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Integrated Phytochemical Characterization, Nutritional Profiling, Bioactivity Evaluation and in-Silico Molecular Docking Analysis of *Nigella Sativa* Seed Oil and Flour Extracts

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Abstract

Background*: Medicinal plants, particularly those exhibiting diverse biological activities, are of significant interest. Nigella sativa is utilized globally for the management of several ailments. These discoveries were inspired by the statements of the Prophet of Islam, Muhammad (SAW), who asserted that N. sativa possesses all forms of medicines except for death.*

Methods*: This study assesses the in-vitro antioxidant capacity of N. sativa seed oil and flour utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The phytochemical composition, proximate analysis, and mineral content were quantitatively assessed using conventional methodologies. The anti-inflammatory activity of the seed oil extract was evaluated utilizing the carrageenan-induced paw edema model. Molecular docking studies were undertaken for insight into mechanistic evidence and structure-activity relationship.*

Results*: N. sativa seed oil extract possessed greater efficacy in scavenging free radicals. The phytochemicals revealed that phenol had the highest concentration. The proximate analysis indicated crude fat, fiber, and carbohydrate concentrations of 32.16%, 6.28%, and 22.78%, respectively. Among the vitamins determined, the vitamin A level of N. sativa was most significant at 284.795 ± 0.815 mg/kg, while the mineral content showed Potassium demonstrating the greatest concentration at 1170.7 ± 0.1 mg/kg. The resolution of edema during inflammation is a time-and dose-dependent process showing varying degrees of edema resolution, with group 1 exhibiting a 100% resolution rate.*

Conclusions*: The result suggests that N sativa seed oil contains bioactive compounds responsible for its use as an antioxidant and therapeutics. Also, administering 100% of the oil orally is superior to its combination with substances that reduce its inhibitory efficacy to around 39.6%. Docking results revealed links between bioactive compounds and diseases. This study demonstrates that the plant's seed oils are an exceptional source of nutritional compounds due to their bioactive contents.*

Keywords: *Phytochemicals, Antioxidants, Inflammation, Nutrition, Nigella sativa, Molecular docking..*

1. Introduction

Medicinal plants, particularly those exhibiting diverse biological activities, are of significant interest. *Nigella sativa*, also known as black cumin, is a species within the *Ranunculaceae* family, with significant properties due to its extensive culinary and medicinal uses [1 - 4]. *Nigella sativa* is utilized globally for the management of several ailments. These discoveries were inspired by the statements of the Prophet of Islam, Muhammad (SAW), who asserted that black seeds possess all forms of medicines except for death [5 - 6].

In traditional medicine, *Nigella sativa* seeds have been utilized to alleviate despondency, weariness, and chronic headaches. Black cumin contains a variety of bioactive phytochemicals with significant pharmacological properties, including thymoquinone (TQ), dithymoquinone, thymohydroquinone, t-anethol, alkaloids (nigellicines and nigelledine), saponins (α-hederin), flavonoids, and monoterpenes such as p-cymene and α-pinene [7 - 9]. *Nigella sativa* comprises significant components such as oils, vital fatty acids, vitamins, carbohydrates, minerals, proteins, and essential amino acids. These bioactive substances show activities that support cardiovascular health, combat diabetes, inhibit cancer, alleviate pain, reduce inflammation, prevent epilepsy, provide antioxidant effects, combat schistosomiasis, modulate the immune system, protect the gastrointestinal tract, safeguard the liver, and protect the kidneys [9]. The compositional versatility in seeds of *Nigella sativa* has been observed [10 - 11] to contain potassium, sodium, phosphorus, magnesium and iron as the major minerals. However, zinc, copper, calcium and manganese are found at relatively low concentrations. Traces of cadmium, lead, nickel, and molybdenum have also been reported in *N. sativa* seeds [12]. The essential oil obtained from *N. sativa* seeds has well-established medicinal significance due to the presence of some vital volatile components.

Evidence abounds about phytochemical research's numerous beneficial compounds in *N. sativa* seeds that enhance the therapeutic benefits and pharmacological effects of the seeds [13]. Black cumin seeds are distinguished by their protein and lipid content, particularly important fatty acids such as linoleic acid (omega-6). They also supply a significant quantity of vital amino acids, vitamins, and minerals, enhancing overall health and immunity [14].

The conventional application of seeds in enhancing health and preventing illness is substantiated by the amalgamation of these nutrients and phytochemicals. The seeds have historically been valued for their applications in culinary and medicinal practices. Recent studies have shown the seeds' substantial mineral richness, phytochemical diversity, and nutritional significance [15]. The essential oil of *N. sativa* demonstrates a multitude of pharmacological effects, including anti-inflammatory [16], anti-Alzheimer [17], anti-cancer [18], anti-microbial [19], anti-oxidant [20], anti-schistosomiasis [21], anti-diabetic [22], anti-stress [23], cardiovascular protective [24], and nephron-protective effects [25].

The predominant actions are mostly ascribed to its TQ, thymol, DTQ, α-pinene, β-pinene, p-cymene, and thymohydroquinone [26].

Research indicate significant levels of calcium, potassium, magnesium, and phosphorus, which are vital for maintaining healthy bones, facilitating muscle contraction, and supporting enzymatic functions [27]. Additionally, trace elements such as zinc, iron, and copper are present in notable quantities, enhancing immune function and mitigating oxidative stress [28]. The high bioavailability of these minerals amplifies their therapeutic potential, particularly in addressing deficiencies prevalent in developing regions. The composition of black cumin seeds is notably rich and varied [26], influenced by factors such as cultivar, maturation stage, geographical, climatic conditions, and extraction methods [29].

Thymoquinone, the most extensively studied phytochemical of *N. sativa*, exhibits well-documented antioxidant, anti-inflammatory, and anticancer properties [5]. Additional crucial constituents comprise carvacrol, thymohydroquinone, thymol, and alkaloids like nigellidine and nigellicine, each exhibiting distinct biological effects. Their elevated protein content provides critical amino acids necessary for tissue growth and repair [6]. The seeds possess substantial amounts of dietary fiber, enhancing regular bowel movements and mitigating constipation. Moreover, its lipid composition has important fatty acids such as oleic acid and linoleic acid (omega-6), which are crucial for sustaining cardiovascular health and mitigating inflammatory responses [30].

Furthermore, the seeds have a high concentration of vitamins, which are necessary for neurological and energy metabolism. Because of their nutritional profile, they are increasingly found in functional foods and dietary supplements designed to help manage chronic conditions and improve overall health [26]. The phytochemicals' antibacterial activity lends support to their traditional use in the treatment of infections. Its value as a dietary supplement is further strengthened by its high nutritional profile, which is particularly required in populations prone to vitamin deficiency.

Despite the extensive traditional utilization and documented pharmacological effects of *Nigella sativa*, comparative and integrative studies assessing both seed oil and defatted flour concurrently, particularly regarding bioactive compound synergy, nutritional potential, and molecular interactions with biological targets, are scarce. Most current research concentrates on either oil or flour alone, neglecting the synergistic potential of their combined profiles in health enhancement or medicinal uses [27].

Moreover, although molecular docking studies have been performed on specific *Nigella sativa* compounds, there is an absence of thorough *in-silico* assessments that correlate phytochemical composition with established molecular targets, especially concerning antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities.

This study, therefore, aims to conduct a comprehensive analysis of *Nigella sativa* seed oil and flour, given the plant's well-documented functional and therapeutic benefits. The investigation will focus on evaluating the mineral and vitamin composition, phytochemical constituents, and overall nutritional profile. Molecular docking analyses will also be performed to explore potential bioactive interactions at the molecular level.

2. Materials and Methods

2.1 Materials

N. sativa seeds were purchased from Nigorite Pharmacy and Diagnostic shop in Anyigba, Kogi State Nigeria and identified by a staff member of the Plant Science and Technology department of Kogi State University, Anyigba Nigeria. The *N. sativa* L. seeds were pressed at room temperature (25˚C) by mechanical pressing without any heat treatment according to [31]. Crushed seeds were stored overnight at room temperature to separate the oil phase from fibers, and then the oil was filtered using Whatman #4 filter paper and a glass funnel. Analytical grade reagents were acquired from local vendors and used without further purification.

2.2 Methods

2.2.1 Phytochemical Composition Determination

Total Phenolic Content (TPC) was determined using Folin–Ciocalteu reagent and expressed as gallic acid equivalents [32]. Total Flavonoid Content (TFC) was determined by aluminum chloride colorimetric assay and expressed as quercetin equivalents [33]. Tannins were determined by Folin-Denis method with absorbance read at 700 nm.

2.2.2 Vitamin Composition Determination

Vitamin C (Ascorbic Acid) was estimated using 2,6-dichlorophenolindophenol titration [35]. Vitamin A (Retinol) was determined by HPLC with UV detection at 325 nm [36]. B-complex vitamins were determined using microbiological assay. Vitamin E (Tocopherol) was determined by Fluorescence detection via HPLC at 295 nm excitation and 330 nm emission [37].

2.2.3 Mineral Content Determination

Mineral contents were analyzed from a sample prepared by dry ashing and acid digestion [38]. Atomic Absorption Spectrophotometry (AAS) was used for Ca, Mg, Fe, Zn, and Cu [39]. Flame Photometry was used for Na and K. UV-Vis Spectrophotometry was used for Phosphorus (molybdate blue method). Quantification was performed using calibration curves of standard solutions.

2.2.4 Proximate Composition Determination

Following [34] methods, Moisture Content was determined by the drying oven method at 105°C until constant weight. Ash Content was determined by ashing at 550°C in a muffle furnace to determine total mineral residue. Crude Protein was evaluated by the Kjeldahl method for nitrogen estimation; conversion factor = 6.25. Crude Fat was estimated by Soxhlet extraction using petroleum ether. Crude Fiber was found by the acid-base digestion method followed by filtration and incineration. Carbohydrate was calculated by difference: 100 - (% moisture + % protein + % fat + % ash + % fiber).

2.2.5 Antioxidant activity determination

2.2.5.1 DPPH Radical Scavenging Assay

DPPH Radical Scavenging Assay was evaluated using the stable radical, 2, 2-diphenyl-1-picrylhydrazyl (DPPH*) according to the method described by [40] and [41]. To 1ml of various concentrations of the samples or the reference compounds, Quercetin and vitamin C (31.25, 62.50, 125.00, 250.00 and 500µg/ml) in test tubes were added 1ml of 0.3mM DPPH* in methanol. Absorbance was measured after 30 minutes at 517nm against a DPPH* control containing only 1ml of methanol. The percentage scavenging activity was calculated by the following formula:

$$\%\ scavenging\ activity\ = Absc\ - \frac{Abss}{Absc}\ X\ 100$$

where Absc is the absorbance of the control and Abss is the absorbance of test sample.

The % scavenging activity was plotted against the log concentration of the extracts (µg/ml) to determine the concentration of extract that reduced DPPH activity by 50% (IC_{50}). All determinations were performed in triplicate.

2.2.5.2 Ferric Reducing Antioxidant Power

Ferric Reducing Antioxidant Power (FRAP) Assay [42] was also used to measure antioxidant activities. Briefly, Acetate buffer (300mM) at pH 3.6 was prepared by dissolving 3.1g of sodium acetate tri-hydrate in 16ml of glacial acetic acid and making the volume to 1L with distilled water. 2,4,6-tripyridyl-s-triazine (TPTZ) (10mM) was dissolved in 40mM of HCl and 20 mM of $FeCl_3.6H_2O$. The working FRAP reagent was prepared by mixing the three (3) solutions in the ratio of 10:1:1 respectively at the time of use. L- Ascorbic acid was used as the standard. Samples were mixed with 3ml of working FRAP reagent, vortexed and absorbance (593nm) was measured at 0 minute. The samples were then placed in the water bath at 37°C and absorbance was again measured after 4 minutes. The absorbance of standard was processed in the same way. The results were calculated as follows.

$$FRAP\ (\mu M)\ = \frac{change\ in\ Absorbance\ of\ sample\ from\ 0\ to\ 4\ minutes}{change\ in\ Absorbance\ of\ standard\ from\ 0\ to\ 4\ minutes}\ X\ FRAP\ standard\ value$$

Results are compared against the ascorbic acid standard.

2.2.6 Anti-inflammatory activity

2.2.6.1 Carrageenan-induced paw edema

This method was carried out as described by [43] with slight modifications from more recent sources [44]. The animals were divided into five groups (n = 5), deprived of food overnight but given water ad libitum prior to the experiment.

One hour after carrageenan induction when test rats were challenged by a subcutaneous injection of 0.1ml of 1% solution of carrageenan in sodium chloride into the sub-plantar side of the left hind paw, following inhalation of 5% isoflurane (v/v) for 15 seconds while the vehicle control group was injected with the carrageenan solution. *Nigella sativa* was administered for 2 weeks. The basal paw thickness was then measured using a vernier caliper [45]. The paw thickness was measured again at 1, 2, 3, 4, and 24 hours after the challenge. The increase in paw thickness was then calculated as a percentage compared with the basal thickness (the zero hour). The difference of average values among treated and untreated groups was calculated for each time interval and evaluated statistically. The percent inhibition was calculated using the formula as follows:

$$\%edema\ (inhibition) = 1 - \frac{T_t}{T_c}\ X\ 100$$

(Tt and Tc are edema thickness in the test and control groups, respectively).

2.2.7 Molecular Docking

The crystal structure of the human KEAP1 Kelch domain (PDB ID: 1CB4) was retrieved from the Protein Data Bank and prepared for docking using PyRx version 0.8. The protein was energy-minimized, and all water molecules, ions, and non-essential heteroatoms were removed. Polar hydrogens were added, and Gasteiger charges were assigned using AutoDock Tools version 1.5.4. The ligand structure was energy-minimized using the MMFF94 force field prior to docking.

Docking simulations were carried out with AutoDock Vina integrated into PyRx, targeting the KEAP1 binding pocket. A grid box was applied to encompass the known active site residues, ensuring adequate coverage of the binding cavity for flexible ligand positioning. Multiple poses were generated and ranked according to predicted binding affinity (kcal·mol^{-1}). The best-scoring pose, defined by the lowest binding energy and an RMSD < 3.0 Å, was selected for further analysis. Ligand–protein interactions were analyzed using BIOVIA Discovery Studio Visualizer version 21.1, identifying π–alkyl hydrophobic interactions, carbon-hydrogen bonds, and van der Waals contacts. Distances between interacting atoms were measured in Å, and interaction diagrams were generated in both 2D and 3D formats for visualization.

3. Statistical analysis

The % scavenging activity was plotted against the log concentration of the extracts (µg/ml) to determine the concentration of extract that reduced DPPH activity by 50% (IC_{50}). All determinations were performed in triplicate. The statistical analysis was carried out using Graphpad Instat version 5 statistical solution software. Results were presented as mean ± SD of three replicate determinations. Statistical analysis among treatments were determined at the significance level of $p \leqslant 0.05$.

4. Results and Discussion

Figure 1 illustrates the phytochemical components of *N. sativa*.

The illustration delineates six categories of phytochemicals: Tannins, Total Phenols, Flavonoids, Saponins, Alkaloids, and Cardiac Glycosides.

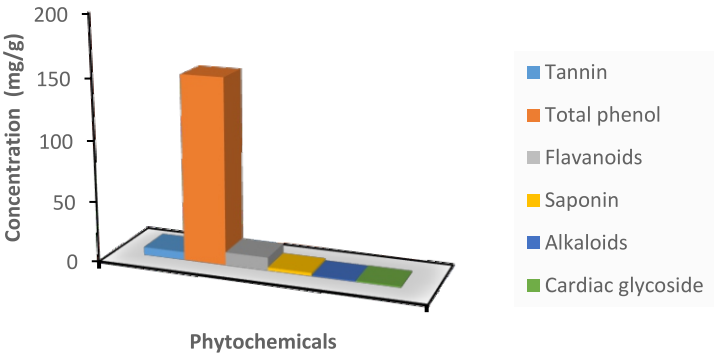

Fig 1: The proportions of various phytochemical components of N. sativa extract.

The tallest bar depicted in orange, represents total phenol, accounting for 87% of the total phytochemical content. This substantial percentage indicates that total phenol is the primary phytochemical in the sample. The residual phytochemicals comprise just 13% in total, distributed as Tannin (1%), represented as a blue section, and is the least substantial contributor. Flavonoids (6%), seen in grey, represent the second-largest minor category. Saponins, alkaloids, and cardiac glycosides are represented by yellow, dark blue, and green segments, respectively. Their exact proportions are minimal but are grouped within the remaining few percent.

The prevalence of total phenol suggests that this sample has significant potential as an antioxidant source, possibly contributing to health-related applications, including the reduction of chronic disease risk. Some phytochemicals are present at modest concentrations but are acknowledged for their specific biological effects: Saponins aid in reducing cholesterol levels and include immune-boosting properties. Flavonoids are secondary metabolites that have antioxidant, anti-inflammatory, and potential cardiovascular benefits. Tannins, regardless of their size, contribute to astringency and exhibit antibacterial and anti-inflammatory properties. Alkaloids are often associated with medicinal properties, including analgesic and antimicrobial actions. Cardiac glycosides are acknowledged for their use in treating heart conditions [46].

N. sativa is primarily a significant source of total phenols, making it especially relevant for incorporation into nutritional supplements as a natural antioxidant source. Moreover, as Functional Foods that promote health benefits, the inclusion of supplementary phytochemicals, albeit in minor quantities, amplifies the therapeutic potential, making the sample adaptable in its applications [47] [48 - 49]. This assertion aligns with the opinion of [50].

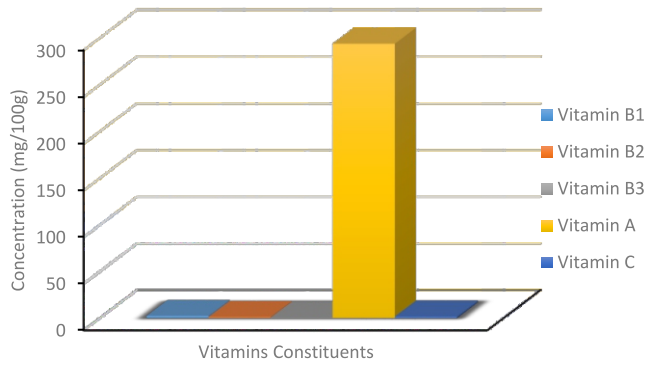

Fig 2: Vitamin Composition (mg/100g) of Nigella sativa extract.

Figure 2 primarily emphasizes the vitamin composition of the *Nigella sativa* sample. Vitamin A predominates in the distribution, resulting in a significant imbalance. The predominance of the orange bar, comprising 99% of the chart, indicates that the sample possesses a substantial quantity of vitamin A. This signifies that this particular component comprises nearly the entirety of the vitamin content per 100g of the sample at 294mm/100g. Merely 1% of the overall vitamin makeup consists of the additional vitamins (B1, B2, B3, and C).

The data reveal that this sample is highly abundant in vitamin A, constituting the predominant portion of its makeup. This sample may be beneficial for situations when vitamin A consumption is essential, such as enhancing immunity or preventing scurvy, as indicated by the minimal quantities of other vitamins [51]. The sample has an unbalanced nutrient supply and necessitates supplementation if included in a diet, as it is deficient in other vitamins, including vitamin C and the B-complex vitamins. This was noted in earlier works of [52]

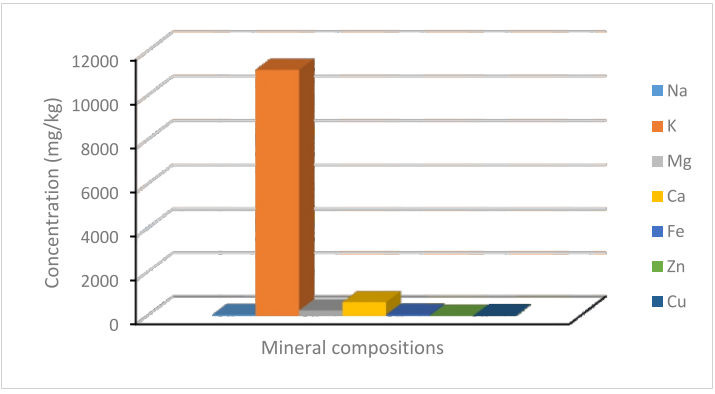

Fig 3: Concentration of various minerals (mg/kg) in N. sativa extract.

In figure 3, the orange bar denoting potassium, with a concentration of 11,170 mg/kg, significantly exceeds the others. This indicates that potassium is the predominant mineral in the sample. Calcium (Ca) and sodium (Na) are found in significantly lesser quantities; however, they are noteworthy in comparison to the others. A yellow bar indicates calcium (Ca), with an estimated value of 632.7 mg/kg. The concentration of blue sodium (Na) is 48.15 mg/kg. Significantly, reduced concentrations of magnesium (Mg), iron (Fe), zinc (Zn), and copper (Cu) are observed.

Given their apparently similar amounts in the data, it seems probable that they are under 300 mg/kg.

Thus, *N. sativa* is a substantial supplier of potassium, a mineral essential for nerve function, muscle contraction, and electrolyte equilibrium. Considering that leafy greens, potatoes, and bananas are rich in potassium, it is plausible to deduce that the sample may originate from analogous sources [53]. Sodium is vital for fluid equilibrium and neuronal transmission, whereas calcium is crucial for skeletal integrity and metabolic functions. Their minimal concentrations suggest that, while not a principal source, the sample may assist in fulfilling these dietary requirements. The insufficient concentrations of magnesium, iron, zinc, and copper suggest that this sample is not a suitable exclusive source for these minerals; however, these micronutrients are vital for several biochemical and enzymatic processes [49], [54 - 55].

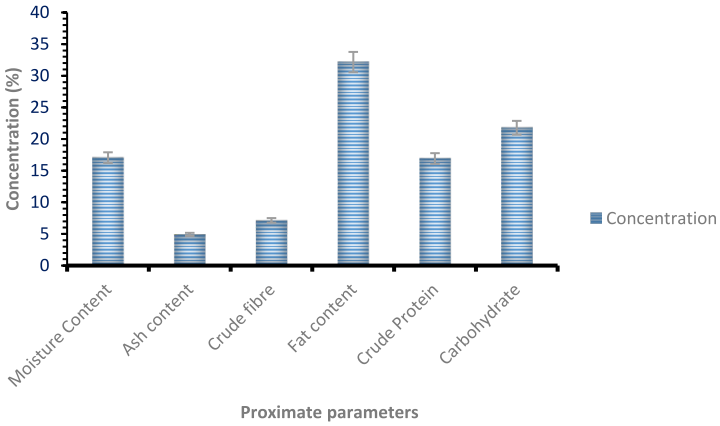

Fig 4: Proximate composition of aqueous extract of N. sativa flour extract

Figure 4 depicts that the proximate composition of *N sativa* is predominantly characterized by fat content, followed by carbohydrate and moisture contents. The components with lower concentrations (ash content and crude fibre) may exert a lesser influence on the sample's overall nutritional or compositional profile. Fat works as a critical energy store. The elevated result shows that this sample may be energy-dense and appropriate for caloric requirements. The variability illustrates that the fat concentration in the sample may vary greatly across measurements. Carbohydrates serve as major energy sources, and this concentration suggests that the sample includes a moderate carbohydrate level. It is the second-largest contributor behind fat. The lower error bars suggest better reliability of results for this component. Overall, the result agrees with literature provisions generally [31], [30], [56 - 57].

Ash denotes the mineral constituents and the low value signifies a minimal contribution to the total composition, suggesting a lack of essential minerals such as calcium, magnesium, or iron limiting usefulness for mineral fortification. A minimal error bar denotes reliable measurements, suggesting trustworthy procedures and a uniform distribution within the sample. The high fat level is characteristic of plant-based samples, suggesting it may derive from an oil-rich plant.

Marginally elevated levels, are however within the allowed parameters for fresh or semi-dry samples. Levels below expected amounts for conventional plant-based sources indicate refined or processed materials [58 - 60]

Table 1: antioxidant activity of Nigella sativa using DPPH assay

Sample	IC_{50} (µg/ml)
N. sativa seed flour	66.37 ± 2.42
N. sativa seed oil	87.42 ± 3.21
Standard ascorbic acid	12.11 ± 0.78

Results are mean ± SD of triplicate determinations (n = 3)

Table 2: FRAP assay of N. sativa seed powder extract

Concentration (µg/ml)	Log concentration (µg/ml)	FRAP value (µM)
12.50	1.10	0.52 ± 0.20
25.00	1.40	0.68 ± 0.10
50.00	1.70	0.81 ± 0.02
100.00	2.00	0.89 ± 0.03
200.00	2.30	0.95 ± 0.01
400.00	2.60	1.03 ± 0.00

Results are mean ± SD of triplicate determinations (n = 3)

Table 3: FRAP values of N. sativa seed oil extract

Concentration (µg/ml)	Log concentration (µg/ml)	FRAP value (µM)
12.50	1.10	0.56 ± 0.08
25.00	1.40	0.71 ± 0.10
50.00	1.70	0.82 ± 0.30
100.00	2.00	0.92 ± 0.10
200.00	2.30	0.97 ± 0.02
400.00	2.60	1.05 ± 0.03

Results are mean ± SD of triplicate determinations (n = 3)

The antiradical activity of *Nigella sativa* powder and oil in scavenging DPPH was assessed using a reduction reaction, shown by a color change from violet to yellow, thereby verifying the antioxidant properties of the extracts. Both samples significantly diminished the stable DPPH radical in a synergistic manner alongside the normal AA. As anticipated, AA exhibited the lowest IC_{50} (12.11 µg/ml), so affirming its antioxidant superiority compared to the powder (66.37 µg/ml) and oil (87.42 µg/ml). This finding supports the research of [6] and [31].

The analysis demonstrated that the FRAP values of both extracts exhibit a directly proportionate relationship with concentration. The maximum FRAP values at the highest concentration for both samples were not statistically significant ($p < 0.05$), measuring 1.03 ± 0.00 % for *Nigella sativa* powder extract and 1.05 ± 0.03 % for *Nigella sativa* oil extract. This outcome aligns with a prior publication by [31], which indicated elevated FRAP values at increased concentrations, a dose-dependent relationship.

Table 4: Carrageenan-induced Edema at various hours during treatment with N. sativa (mm)

Treatment groups	Time (hr)					
	0	1	2	3	4	24
1	4.6 ± 0.10	6.0 ± 0.11	6.3 ± 0.13	5.4 ± 0.12	4.9 ± 0.09	4.6 ± 0.02
2	4.1 ± 0.04	5.6 ± 0.10	6.1 ± 0.17	6.2 ± 0.17	5.6 ± 0.14	4.8 ± 0.08
3	4.1 ± 0.03	5.1 ± 0.07	5.5 ± 0.11	5.7 ± 0.12	5.3 ± 0.90	4.7 ± 0.07
4	4.4 ± 0.12	6.1 ± 0.11	6.9 ± 0.17	7.3 ± 0.19	6.7 ± 0.16	5.6 ± 0.11

Results are mean ± SD of triplicate determinations (n = 3). Group 1 (only N. sativa), Group 2 (mixed), Group 3 (Vehicle control) & Group 4 (untreated)

Table 5: Differences in paw thickness (Edema) after treatment with N. sativa oil extract (mm)

Treatment groups	Time (hr)				
	1	2	3	4	24
1	1.4 ± 0.03	1.7 ± 0.11	0.8 ± 0.03	0.3 ± 0.01	0.0 ± 0.00
2	1.5 ± 0.02	2.0 ± 0.14	2.1 ± 0.12	1.5 ± 0.01	0.7 ± 0.01
3	1.0 ± 0.00	1.4 ± 0.01	1.6 ± 0.02	1.2 ± 0.03	0.6 ± 0.04
4	1.7 ± 0.03	2.5 ± 0.17	2.9 ± 0.20	2.3 ± 0.13	1.2 ± 0.02

Results are mean ± SD of triplicate determinations (n = 3)

Table 6: Percentage inhibition of edema in the rats administered N. sativa oil extract (%)

Treatment groups	Time (hr)				
	1	2	3	4	24
1	17.60	32.00	72.40	87.00	100.00
2	11.80	20.00	27.60	34.80	41.70
3	41.20	44.00	44.80	47.80	50.00

Acute inflammation is the initial phase of the inflammatory response characterized by vasodilation and heightened capillary permeability, leading to fluid exudation and the migration of neutrophils. This transient reaction, lasting a few hours, serves as the body's defense system to eradicate harmful intruders, followed by the evacuation of affected cells and tissues [61 - 62]. Tables 4 and 5 indicate that after the third hour, the resolution of edema began, including in the untreated group 4, indicating that the resolution of inflammation is a time-and-dose-dependent phenomenon. After 24 hours, group 1, which received only *N. sativa* oil, exhibited total edema resolution (Table 6), whereas the test groups receiving combined therapy and the conventional medicine demonstrated varying degrees of resolution.

This notable observation was also documented by [63], who asserted that the exclusive use of oil extract imparts medicinal properties of *N. sativa*, even in the form of a balm. This indicates that utilizing 100% of the oil orally is preferable compared to its formulation with items that diminish its inhibitory activity to around 39.6%. The observed behavior of *N. sativa* may be attributed to its active component, thymoquinone, which suppresses the synthesis of prostaglandins and leukotrienes, thereby preventing lipid peroxidation [64].

In the molecular docking studies, the crystal structure of the human KEAP1 Kelch domain (PDB ID: 1CB4) was retrieved from the Protein Data Bank and prepared for docking using PyRx version 0.8. The protein was energy-minimized, and all water molecules, ions, and non-essential heteroatoms were removed. Polar hydrogens were added, and Gasteiger charges were assigned using AutoDock Tools version 1.5.4. The ligand structure was energy-minimized using the MMFF94 force field prior to docking.

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The best-scoring pose, defined by the lowest binding energy and an RMSD < 3.0 Å, was selected for further analysis. Ligand–protein interactions were analyzed using BIOVIA Discovery Studio Visualizer version 21.1, identifying π–alkyl hydrophobic interactions, carbon hydrogen bonds, and van der Waals contacts.

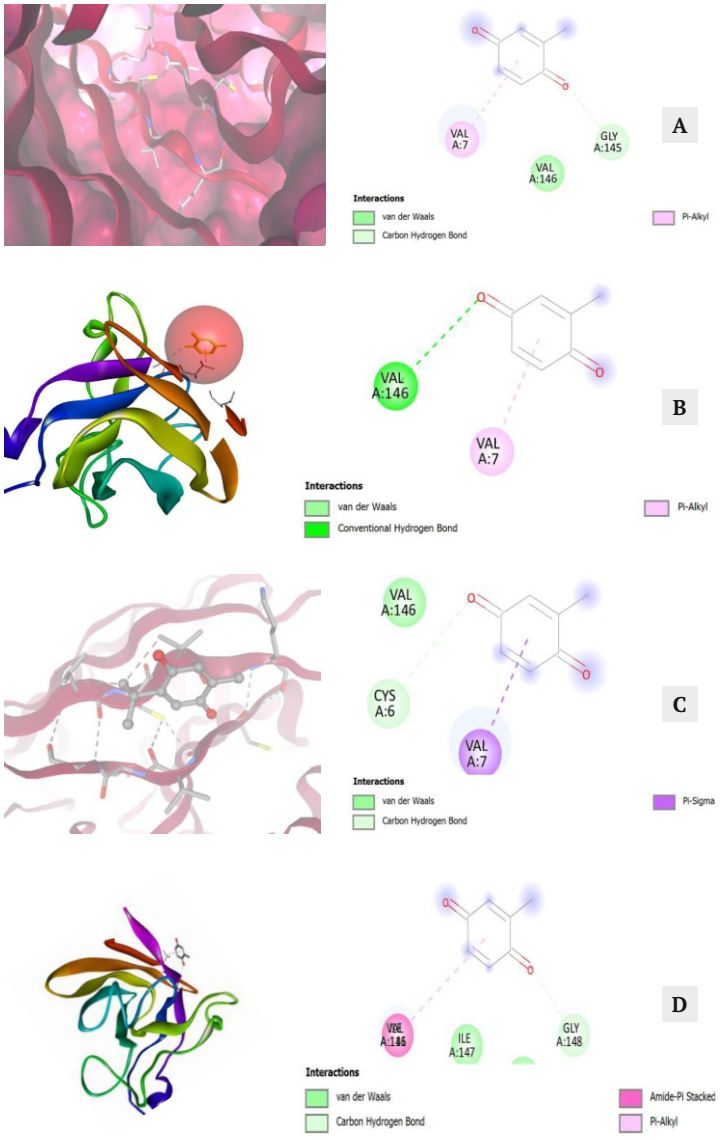

Figure 5(a-d). Three-dimensional visualization of the best-scoring ligand pose within the KEAP1 (PDB ID: 1CB4) binding pocket and Two-dimensional interaction diagram of the docked ligand within the KEAP1 (PDB ID: 1CB4) binding site, generated in Discovery Studio. Pink dashed lines indicate π–alkyl hydrophobic interactions; green dashed lines represent carbon-hydrogen bonds; shaded green areas denote van der Waals contacts. Residues are labeled with their names, chain identifier (A), and residue number. 3D Structures obtained from AutoDock Vina docking in PyRx (binding affinity: −3.693 kcal·mol^{-1}; RMSD < 3.0 Å). The protein is shown in cartoon representation (pink helices) with the ligand in stick form. Hydrogen bonds are depicted as blue dashed lines, illustrating polar contacts that contribute to ligand orientation within the hydrophobic cavity.

Interaction profiling in Discovery Studio revealed (Fig 5a-d) that the ligand engages in a π–alkyl hydrophobic interaction with VAL7, a carbon-hydrogen bond with GLY145, and a van der Waals contact with VAL146. The absence of strong conventional hydrogen bonds or electrostatic interactions suggests that binding is predominantly driven by hydrophobic and weak polar forces, which may limit thermodynamic stability under physiological conditions. Given KEAP1's regulatory role in the NRF2 signaling pathway, the identified pose may serve as a weak starting fragment for further structure-based optimization aimed at enhancing hydrogen-bonding potential and overall pocket complementarity. Several non-covalent interactions stabilize the binding of Thymoquinone to the protein, as outlined in the table below.

Table 7: Ligand-Protein interaction of compounds with PDB ID: 1CB4 and 5IKR

Target Protein (PDB ID)	Interaction Type	Interaction Name	Ligand Atom / Group	Protein Residue	Approximate Effect
1CB4 (Thrombin)	Pi–Alkyl	Hydrophobic interaction	Aromatic ring	VAL7	Contributes to van der Waals stabilization and positioni in pocket
1CB4 (Thrombin)	Carbon Hydrogen Bond	Polar contact interaction	Carbonyl oxygen	GLY145	Weak hydrogen-bond-like interaction aiding orientation
1CB4 (Thrombin)	van der Waals	Non-specific interaction	Aromatic/Carbonyl	VAL146	General stabilization in hydrophobic microenvironmen
5IKR (KEAP1)	Conventional H-bond	Polar contact interaction	Carbonyl oxygen (quinone)	ARG:A:376	Stabilizes ligand via polar interaction
5IKR (KEAP1)	π–π Stacked	Aromatic interaction	Aromatic ring	PHE:A:142	Enhances aromatic ring anchoring
5IKR (KEAP1)	van der Waals	Non-specific interaction	Aromatic ring face	LEU:A:145	Contributes to hydrophobic packing
5IKR (KEAP1)	van der Waals	Non-specific interacton	Aromatic ring face	GLN:A:374	Adds weak stabilizing contact

Molecular docking of thymoquinone with human 5IKR using AutoDock Vina (version 0.8) yielded a best binding pose with a binding affinity of −4.303 kcal·mol^{-1} and RMSD < 3 Å relative to the lowest-energy conformation. The ligand was positioned within the active site pocket, engaging in a conventional hydrogen bond with ARG376 and a π−π stacking interaction with PHE142. Additional van der Waals contacts were observed with LEU145 and GLN374. The hydrogen bond with ARG376 likely stabilizes the quinone moiety, while π−π stacking with PHE142 may enhance aromatic ring anchoring within the binding site, collectively contributing to ligand affinity. The binding pocket itself appears to be largely hydrophobic, contributing further to ligand stabilization.

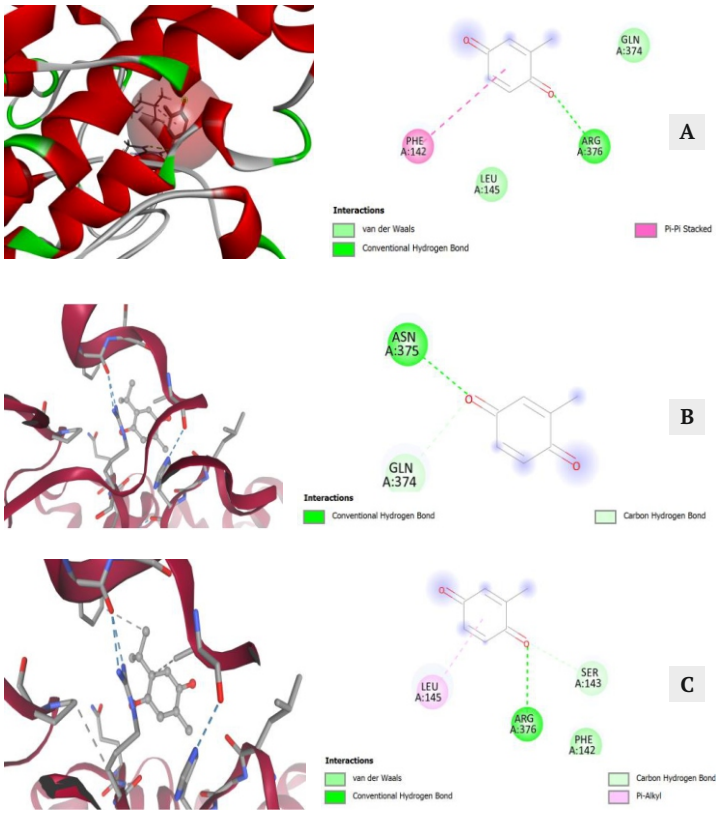

Figure 6: 2D and 3D view of thymoquinone bound to 5IKR (best AutoDock Vina pose; ΔG = −4.303 kcal·mol^{-1}; RMSD < 3.0 Å). The protein is shown in cartoon representation (red helices) and the ligand in sticks. Blue dashed lines indicate putative hydrogen bonds between quinone oxygens and nearby protein donors; grey sticks highlight hydrophobic side chains forming the binding cleft.

Figure six (6) is a depiction of 3D and 2D views of the molecular interaction of a ligand with specific amino acid residues within a protein binding pocket. Several important residues are involved in stabilizing the ligand within the pocket likely involved in π−π stacking interaction with the aromatic ring of the ligand. The combination of hydrogen bonding, cation−π interaction, and possible π−π stacking contributes significantly to the binding affinity and specificity of the ligand. These interactions are crucial for understanding molecular recognition and for guiding structure-based drug design efforts.

In both figures 5 and 6, the binding modes are biologically meaningful and reflect physiologically relevant interactions in understanding the mechanisms of inhibition or activation and structure activity relationship (SAR) analysis.

5. Conclusion

Black cumin (*Nigella sativa*) has long been utilized across the globe in traditional medicine for the treatment of various ailments. Its therapeutic potential is attributed to a diverse array of bioactive phytochemicals, including thymoquinone, *t*-anethole, alkaloids, and saponins [65]. The regular dietary consumption of *Nigella sativa* seeds and their oil has been linked to the prevention and management of several chronic conditions, as well as the enhancement of overall health and wellbeing. These medicinal benefits are largely due to the plant's rich composition of biologically active compounds and essential nutrients.

Growing consumer interest in the nutraceuticals value of *Nigella sativa* has prompted renewed scientific attention towards its compositional and functional properties [66]. Historically revered, *Nigella sativa* is considered one of the most significant nutrient-dense herbs, with around 20 varieties distributed across regions from the Mediterranean to West Asia. It is notably endorsed in *Tibb-e-Nabawi* (Prophetic medicine) for regular use and has also been referred to in ancient texts—known as the curative black cumin in the Holy Bible and as *Melanthion* by Hippocrates [67].

In conclusion, the enduring reverence and expanding scientific validation of *Nigella sativa* underscore its role as a potent natural remedy and functional food. Continued exploration of its phytochemical profile and health-promoting properties may pave the way for innovative applications in modern integrative medicine.

Abbreviations

SAW = Salahu Alaihi Wasalam
DPPH = 2,2-diphenyl-1-picrylhydrazyl
FRAP = Ferric Reducing Antioxidant Power
TQ = Thymoquinone
DTQ = Dithymoquinone
TPC = Total Phenolic Content
TFC = Total Flavanoid Content
TPTZ = 2,4,6- tripyridyl-s-triazine
KEAP = Kelch-like ECH-Associated Protein
PDB = Protein Data Bank
MMFF = Merck Molecular Force Field
RMSD = Root Mean Square Deviation

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Author's contributions

OJA, SMS, OA and ESA: Conceptualization. AOP, EKO, SFA, AF and ALO: Experimentation. OJA, NPM and OA: Investigation/authentication. ODO, OJA and AE: Software. OJA, ESA and AE: Validation. SMS, OA and OJA: First draft of manuscript. All authors read the final manuscript and approved.

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