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***Review Paper***

# A comprehensive review on secondary metabolites of *Nigella sativa* L (Seeds)

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## Abstract

*Nigella sativa L. synthesizes and accumulates a variety of biological active secondary metabolites. Due to various substances isolated and identified the claim made in traditional medicinal system. Seeds are generally called kalongi (Ranunculacae) is an annual flowering plant, originate from S. W. Asia. Seeds and NSO showed as a folk medicine for a prehistoric usage in all systems of medicines and are used as food also. This comprehensive account provides a botanical distribution of plant, chemical constituents and pharmacological potential are reviewed and summarized the information focusing on its wide spectrum biological and medicinal properties including anti-tumor, antipyretic, analgesic, antinematode, antihypertensive, antidiabetic, antiulcerogenic and anti- bacterial as well as dietary nutritive supplements and also focused on synthesis and properties of nanopaticles due to secondary metabolites show reducing and stabilizing activity.*

**Keywords:** *Nigella sativa*, seeds, volatile oil, chemical constituents, pharmacological properties, nanoparticles.

## Introduction

Nature has gifted herbal power for healthy and disease free life. Medicinal plants serve as a biofactory having kinds of secondary metabolites, amongst several herbs in traditional system of medicine; one of miraculous herb is Nigella and its13 species found in Turkey; *N. arvensis, N. demascena, N. unguicularі*, *N. glandulifera* and *N. sativa* are of medicinal and horticulture interest[1]. The seeds of *Nigella sativa* L. (*N. indica,* Roxb), is commonly known as kalonji or kalajira / black cumin in Hindi, *Semen Nigellae* in Pharmacy, belongs to buttercup family Ranunculaceae. It is supposed to be native to the Mediterranean countries and has been farming throughout India and other tropical area. It is one of the native plants that are widely distributed in Egypt[2]. The seeds of this herb are responsible to controlled different disorders and treat as a natural remedy. For example, It has been used to control various disfunctions which originate in human bodies (liver, lung, kidney, prostate, breast, cervix, skin and cardiac disorder etc)[3], for a long time of history. Due to its supernatural power of therapeutic, Kalongi seeds have top ranked herbal medicines[4,5], amongst.

We have to discuss and summarized its wide spectrum biological activities, medicinal properties and active principles from seeds and focused attention on nutritive value.

## Chemical constituents

Black seeds contain 0.5-1.5% yellowish volatile essential oil, 37.5% reddish fixed oil, albumin, sugars, mucilage, organic acid, metarbin, toxic glucoside, toxic saponin metanthin resembling helleborin, 5% ash, carvone(an unsaturated ketone), terpene or d-limonene also called carvene and cymene[6]. The occurrence of fats and fatty acids, essential oils[7], enzymes, proteins, peptides, alkaloids[8], saponins[9], flavonids[10,11], phenols and polyols has been reported in *Nigella* species[12,13]. Numerous phytoconstituents have been extracted from it and characterized by 1D and 2D spectroscopic techniques and elemental analyses, such as alkaloids: viz. nigellidine, nigellimine, nigellicine and nigeglanine; sterols like cholesterol, campesterol, stigmasterol, stigma-7-en-3β-ol, β-sitosterol, α-spinasterol; saponins, terpenes, flavonol triglycosides and an isobenzofuranone derivative[14-31]. Various aliphatic constituents saturated and unsaturated fatty acids, fatty esters, glycosides and sugars have been isolated from unsaponifiable matter from fixed oil of seeds Figure-1[32]**.** Moreover, the content of total polyphenols and tocopherols of the fixed oil were determined together with water soluble vitamins and minerals in seeds[33-35]. Melanin is a macromolecule composed of 5, 6-dihydroxy indole (DHI) and 5,6-dihydroxy indole-2-carboxylic acid (DHIC), has been extracted from its seed coats. Immunological study of herbal melanin (HM) was also evaluated[36-38].

Rich source of nutritional and healthy supplements: Oil contains several ingredients with potential value and prescribed for disorders originate from skin to rejuvenate the body. The active nutrients and constituents of fixed and volatile oil of seeds from Iran[28] given in Table-1, 2.

Reported study from fixed oil from Iraq, generally regarded as safe (GRAS) was determined by GC/MS analysis and found that twenty six fatty acids (95 %) present in it, in which major fatty acids were identified as linoleic acid (42.76%), oleic acid (16.59%), Palmitic acid (8.51%), eicosatrienoic acid (4.71%),

eicosapentaenoic acid (EPA 5.98%) and docosahexaenoic acid (DHA 2.97%). In the diet DHA taken along with EPA, improving learning ability which is a part of several health foods[39] and similar as fish oil having n-3 polyunsaturated fatty acid (PUFA) which is also show low risk of atherosclerosis and cardiovascular diseases[40]. Peroxidizability index for the oil found to be 118.21% and unsaturated to saturated ratio was 5.27, due to five time more unsaturation, therefore it shows resistance towards oxidation and beneficial for human health. High valued omega-3 fatty acid present in seeds and more people are aware that seeds used as health foods and nutraceutical preparations and can control various diseases including Lowering blood cholesterol, eczema, dermatitis, pre-menstrual tension, hypertension, arthritis, atherosclerosis, diabetes mellitus, myocardial infarction, thrombosis and cancers etc[41].

Monosaccharides (glucose, rhamnose, xylose, arabinose) and non-starch polysaccharide component were reported and show an useful sources of fuel and as dietary fiber. Seeds contain carotene, which further converted via liver into vitamin A, which known for its anti-cancer activity.

Since, seeds rich in essential fatty acids (linoleic and linolenic acids), and both act as precursors of prostaglandins. Both must be taken as supplements from healthy foods. Their lower concentration enough effective for wide spectrum effect in biological functions. Thus intake of the seeds will results increased prostaglandin level and showing remarkable action on various parts of the body (Increasing cardiac and renal output, smooth muscles, reproductive system, G.I.T. tract and respiratory tract, central nervous system, endocrine system) and metabolism.

Fifteen amino acids present in seeds, including eight essential amino acids as well as also contain arginine, which is essential for infant growth. Seeds are source of metals; calcium, iron, sodium, and potassium and metals may present as micro/macronutrients, their main role as essential cofactors in various enzymatic functions including their biochemistry. Hence According to tradition, the use of the black seed as a natural remedy to prevent various disorder and helpfulness for all illnesses, in human being.

## Pharmacological properties

Biological activities are exhibited due to presence of various phytoconstituents present. Different extracts of seeds and active principles have been shown wide spectrum activities, may be single component effect or cumulative effects.

**Analgesic, antipyretic and anti-inflammatory activity (NSAIDS):** Anti-inflammatory action is caused through inhibition of 5-LO or blocked lipooxygenase pathway. A reported study indicated that in a concentration range of NSO (12.5-50 mg/ml), suppressed formation of 5-LO and 5-hydroxy-eicosa-tetraenoic acid (5-HETE) products from PMNL and produced with half maximal effects ($IC_{50}$) at concentration range 25.0±1.2µg/ml and 24±1.4µg/ml, respectively. Nigellone which present in seeds caused concentration related suppression of 5-HETE formation ($IC_{50}$:11.9±0.3µg/ml). Thymoquinone which also present in it, also responsible for inhibition the formation of 5-LO ($IC_{50}$:0.26±0.02µg/ml) and 5-HETE production ($IC_{50}$: 0.36±0.02µg/ml). These results indicated that NSO and Nigellone/THQ shows anti- inflammatory action and they can be used inflammatory associated diseases[42]. Other reported study showed that it is inhibited eicosanoid generation in leukocytes and membrane lipid peroxidation[43].

Study reported that when acetaminophen with NSO exposed in adult rats to maintain arterial blood pressure and renal function, due to various compounds present in oil[44].

Extracts of aqueous and methanolic defatted of seeds, reported analgesic, antipyretic and anti-inflammatory activity, *in vivo*. The extract showed inhibitory effects on Carrageenan induced paw edema and reported study suggested anti-inflammatory action. In other study, hot plate reaction time is significantly increased in mice, again NSO indicating analgesic effect[45]. Ethanolic extract showed antipsoriatic activity *in vivo* by using mouse tail model and and *in vitro* by using sulforhodamine B assay employing HaCaT human keratinocyte cell lines[46]. Antiinflammatory effect is shown by Thymoquinone during the allergy in the lung due to may be inhibition of synthesis of prostaglandin PGD2 and Th2-driven immune response[47]. Reported study suggested THQ act as antitussive activity through anti-inflammatory effect.

**Table-1:** Fatty acid composition of the fixed oil of Nigella sativa L.[28].

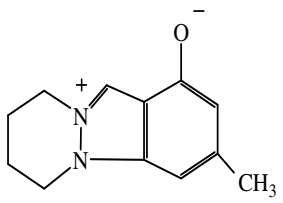
| Fatty acid | RT | Percentage | Fatty acid | RT | Percentage |
|---|---|---|---|---|---|
| Lauric acid | 4.68 | 0.6 | Linoleic acid | 10.52 | 55.6 |
| Myristic acid | 5.91 | 0.5 | Linolenic acid | 11.95 | 0.4 |
| Palmitic acid | 7.48 | 12.5 | Eicosadienoic acid | 12.71 | 3.1 |
| Stearic acid | 9.37 | 3.4 | Oleic acid | 9.79 | 23.4 |

Total fatty acids 99.5 %

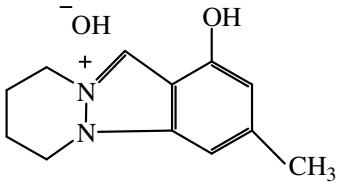
**Table-2:** Chemical composition of the volatile constituents of *Nigella sativa* L.[28].

| Compound | RI | Percentage | Compound | RI | Percentage |
|---|---|---|---|---|---|
| n-Nonane | 901 | 1.7 | α-Thujene | 928 | 2.4 |
| 3-Methyl nonane | 931 | 0.3 | α-Pinene | 935 | 1.2 |
| 1,3,5-Trimethyl benzene | 969 | 0.5 | Sabinene | 975 | 1.4 |
| n-Decane | 1001 | 0.4 | b-Pinene | 979 | 1.3 |
| 1-Methyl-3-propyl benzene | 1052 | 0.5 | Myrcene | 992 | 0.4 |
| 1-Ethyl-2,3-dimethyl benzene | 1087 | 0.2 | α-Phellandrene | 1007 | 0.6 |
| n-Tetradecane | 140 | 0.2 | p-Cymene | 1026 | 14.8 |
| n-Hexadecane | 1600 | 0.2 | Limonene | 1030 | 4.3 |
| **Nonterpenoid hydrocarbons** | | **4.0** | γ-Terpinene | 1059 | 0.5 |
| | | | **Monoterpenoid Hydrocarbons** | | **26.9** |
| Fenchone | 1097 | 1.1 | Terpinen-4-ol | 1179 | 0.7 |
| Dihydrocarvone | 1206 | 0.3 | p-Cymene-8-ol | 1186 | 0.4 |
| Carvone | 1245 | 4.0 | Carvacrol | 1302 | 1.6 |
| Thymoquinone | 1251 | 0.6 | **Monoterpenoid Alcohol** | | **2.7** |
| **Monoterpenoid hydrocarbons** | | **6.0** | | | |
| α-Longipinene | 1353 | 0.3 | Estragole | 1200 | 1.9 |
| Longifolene | 1408 | 0.7 | Anisaldehyde | 1255 | 1.7 |
| **Sesquiterpenoid Hydrocarbons** | | **1.0** | trans-Anethole | 1289 | 38.3 |
| **Total compounds** | | **86.7 %** | Myristicin | 1523 | 1.4 |
| | | | Dill apiole | 1627 | 1.8 |
| | | | Apiole | 1684 | 1.0 |
| | | | **Phenyl propanoid compounds** | | **46.1** |

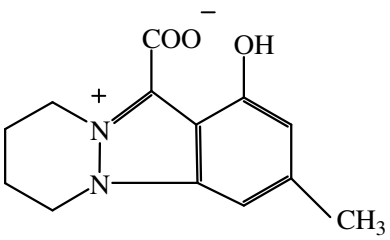
| Vitamins | Concentration | Metals | Concentration |
|---|---|---|---|
| Protein | 208 µg/g | Calcium | 1.859 mg/g |
| Thiamin | 15µg/g | Iron | 105 µg/g |
| Riboflavin | 1 µg/g | Copper | 18 µg/g |
| Pyridoxine | 5µg/g | Zinc | 60 µg/g |
| Niacin | 57 µg/g | Phosphorus | 5.265 mg/g |
| Folacin | 610 IU/g | | |



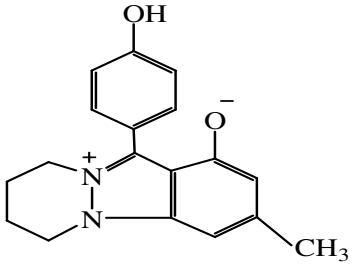
Nigeglanine



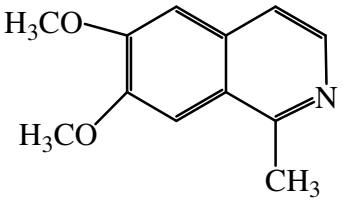
Synthetic alkaloid



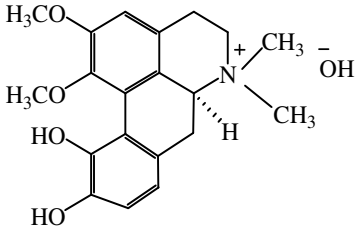
Nigellicine



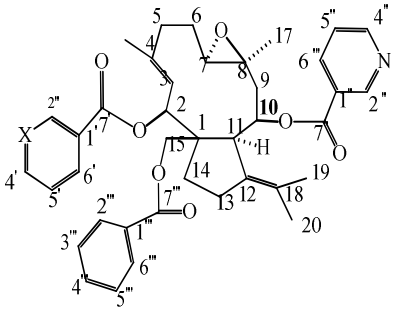
Nigellidine

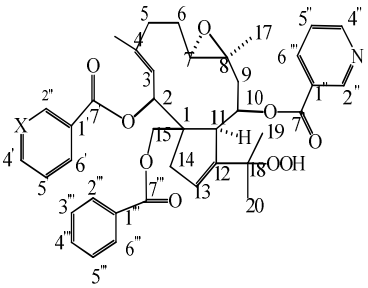


Nigellimine

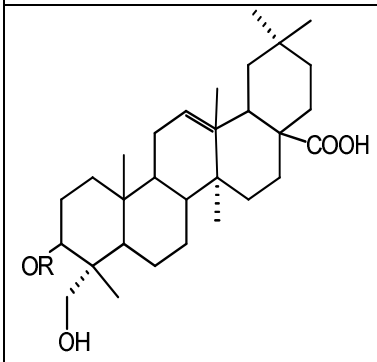


Fuzitine



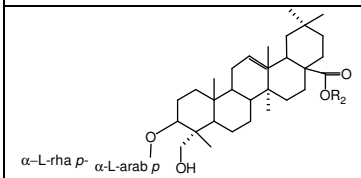


Nigellamines : A1: X = CH A2: X = N B1: X = CH B2: X = N

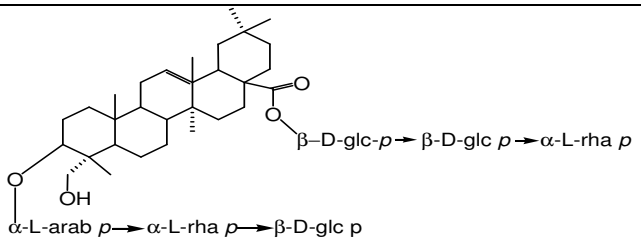


Kalopanax saponin A:
R = (3-O[L-rham p- (1→2)-a-L-arab p]-hederagenin

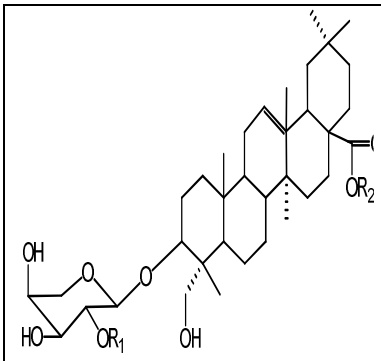
Kalopanax saponin I:
R = (3-O b-D-xyl p- (1→3)-a-L-rham p-(1→2)-a-L-arab p]-hederagenin



α -hederin

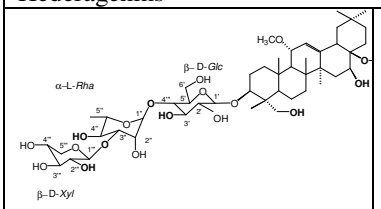


Nigellone

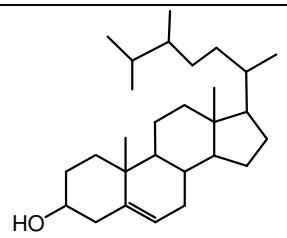


| $R_1$ | $R_2$ |
|---|---|
| β–D-xyl-*p*(1➔ 3)- α-L-rha *p* | α-L-rha *p* (1➔ 4)- β-D-glc *p* (1➔ 6)- β-D-glc *p* |
| α-L-rha *p* | α-L-rha *p* (1 ➔ 4)- β-D-glc *p* (1 ➔ 6)- β-D-glc *p* |
| β–D-xyl-*p*(1➔3)- α-L-rha *p* | H |
| β-D-xyl-p(1➔3)- α-L-rha p | α-L-rha p (1➔ 4)- β-D-glc p (1➔ 6)- β-D-glc p |
| α-L-rha p | α-L-rha p (1➔ 4)- β-D-glc p (1 ➔ 6)- β-D-glc p |
| β-D-xyl-p(1➔3)- α-L-rha p | H |

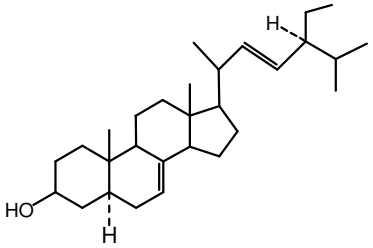
Hederagenins



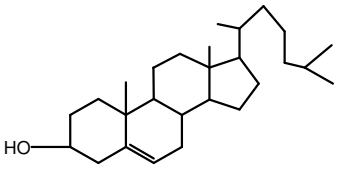
3-O-[β-D-xylopyranosyl-(1 ➔ 3)-a-L-rhamnopyranosyl-(1 4)-β-D-glucopyranosyl]-11-methoxy-16-hydroxy-17-acetoxy hederagenin



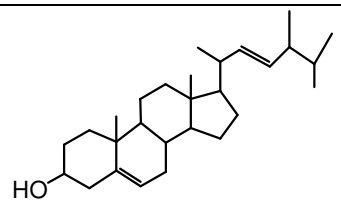
Campesterol



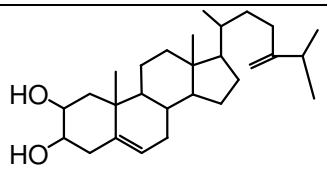
α-spinasterol



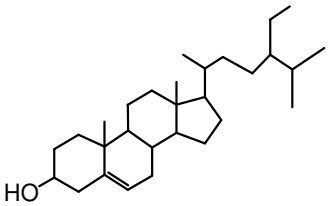
Cholesterol



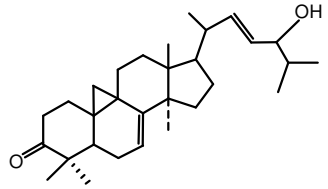
Stigmasterol



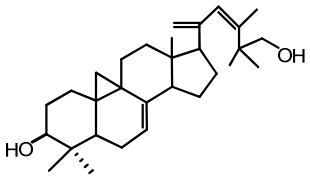
Ergosta-5,24 (28)-dien-2,3-cis-diol



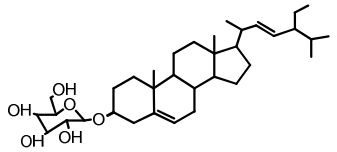
β-sitosterol



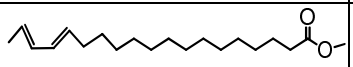
Cycloart-3-one-7,22-diene-24-ol



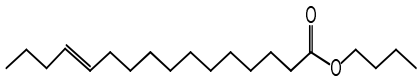
Cycloart-23-methyl-7,20,22-triene-3b,30-diol



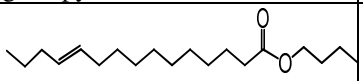
Stigma-5,22-diene-3-O-β-D-glucopyranoside



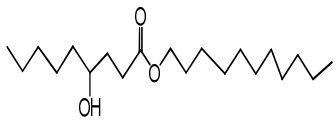
Methyl nonadec-15, 17-enoate



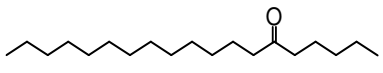
Butyl hexadec-12-enoate



Pentyl pentadec-11-enoate



4-hydroxy undecyl nonanoate



6-Nonadecanone

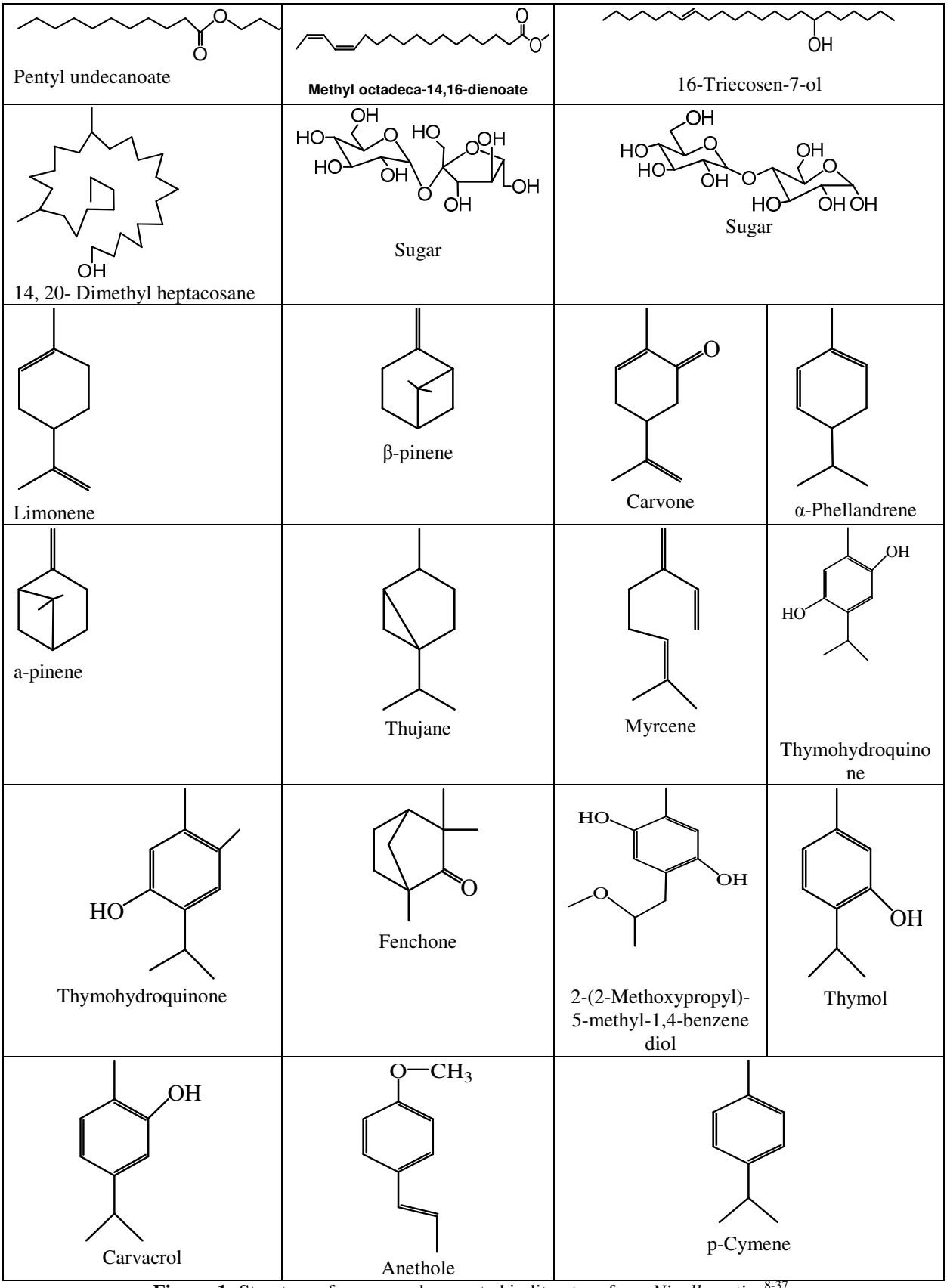



**Figure-1:** Structure of compounds reported in literature from *Nigella sativa*[8-37].

Black seed-extracted and pure THQ on CAT were studied with aPTT assay using pancreatic cancer cell lines for TF, and with TEG assay with lipopolysaccharide (inflammatory trigger) and inactivation factors IIa and Xa were also assessed. Luciferase assay was also studied with THQ's, since TNF-α facilitates crosstalk between inflammation and thrombosis by triggering the NF-κB pathway.

Results suggested that pure THQ reversed CAT initiated by both TF and inflammation to basal levels ($p<0.001$). Mechanistically, while THQ had negligible to no affect on factors IIa and Xa inactivation, but it strongly reduced the effects of TNF-α on NF-κB elements ($p<0.001$).

THQ has insignificant effect on blood coagulation and can reverse CAT in vitro, maybe through interfere with the crosstalk between inflammation and coagulation; therefore it can act as a supplement to existing chemotherapies and anticoagulant therapies[48].

**Gastroprotective (anti-ulcer) activity:** Thymoquinone (24-25%), main constituent of NSO showed gastroprotective property, i*n vitro*. The study showed that mucin contents and glutathione level significantly increased when NSO administered in rats but a significant reduce in mucosal histamine contents while ethanol administered produce 100% ulcer induction with an ulcer score of 12.62±1.35 (means ± S.E., n=8) and caused reduce in free acidity and glutathione level mucosal histamine contents increased. When NSO administered before induction of ulcer there was glutathione level, mucin content and free acidity significantly increased and gastric mucosal histamine contents significantly decreased by means of a protection ratio of 53.56% as compared to the ethanol induced ulcer in rats.

This result suggested that anti ulceration property may be due to presence of THQ in NSO[49] and also responsible for increasing concentration of PGE which protected gastric mucosa against different types of gastric lesion, when NSO taken orally.

Alcoholic extract of seeds also showed anti-ulcer activity in rats by use of two models; pyloric ligation and aspirin-induced gastric ulcer with taking various parameters; volume of gastric secretion, free acidity, total acidity and ulcer index and consequences observed that extract significantly decreases ($p<0.001$) all parameters with respect to control[50].

Other study indicated that NSO (800 mg/Kg) taken orally for 4 weeks in rats, serum lipid profile lowered and serum albumin and fibrinogen levels were increased when powdered seeds administered in rats and also improves liver and pancreas tic cells as well as blood glucose level in rats[51].

THQ also shows chemo-sensitizing effect, study was reported in the treatment of 5-Fluorouracil induced gastric cancer[52].

**Anticonvulsant activity:** The anticonvulsant effect also shown by thymoquinone (NSO) and study has been reported on pentylene tetrazole (PTZ) and maximal electroshock test (MES) in petitmal and grandmal epilepsy models in mice, respectively. 40 and 50mg/kg doses of thymoquinone injected (i.p.) in mice which have PTZ- induced convulsion, results suggested prolonged the onset of convulsion. And the projection activity of thymoquinone against mortality was found 71.4% and 100% in the mentioned doses respectively.

Thymoquinone when applied for MES, it failed to reduce the duration convulsion while exhibited a complete protection against mortality. Further study reported on taking flumazenil (10 mg/kg, i.p.) which is antagonist of benzodiazepine site and $ED_{50}$ of diazepam was optained 1.13 mg/kg (95% cL : 0.89 – 1.44), in PTZ test.

These results suggested that thymoquinone has shown anticonvulsant activity (Petitmal epilepsy) through benzodiazepine receptors[53,54].

**Effects of thymoquinone on smooth muscle contraction:** Study has been reported that THQ inhibit the contractile responses to exogenous norepinephrine NE (100 μm) and KCl (80 mm) in a concentration dependent manner furthermore it is reduced the amplitude of electric evoked contraction of vas deferens in a concentration based manner. Concentration of $CaCl_2$ (0.1-10mm) is cumulative added to tissue bath; it has been failed to enlarge the amplitude of contractile responses to electric field stimulation in the presence of thymoquinone (80 μm). Therefore THQ enhanced non selective and concentration dependent inhibition of contractile responses to NE, KCl and electric-field stimulation, result suggested that alkaloid may interfere with the mobilization of $Ca^{2+}$ required for smooth muscle contraction[55].

**Immunomodulating and cytotoxic properties:** In reported study, a rat model was design to examine the effect of volatile oil on selected immune components. Specific antigen (typhoid TH) of Cong Evans rats were treated with Oil and effects were analyzed in their serum antibody titer along with the splenocytes and peripheral immune cells. Antibody titer for the experimental animal was found 1280 as compared to the 2560 in the control rats. Results indicated that there was significant ($p<0.05$) decrease in splenocytes and neutrophill counts, but increased peripheral lymphocytes and monocytes.

Cell mortality has been estimated using MTT assay in which vinblastine sulphate and mitomycin C used as the positive control. $LC_{50}$ values have been obtained for NSVO: 155.02± 10.4, 185.77±2.9, 120.40±20.5, 384.53±12.8 and 286.83±23.3 μg/mL respectively against the cancer lines (SCL, SCL-6, SCL-37'6, NUGC-4) and fibroblast line(3T6) and Results indicated that immunosuppressive cytotoxic activity is shown by the NSVO[56].

The splenocyte proliferation, macrophage function, and NK cells anti-tumor activity of NS have revealed the potent immunomodulatory properties of the Nigella seeds[57].

In vitro screening of seeds extracts indicated that only ethyl acetate fraction appeared to be cytotoxic against the tumor cells. The $ED_{50}$ values of EAF against Molt 4, P388, J82, Wehi 164, LL\2, SW620 and Hep G2 were found that 12, 17, 22, 14, 16, 18 and 11 µg/ml, respectively. At the concentration of 50 and 100 µg/ml the EAF completely killed all cell types tested. The differential sensitives of the cell lines were found in order: Hep G2 > Molt 4> Wehi 164 > LL/2 > SW620 > J 82[58,59].

Two triterpenes have been isolated from seeds of *N. glandulifera*: Kalopanax saponins A and I. and they have shown antitumor activity against the tumor cells (Hep G2) and drug resistant (Hep G2) and primary cultured normal mouse hepatocytes. Reported study suggested that A and I possibly potential therapeutic agent for treatment of parental and drug resistant hepatoma[9]. NSVO contain thymoquinone and dithymoquinone, were shown to be cytotoxic to both parental and multi drug resistant tumor cells *in vitro*. Nigellone is also carbonyl polymer of thymoquinone, was effective in inhibiting histamine release from rat peritoneal (mast cells) by different secretagogues, in relatively low concentration, *in vitro*[60].

**Anti-cancer activity:** Thymoqunone induced cytotoxicity against various cell lines was studied, by using a proliferation assay (MTT assay) and apoptosis assay. Result revealed that cells cycles arrest at G1 and attributed that TQ kills cancerous cells through apoptosis process and arrest cell cycle but non cancer cells are relatively resistant to TQ. This chemopreventive effect of ENS may be due to the synergistic actions of various constituents present in it, during the initiation phase[61].

Ethanolic extract of indigenous *Nigella sativa* seeds (ENS), equivalent to 150 mg of powdered seeds/ kg body weight, administered during the initiation phase, showed chemopreventive effect against DMH-induced colon carcinogenesis even with its relatively low content of THQ. Based on reported study, results suggested that ENS is proficient of delay progression; restrict incursion and attenuating antagonism of colon tumors in a rat model. On the contrary, in post initiation phase, ENS was found to be poor effect of chemopreventive and tumorigenesis inhibition[62,63].

**Relaxant effect on uterine smooth muscles:** Thymoquinone has been tested on spontaneously contracting and on agonist - induced contraction in isolated rat uterine muscles, in literature. THQ (37.5-300 µM) reduced spontaneously contraction of isolated non pregnant rat uterus in concentration based manner. It (150 or 300 µM) reduced prostaglandin $F_{2\alpha}$ oxytocine and acetylcholine induced uterine concentration. This was shown to shift the non cumulative log concentration response $E_{max}$ of the tissue to all selected uterine stimulants and elevation of $EC_{25}$. Oral administration (THQ: 10 or 20 mg/kg) daily for three weeks inhibited the contraction evoked by previous oxytocic agents (PROS. $F_{2\alpha}$ oxytocine and acetylcholine) on uterine muscle. It also shifted the non cumulative log concentration response curve of $CaCl_2$ in a concentration dependent manner without a reduction in $E_{max}$. This effect was similar to that produced by verapamil. It also inhibited $K^+$ induced contraction in a concentration dependent action. These results suggested that THQ, caused embarrassment of uterotonic potencial of various oxytocic agents and may blocking $Ca^{2+}$ channels[64].

**Cardio vascular effect:** Administration of the dethymoquinonated volatile oil (DE-THQ), α-pinene or p-cymene in the dose range (2-16 µl /kg) decreased both the arterial B.P. and the heart rate. These effects were significantly antagonized by treatment of the animals with hexamethonium and by spinal pithing of the rats. The induced bradycardias were also blocked by atropine. Thus the induced cardiovascular depressant actions seemed to be mediated via central mechanisms that probably involved the vasomotor centers in the medulla with the resultant decrease in the heart and blood vessels. Furthermore treatment of rat platelets *in vitro* with DE-THQ, α-pinene or p-cymene in doses of (5-20 µL/ml) produced no significant and non dose dependent, inhibition of ADP-induced aggregation. It can be concluded that deprivation of the whole volatile oil and its constituent's thymoquinone which is known for its bronchoconstricting and gastric ulcerogenic actions did not abolish the cardiovascular depressant actions of the oil[65].

**Hypolipidemic activity:** Various alkaloids influence human physiology, they are phytoconstituents obtained from various part of plants eg. atropine, nicotin. Structure of nigellamine-n-oxide is similar to quinolone, this group shows possibly function in the treatment of malaria and for the prophylactic treatment of cardiac arrhythmiasis, vasodilator effects and also tend to have anti-psychotic effects.

Dolabellane type diterpene alkaloids nigellamines $A_1$, $A_2$, $B_1$ and $B_2$ in Figure-1; isolated from the methanolic extract have been reported to show hypolipidemic action and showed potent lipid metabolism promoting activity in primary cultured mouse hepatocytes. Hepatocytes were isolated from male ddy mice using the collagenase perfusion method. Effect of nigellamines on stored triglyceride in primary cultured mouse hepatocytes were examined and reported to show potent reduction of triglyceride levels[inhibition (%) at 0.1 µM]; $A_1$ (64 ±4), $B_1$ (70±2%) and $B_2$ (79±2%). Their activities were equivalent to that of a PPAR-α-agonist, hypolipidemic medicine; clofibrate (64±5%)[66].

**Anti-angiogenic, anti-oxidant and anti-viral activity:** Seeds of *Nigella sativa* and Green Tea have been reported antiviral activity against Infectious Laryngotrachietis Virus (ILTV) at concentration of 35 and 4.22 µM, respectively and both plants protected Diethylnitrosamine (DEN) induced hepatocellular adenoma. Further, study was reported on Hella and Vero cells and indicated that NS showed inhibition rate of 80 and 65% at concentration of 80µM, while GT showed 75 and 45% at concentration of 90 µM, respectively.

Both also reported anti-angiogenic activity as well as antioxidant activity on endothelial cells of rat's aorta and Diphenyl Picryl Hydrazyl (DPPH) radical scavenging activity, Nitric Oxide (NO) radical inhibition assay and lipid peroxidation assay, respectively[67].

Both species of *N. sativa* and *N. damascenea* comparatively reported antioxidant potential through electron transfer assays and results suggested that *N. damascena* exhibiting a higher free radical scavenging activity. Diuretic activity was not shown by *damascena* species but significantly increased urine volume when ethanolic extract of *N. sativa* (100 $mg \cdot kg^{-1}$) administered but found less than the reference drug.

Reported study is shown that extract of *N. sativa* have a greater natriuretic effect than kaliuretic with reference to the elimination of $Na^+$, $K^+$ and uric acid and a similar uricosuric effect with control while decreases $Na^+$ excretion, the $Na^+/K^+$ was sub unitary for *N. damascena* and no increases of kaliuretic effect[68].

When intraperitoneally NSO is given to mice infected with murine cytomegalovirus for 10 days, the virus was undetectable in the liver and spleen, while it was still detectable in the control mice. This pharmacological response was considered to be related to enhance in the number and function of M-phi and CD4 +ve T cells and greater than before production of INF-gamma. Study suggested that seeds used as antiviral agent against murine cytomegalovirus infection[69].

Antioxidant activity of extracts of seeds was studied due to presence of Phenolic compounds[70].

**Antibacterial Antifungal and other activities:** Seed extract has shown inhibition of growth of the bacteria *Escherichia coli, Bacillus subtilils, Streptococcus faecalis* and pathogenic yeast *Candida albicans.* Methanolic, ether and aqueous extracts of seeds were tested in different dilution against standard strain of *Staphylococcus aureus*, *Pseudomonas aerugenosa* and *E. coli* (NCTC and ATCC strain) using disk-agar diffusion technique on Muller-Hinton agar plates. The aqueous and methanolic extract exhibited significant inhibition against *S. aureus* and *Pseudomonas spp.* whereas ether extracts did not show any activity against any one. Commercial kalongi oil showed good activity against both bacteria. Reported study showed that Gram positive organism were more sensitive than gram negative on seeds viz., *S. aureus*, *S. aureus* (ATCC), *S. faecalis*[71]. Seeds and oil have shown antibacterial activity against different multi-drug resistant bacteria and can be used as an alternative and cheap medicine associated with diarrhea, enteric diseases, urinary tract infections, neonatal infection, and wound infection[72,73].

Extract of it showed almost similar results to topical mupirocin in the treatment of neonates with *staphylococcal pustular* skin infections with no side effects[74]. The ether extract was reported to inhibit the growth of Candida yeasts in several organs in experimental animal infections[75]. *In vitro* study showed that THQ inhibit *Aspergillus niger* and *Fusarium solani* and the activity was comparable to amphotericin-B[76,77]. THQ is more effective than amphotericin-B and griseofulvin against *Scopulariopsis brevicaulis* growth *in vitro*. There was 100% inhibition of the growth of *S. brevicaulis* with thymoquinone 1 mg/ml, while amphotericin-B 1 mg/ml inhibited only 70% growth. However, clotrimazole was much more effective than the above mentioned drugs, with an MIC of 0.03 mg/ml[78].
Anti-nociceptive activity shown by water suspension in mice compared with naproxen, significantly ($P<0.05$) reduced nociceptive reaction both at early and late phases whereas naproxen attenuated the nociceptive response in late phase of test only. Pretreatment with naloxone the analgesic effect of *Nigella sativa* reversed at late phase only ($P<0.05$), due to opioid activity of constituents mainly, THQ[79].

*In Vitro* study reported that seeds powder and its oil showd hypocholesterolemic and **anti**atherogenic cardioprotective properties and significantly reduce ($P<0.05$) total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) levels but enhance high-density lipoprotein cholesterol (HDL) levels after treatment for 2, 4, 6 and 8 weeks compared to the PC group[80].

**Multiple sclerosis:** The composition of black cumin oil (150 ml, NSO), flax oil (750ml, *Oleum lini*), borage oil (100ml, *Borago officinalis*), vitamins and minerals taken internally, this composition (20 ml- 40ml/daily) has been used for treatment of multiple sclerosis[81]. This study provides α-linolenic acid 45.5%, linoleic acid 23.6%, oleic acid 17.9%, γ-linolenic acid 2.2%, palmitic acid 5.6%, stearic acid 3.5%, other fatty acids 2.1% and aromatic oils 0.3%. The composition also comprised 0.1g vitamin $B_6$, 0.1g vitamin $B_5$, 5.0 g vitamin C, 14g Mg and 0.6g Zn[82].

**Green synthesis and activity of Nanoparticles from Nigella sativa:** Green synthesis of nanoparticles are cost effective, cheap and eco-friendly without any harm and particles are characterized using change in absorbance on UV-Visible spectrophotometer, FT-IR, XRD and SEM/TEM techniques. Plants have secondary metabolites like phenols, acids, tannins, steroids, terpenes etc and reduce metal solution and nanoparticles show various acivities eg. Antibacterial, antitumor, optical properties and can act as biosensor.

Silver nanorods prepared from 1mM $AgNO_3$ solution and seed extract of it. Extract shows reducing as well as capping agent, properties. Nanorods are characterized using instrumental analyses and showed uniformly sized nanorods, and also showed antidiabetic property, *in vitro*[83]. They also prepared from leaf extract and MTT assay was studied on stem cells of mice, results suggested that it is less toxic than chemical method[84]. Synthesized nanoparticles and their activities are shown in Table-3.

**Table-3:** Activity and synthesis of nanoparticles from *Nigella sativa*

| Synthesis | Activity |
|---|---|
| Synthesis of AuNPs From seeds extract | *In vivo* imaging and therapy[85] |
| Silver Nanoparticles From seeds extract | Antimicrobial lotions, in drug delivery and biomedical applications[86] |
| Thymoquinone Poly (lactide-co-glycolide) NPs | Anti-proliferative, anti-inflammatory, chemosensitizing[87] |
| Silver NPs from essential oil | Inhibitory activity against pathogenic from aquatic environment[88] |
| Silver Nanorods from aqueous seeds extract | Antidiabetic activity[83] |
| Silver NPs from leaf extract | MTT assay[84] |

Poly shaped crystalline gold nanoparticles AuNPs have been prepared from aqueous chloroauric acid solution and seed extract of *N. sativa*, reported in the literature using microwave irradiation and thermo-induced procedures and reaction temperature controlled. The XRD studies indicated that gold nanocrystals are highly anisotropic in nature, mainly triangular and hexagonal shapes, and that the particles are (111) oriented and may be applied future *in vivo* imaging and therapy[85].

One-pot green synthesis method to produce bio-entity capped silver nanoparticles, using plant extracts of *Nigella sativa*, *Dioscorea alata* and *Ferula asafetida* and found crystalline size between 4–17 nm by varying the reaction temperatures and time, thymoquinone, dioscorin and ferulic acid used as capping agents, respectively. The prepared nanoparticles show surface plasmon resonance peaks in the wavelength range of 400 to 453 nm. Silver nanoparticles obtained *Nigella sativa* and *Dioscorea alata* are crystalline, while using *Ferula asafoetida* are amorphous due to excess amount of capping agent. Heat flow curves of all nanoparticles exhibit exothermic peaks at temperatures where sharp weight reduction is observed for the nanoparticles. TEM images show spherical particle morphology. These nanoparticles may find use in various antimicrobial lotions, drug delivery and other biomedical applications due to presence of thymoquinone, dioscorin and ferulic acid as capping agents[86].

Effectiveness and bioavailability of Thymoquinone (TQ), from seeds of *N. sativa* is increased, using polymer-based nanoparticle approach. Polymer (lactide-co-glycolide) (PLGA) and the stabilizer polyethylene glycol (PEG)-5000, encapsulated on TQ and 97.5 % increased biodegradable efficiency stabilizer polyethylene glycol (PEG)-5000. Encapsulation of TQ into nanoparticles enhances its anti-proliferative, anti-inflammatory, and chemosensitizing effects[87].Silver nanoparticles synthesis (EO Ns-AgNPs) prepared using essential oil of *N. sativa*, showing inhibitory activity against pathogenic *Vibrio harveyi* and *V. parahaemolyticus* isolated from aquatic environments. Results suggested that increasing concentrations of EONs-AgNPs (30 µg/ml) effectively reduced the number of colonies of *Vibrio harveyi* and *Vibrio parahaemolyticus*. Among the two bacterial strains, EONs-AgNPs exhibited greater antibacterial activity against *V. harveyi* than *V. parahaemolyticus*.

EONs-AgNPs showed better inhibition of biofilm formation of *V. harveyi* followed by *V. parahaemolyticus* at 80 µg/ml after 24 h. Therefore it can be used against pathogenic microbes of aquaculture importance[88].

## Conclusion

The pursuit of discovering various pharmacological properties and applications based on herbs *Nigella sativa*, included recently. Intake of Seeds support for balanced health in our daily life and socio-economic importance connect in applied field may use of nano-herbal medicinal products. Recently, attention focused on nanoparticles preparations and applications in drug-delivery system.

Various terpenes, thymol, thymoquinone, limonene and pinene etc. and specific alkaloid may be further investigated on formulation of nanoparticles and may enhance their biological activities.

**Abbreviations:** GIT, gastrointestinal tract; GRAS, generally regarded as safe; GC/MS,gas chromatography-mass spectrometry; EPA, eicospentaenoic acid; DHA, docosahexaenoic acid; LO, lipooxygenase; HETE , hydroxyl eicosa-tetraenoic acid; PMNL,polymorphonuclear leucocyte; $IC_{50}$ , inhibitory concentration at 50 %; DPPH, diphenylpicryl hydrazide; THQ, thymoquinone; DE-Tq, dethymoquinonated volatile oil ; TEG, thromboelastography; CAT, catalase; aPTT , activated partial thromboplastin time; $E_{max}$ , electrode potential; TNF-α, tumor necrosis factor-α; NF-κB,nuclear factor-kappa B; PGE, .7prostagalandin E; PTZ, pentylene tetrazole; MES, maximal electroshock test; $LC_{50}$, lethal concentration; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide; LDLC, low density lipoprotein cholesterol; HDLC, high density lipoprotein cholesterol; ED50, effective dose at concentration 50 %; NSO, *Nigella sativa* oil; NSVO, *Nigella sativa* volatile oil; NE, norepinephrine; EAF, ethyl acetate fraction; EAC, Ehrlich ascites carcinoma; DLA, Dalton's lymphonia ascites; S-180, sarcoma-180; ENS, ethanolic extract of indigenous *Nigella sativa*; DMH, 1,2-dimethyl hydrazine; $EC_{25,}$ effective concentration at 25% ; DF, defatted flour; PC, protein concentration; PI, protein isolate; SPI, soybean protein isolate; ADP, adenosine diphosphate; TC, total cholesterol; $EC_{25}$, INF-gamma, interferon-gamma; GT, green tea; ILTV, infectious laryngotrachietis virus; DEN, diethylnitrosamine; NO, nitric oxide; MIC, minimum inhibition concentration; NPs, nanoparticles.

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