was evaporated by rotator then dried the oil and weighted. The weights were recorded and percentage of oil was calculated as:

$$\text{Oil content\%} = \frac{\text{weight of oil(g)}}{\text{Weight of seed(g)}} \; 100\%$$

2.2.1.6- Carbohydrates

The content of carbohydrates was calculated as a percentage by difference.

Carbohydrates   = 100 – (Oil % + Ash% + Moisture% + Protein% + Fibre %).

2.2.2- Physiochemical properties

2.2.2.1- Extraction of oil by solvent

The seeds of *Nigella sativa* were grounded; using a mechanical grinder, about 300 g of powder was put in soxhlet extractor using hexane as a solvent to eight hours. The product was collected in round bottom flask and the solvent was evaporated. The oil was filtered and kept in dark and cold place.

2.2.2.2- Determination the Color (Lovinod method)

The color was determined by Tintometer type D (made in Sali Bury England). 2 ml from *Nigella sativa* oil was placed in a standard- sized glass cell and visually compared with red, yellow, blue, and neutral color standards. Results were expressed in terms of the numbers associated with the color standards.

2.2.2.3- Determination of refractive index

Refractive index was determined (at 32 °C) with a refractometer (Bellingham & Stanliy lond, N 918095- England).

2.2.2.4- Determination of Density and specific gravity

An improved specific gravity bottle was washed and rinsed with acetone and dried in an oven. The bottle was cooled at room temperature in a desiccators and the weight of empty bottle was determined using an electronic weighting balance. The weight of

bottle filled with water was recorded, then the water poured out and the bottle rinsed with acetone and was dried in the oven. The procedure was repeated with the *Nigella sativa* oils (NMP and NSE).

2.2.2.4.1- Determination of density

The density of *Nigella sativa* oil was determined according to the formula:

$$\rho = \frac{W2 - W1}{V}$$

Where:

ρ density.

W_2= weight of bottle and oil.

W_1= weight of oil.

V= volume of oil.

2.2.2.4.2- Determination of specific gravity

Specific gravity for *Nigella sativa* oils were calculated as:

$$\text{Specific gravity} = \frac{weight\ of\ oil(g)}{Weight\ of\ water(g)}$$

2.2.2.5- Determination the viscosity

Viscosity of NSE and NMP oils was determined by used Ostwald Viscometer. The viscometer was washed by water and ethanol then dried in oven; the viscometer was filled with the water, clicked the start button and simultaneously clicked the start button on stop watch, when the water was reached label (D), then the flow time on the stop watch was noted and 25 ml of water was weighted in digital balance, viscosity of water was calculated, this method was repeated with NMP and NSE. The viscosity of oils was calculated used water as reference liquid by the relation below:

$$= \frac{\eta}{\eta_r} = \frac{m \times t}{m_r \times t_r}$$

Where:

η : viscosity coefficient of oil (Cp).

$η_r$: viscosity coefficient of water(Cp).

m : mass of oil(g).

m_r: mass of water(g).

t: time flow of oil(sec).

t_r: time flow of water(sec).

2.2.2.6- Determination of Acid value and FFA

2 g of *Nigella sativa* oil was put in conical flask, 50 ml of ethanol was added to it. About 3 drops of phenolphthalein were added to solution, and then titrated against (0.1 N) Sodium Hydroxide with constantly shaking until a pink color which persists for 15 sec was obtained, the volume consumed from Sodium Hydroxide was recorded. Then 50 ml of ethanol was put in conical flask added 3 drops from phenolphthalein as indicator was added then titrated versus (0.1 N) sodium hydroxide, and used as blank. The acid value was calculated as:

$$\text{Acid value} = \frac{(b-a) \times 0.1 \times 40}{W(g)}$$

Where:

(b) is the volume of sodium hydroxide consumed to titration the blank.

(a) is the volume of sodium hydroxide consumed to titration the sample.

W (g) is weight of sample by gram.

40 is equivalent weight for Sodium Hydroxide.

Free fatty acid was usually calculated as oleic acid (1 ml of 0.1 N 0.0282 g oleic acid, i.e. the acid value = 1.99 FFA.

2.2.2.7- Determination of Peroxide value

2 g of *Nigella sativa* oil was put in conical flask, 15 ml of Acetic acid glacial and 10ml of triChloromethane (Chloroform) were added to it. 1ml of saturated Potassium Iodide was added. The solution was kept in dark placed to five minutes, after that 30 ml of distill water and three drops from starch 1% were added to it, then titrated versus (0.1 N)

Sodium thiosulphate until the end point. The volume of base was recorded. This experiment was repeated except addition of oil; the volume consumed was recorded and used as blank. The peroxide value was calculated according this relation:

Peroxide value = $\dfrac{(b-a) \times 0.01 \times 1000)}{W(g)}$

Where:

b: volume of (0.1N) sodium thiosulphate consumed by titration sample.

a: volume of (0.1N) sodium thiosulphate consumed by titration the blank.

W (g): weight of sample by gram.

2.2.2.8- Determination of Iodine value

Iodine value was determined by Wijs' method

-2.2.2.8.1Wiijs solution

8g of iodine trichloride was dissolved in200 ml of glacial acetic acid 9 ,g of iodine was dissolved in 300 ml of carbon tetrachloride, and the two solutions were mixed and diluted to 1000 ml with glacial acetic acid. A method for checking the iodine/ Chlorine ratio of Wijs' solution has been described

2.2.2.8.2- Method

The oil was put into small beaker, small rod was added, 2 g of *Nigella sativa* oils was weighted into dry glass-stoppered bottle of about 250 ml capacity. 10 ml of carbon tetrachloride was added to the oil and dissolved it, 20 ml of Wijs' solution was added, the stoppered was inserted (previously moistened with potassium iodide solution) and allowed to stand in the dark for 30 min. 15 ml of potassium iodide solution (10%) was added and 100 ml of water, the components were mixed and titrated with 0.1 Sodium thiosulphate solution using starch as indicator just before end point. The blank was carried out at the same time commeneing with 10 ml of carbon tetrachloride. The iodine value was calculated as:

Iodine value $\dfrac{(b-a) \times 0.1 \times 126.9}{\text{Weight of sample (g)}}$

Where:

(b) is volume of base consumed to titration the blank.

(a) is volume of base consumed to titration the sample.

126.1 is equivalent weight for iodine.

-2.2.2.9Determination of Saponification value

Alcoholic solution potassium hydroxide 20 g of potassium hydroxide was dissolved in 10 ml of water and diluted to 500 ml with alcohol (95%). The solution has been allowed to stand overnight and decanted off the clear liquid.

Method

1 g of *Nigella sativa* oil was weighted into round bottom flask; exactly 50 ml of alcoholic potassium hydroxide solution was added to it. A reflux condenser was attached and the flask was heated in boiling water for 1 hour, shaken frequently. 1 ml of phenolphthalein (1%) solution was added and titrated hot the excess alkali with 0.5 N hydrochloric acid. The blank was carried out at the same time. The volume of hydrochloric acid was recorded; the titrated liquid has been retained to determine the unsaponifiable matter. The saponification value was calculated according to:

$$\text{Saponification value} = \frac{(b-a) \times 0.5 \times 56.1}{W(g)}$$

Where as:

(b) volume of 0.5N HCl used to titration the blank.

(a) volume of 0.5N HCl used to titration the sample.

W(g): weight of sample by gram.

56.1: equivalent weight for potassium Hydroxide.

2.2.2.10- Determination of unsaponifiable matter

After the titration of saponification value, the liquid made the neutralized by added 1 ml of3 N potassium hydroxide solution. The liquid was transferred to a separator funnel and washed with water. The solution was extracted while still just warm 3 times with 60 ml quantities of trichloro methane. The extract was poured into separator containing 20 ml of water. After the third extract has been added, the combined of ether extracts were

shaken with 20 ml of water and then vigorously with two further 20 ml of quantities. The extracts were washed twice with 20 ml of aqueous 0.5 N potassium hydroxide solutions and at least twice with 20 ml quantities of water until the washed water was no longer alkaline to phenolphthalein. The extract was poured into a weighted flask, the solvent was evaporated off, and the residue was dried at^0 70 C and weighted, the weight was recorded and percentage of unsaponification was calculated .

2.2.2.11- DPPH radical scavenging assay

The DPPH radical scavenging was determined ,with some modification ,according to the method of shimada *et al* .(1992) ,.In-96 wells plate, the *Nigella sativa* oils were allowed to react with 2.2 Di-4) tert-octylphenyl-1-(picryl-hydrazyl stable free radial (DPPH) for half an hour at^0 37 C .The concentration of DPPH was kept as 300) µM). The *Nigella sativa* oils were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplated reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with DMSO treated control group. All tests and analysis were run in triplicate.

The absorbance values were measured at 517 nm and converted into percentage antioxidant activity using the following equation: % Inhibition =   100%

Where:

A_B: absorption of blank sample;

A_A: absorption of *Nigella sativa* oils

2.2.2.12- GC/MS for *Nigella sativa* oils

2.2.2.12.1- Sample preparation (Methylation)

2ml from *Nigella sativa* oils (NMP and NSE) were taken in to test tube, 7 ml from alcoholic Sodium Hydroxide that prepared by dissolve 2 g Sodium Hydroxide in 100 ml methanol were added to it, 7 ml from alcoholic Sulphuric acid 1% prepared by mix 1ml conc. Sulphuric acid plus 99 ml methanol were added to it, the mixture were shaken by vortex for three minutes, the content was leaved to overnight. 2 ml from supersaturated Sodium Chloride were added to it. 2 ml from normal hexane was added, shaken for three minutes and the hexane layer was collected. 2L from hexane collected was taken and

diluted it with 5ml diethylether.1g from Sodium Sulphate were added to it as drying agent. Through syringe filter 0.45 m were filtrated. The filtrate directly was transferred to the GC/MS vial. 1 ml directly was injected to the GC/MS.

2.2.2.12.2- GC/MS conditions

The qualitative and quantitative analysis of the *Nigella sativa* oils were carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from japans ˈSimadzu Companyˈ with serial number 020525101565SA and capillary column (Rtx-5ms-30×0.25mm×0.25µm). the *Nigella sativa* oil was injected by using split mode, helium as carrier gas passed with flow rate 1,61ml/min, the temperature program was started from 60 ^{0}C with rate 10^{0}C /min to 300 ^{0}C as final temperature degree with 2 minutes hold time, the injection port was 300 ^{0}C, the ion source temperature was 200^{0}C and the interface temperature was 250^{0}C. The *Nigella sativa* oil was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 26 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the National Institute of Standards and Technology (NIST). The results were recorded. This method using to analyze *Nigella sativa* oils extraction by two different methods (mechanical pressing and solvent extraction).

Peroxidability index

It means lipid peroxidation susceptibility, which is based on the number of unsaturatations present in the fatty acid chain. It was calculated on the basis of their free acid composition, according to the following formula:

PI = (% monoenoic × 0.025) + (% Dieonic × 1) + (% trienoic × 2) + (% tetraenoic × 4) + (% pentaenoic × 6) + (% hexaenoic × 8)

3- Results and discussion

3.1- Proximate analysis

Table (3.1): proximate analysis for *Nigella sativa* seeds

Percentage (MTS.D.)	Characteristics
39.4 ± 0.02	Oil content
6.52 ± 0.47	Moisture
21.78 ± 0.1	Crude Protein
4.75 ± 0.19	Ash content
8.56 ± 0.15	Fibre content
18.99	Total Carbohydrate

MTS.D = mean of triplicate samples T standard deviation.

Total carbohydrates were calculated by difference.

Proximate analysis of *nigella* seeds showed that crude protein (21.78%), moisture content (6.52%) and ash (4.75%) within the range reported in the literature and Indian specifications, while, carbohydrate (18.99%) of the seeds were lower than those previously reported in the literature, these differences may be related to the climate condition and variations of cultivated regions or geographical land zones.

Crude oil extracted from *Nigella* seed by cold press was lower than that gained by solvent extraction. This may be attributed to the greater ability of the organic solvent (compared to cold pressing) to extract most of the lipid-components, including

oleoresins that are present in *Nigella* seed (Atta, 2003 and salem, 2001). Nevertheless, these results conflict with those reported by Ustun et al. (1990), who found no difference between the amounts and characteristics of crude oils extracted from *Nigella* seed by two different methods (cold pressed and solvent extraction).

Crude oil extracted by hexane (NSE) as a solvent was higher than those extracted by ether as a solvent (according to Indian specifications for *Nigella sativa*). That may be due to the polarity of solvent, where as hexane was non polar solvent it could be extracting more lipid than polar solvent. Also, this result was higher than reported by Ali, and Atta, (2003) who extracted by different solvent (light petroleum ether). Also, this result was lower than these determined by Ebrahim, (1998) this difference due to who used hot petroleum ether.

Crude oil extraction by hot petroleum ether (boiling range 60 - 80˚C) is more efficient procedure that affords the oil in a higher yield 42.08% determined by Ebrahim, (1998).

3.2-Physiochemical properties

Table (3.2) Physical and chemical properties for *Nigella* seed oil as affected by extraction method

Sample(MTS.D.) Characteristics	NMP	NSE
Specific gravity at 25 °C	0.887± 0.005	0.909± 0.003
Density (g/cm^3)	0.9102± 0.002	0.9158± 0.02
Viscosity coefficient (Cp)	33.66	20.82
Refractive index at 32 °C	1.484± 0.001	1.476± 0.001
Color (º)	Red: 8.5 Yellow: 20.3 Blue: 1.3	Red: 7.3 Yellow: 20.3 Blue: 1.2
Iodine value (g of I$_2$/100 g of oil)	96.24± 0.84	89.25± 1.689
Saponification value (mg of KOH/g of oil)	183.16± 1.77	3.00±120.46
Acid value (mg of KOH/g of oil)	14.66±1.06	23.26± 0.07
Free fatty acids (%) as oleic	7.36 ± 0.8	11.73± 0.1
Ester value (mg of KOH/g of oil)	168.5	97.2
Unsaponifiable matter (g/100 g)	0.48±0.02	0.83± 0.07
Peroxide value (meq O$_2$/kg of oil)	6.43± 0.08	14.2± 0.7
Antioxidant activity	62± 0.05	90± 0.01

Values given are the mean ± standard deviation of three replications.

Color values were slightly different, This may be related to the ability of organic solvent to extract most lipid-soluble pigments and oleoresins present in *Nigella* seed.

Refractive index (RI) is related to the amount of saturation in a lipid; the refractive index of *Nigella sativa* extracted by mechanical pressing has higher than those extracted by solvent extraction (NSE) it was expected result due to refractive index increases linearly as iodine value (a measure of total unsaturation) increases. RI also is used as a measure of purity, this result agreement with peroxide value and the difference from oil extracted by two different methods may be related to heating.

Nigella sativa oils were found to be most unsaturated compared with *Menhaden* oil (RI = 1.472) and Coconut oil (RI = 1.448 – 1.450) have a relatively unsaturated lipid and a relatively saturated lipid respectively.

The viscosity coefficient of NMP oil was found higher than viscosity of NSE the expected result due to effect of tempereture.

Both Free fatty acid content and Peroxide value of oil are valuable measures of oil quality. The FFA content of mechanical pressing and solvent extracted crude oils were 7.36% and 11.73% (as oleic acid), respectively. These results are lower than those determined by Gad et al., (1963) and Ustun et al., (1990). The high acidity of oil may be related to the nature of *Nigella* seed, whereas many oil-bearing seeds, such as olive, palm and rice bran, contain high acidity oils (Atta, 2003). The acidity of *Nigella sativa* oil extracted by solvent was high acidity than those extracted by mechanical pressing this may be resulted of the enzyme lipase which is present in the raw seeds hydrolyzing the fatty acid ester. The oil extracted by mechanical pressing has low FFAs than those extracted by solvent extraction these results conflicted with Atta (2003) and indicated to the stability; shows it is edible and could have a long shelf life of mechanical press oil and arise of FFAs of oil extracted by solvent extraction may be related to heating.

The peroxide value of mechanical pressing (6.43 meq/ kg) and solvent extracted (14.2 meq/kg) nigella oils this expected result. The peroxide value of *Nigella sativa* extracted

by mechanical press was lower than those extracted by solvent extraction this indicated to purity of oil.

The result of peroxide value achieved by solvent extraction were found to be lower than those reported by Abdel-Aal and Attia (1993) and higher than those reported by Cheick *et al*. (2007), Salem (2001) and Atta and out of range specified by specification of Natural Sourcing specialists in cosmeceutical Ingredients.

Peroxide value of oil achieved by mechanical pressing were found lower than those reported by Atta (2003) and Argon (2016) these may be related to different cultivation region.

From the peroxide value, oils extracted by mechanical pressing and solvent extraction have medium and high levels of oxidation respectively.

The USM% of vegetable oils is considered to be natural antioxidants that are able to minimize oil oxidation during handling and storage. The estimated USM% of both mechanical pressing (0.55%) and solvent-extracted (0.83%) oils were found within range assigned by Aromatic Natural Skin care Specification and were slightly different than those reported by Gad et al. (1963) and lower than those reported by Atta (2003) and much lower than those reported by Ustun et al. (1990). These variations could be related to the diversity in maturity of seeds and the agricultural conditions of the cultivated area. Oil extracted by solvent extraction was found richer in USM than the mechanical pressing, These difference could be due to the effect of extraction methods which may be correlated with potentiality of solvent to extract most of the lipid-associated substances, including phospholipids, sterols, fat-soluble, vitamins, hydrocarbons and pigments (Atta, 2003).

Iodine value (IV) of oils extracted by two methods were found to be lower than those reported by Atta (2003), Salem (1998), Argon (2016) and *Gad et al.* (1963) and agreement with Solati *et al*., (2013). but as generally the iodine value of *Nigella sativa* oil is high than other vegetable oil that indicated to oil contains high levels of oleic and linoleic acids. The oil extracted by solvent extraction was found lower than those extracted by mechanical pressing that means high level of saturated fatty acids in the composition of NSE oil and confers more stability.

Saponification value (SN) of *Nigella sativa* oils extracted by two methods (mechanical pressing and solvent extraction) were found to be lower than those reported by Cheikh *et al.* (2007), Abdel-Aal and Attia (1993), Argon (2016), Atta (2003) and Gad *et al.* and agreement with that determined by Babayan this different may be related to variation of region of cultivation.

SN value for *Nigella sativa* oil extracted by mechanical pressing was higher than those extracted by solvent extraction this agreement with result of GC/MS, oil extracted by solvent has component higher than oil extracted by mechanical pressing that means high mean molecular weight, i.e this result was expected.

NSOs were exhibited antioxidant activity, NSE oil exhibited higher antioxidant activity than that NMP oil, possibly due to difference in composition derived from extraction conditions applied and may be related to exist of thymoquinone in NSE and hidden in NMP.

3.3- Components of *Nigella sativa* oils analysis by GC/MS

The major fatty acids in *Nigella* seed oil were palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) as saturated fatty acids, however oleic ($C_{18:1}$), linoleic ($C_{18:2\,n-6}$) and Eicosadienoic acid
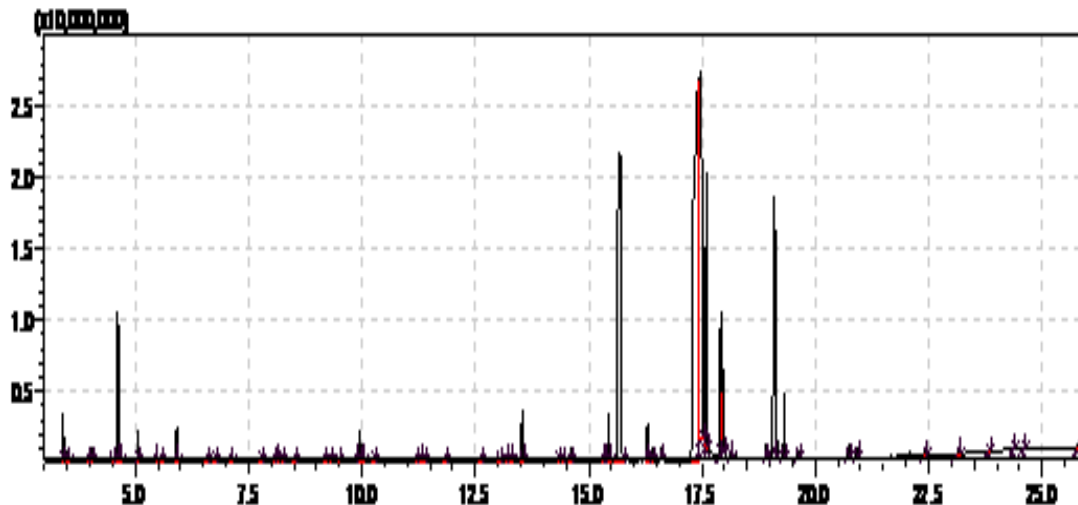

Figure (3.1): *Nigella sativa* **oil extracted by solvent extraction analysis by GC/MS.**
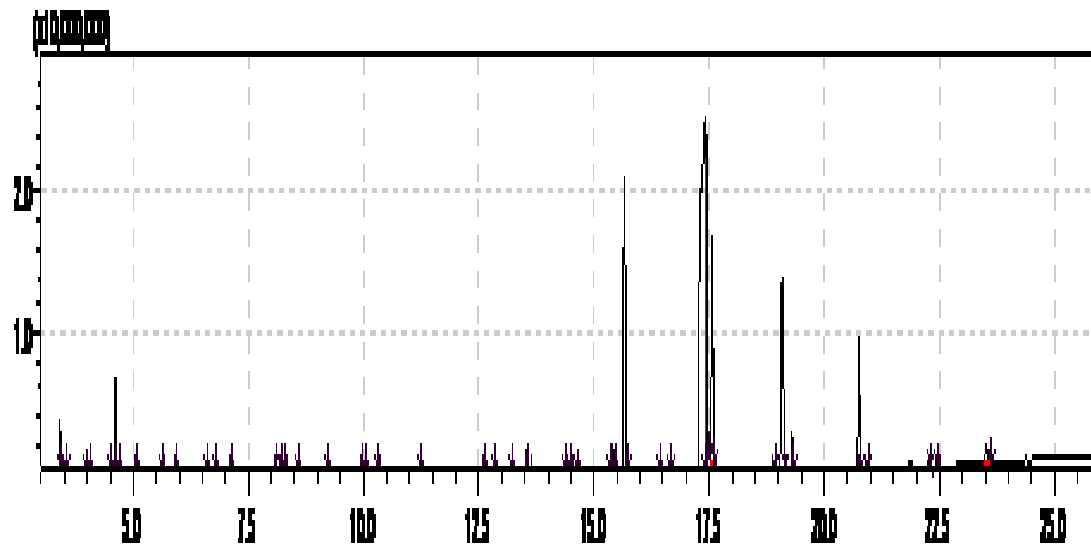
Figure (3.2): **oil extracted by mechanical press analysis by GC/MS.**

Table (3.3): Components of Nigella sativa oils extracted by different methods

NMP	NSE	Component
0.53	0.88	Myristic acid
2.23	2.72	p-Cymene
O.53	0.89	Palmitoleic acid
15.35	15.35	Palmitic acid
0.19	0.24	Margaric acid
39.59	35.49	Linoleic acid
13.06	14.76	Oleic acid
8.17	7.09	Stearic acid
-	2.98	9,12-Octadecadienoic acid, ethyl ester
-	1.68	Oleic acid, ethyl ester
9.77	9.25	cis-11,14-Eicosadienoic acid
1.00	1.24	Methyl 18-methylnonadecanoate
-	0.30	10-Octadecynoic acid
0.21	0.21	Pehenic acid
0.15	0.17	Lingoceric acid
-	0.11	Squalene
4.55	-	13-Docosenoic acid, methyl ester, (Z)-
1.26	-	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
24.61	23.96	Saturated fatty acids
53.18	51.14	Unsaturated fatty acids
1.38	2.17	Trace (less than 0.2%)
49.38	48.38	PI

Fatty acid composition of *Nigella* seed oil is given in Table (3.3), which shows that linoleic, oleic and palmitoleic acids account for more than 51.14% of the total fatty acids for NSE oil and 53.18% for NMP oil. They represent the main unsaturated fatty acids. This result agreement with iodine value those reported previously. The ratio of linoleic acid to oleic acid was more than 2:1 for NSE, This result agreed with those reported in soybean oil (C18:2 = 52%, C18:1 = 25%) and in corn oil (C18:2 = 58.7%, C18:1 = 26.6%) (Ramadan and Morsel, 2002), 3:1 for NMP. Atta (2003) showed that the oils of black cumin varieties contained oleic and linoleic acids at relatively high levels (18.9–20.1 and 47.5–49.0, respectively) but these are lower than those corresponding to the Tunisian variety (25.0 and 50.3, respectively) and to the Iranian variety (23.7 and 49.2, respectively), In this study, saturated acids accounted for 23.96% and 24.61% of total fatty acids, for NMP and NSE oils, respectively. Among them, the main saturated normal chain fatty acids were palmitic, stearic and myristic with minute amounts of margaric, behenic and lignoceric acids. Margaric and margaroleic acids were not detected in previously published data (Abdel-Aal and Attia, 1993; Atta, 2003; Babayan et al., 1978; Gad et al., 1963). And unsaturated was slightly higher than those reported by Atta (2003) and Cheikh (2007). The source of variability may be genetic (plant cultivar, variety grown), seed quality (maturity, harvesting-caused damage and handling/storage conditions), oil processing variables, or accuracy of detection, lipid extraction method and quantitative techniques (Ramadan and Mörsel, 2002).

Some components was found in NSE but not detected in NMP such as thymoquinone, squalene, grindelne and tetrahydroilludin M. This may be related to high effectiveness of solvent to extracted more components than mechanical pressing, and may be due to polarity of solvent. The percentage of thymoquinone was found 0.11% it was lower than those recorded by Solati (2014) due to used different methods for extraction.

The PI for NMP was calculated equal 49.87 it was found high than PI for NSE (48.38) that means NMP was most unsaturated than NSE.

The results of GC/MS showed that NMP oil has 46 components whereas NSE oil contains 64 components this may be related to the effectiveness of solvent to extract more components than mechanical pressing.

Fatty acid compositions show that NSE has high value of oleic acid that NMP means the oil was on rancidity and has long shelf life although peroxide value was found as high than NMP that may be NMP was exposure to humidity or may be due to the condition which the experiment carried out.

Conclusion

This study has revealed the general compositions of the whole seed of *Nigella sativa* as well as the comparison between the properties of its oil extracted by two different methods namely mechanical pressing and solvent extraction. The following are the conclusions drawn out from this study:

- *Nigella sativa* seeds are rich source oil and protein. From a proximate analysis *Nigella sativa* seed are rich of oil (39.4%), protein (21.78%) and mild percentage of carbohydrates (19.0%) and fibre (8.6%).

- They constitute a good alternative source of essential fatty acids compared with common vegetable oils.

- This preliminary study shows that *Nigella* seed oils contain high relative percentages of linoleic, oleic and palmatic acids.

- Nigella Sativa extracted by hexane solvent and those extracted by mechanical pressing oils have different properties.

Recommendation

- *Nigella Sativa* oil extracted by hexane solvent was preferred for usage as antioxidant.

- The physiochemical properties analyzed of the oil show high saponification value (183.18 mg KOH/g for *Nigella sativa* extracted by mechanical pressing and 120 mg KOH/g for *Nigella sativa* extracted by solvent), which explore their potential usage in the soap making but *Nigella Sativa* extracted by mechanical pressing was preferred.

- *Nigella Sativa* oil extracted by solvent has other components such as thymoquinone and alpha hyderein was recommended in pharmaceuticals industry.

- Nigella Sativa extration by solvent contains squalane, it may be preferable in topical skin lubrication and protection.

- Study the effect of different solvents on *Nigella sativa* for the production of the oil and its properties.

- It may be useful in pharmaceutical industry as skin medicine.

- *Nigella sativa* oil extracted by solvent has high chemical stability so shelf life is very long and it can be useful in Industry

Suggestion for further studies

- Choosing different solvent to study optimum yield production and properties.
- Study the Isolation of thymoquinone from the *Nigella sativa* oil since it has great application in pharmacology industry.
- Study the effects of *Nigella sativa* cultivation on antibacterial and antimicrobial activities of the extracted oil.

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