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Evaluation of Phytochemical Antioxidant Properties and Quantification of Thymoquinone in *Nigella sativa* L. Seed Extract

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Abstract: ***Herbal drugs have been considered to be a beneficial remedy for various diseases. They are considered to have fewer side effects compared to modern medicines. In the world of herbal medicine, Nigella sativa L (Ranunculaceae), is one such herb held in high esteem owing to its notable pharmacological efficacies like neuroprotective and antioxidative properties. The present study aims to analyse the polyphenolic content, and anti-oxidant properties, and to quantify Thymoquinone (TQ) in N.sativa extract. Ethanolic extract was screened for possible antioxidant activity against 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Hydroxyl radical, Superoxide anion radical and Hydrogen peroxide. Further, quantification of TQ was performed by HPLC. The results confirmed that N. sativa extract possesses positive antioxidant properties, which can be attributed to the presence of bioactive compounds. Thus, this extract can be considered as an effective agent with high therapeutic value in preventing cellular damage. Exploration of these chemicals might lead to novel drug discovery which might be an effective remedy for neurological disorder.***

Keywords: *Nigella sativa,* Antioxidant activity, Thymoquinone, HPLC, Ethanolic extract

1. Introduction

Reactive oxygen species (ROS) have been one of the leading mechanisms that correlates with oxidative damages caused by generation of free radicals and leads to chronic diseases like diabetes, cancer, neurodegenerative disease and aging [1]. For many years, researchers have been looking for effective and non-toxic antioxidants from natural resources, especially medicinal plants for the prevention and treatment of ROS mediated diseases. A special emphasis has been laid on antioxidant therapy that can inhibit the oxidation of lipid and other molecules by inhibiting the initiation of oxidative chain reactions [2]. Thus, attention has been increasingly paid towards development and utilization of more effective and non-toxic antioxidants [3].Nature has been a resource of medicinal treatments for thousands of years. There has been an increase in the use of products derived from plants and their purified bioactive compounds as alternative medicines which show favourable therapeutic advantages [4**,** 5]**.** *Nigella sativa* Linn(Black cumin or Black seeds) belonging to the family Ranunculaceae is an annual herbaceous plant commonly found in the Mediterranean countries, Middle East, Eastern Europe and Western Asia [6]. N.*sativa* seeds are a source of traditional herbs used in the Middle East as folk medicine and natural remedies due to its various biological properties [7]. Phenolic and flavonoid compounds are primarily responsible for the free radical scavenging and antioxidant activities of plants which play a beneficial role in medicine and have extensive pharmacological properties [8].

The seeds of N.*sativa* have been subjected to a wide range of pharmacological investigations due to the presence of phenolic and other active components such as carvacrol, thymol, thymoquinone, dithymoquinone and nigellin which exhibit a wide range of biological effects, including anti-oxidative, neuroprotective, anti-tumour, anti-fungal, analgesic, and anti-inflammatory [9,10]. Thymoquinone (2-methyl-5-isopropyl 1-1, 4-benzoquinone, also called TQ) is the majorconstituent of N.*sativa* seeds and is also the main oxidation product of carvacrol and thymo [11]. It is reported that these compounds have free radical scavenging properties and a good reducing power. The extraordinary biological activity of N.*sativa* is attributed to its oil component and thymoquinone [12, 13].

The remarkable advances in understanding the mechanisms of action of antioxidants against oxidative stress have brought their importance on human health to light. Natural antioxidants are also in high demand for application as nutraceutical or functional foods and biopharmaceuticals due to its consumer preferences and their therapeutic effect. Additionally , some human epidemiological studies have shown that natural antioxidant have potential health effects [14]. However, there is a dearth of scientific information on antioxidant potential of N.*sativa* seed to justify its continuous use in traditional folk medicine. Therefore, the present study aims to evaluate the quantity of Thymoquinone, polyphenolic contents and free radical scavenging potential of *Nigella sativa* L. ethanolic seed extract with a view to determine its potential health benefits.

2. Materials and Methods

Plant material

The *Nigella sativa*seeds were purchased from a local market in Bangalore, Karnataka. The seeds were dried at an ambient condition and were powdered. Powdered seeds were subjected to Soxhlet extraction using ethanol as a solvent. It was then filtered and dried by using rotary evaporator, and preserved at -4°C until further analysis.

Chemicals

Gallic acid, Trichloroacetic acid (TCA), ferric chloride, nicotinamide adenine dinucleotide (NADH), butylated toluene (BHT), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), quercetin, ascorbic acid, thymoquinone were obtained from Hi media, Sigma Aldrich India ltd

Phytochemical Screening

Estimation of Total phenolic (TP) content:

The amount of total phenolic content was determined according to the modified method of Prabhu *et al*., using by Folin- Ciocalteu reagent [15]. Gallic acid (0.5mg/mL) was used as the standard. 1 mLaliquot of various concentrations of gallic acid ranging from (1-125mg/ mL) and 5mL of FC reagent (1:10 diluted with distilled water) were mixed. After 5 min, 4mL of sodium carbonate solution was added and the solution was incubated for 30 min at a room temperature. The Absorbance of the samples was measured at 765nm using a UV Vis spectrophotometer. The contents were calculated in gallic acid equivalents (GAE) using the following formula.

X=C.V/m

Where

X= Phenol content, mg/g plant extract in Gallic equivalents;

C= Gallic acid concentration from the calibration curve in mg/g

V= Volume of plant extract, mL

m= Weight of the plant extract, g

Estimation of Total flavonoid (TF) content:

The amount of total flavonoid content was estimated spectrophotometrically by following the modified protocol given by Bhatti *et al*., [16].This method is based on the formation of flavonoid aluminium complex. 0.5mL of the sample was mixed separately with 0.1mL of aluminium chloride solution (2% w/v). After incubation at room temperature for 15min, the absorbance of the reaction was measured at 510nm. The flavonoid content was calculated from the standard Quercetin curve and the results are expressed as quercetin equivalent (QE) using a similar formula used for total phenols.

Estimation of Total tannin content

The amount of tannin content was estimated by modified method of Stalin and Sudhakar [17]. The method is based on the mechanism by which phenols reduce potassium ferrous cyanide to produce ferrous ions; these ferrous ions in turn reacts with ferric chloride in the presence of dilute HCl to form a Prussian blue coloured complex, which can be measured spectrophotometrically at 700nm. The tannin content was calculated from the standard tannic acid curve and results the expressed as tannic acid equivalent (TAE) using a similar formula used for total phenols and flavonoids.

DPPH radical scavenging activity

The DPPH quenching ability of N.*sativa* ethanolic extract was measured by DPPH radical scavenging assay described by Rehman*et al*., [18].This method depends on the reduction of purple 1,1-diphenyl-2-picrylhydrazyl (DPPH) to a yellow coloured diphenyl- picrylhydrazine and the remaining DDPH which showed maximum absorption at 517nm which was measured spectrophotometrically. 1mL of extract of varying concentrations (1-100µg/mL) and quercetin standard were mixed with 3mL of freshly prepared DDPH in methanol. An equal amount of methanol and DPPH were served as control. The solutions were mixed thoroughly and incubated in dark for 15 min at room temperature. The absorbance of sample and standard was measured at 517nm. The decrease in absorbance ofDPPH solution indicates an increase in DPPH radical scavenging activity. The ability to scavenge the DPPH was calculated using the following equation.

DPPH radical scavenging activity = $(A_o-A_1)/A_o \times 100$

Where:

A_o is the absorbance of the control (DPPH) and A_1 - is the Absorbance of the sample.

Percentage radical activity was plotted against the concentration of the corresponding antioxidant substance to obtain the IC_{50} value. IC_{50} is defined as the amount of antioxidant substance required to scavenge 50% of free radicals present in the assay system. IC_{50} values are inversely proportional to the antioxidant potentials. The analysis of the sample was done in triplicates.

Superoxide radical scavenging activity

The scavenging activity of N.*sativa* extract was measured by the reduction of NBT (Nitro blue tetrazolium) [19]. The non-enzymatic phenazine methosulphate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce NBT to a purple formazan. Various concentration (0-100µg/mL) of extract and standard were mixed with 1mL of reaction mixture consisting phosphate buffer (100Mm, pH 7.4), NBT (156µm), and NADH (73µM). The reaction was initiated by adding PMS (60mM). The reaction mixtures was incubated for 5min and measure the absorbance at 560nm against standard ascorbic acid. The percentage inhibition was then compared with that of the reference compound.

Hydroxyl radical scavenging assay

The 2- deoxyribose assay was used to determine the effect of the extract on –OH radical [20]. Various concentrations of 0.4mL extract were mixed with 0.6mL of deoxy ribose (1mM) and the volume was made to 1.6mL using phosphate buffer. The reaction mixture was incubated for 10 min at an ambient temperature and 0.4mL of phenyl hydrazine hydrochloride (0.2mM) was added. The reaction mixture was incubated for 1hour after adding 1mL each of 2.8% TCA and 1% TBA. It was then heated on boiling water bah for 10 min and absorbance was measured at 532nm. The negative control without any antioxidant was considered 100% deoxyribose oxidation. The percentage of hydroxyl radical scavenging activity of extract was determined by comparing with the negative control. Ascorbic acid was used as the standard.

Hydrogen peroxide scavenging assay

Hydrogen peroxide radical scavenging activity of N.*sativa* extract was determined by adding 1mL of 1M hydrogen peroxide to 1mL of extract. 3% of ammonium molybdate was added followed by 2M sulphuric acid and 1.8mM

potassium iodide. The mixture was titrated against 5.09mM sodium thiosulphate. The end point was yellow to colourless. The reaction mixture without extract was used as control. Ascorbic acid was used as the standard [21].

The percentage inhibition was calculated using the formula,
% Inhibition = $((V_0-V_1)/V_0) \times 100$
Where, V_0 - Volume of sodium thiosulphate used by control
V_1 – Volume of sodium thiosulphate used by extract.

Quantification of Thymoquinone (TQ) by HPLC:
Preparation of Thymoquinone standard for HPLC analysis. Thymoquinone (99.9% purity), standard was procured from Sigma Aldrich, Bengaluru. Standard solutions of TQ 0.2%, 0.4%, 0.6% and 0.8% w/v were prepared in methanol by dilution from stock solutions and were used for verification of retention time and preparation of calibration curve.

Chromatographic procedure
Thymoquinone was quantified by HPLC (High Pressure Liquid Chromatography) in a Shimadzu HPLC model. The elution was carried out in Luna 5m C18 (2)100 Å column with dimension of 250 ×4.6mm, 5µ, Acetonitrile: HPLC grade water (55:45, v/v) is used as mobile phase with flow rate of about 1mL/min. PDA (ë=254nm) is used for quantification of thymoquinone. The total run time was for 14 min. About 20 µL of the standard (Thymoquinone) and N. *sativa* extract and was injected separately and respective chromatograms were analysed [22].

Statistical Analysis:
All data were reported as mean ± standard deviation of means of three replicates. The IC_{50} were calculated using Graph Pad Prism 6 Software.

3. Results

Total phenolic, flavonoid and tannin content
The total phenolic content of N.*sativa* extract was expressed in terms of gallic acid equivalent (GAE). The concentration of total phenol is expressed as mg of GAE/g extract, the total phenolic content in the extract was found to be 8.248 ±0.33 mg GAE/g.

The total flavonoid content of N.*sativa* extract is expressed in terms of quercetin equivalent (QE). The concentration of total flavonoid is expressed as mg of QE/g of extract. The total flavonoid content in the extract was 4.62±0.01 mg QE/g.

The total tannin content of N.*sativa* extract is expressed in terms of tannic acid equivalent (TAE). The concentration of total tannin is expressed as mg of TAE/g of extract. The total tannin content in the extract was 0.59±0.01 mg TAE/g.

DPPH radical scavenging assay
The antioxidant potential of the ethanolic extract of N.*sativa* was analysed by DPPH radical scavenging assay. Fig 1 suggests that the extract exhibits a dose dependent activity, i.e. DPPH scavenging activity increases proportionally with increase in concentration of the extract. The IC_{50} value of the extract was found to be 46.01 µg/mL and that of standard quercetin was 2.02 µg/mL. The ethanolic extract of N.*sativa* showed moderately significant scavenging effects on DPPH radical.

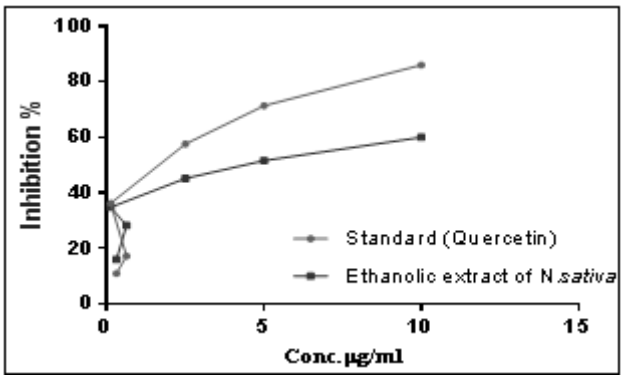

Figure 1: DPPH radical scavenging activity of N.*sativa*

Superoxide radical scavenging assay:
The percentage inhibition of super oxide radical generation of N.*sativa* ethanolic extract was found to be increasing in a concentration dependent manner as illustrated in fig 2. The IC_{50} value and the standard ascorbic acid value was found to be 46.26 µg/mL, 80.46 µg/mL respectively

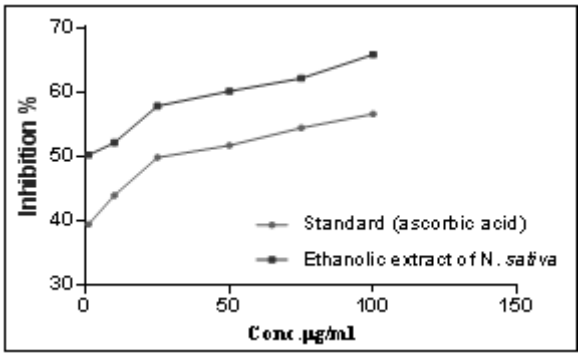

Figure 2: Superoxide radical scavenging activity of N.*sativa*

Hydroxyl and Hydrogen peroxide radical scavenging assay
This assay shows the ability of N.*sativa* extract and standard ascorbic acid to inhibit hydroxyl radical mediated deoxyribose degradation. The results indicate that the extract inhibition occurs in a dose dependent manner as per Fig 3. The IC_{50} value of the extract at 65.24 µg/mL was less than that of standard 56.21 µg/mL. The result obtained from the hydroxide scavenging assay shows that the inhibition of N.*sativa* at 53.53% was less than that of standard Ascorbic acid at 46.1%.

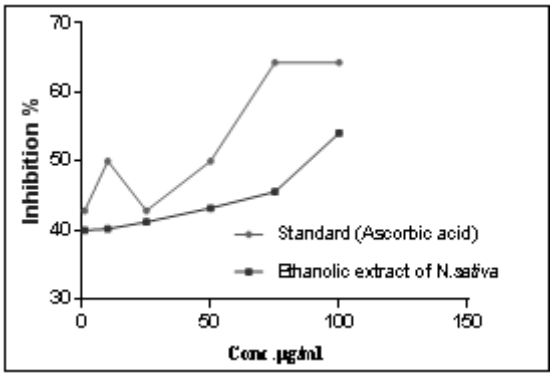

Figure 3: Hydroxyl radical scavenging activity of N.*sativa*

Quantification of Thymoquinone (TQ) by HPLC

The ethanolic extract of N.*sativa* was subjected to HPLC analysis to quantify the amount of TQ present. This analysis demonstrated the presence of TQ, the bioactive compound. Its quantity was found to be 0.32%. The linear regression equation was obtained in the form of Y=mx+c, where y and x corresponds to peak area and concentration, respectively (Fig 4a). A comparison of HPLC profiles of the ethanolic extract with that of thymoquinone showed a retention time of 10.53min (Fig4b and Fig4c).

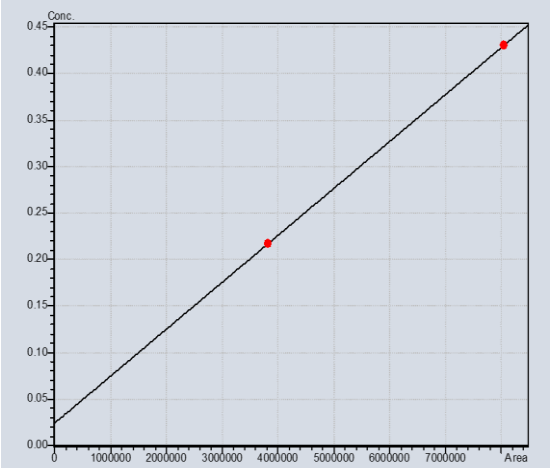

Figure 4 (a): Calibration curve for standard Thymoquinone

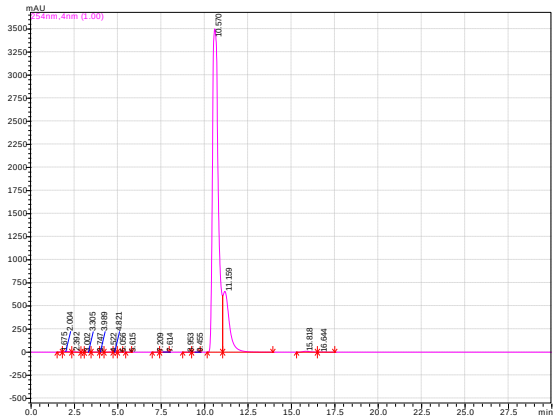

Figure 4 (b): HPLC peak for standard Thymoquinone.

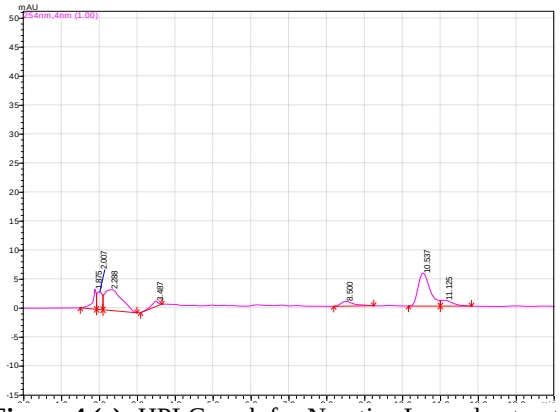

Figure 4 (c): HPLC peak for N.*sativa* L. seed extract

4. Discussion

Recently appropriate assessments of antioxidant activity as well as availability of resources in the form of food and medicinal plants are drawing great attention in food science and nutrition. Plants are a good source of antioxidants, the presence of polyphenols such as phenols, flavonoid and tannins play a major role in scavenging free radicals [23,24].Free radicals are responsible for unregulated chain of oxidation and reduction reactions. Oxidative stress in the form of reactive oxygen species (ROS) is generated due to various environmental factors such as pollutants, pathogen invasion, toxins and ultra violet radiation. The presence of ROS is extremely damaging to the organism at high concentration. The increased production of ROS due to environmental stress leads to various disease conditions such as cancer, neurodegerative diseases, diabetes and other inflammatory diseases [25]. Many synthetic drugs protects against oxidative damage but they have adverse side effects, substitute to this problem is to consume natural antioxidants.

Plants metabolite includes aromatic compounds such as phenols. Phenols exhibit anti-aging, anti-inflammatory and anti-apoptotic properties. Flavonoids are potent antioxidants. The potency depends on the molecular structure and the position of hydroxyl group within the chemical structure. Tannins are medicinally used for their antiviral, antibacterial and anti-tumour activities. Certain kind of tannins can reduce the mutagenicity of a number of mutagens and also exhibit antioxidant activity [26,27]. It is evident from the study that N.*sativa* extract shows a higher phenolic, flavonoid and tannin content (8.248 ±0.33 mg, 4.62±0.01mg and 0.59±0.01 mg) and is a good source of polyphenols. Toma*et al*, [28] reported the presence of total phenols 4.12mg and flavonoid 2.01mg content in the crude ethanolic extract of N.*sativa*. Similarly, Goga *et al*, [29] reported 5.58mg of phenolic content and 3.99mg of flavonoid content in crude ethanol and 31.5mg of phenolic and 16.34 mg flavonoid content in n-hexane extract of N.*sativa* respectively. Hence, the present study is in line with observations from earlier studies. Further, the role of these secondary metabolites in the maintenance of anti-oxidative approach is well elucidated.

The antioxidant activity of N.*sativa* extract was determined using DPPH, a stable radical commonly used to determine scavenging activity. In the present study, it was found that the scavenging activity increased with increase in concentration. The results from the studies on anti-oxidant property of N.*sativa* extract demonstrated a significant scavenging activity. The IC_{50} value was found to be 46.01 µg/µL when compared to the standard quercetin 2.02 µg/ µL. Similar studies in petroleum ether, distilled water and methanol revealed a dose dependent increase in scavenging activity [30]. Toma*et al* highlight the scavenging ability of N.*sativa* crude ethanolic extract that showed IC_{50} = 624.7 ± 12.77 µg/ µL [28]. The result of the present study confirms that the ethanolic extract of N.*sativa* contains sufficient phytochemical constituents with the ability to donate 'H^+' to DPPH free radical and reduce it to a non-free radical form viz., DPPH-H.

Superoxide anion radical ($O_2^{\cdot}$) is generated by four electron reduction of molecular oxygen in to water. This radical also forms in aerobic cells due to electron leakage from the electron transport chain. $O_2^{\cdot}$ radicals are very harmful to cellular components and increase in number under stress conditions [31] . Inhibition of superoxide anion radical derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT in PMS/NADH-NBT system. It is reported that flavonoids are effective antioxidants mainly because they scavengesuperoxide anions. In this study an assessment was made to confirm invitro super anion scavenging activity in *N.sativa* seed extract. The IC_{50} value was found to be 80.46 µg/mL when compared to standard ascorbic acid (46.26 µg/mL). Rather *et al* showed moderate to strong free radical scavenging activity of N.*sativa* oil [32]. Kamili [33] reported that petroleum ether extract of N.*sativa* exhibits good superoxide anion radical scavenging activity (85%). The present study has shown that superoxide radical activity of N.*sativa* and standard ascorbic acid are markedly increased with increase in concentration and suggests that the extract possesses notable quenching activity due to the presence of phenolic compounds.

Hydrogen peroxide (H_2O_2) is a weak oxidising agent that directly inactivates some enzymes, usually by the oxidation of essential thiols (-SH) groups. H_2O_2 plays a major role in signalling and regulates a wide variety of biological functions. It can cross the cell membranes rapidly, and can react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radicals which is the origin of many toxic effects [34]. An earlier study by Nejdet *et al*, reported that the reducing power of N.*sativa* in methanolic extracts increased with increasing concentration [35]. The present study, demonstrate that the ethanolic extract shows of N.*sativa* exerted significant H_2O_2 scavenging activity on peroxide radicals. N.*sativa* showed activities of 53.5% and 46.1%, respectively.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and oxidative damage of DNA. The interaction of ferrous or cupric metal ions with hydrogen peroxide leads to the formation of hydroxyl radical in biological systems [36,37]. The hydroxyl radical generated by the Fe^{2+} ascorbic acid and EDTA $–H_2O_2$ system was scavenged by the extract and the standard in a concentration dependent manner. This observation suggests that N.*sativa* can be used as an alternative remedy to synthetic antioxidants in combating the oxidative activity of hydroxyl radical.

The HPLC analysis in the present study revealed a 0.32% composition of thymoquinone in the ethanolic extract of N.*sativa*. According to Jumah *et al*, the lowest percentage composition of thymoquinone was observed in the aqueous extract. The solubility and stability of thymoquinone were studied and it was suggested that the content of thymoquinone may differ as a result of various factors of extraction [22]. A lesser content of thymoquinone in ethanol and methanol extracts was reported [38,39]. As per Ashraf *et al.*, the antioxidant potential of thymoquinone varies depending upon the choice of solvent or extraction procedure [40].

5. Conclusion

Phytochemical investigation of ethanolic extract of N.*sativa* revealed the presence of polyphenolic compounds and favourable free radical scavenging activity. N.*sativa* extract showed the presence of Thymoquinone, which is the main bioactive compound, used traditionally in the treatment of various diseases. Hence, this plant alone or in combination may exhibit a distinguished antioxidant activity and help to curb free radicals such as ROS and is recommended a substitute for synthetic drugs to handle oxidative damage caused to various biological macromolecules, including DNA, lipids and proteins. Further isolation of bioactive compounds and evaluation of therapeutic values in living models is required for novel drug discovery.

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References

[1] Ali SA, Meitei K V. Nigella sativa seed extract and its bioactive compound thymoquinone: The new melanogens causing hyperpigmentation in the wall lizard melanophores. *J Pharm Pharmacol*. 2011;63(5):741-746. doi:10.1111/j.2042-7158.2011.01271.x

[2] De Brum TF, Zadra M, Piana M, et al. HPLC analysis of phenolics compounds and antioxidant capacity of leaves of Vitex megapotamica (Sprengel) moldenke. *Molecules*. 2013;18(7):8342-8357. doi:10.3390/molecules18078342

[3] Moein MR, Moein S, Ahmadizadeh S. Radical scavenging and reducing power of salvia mirzayanii subfractions. *Molecules*. 2008;13(11):2804-2813. doi:10.3390/molecules13112804

[4] Venkatachallam SKT, Pattekhan H, Divakar S, Kadimi US. Chemical composition of Nigella sativa L. seed extracts obtained by supercritical carbon dioxide. *J Food Sci Technol*. 2010;47(6):598-605. doi:10.1007/s13197-010-0109-y

[5] Mahesar SA, Kandhro AA, Khaskheli AR, Talpur MY, Sherazi STH. SB-ATR FTIR spectroscopic monitoring of free fatty acids in commercially available Nigella sativa (Kalonji) oil. *J Spectrosc*. 2014;2014. doi:10.1155/2014/510890

[6] Fahmy HM, Noor NA, Mohammed FF, Elsayed AA, Radwan NM. Nigella sativa as an anti-inflammatory and promising remyelinating agent in the cortex and hippocampus of experimental autoimmune encephalomyelitis-induced rats. *J Basic Appl Zool*. 2014;67(5):182-195. doi:10.1016/j.jobaz.2014.08.005

[7] Seghatoleslam M, Alipour F, Shafieian R, et al. The effects of Nigella sativa on neural damage after pentylenetetrazole induced seizures in rats. *J Tradit Complement Med*. 2016;6(3):262-268. doi:10.1016/j.jtcme.2015.06.003

[8] Beheshti F, Hosseini M, Vafaee F, Shafei MN,

Soukhtanloo M. Feeding of Nigella sativa during neonatal and juvenile growth improves learning and memory of rats. *J Tradit Complement Med*. 2016;6(2):146-152. doi:10.1016/j.jtcme.2014.11.039
[9] Imam A, Ajao MS, Ajibola MI, et al. Black seed oil ameliorated scopolamine-induced memory dysfunction and cortico-hippocampal neural alterations in male Wistar rats. *Bull Fac Pharmacy, Cairo Univ*. 2016;54(1):49-57. doi:10.1016/j.bfopcu.2015.12.005
[10] Elkhateeb A, El Khishin I, Megahed O, Mazen F. Effect of Nigella sativa Linn oil on tramadol-induced hepato- and nephrotoxicity in adult male albino rats. *Toxicol Reports*. 2015;2:512-519. doi:10.1016/j.toxrep.2015.03.002
[11] Badary OA, Taha RA, Gamal El-Din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. *Drug Chem Toxicol*. 2003;26(2):87-98. doi:10.1081/DCT-120020404
[12] Radad K, Hassanein K, Al-Shraim M, Moldzio R, Rausch WD. Thymoquinone ameliorates lead-induced brain damage in Sprague Dawley rats. *Exp Toxicol Pathol*. 2014;66(1):13-17. doi:10.1016/j.etp.2013.07.002
[13] Gupta B, Ghosh KK, Gupta RC. *Thymoquinone*. Elsevier Inc.; 2016. doi:10.1016/B978-0-12-802147-7.00039-5
[14] Aruoma OI, Bahorun T, Jen LS. Neuroprotection by bioactive components in medicinal and food plant extracts. *Mutat Res - Rev Mutat Res*. 2003;544(2-3):203-215. doi:10.1016/j.mrrev.2003.06.017
[15] Prabu K, Rajasekaran A, Bharathi D, Ramalakshmi S. Anti-oxidant activity, phytochemical screening and HPLC profile of rare endemic Cordia diffusa. *J King Saud Univ - Sci*. 2018:0-3. doi:10.1016/j.jksus.2018.04.025
[16] Bhatti MZ, Ali A, Ahmad A, Saeed A, Malik SA. Antioxidant and phytochemical analysis of Ranunculus arvensis L. extracts. *BMC Res Notes*. 2015;8(1):1-8. doi:10.1186/s13104-015-1228-3
[17] N S, P SS. Screening of phytochemical and pharmacological activities of Syzygium caryophyllatum (L.) Alston. *Clin Phytoscience*. 2018;4(1). doi:10.1186/s40816-017-0059-2
[18] Rahman MM, Islam MB, Biswas M, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC Res Notes*. 2015;8(1):1-9. doi:10.1186/s13104-015-1618-6
[19] Chakraborthy GS. Free radical scavenging activity of Aesculus indica leaves. *Int J PharmTech Res*. 2009;1(3):524-526.
[20] Abhirama BR, Shanmuga Sundaram R, Sambath Kumar R. Studies on in vitro antioxidant and free radical scavenging activities of ethanol extract of whole plant Biophytum sensitivum (Linn.) DC. *Asian J Chem*. 2017;29(3):519-524. doi:10.14233/ajchem.2017.20216
[21] Maswada HF. Assessment of total antioxidant capacity and antiradical scavenging activity of three Egyptian wild plants. *J Med Sci*. 2013;13(7):546-554. doi:10.3923/jms.2013.546.554
[22] Salmani JMM, Asghar S, Lv H, Zhou J. Aqueous Solubility and Degradation Kinetics of the Phytochemical Anticancer Thymoquinone; Probing the. 2014:5925-5939. doi:10.3390/molecules19055925
[23] Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J Bot*. 2012;2012:1-26. doi:10.1155/2012/217037
[24] Kasote DM, Katyare SS, Hegde M V., Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. *Int J Biol Sci*. 2015;11(8):982-991. doi:10.7150/ijbs.12096
[25] Xu DP, Li Y, Meng X, et al. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. *Int J Mol Sci*. 2017;18(1):20-31. doi:10.3390/ijms18010096
[26] Mengesha Yessuf A. Phytochemical Extraction and Screening of Bio Active Compounds from Black Cumin (Nigella Sativa) Seeds Extract. *Am J Life Sci*. 2015;3(5):358. doi:10.11648/j.ajls.20150305.14
[27] Elgailani IEH, Ishak CY. Methods for Extraction and Characterization of Tannins from Some Acacia Species of Sudan. *Pakistan J Anal Environ Chem*. 2016;17(1):43-49. doi:10.21743/pjaec/2016.06.007
[28] Toma CC, Olah NK, Vlase L, Mogoşan C, Mocan A. Comparative studies on polyphenolic composition, antioxidant and diuretic effects of nigella sativa L. (black cumin) and nigella damascena L. (Lady-in-a-Mist) seeds. *Molecules*. 2015;20(6):9560-9574. doi:10.3390/molecules20069560
[29] Goga, A., Hasić, S., Bećirović, Š. and Ćavar S. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina Phenolic Compounds and Antioxidant Activity of Extracts of Nigella sativa L .*. *Bull Chem Technol Bosnia Herzegovina*. 2012;39:15-20.
[30] Reddy SH, Al-Kalbani AS, Al-Rawahi AS. Studies on Phytochemical Screening - Gc-Ms Characterization, Antimicrobial and Antioxidant Assay of Black Cumin Seeds (Nigella Sativa) and Senna Alexandria (Cassia Angustifolia) Solvent Extracts. *Int J Pharm Sci Res*. 2018;9(2):490-497. doi:10.13040/IJPSR.0975-8232.9(2).490-97
[31] Free Radical Scavenging And Antioxidant Activity Of Plant Fla Vonoids Chithan Kandaswami and Elliott Middleton , Jr . Department of Medicine School of Medicine and Biomedical Sciences State University of New York at Buffalo Buffalo , NY 14203 INTRODUCTION. 1994:351-376.
[32] Rather AA, Jain K. UK Journal of Pharmaceutical and Biosciences Available at www.ukjpb.com Pharmacognostic and Physicochemical Standardization of Nigella sativa and Allium cepa Seeds. 2017;5(6):35-40.
[33] Kamili AN. Antimicrobial and antioxidant activity of Nigella sativa Linn seeds. 2013;6(2):190-195.
[34] Hassan W, Noreen H, Khalil SU, et al. Ethanolic extract of Nigella sativa protects Fe(II) induced lipid peroxidation in rat's brain, kidney and liver homogenates. *Pak J Pharm Sci*. 2016;29(1):231-237.
[35] Şen N, Kar Y, Tekeli Y. Antioxidant activities of black cumin (Nigella sativa L.) seeds cultivating in different regions of Turkey. *J Food Biochem*. 2010;34(SUPPL. 1):105-119. doi:10.1111/j.1745-4514.2009.00309.x
[36] Treml J, Šmejkal K. Flavonoids as Potent Scavengers of Hydroxyl Radicals. *Compr Rev Food Sci Food Saf*.

2016;15(4):720-738. doi:10.1111/1541-4337.12204
[37] Lipinski B. Hydroxyl radical and its scavengers in health and disease. *Oxid Med Cell Longev*. 2011;2011. doi:10.1155/2011/809696
[38] Houghton PI, Zarka R, Heras B De, Hoult IBS. Fixed Oil of Nigella sativa and Derived Thymoquinone Inhibit Ficosanoid Generation in Leukocytes and Membrane Lipid Peroxidation. 1994;(6):33-36.
[39] D Kehep, Bakeet DM. The Black Seed Nigella sativa Linnaeus - A Mine for Multi Cures : A Plea for Urgent Clinical Evaluation of its Volatile Oil. *J Taibah Univ Med Sci*. 1(1):1-19. doi:10.1016/S1658-3612(06)70003-8
[40] Ishtiaq S, Ashraf M, Hayat MQ, Asrar M. Phytochemical analysis of nigella sativa and its antibacterial activity against clinical isolates identified by ribotyping. *Int J Agric Biol*. 2013;15(6):1151-1156.