

Pharmacological Characterization of *Nigella sativa* Extract and the Compound Beta-caryophyllene

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Traditional medicines made from medicinal plants are the most abundant biosources of medications. Pharmaceutical medications, synthetic pharmaceuticals, Siddha, Ayurveda, nutraceuticals, and dietary supplements all come from plant sources. In spite of several studies using herbal plants that have shown a strong link between phytochemical, anti-diabetic, and anti-inflammatory content, *Nigella sativa*—a spice plant of the Ranunculaceae family—showed more significant qualities than its competitors. Numerous pharmacological properties were discovered in both the *Nigella sativa* essential oil and the seeds. *Nigella sativa* is considered to be the most powerful antioxidant and medicinal plant with known medicinal properties and therapeutic applications, and it is used as traditional medicine for respiratory, gastrointestinal, rheumatic, and inflammatory disorders. The study was conducted using *Nigella sativa* and compound Beta caryophyllene to determine the anti-oxidant, invitro-anti-diabetic, anti-inflammatory, and enzymatic and non-enzymatic activity of the ethanolic seed extract of *Nigella sativa* and compound Beta caryophyllene on Alzheimer's disease because there were no toxic effects or serious side effects observed using animal models or in clinical trials. The antioxidant activity of the compound and the extract was examined to study the qualitative and quantitative analysis of the extract and the compound beta-caryophyllene. Phytochemical screening exhibited secondary metabolites of the extract, such as phenols, flavonoids, steroids, alkaloids, and cardiac glycosides. *Nigella sativa* extract and its constituents such as beta-caryophyllene, possess a protective effect against toxicity, which is caused by drugs used for treating cancer as well as neurodegenerative disorders. Further, the extract prevents a decrease in hemoglobin levels as well as leukocyte counts. The purpose of the research is to demonstrate that *Nigella sativa* and the chemical beta-caryophyllene may be employed as medicinal agents and that beta-caryophyllene may have potential cholinesterase inhibitory effects.

Keywords: Alzheimer's; Anti-inflammatory; Beta-caryophyllene; Cholinesterase; *Nigella sativa*; Pharmacological activity.

Medicinal plants have various forms like extracts, oils, and compounds that are identified to have anti-AD, anti-diabetic, and anti-inflammatory properties and have also undergone many clinical and pre-clinical studies. Dementia is defined as an acquired deterioration in cognitive abilities that impairs the successful performance of activities

of daily living. The memory impairment in Alzheimer's disease (AD) patients is due to the loss of cholinergic neurons, while those in non-AD dementia are due to the loss of serotonergic and glutaminergic neurons. While in the former, memory impairment is primary, in the latter, behavioral symptoms are primary, leaving memory

relatively spared. The authors suggestions have led several researchers to conclude that the presence of certain types of alkaloids, saponins, and glucosides, as well as antioxidant activity, possess a neuroprotective effect against A β production, causing AD, which tends to be high. As medicinal plants are rich biosources of drugs from plant sources, they have been widely used in many research studies and nutraceuticals, the food industry for preparing food supplements, the drug manufacturing industry, and also as Siddha and Ayurvedic medicines. The most common and native treatment used in India is Ayurvedic medicine. The treatment and cure for diseases are through medicinal plants, which are evaluated and designed for potential cure ¹.

For many health-related diseases and disorders various phytotherapies are used, which are obtained from herbal medicines. It causes a major change in the outlook for treatment around the world. According to research, herbal plants are considered to be the best remedy and have holistic therapy, which consolidates the physiological status of the patients ². The efficient and effective herbal medicines contain several compounds that actively interact with the *in vivo* environment and change the physiological status of the body through certain alterations in the biochemical processes without any side effects ³. Efficient and effective herbal medicines contain several compounds that actively interact with the *in-vivo* environment and change the physiological status of the body through certain alterations in the biochemical processes without any side effect. ³

Nigella sativa, a well-known spice plant (black cumin) of the Ranunculaceae family, exhibited many significant therapeutic properties. The active compounds of *Nigella sativa* have made it to be used as a medicinal plant for thousands of years and also as medicinal ailments ⁴. The drugs that are formulated are from the plant's stem, bark, flowers, leaves, roots, seeds, and fruits. The phytochemical constituents are the most important sources for designing a drug ^{5,6}. Various essential oils and extracts, which are the richest source of phytochemicals and contain tannins, carbohydrates, alkaloids, terpenoids, phenolics, flavonoids, and steroids ⁷. Many studies are correlated with herbal extracts, which are a good source of phytochemical constituents, have

anti-diabetic and anti-inflammatory activities, and have good potential for enzymatic assays. The generation of reactive oxygen species causes adverse effects in aerobic organisms, but the metabolism of oxygen has more beneficial effects. The macromolecules mostly undergo oxidative reactions that are mediated by reactive oxygen species. Biomedical research mainly focuses on the adverse effects of reactive oxygen species, which act upon the biological system ⁸. Based on the experimental studies and clinical studies, pharmacological activity occurs due to the antioxidant activity of the extract. Because it has the ability to scavenge free radicals and inhibit lipid peroxidation ⁹. Many studies and researches have concluded that the seed extract of *Nigella sativa* and its derivative products were used as a treatment for many diseases, like liver diseases, rheumatism, as well as in the treatment of inflammatory disorders and their relevant consequences ¹⁰. Further, the constituents of the extract are found to be effective, and this is evident as it decreases the nephrotoxicity induced by cisplatin in rodents and possesses high anti-tumor activity ¹¹. Most of the therapeutic properties are due to the presence of some phenolic compounds in the seed, especially thymoquinone, beta-caryophyllene, etc which are known to be the major bioactive component. The present study was carried out using *Nigella sativa* extract and the compound beta-caryophyllene to phytochemically analyze the effects of the extract and the compound on Alzheimer's disease.

MATERIAL AND METHODS

Reagents

Amyloid peptide, Thymoquinone, beta-caryophyllene, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was purchased from Sigma Aldrich; 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, Dimethyl Sulfoxide (DMSO), Triton X 100, and p-Nitrophenyl - α -D- glucopyranoside were obtained from AR Teck Pvt. Ltd., India. All the chemicals used for the experiments are of analytical grade.

Anti-oxidant activity of ESENS and compound beta-caryophyllene

The antioxidant activity of compounds such as 2,2 -diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide, and nitric oxide was studied.

The DPPH radical scavenging capacity was measured using the Brand-William method¹². Similarly, hydrogen peroxide and nitric oxide were measured using Ruch and Marcocci methods^{13,14}.

2,2 Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay of ESENS and the compound beta-caryophyllene

Ethanol seed extract of *Nigella sativa* (ESENS) and compound beta-caryophyllene obtained through HR-LCMS were pre-dissolved using DMSO and used for the study. The compounds and the extract in various concentrations were taken and added to 100 μ l of 0.1 mM DPPH, which was freshly prepared using ethanol. DPPH alone serves as a blank, and ascorbic acid is the standard. The solution mixture was further incubated for 30 minutes. The incubated mixture was studied using an absorbance of 517 nm using a UV-Vis spectrophotometer. The triplicates obtained were used to calculate the percentage of radicals that were being scavenged.

Hydrogen peroxide scavenging assay

Based on the Ruch and Marcocci method, using phosphate buffer, 40 mM of hydrogen peroxide was freshly prepared, and the pH was adjusted to 7.4. Various concentrations of the ESENS and the compound beta-caryophyllene were added to the freshly prepared hydrogen peroxide, which was further incubated for 30 minutes at RT. Phosphate buffer alone serves as a blank, and ascorbic acid is used as a standard. After the incubation period, the samples were measured at 560 nm using UV-Vis spectrophotometry. Triplicates obtained were used to calculate the percentage of inhibition that the compounds beta-caryophyllene and ESENS had undergone.

Nitric oxide radical scavenging assay

Based on modified protocol¹⁵, the nitric oxide radical scavenging assay was performed for the various concentrations of ESENS and the compound beta-caryophyllene. Equal amounts of Griess reagent were mixed with sulphanilamide (1%), which was prepared from 2.5% phosphoric acid, and naphthylethylene diamine dihydrochloride (0.1%) in 2.5% phosphoric acid. To different concentrations of Ethanol Seed Extract of *Nigella sativa* (ESENS) and compound beta-caryophyllene, 0.5 ml of 10 mM sodium nitroprusside, which was prepared using phosphate buffered saline, was added. Further, the tubes containing the reaction

mixture were incubated at 25°C for 180 minutes, followed by the addition of an equal amount of freshly prepared Griess reagent. For control, the reaction mixture was prepared with buffered saline without ESENS and the compound beta-caryophyllene. The samples were read at 546 nm using UV-Vis Spectrophotometers. Ascorbic acid is used as a standard. The percentage of inhibition was observed and recorded to analyze the percentage of nitrite scavenging activity of the extract, compound, and standards.

Anti-*in vitro* studies of ESENS and the compound beta-caryophyllene

Increasing levels of proinflammatory cytokines cause metabolic disorders that lead to cell death. The ESENS and the compound beta-caryophyllene were studied to find out how they stop the proinflammatory cytokines that cause metabolic disorders.^{16,17}

HRBC membrane stabilization assay of ESENS and the compound beta-caryophyllene

To the tube containing EDTA, 100 μ l of blood sample and various concentrations of ESENS and the compound beta-caryophyllene were added. Triton x 100 serves as a positive control, while a blood sample alone serves as a negative control. The tube containing the reaction mixture was incubated at 37°C for 30 minutes. Followed by the centrifugation of the sample at 5000 rpm for 15 minutes. The supernatant is collected in a separate tube for analysis and stabilization using UV-Vis Spectrophotometer at 517 nm.

Cytotoxicity assay of ESENS and compound the beta-caryophyllene

The isolated lymphocyte cells are cultured in humidified 5% (v/v) CO₂ / air at 37°C in Dulbecco's Modified Eagle Medium (DMEM), which is supplemented with 10% fetal bovine serum and 100 U/ml of penicillin. In 96-well plates, 5x10⁴ cells/ml were cultured. Simultaneously, amyloid beta fibrils were prepared by incubating the amyloid monomer at room temperature. The pre-formed amyloid beta fibrils with ESENS and compound beta-caryophyllene and without ESENS and compound are diluted in freshly prepared DMEM medium and added to a microtiter well plate; the wells final concentration was made to 2im μ l/L. The same volume of medium is added to the control well. The plates were then incubated at

37°C for 48 hours. Cell viability was determined by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) toxicity assay. The MTT was prepared at a concentration of 5mg/ml; added to each well, and incubated further for 3 hours at 37°C. In the medium that was removed, DMSO was added to each well. The samples in the wells were mixed well, and the samples were read using a microplate reader at 490 nm.^{18,19}

Enzyme linked immune-sorbent assays of ESENS and the compound beta-caryophyllene

Interleukin – 1 – beta were analyzed using lymphocyte cell culture supernatant and studied using commercially available ELISA kit protocols. The lymphocytes were collected, and 100 µl of the sample was added to each well of the 96-well microtiter plates along with an equal volume of DMEM medium. The samples were further incubated for 2 hours at 37°C. The incubated samples were again aspirated and washed 3 times. Further, 100 µl of detection reagent B was added and further incubated for 30 minutes at 37°C. The samples are washed 5 times. To achieve this, 90 µl of substrate solution was mixed and incubated for 10–20 minutes at 37 °C. To terminate the reaction, 50 µl of the stop solution was mixed, and the absorbance was measured at 450 nm immediately after mixing the stop solution.

Anti-diabetic activity of ESENS and the compound beta-caryophyllene

Anti-diabetic studies such as the α -amylase inhibitory assay and the α -glucosidase inhibitory assay were studied.

α -amylase inhibitory assay

Based on the protocol, ESENS and beta-caryophyllene of various concentrations were prepared and mixed with di-methyl sulfoxide solution (DMSO). Simultaneously, alpha-amylase was prepared by mixing it with phosphate buffer (pH 6.8). The reaction mixture prepared was incubated, after which 1% starch solution was added to all the tubes. In addition, the tubes were again incubated for another 15 minutes. The reaction was stopped by adding 1ml of di-Nitro Salicylic acid (DNS) reagent, and the tubes were boiled in a water bath for 10 minutes. The contents were then cooled, and 10 ml of distilled water was added to all the tubes. Absorbance at 540 nm was measured with acarbose as a positive control.^{20,21}

α -glucosidase inhibitory assay

α -glucosidase inhibitory assay was studied based on the protocol¹⁶. 112 il of potassium phosphate buffer at pH 6.8 was mixed with 20 il of enzyme solution, 8 il of the extract, and beta-caryophyllene and incubated for 15 minutes at 37°C. Following the incubation, 20 il of NPG was added and again incubated for 15 minutes at 37°C. The reaction was then terminated using 80 il of Na₂CO₃ solution. The sample absorbance was measured at 450 nm. For control, 8 il of Dimethyl Sulfoxide (DMSO) was added to the reaction mixture instead of extract and compound. Acarbose serves as a standard.

Enzymatic and non-enzymatic assays of ESENS and the compound beta-caryophyllene

The enzymatic assays such as Acetyl cholinesterase (AChE) hydrolysis²² and the non-enzymatic assays such as Metal chelating assay²³ were studied using the Ethanolic Seed Extract of *Nigella sativa* (ESENS) and beta-caryophyllene to analyze the effectiveness of the compound and the ESENS in preventing the hydrolysis of the enzyme AChE, which is a neurotransmitter.

Enzymatic assay- Acetyl cholinesterase assay

The acetylcholinesterase assay of the Ethanolic Seed Extract of *Nigella sativa* (ESENS) and the compounds were performed by adding 40 il of 0.28 U/ml acetylcholine enzyme and 140 il of 3.3 mM 5,5-dithiobis-(2-nitrobenzoic) acid, which is prepared using a 0.1 M phosphate buffered solution of pH 7.0 that also contains 6 mM of NaHCO₃. The extract, the compounds of various concentrations, and 80 il of phosphate buffered saline of pH 8.0 were added to the reaction mixture. The solution was incubated for about 20 minutes at 25°C. 40 il of 0.5 mM acetylthiocholine iodide was further added to each well containing the solution. After the addition of substrate, the reaction mixture was measured at 412 nm using UV-Vis spectrophotometry.

Non-enzymatic assay – Metal chelating assay

To the mixture containing 168 il of 0.1 M tris-HCL, 218 il of 0.8% w/v sodium chloride, 150 il of 500 iM freshly prepared FeSO₄, further, various concentrations of extract and compounds were added, followed by incubation for about 20 minutes. To the incubated mixture, 3 il of 0.25% of 1, 10 - Phenanthroline was added to read the absorbance of the mixture at 510 nm.

RESULTS

Anti-oxidant activity of ESENS and the compound beta-caryophyllene 2,2 Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay

Diphenyl-1-picrylhydrazyl (DPPH) is more stable for estimating free radicals. It is

due to the ability to donate hydrogen molecules. It takes up H molecules to form a dimagnetic compound, which is more stable. The ESENS showed good activity as compared to the control ascorbic acid, whose IC₅₀ value is 11.26±0.93ig/ml and for ascorbic acid it is 2.46±0.41ig/ml. Beta-caryophyllene exhibited good potential, and its IC₅₀ value is 14.02±0.71ig/ml. This result suggests

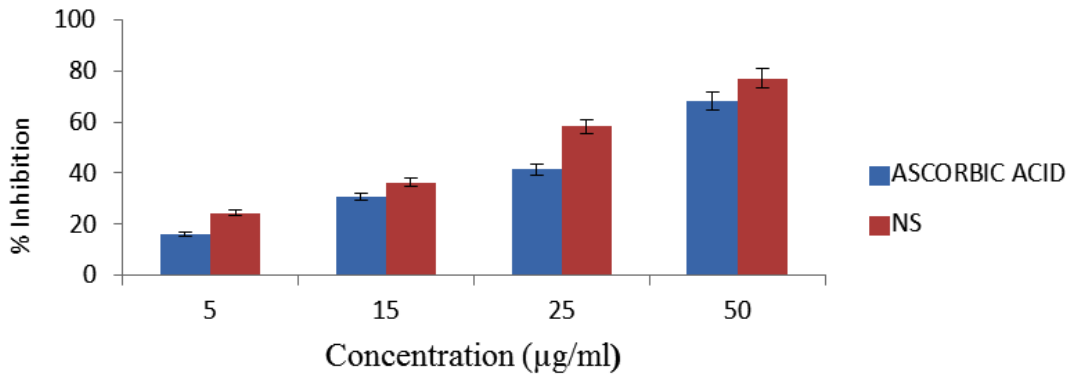


Fig. 1. DPPH radical scavenging activity of ESENS

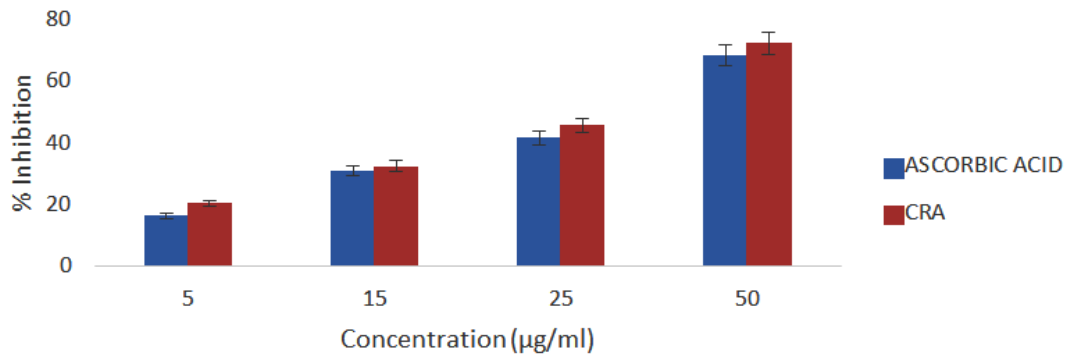


Fig. 2. DPPH radical scavenging activity of compound beta-caryophyllene

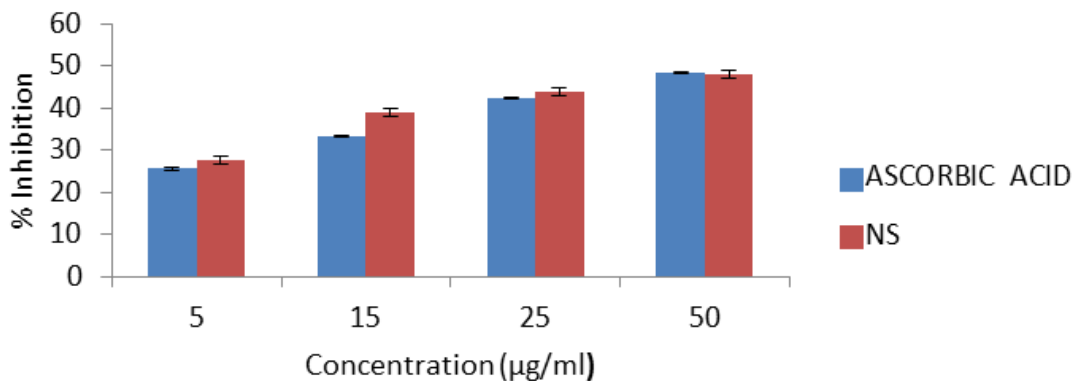


Fig. 3. Hydrogen peroxide radical scavenging assay of ESENS

that ESENS and beta-caryophyllene exhibit good potential activity (Fig. 1,2).

Hydrogen peroxide scavenging assay

The hydrogen peroxide activity exhibited is dose dependent when compared to the control

Ascorbic acid, whose IC₅₀ values are found to be 17.50±0.11µg/ml for Ascorbic acid, 95.14±0.66µg/ml for beta-caryophyllene. This reveals that the ESENS exhibited good radical scavenging activity when compared to the control where Beta

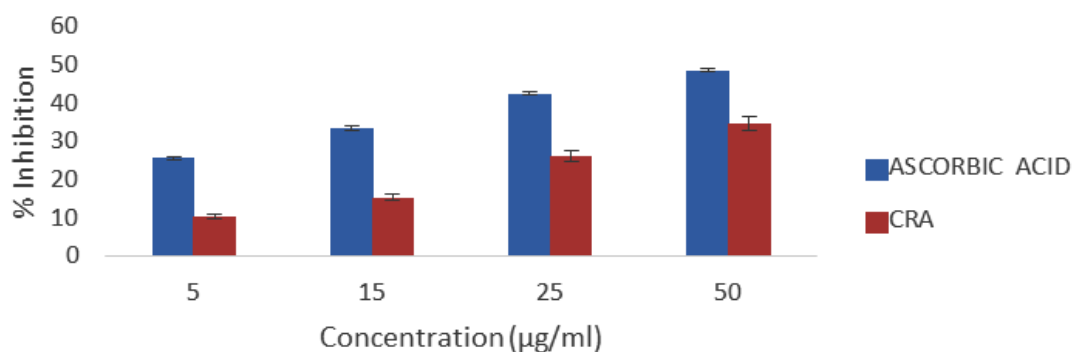


Fig. 4. Hydrogen peroxide radical scavenging assay of compound beta-caryophyllene

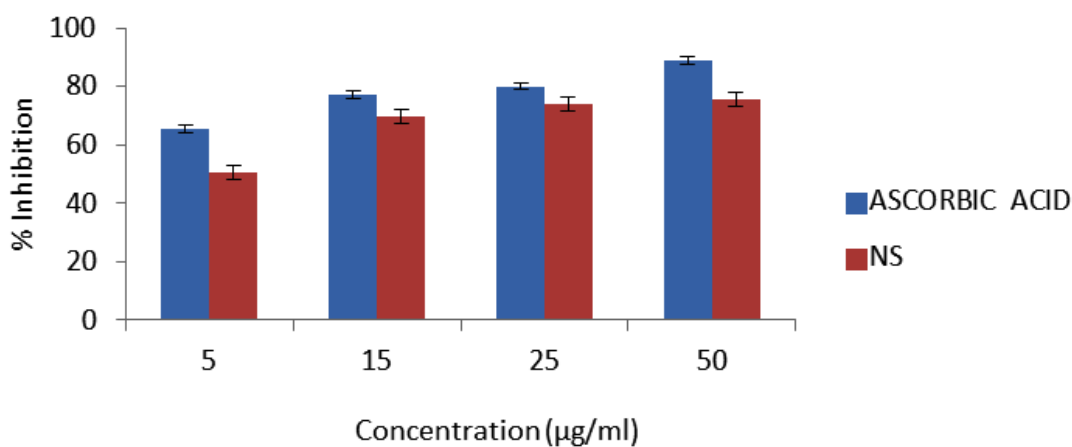


Fig. 5. Nitric oxide radical scavenging assay of ESENS

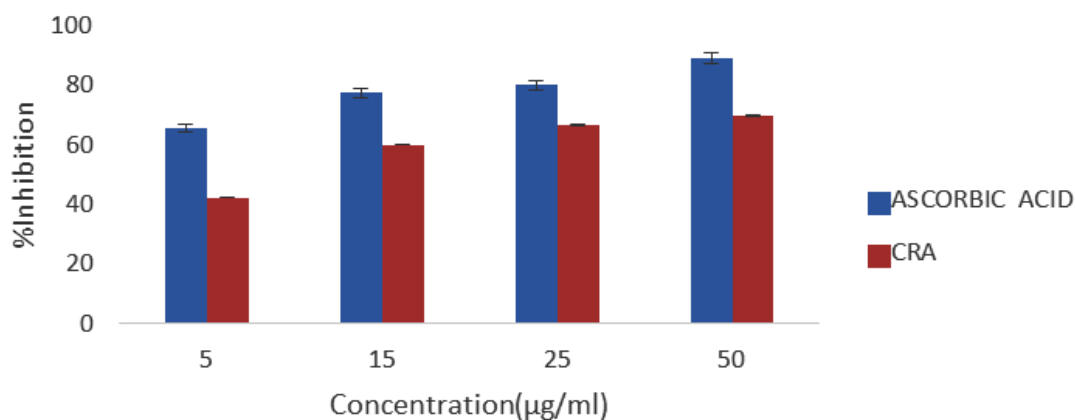


Fig. 6. Nitric oxide radical scavenging assay of compound beta-caryophyllene

caryophyllene exhibited less radical scavenging activity (Fig. 3,4).

Nitric Oxide Radical scavenging assay

ESENS and beta-caryophyllene significantly inhibit nitric oxide in a dose-dependent manner at a concentration of $122 \pm 0.11 \mu\text{g/ml}$ of ESENS and $132 \pm 0.21 \mu\text{g/ml}$ of beta-caryophyllene,

respectively, when compared to ascorbic acid. The result suggests that the extract and the compound beta-caryophyllene are capable of inhibiting nitric oxide and proves that they can be used as drugs in the indigenous system for treating various diseases and disorders (Fig. 5,6).

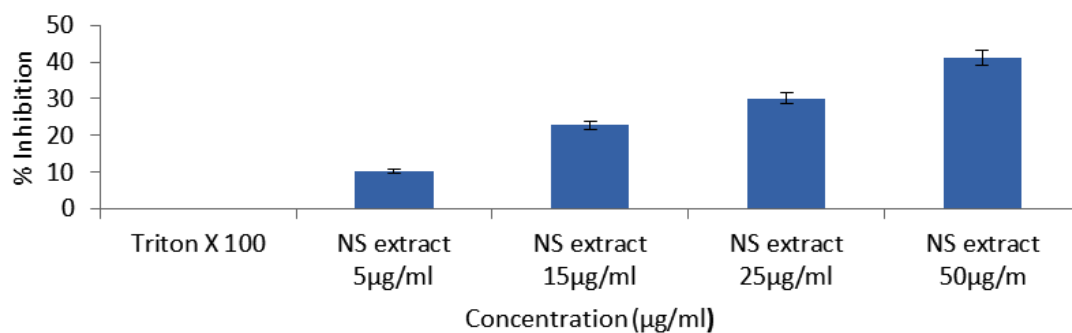


Fig. 7. Membrane stabilization assay of ESENS

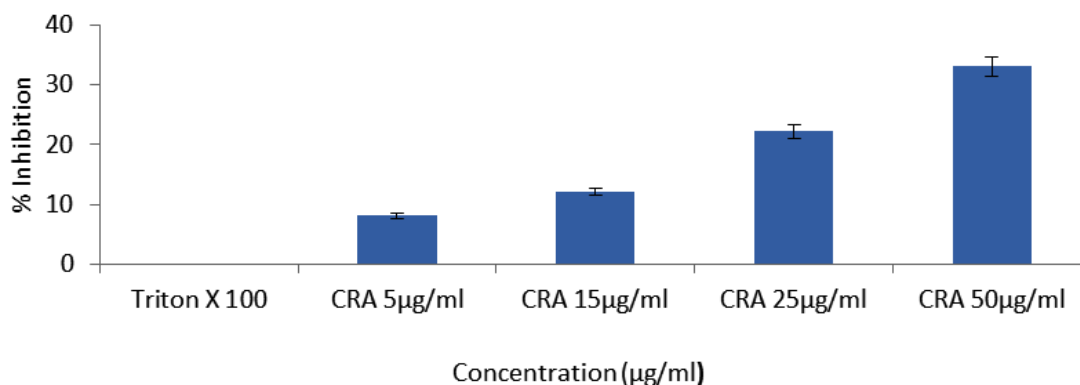


Fig. 8. Membrane stabilization assay of compound beta-caryophyllene

Table 1. Cytotoxicity assay of ESENS

Incubation time (Hours)	IC ₅₀ Value (mg/ml)- Trypan blue	IC ₅₀ Value (mg/ml) -MTT
24	6.83 ± 0.71^a	$8.07 \pm 0.52^*$
48	4.93 ± 0.25^b	$5.20 \pm 0.10^{**}$
72	3.70 ± 0.10^c	$4.47 \pm 0.12^{**}$

Table 2. Cytotoxicity assay of compound Beta caryophyllene

Incubation time (hours)	IC ₅₀ Value (mg/ml)- Trypan blue	IC ₅₀ Value (mg/ml)- MTT
24	6.93 ± 0.51^a	$10.07 \pm 0.42^*$
48	4.83 ± 0.45^b	$7.30 \pm 0.20^{**}$
72	2.90 ± 0.11^c	$6.37 \pm 0.14^{**}$

Anti-*invitro* studies of ESENS and the compound beta-caryophyllene**HRBC membrane stabilization assay**

Different concentrations of ESENS and beta-caryophyllene were analyzed to study the

prevention of hemolysis, which is determined by the HRBC membrane stabilization assay. Triton X 100 is a detergent that destabilizes the RBC membrane and leaks hemoglobin, and the results suggest that ESENS and beta-caryophyllene

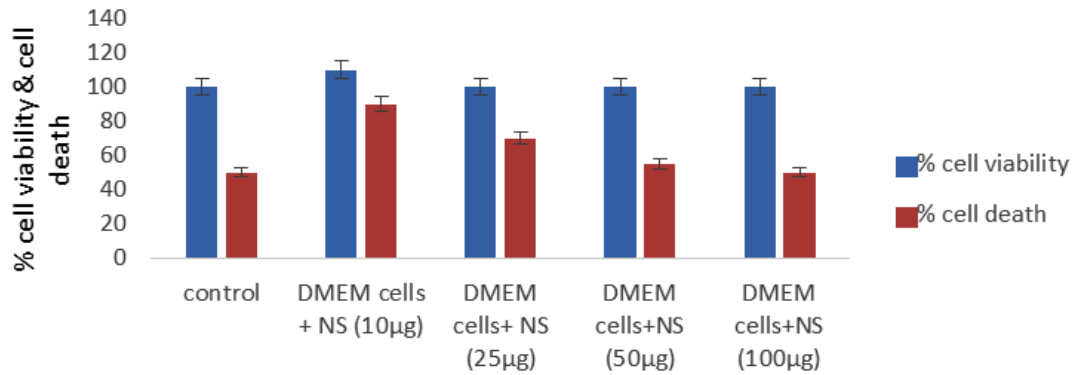


Fig. 9. Cell viability assay of ESENS

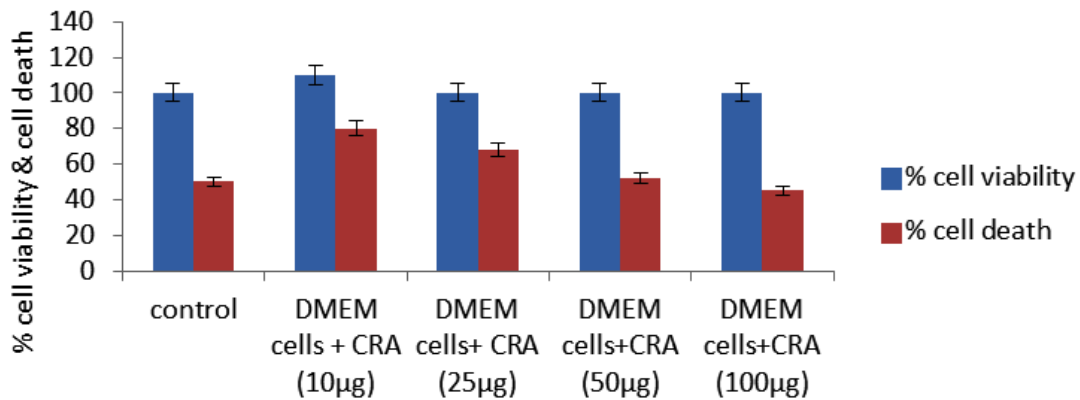


Fig. 10. Cell viability assay of compound beta-caryophyllene

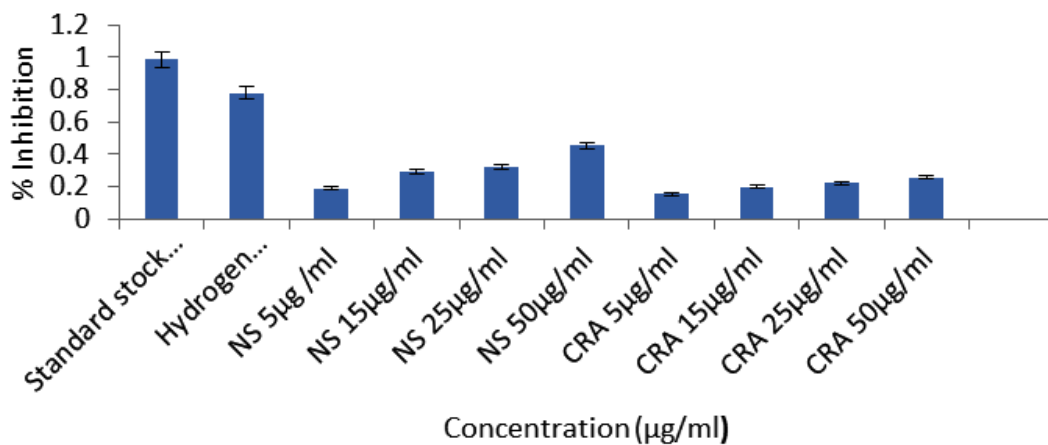


Fig. 11. Anti-inflammatory activity of ESENS and the compound beta-caryophyllene

prevent the leaking of hemoglobin and prevent the integrity of the RBC; furthermore, it is proved to be non-toxic in nature and can be used in biological

studies (Fig. 7,8). Increased levels of IL-1 beta cytokines are the major cause of various metabolic disorders. The compound beta-caryophyllene is

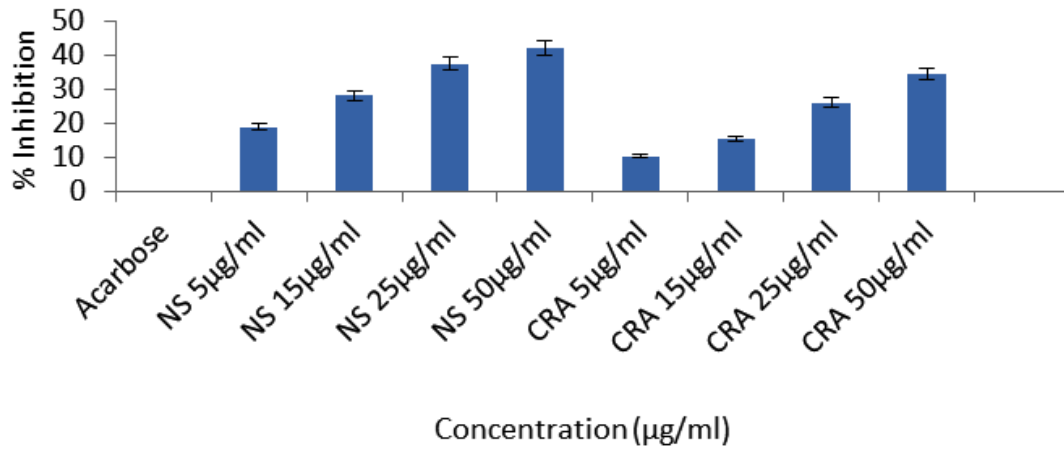


Fig. 12. α-amylase activity of ESNS and the compound beta-caryophyllene

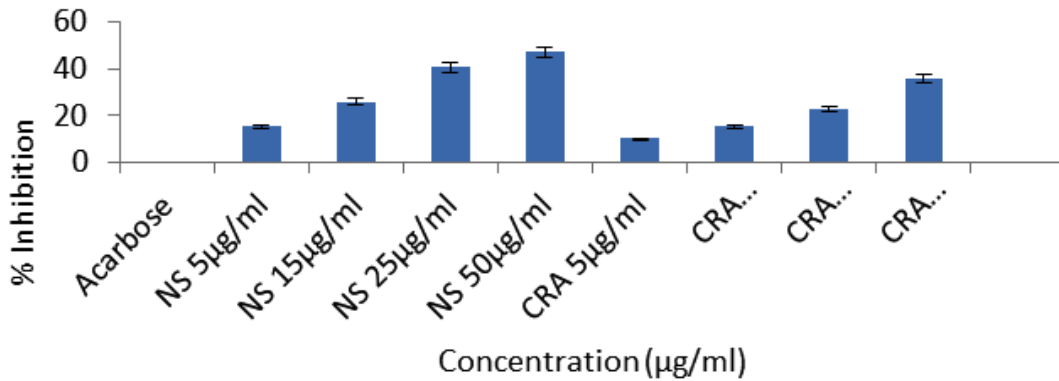


Fig. 13. α-glucosidase activity of ESENS and the compound beta-caryophyllene

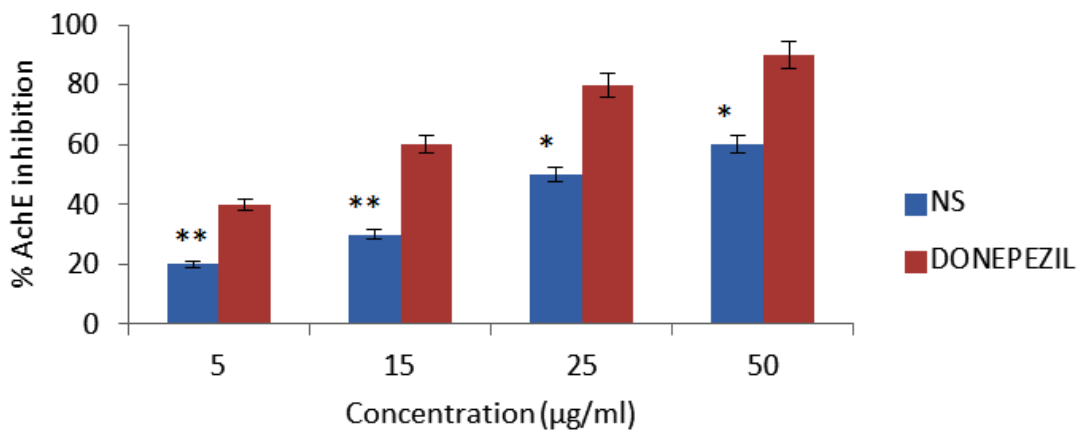


Fig. 14. Enzymatic assay – Acetyl cholinesterase assay of ESENS VS drug Donepezil

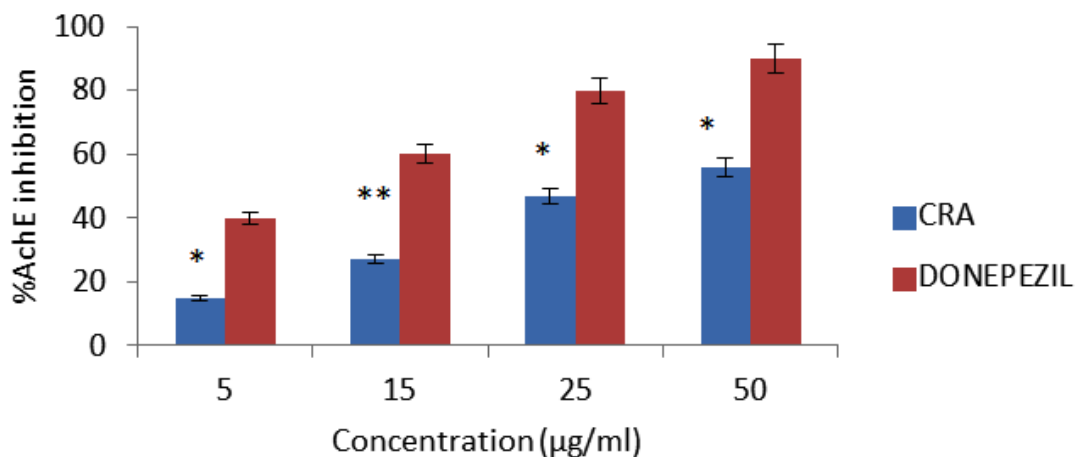


Fig. 15. Enzymatic assay – Acetyl cholinesterase assay of compound beta-caryophyllene VS drug Donepezil

Table 3. Non-Enzymatic assay – Metal chelating assay of ESENS and compound Beta Caryophyllene VS drug Donepezil

S. no	Parameters assessed	Nigella sativa	Beta caryophyllene
1	5	4.28±0.31*	7.11±0.93*
2	15	3.12±0.22*	6.04±0.88*
3	25	2.06±0.21*	5.89±0.67*
4	50	1.75±0.17*	5.67±0.53*

found to be more potent when compared to ESENS.

Cytotoxicity assay

The isolated lymphocytes were treated with ESENS and beta-caryophyllene to study the cell's toxicity. The ESENS exhibited 50% cell death, and beta-caryophyllene exhibited 40% cell death when compared to the control sample (Table 1,2). This result suggests that the extract and compound exhibited less cell proliferation (Fig 9, 10).

Enzyme linked immune-sorbent assay of ESENS and the compound beta-caryophyllene

The ESENS and the compound beta-caryophyllene in inhibition of IL-1 Beta were analyzed. The effects of ESENS and the compound beta-caryophyllene are expressed in Fig. 11. Based on the result, it is suggested that the compound has a high potency in inhibiting IL-1 Beta when compared to the extract ESENS, which showed a good potential effect. The samples are compared with the standard of IL-1 Beta and the lymphocyte

cells that are treated with hydrogen peroxide. The compounds have exhibited good potential effects individually when compared to the ESENS; it is revealed that the compound beta-caryophyllene has anti-inflammatory activity.

Anti-diabetic activity of ESENS and the compound beta-caryophyllene

α-amylase inhibitory assay

The α-amylase inhibitory assay of the ESENS and beta-caryophyllene was used to analyze the inhibitory activity of the extract and the compound. The extract and the compound at various concentrations determined exhibited the highest inhibition rate of about 50.84% and the lowest inhibition rate of 15.49% for ESENS and beta-caryophyllene, respectively, at 55.81% and 13.04%. The extract and the compound are compared with the control acarbose. Based on the results, the compound beta-caryophyllene exhibited effective inhibitory activity when compared to ESENS, and it showed dose-dependent activity (Fig. 12).

α-glucosidase inhibitory assay

α-glucosidase inhibitory activity of ESENS and beta-caryophyllene was analyzed and determined using the substrate p-NPG, which was then compared with the control acarbose. ESENS and the compound beta-caryophyllene of various concentrations were studied, and the result suggests that the compound beta-caryophyllene exhibited 40% inhibition when compared to ESENS and the control. Based on the result, it is suggested that the

compound beta-caryophyllene exhibited a good potential effect on the inhibitory action (Fig. 13).

Enzymatic and non-enzymatic assay of ESENS and the compound beta-caryophyllene

Enzymatic assay- Acetylcholinesterase assay

It is an *in vitro* assay that is used to study the enzyme cholinesterase, which plays a major role in neurotransmittance in Alzheimer's disease. This reveals the role of ESENS and beta-caryophyllene, where beta-caryophyllene is $42.06 \pm 2.11 \text{g/ml}$ and ESENS is $84.7 \pm 4.3 \text{g/ml}$. The compound exhibited higher inhibition of the enzyme cholinesterase when compared to ESENS (Fig. 14,15).

Non-enzymatic assay – Metal chelating assay

The results obtained from non-enzymatic assays suggest that the ESENS of various concentrations chelates the ferric ion (Fe-II) and is found to exhibit $1.75 \pm 0.17^*$ at $50 \mu\text{g/ml}$ when compared to the compound beta-caryophyllene of about $5.67 \pm 0.53^*$. The results revealed that the ESENS have high scavenging activity when compared to the compounds. But Beta caryophyllene is found to have the same efficacy as beta-caryophyllene (Table 3).

DISCUSSION

The medicinal plants are used as the most abundant biosource for medication production. Numerous pharmacological properties of *Nigella sativa* have been discovered, including anti-analgesic, anti-ulcer, anti-inflammatory, anti-bacterial, anti-microbial, anti-cancer, and anti-diabetic properties. The qualitative analysis of the ESENS revealed positive relevance in both therapeutic and physiological activities, according to the current research. The isolation and identification of these bioactive substances pave the way for the creation of novel medications that may be used to treat a wide range of illnesses and ailments. Tannins have medicinal benefits for anti-diarrhoea, anti-haemostatic, and a broad spectrum of anti-microbial activity against viruses, bacteria, and fungi. Alkaloids are the largest group of compounds that have been linked to many medicinal properties for years and have a potential for cytotoxicity. Steroids have antibacterial properties, whereas flavonoids are hydroxylated phenolic compounds made from plants that have

potent antioxidant and anti-cancer properties²⁴. Flavonoids also have a favorable response to microbial infection. Terpenoids are an example of an essential lipid that has an aromatic flavor and performs activities including controlling growth and color. Metabolites of phenols exhibit biological features, including anti-apoptosis, anti-aging, and anti-inflammatory effects. It is a compound that protects the heart and enhances endothelial health. The blood pressure is also lowered and controlled by glycosides²⁵.

Essential oils and other plant extracts antioxidant properties are gaining increased attention in scientific circles as well as in the food, cosmetics, and pharmaceutical sectors. By using recognized antioxidants like ascorbic acid in DPPH radical scavenging assays, hydrogen peroxide scavenging assays, and nitric oxide assays, researchers were able to assess the anti-radical or free radical scavenging capabilities of plant extracts or essential oils. Antioxidants' ability to cause a decrease in absorbance at 517 nm was utilized to gauge their potential to lower DPPH radical generation²¹. The ability to transfer hydrogen is the justification. ESENS and the substance beta-caryophyllene demonstrated effective nitric oxide scavenging abilities. The substance showed promise for scavenging free radicals in the DPPH test as well. According to research²⁶, beta-caryophyllene exhibited reduced activity, and hydrogen peroxide was shown to be dosage-dependent.

When compared to the crude extract of *Nigella sativa*, the *in vitro* investigations showed that the compound beta-caryophyllene has a large number of phytochemical elements that are medicinally relevant. The isolated component beta-caryophyllene and the crude extract of *Nigella sativa* both exhibit anti-inflammatory and anti-diabetic properties, which were shown by the IL-1 beta test, alpha amylase, and alpha glucosidase inhibitory assays. While the crude extract of *Nigella sativa* and the compound beta-caryophyllene (CRA) showed less effectiveness in hydrogen peroxide scavenging activity, the molecule beta-caryophyllene is more efficient than ESENS in DPPH radical scavenging, the nitric oxide test, the acetyl cholinesterase assay, and the metal chelating assay.

CONCLUSION

The *in vitro* studies revealed that the ESENS have many phytochemical properties that have been found to be medically significant. The compound beta-caryophyllene tends to have reduced hydrogen peroxide activity when compared to ESENS, and the compound beta-caryophyllene has potential anti-inflammatory, anti-diabetic, AchE, and metal chelating properties. Since the compound is more effective *in vitro*, pharmacological studies have shown it to be successful and promising for the treatment of Alzheimer's disease, which needs an effective therapy to control brain functions and other complications. Due to these qualities, the compound beta-caryophyllene is a prospective candidate for the treatment of Alzheimer's disease. The future prospective of the current study is to conduct biophysical, kinetic characterization and animal studies of the ESENS and the compound beta-caryophyllene.

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Conflict of Interest

The authors have no conflict of interest to declare. The co-author has seen and agreed with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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