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COMPREHENSIVE PHYTOCHEMICAL SCREENING AND MOLECULAR DOCKING OF NIGELLA SATIVA EXTRACT FOR POTENTIAL ACTIVITY

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ABSTRACT –
This study evaluate the impact of solvent such as ethanol and water. As in this study the extraction of flower part is performed in hydro alcoholic solvent, the extract contain a mixture and water, which helps extract both polar and non polar compounds.Additionally, protein ligand modeling studies were conducted to examine how these compounds interacted with therapeutic target proteins. The objective is to investigate how *Nigella Sativa*seeed extract might be used for development of herbal formulations havingwide range of medicinal benefits. Ascertain the presence of distinct phytochemicals using both quantitative and qualitative tests, the seeds extract was made using ethanol, water, and hydro-alcoholic solvent system. Ethanol extracts generally yield moderate amounts of alkaloids and flavonoids, while water extracts excel in flavonoids and saponins. Hydroalcoholic extracts often show slightly higher yields for compounds like saponins and terpenoids and flavonoids compared to ethanol and water extracts. Water and hydroalcoholic extracts have relatively similar yields for tannins, with water showing a marginally higher yield. Glycosides and steroids, however, are more effectively extracted in hydroalcoholic solvents. Overall, water is particularly effective for saponin and tannins, while hydroalcoholic extracts provide a balanced yield across various compounds, showing better extraction for flavonoids, terpenoids and steroids. The Docking study were performed on all the molecules with the PdB IDs (3VXI, 4K7A, 2XFH) and there results are arranged as per the dock score is on the top. Based on their docking scores and binding energies, which are similar to those of the reference molecules, the compounds shows potential as Anti-oxidant, Anti-Alopecia and Anti-fungal activity agents. Thymoquinone showed the strongest binding affinity, significantly surpassing Ascorbic acid, for Anti-alopecia activity Thymoquinone outperformed Minoxidil, indicating its potential as a better agent for alopecia treatment and for Anti-fungal activity Thymoquione again showed the highest docking score, surpassing Clotrimazole in binding affinity. However, the compounds showed some limitations in terms of ADME properties, which could hinder their oral bioavailability. Further studies, including in vitro and in vivo testing, are required to validate the pharmacological potential of these compounds and to address their bioavailability challenges.

KEY WORDS -Nigella Sativa, Phytochemical Screenning, extraction, Ligand binding, Molecular docking

1. INTRODUCTION –

The plant commonly known as black seed or black cumin, is a flowering plant that belongs to Ranunculaceae family found in native parts of western Asia, the Mediterranean region and North Africa. The plant have delicate fern like leaves and the flowers are star shaped blue or white in color.Seeds are small, black and triangular in shape. They have been used for centuries for their medicinal properties. The seeds are most famous for their potential health benefits. They contain various compounds which give various activities like anti-inflammatory, antioxidant, antimicrobial and antifungal activity. It is been traditionally used in herbal medicine for a wide range of aliments, including digestive issues, respiratory conditions and skin disorders. It is also used in cooking mainly at Middle Eastern, Indian and Mediterranean cuisines. In the past years various studies have been explored the health benefits of seeds. They suggested its potential as a natural remedy for various chronic conditions, including asthma, diabetes and hypertension. From past years to till now the interest has been focused on its potential in enhancing hair growth and exhibiting antimicrobial activity. The extract of seed with various solvent has shown good results for hair growth as well as for antimicrobial and antioxidant activity. Extraction in water yields 15% and was rich in flavanoids, phenols and saponins which showing moderate antimicrobial activity against S. aureus and 80% inhibition in antioxidant tests. Extraction in erthanol gave 12 %, containing flavanoid, alkaloid an tannins with antioxidant activity (IC50: 22ug/ml) and antimicrobial activity against E. coli. Methanol produced highest yield at 18%, rich in anthocyanins, phenolic acid and flavanoids, showing antioxidant (85% ZOI) and antimicrobial activity against P. aeruginosa. Hexane (5% yield)extracted terpenoids and steroids that shows weaker antioxidant and antimicrobial property with no hair growth activity. Chloroform extract (8% yield) is primarily rich in alkaloids, flavanoids and terpenoids with medium antioxidant and antimicrobial activity. This study also evaluate the impact of solvent such as ethanol and water. The extraction process or the seed and use of solvent influence the phytochemical quality and quantity. As in this study the extraction of flower part is performed in hydro alcoholic solvent, the extract contain a mixture and water, which helps extract both polar and non polar compounds. The constituents found in the extract include flavanoid such as quercetin, kaempferol and anthocyanins, phenolic compound such as phenoilic acid, gallic acid and ferulic acid, tannins, saponins, alkaloids , anthocyanins, steroids, terpenoids. They contribute in antioxidant, antimicrobial, anti inflammatory, astringent and hair growth activity. Mixture of ethanol and water as a extraction solvent particularly effective because they can extract brooder spectrum of phyochemicals compared to water or alcohol alone, provide a well rounded extract rich in bioactive compounds. This study is performed to elucidate the phytochemical profile of the seed and evaluate its efficacy in promoting hair growth and combating microbial infection. However, more research is needed to fully understand its therapeutic potential.The process of protein ligand binding for a plant extract involves using computer simulations to clarify how the bioactive component in the extract interacts with target proteins linked to certain disorders. The ability of plant-derived compounds to bind to and block the activity of proteins linked to illnesses like cancer or microbial infections has been predicted computationally using this technique. By examining binding affinities and interaction sites and streamlining the drug development process, molecular docking aids in the identification of possible medicinal molecules from natural sources. It is a helpful tool for phytomedicine research, allowing for the investigation of plant extracts for the creation of pharmaceuticals. It offers comprehensive drug discovery, research on natural products, and comprehension of the molecular underpinnings of plant-based therapeutic effects. This strategy has a lot of potential in leveraging traditional knowledge of medicinal plants for modern pharmaceutical applications.

The yield and variety of phytochemicals isolated from *Nigella sativa* seeds are assessed in this study. The purpose of the study is to clarify that the plant seeds contains more and better components when extracted using a hydroalcoholic solvent. Additionally, protein ligand modeling

studies were conducted to examine how these compounds interacted with therapeutic target proteins. The objective is to investigate how *Nigella Sativa* seeed extract might be used for development of herbal formulations havingwide range of medicinal benefits.

2. MATERIAL AND METHOD –

Chemical and reagents –

Thechemicals were used are Water, ethanol, ferric chloride, acidified alcohol, ammonia, chloroform, acetic acid, Mayer's reagent, Dragendroff's reagent, sulfuric acid, sodium hydroxide, fehling's solution, Molisch's regent, hydrochloric acid, zinc dust, magnesium turnings, biuret reagent, folin-Ciocalteu reagent, sodium carbonate, gallic acid solution, acetone and isopropyl alcohol (IPA), methanol, aluminium trichloride, potassium acetate, etc.

Plant material and extraction –

After being gathered from the garden, *Nigella Sativa* seeds were cleaned, dried, and ground into a powder. Soxhlet extraction procedure was used to extract the powder using a hydro-alcoholic , ethanolic and water as solvent. A rotary evaporator was used to concentrate the extract, which was then kept at 40 C for further examination.

Phytochemicals screening –

The spectrum and concentration of bioactive components in plant extracts varies significantly between water, ethanol and hydroalcoholic plant extracts when comparing the phytochemical contents. Hydrophilic compounds such polyphenols like flavanoids, phenolic acid, glycosides, and alkaloids are present in the extract with water as a solvent. These compounds effectively demonstrate anti-inflammatory, antibacterial, and antioxidant properties. While hydroalcoholic solvents, which are a mixture of alcohol and water, effectively extract a wider range of phytochemicals, including both hydrophilic and lipophilic compounds like terpenes, essential oils, saponins, and alkaloids, plant extracts are thought to be safer and more appropriate for therapeutic purposes where hydrophilic compounds are needed. The alcohol present in the solvent enhances the solubility of non polar compounds; provide the extract greater pharmacological activity. So the choice of solvent significantly influences the composition, potency and potential therapeutic effect, its essential to select the appropriate extraction method.

The present investigation employed conventional methods to undertake a comparative confirmatory qualitative phytochemical screening of plant extracts in water, ethanol and hydroalcoholic solvent in order to detect the presence of tannins, saponins, flavanoids, alkaloids, phenols, glycosides, steroids, and terpenoids. In order to ascertain the presence of distinct phytochemicals using both quantitative and qualitative tests, the seeds extract was made using ethanol, water, and a hydro-alcoholic solvent system. Based on chemical reactions, such as color changes or precipitation formation, the test results were recorded. For example, a yellow precipitate for flavonoids or a green-black tint for tannins were observed. Each phytochemical's concentration or yield in the extract was determined by the quantitative and qualitative test and the results were reported as mg/gram or as a percentage yield (w/w or mg/gram). The results are given in the given in the table, providing insight into the phytochemical composition of the flower extracts prepared with different solvent and offering valuable data for further analysis and potential application of these extract.

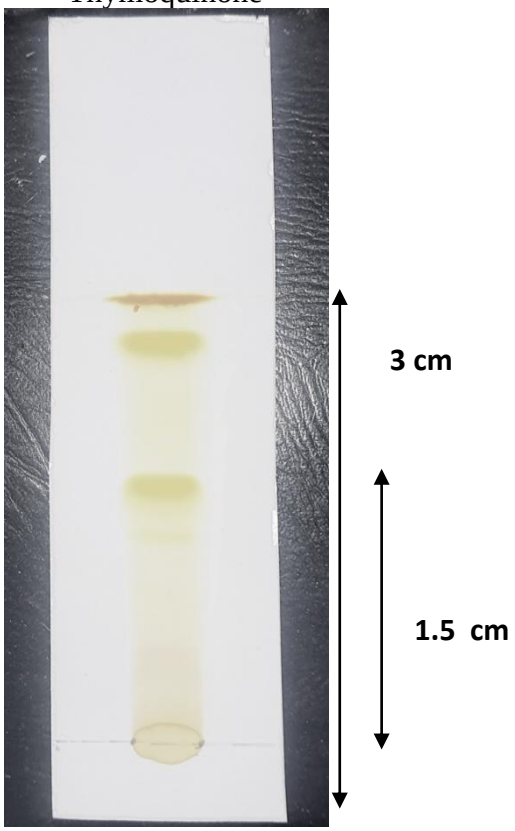
Isolation of Thymoquinone from hydroacoholic seed extract -

Chloroform was used as a solvent to extract the hydroalcoholic seed extract in a separating funnel. To extract as much of the most active components with the highest biological activity as feasible, exhaustive extraction (EE) is performed using a variety of solvents with varying degrees of polarity. As a result, the extract was separated into two separate layers. The two layers were split apart. The lower, milky-white organic layer was allowed to pass through a silica column, producing colorless fractions that were gathered one minute apart. A solvent system consisting of hexane and dichloromethane (7:3) was employed.

Thin layer chromatography –

The original 80% methanolic extract, the upper and the lower organic layers obtained by solvent extraction, and 6 of randomly selected colorless fractions that were obtained by column chromatography of the hydroalcoholic extract were subjected to TLC. TLC was performed using a solvent system consisting of hexane and dichloromethane (1:1) was employed. Until the solvent had traveled three-quarters of the card, the card was left to run. The cards were taken out of the solvent. In order to visualize the cards, they were let to dry. The spots obtained (Figures 1) that were visible were noted in order to calculate Rf values. The Rf value can be understood as the compound's displacement from the beginning point divided by the solvent's displacement from the starting point.

Figure No. – 1 - TLC card for hydroalcoholic extract of Nigella Sativa seeds for the extraction of Thymoquinone



High- performance liquid chromatography –

Reversed phase high-performance liquid chromatography (RP-HPLC) is a straightforward, sensitive, and accurate technique that was utilized to quantify thymoquinone in N. sativa seed hydroalcoholic extracts.

Stock solution and HPLC condition –

To achieve a concentration of 1000 µg/mL, 10 mg of standard thymoquinone (purity 99%) that had been precisely weighed was dissolved in 10 ml of methanol. This solution served as thymoquinone's stock solution. Water and methanol (40:60, v/v) were used as the mobile phase in an isocratic system for RP-HPLC analysis, with a flow rate of 1.5 mL/min and a detection wavelength of 254 nm. Before being injected into the device, the mobile phase and samples were degassed under vacuum and passed through a 0.22 µm Dura PVDF membrane aqueous filter and MillexGVfilterunit.

A chromatogram of standard thymoquinone and that of the sample were obtained. Thymoquinone was discovered during a retention time of 6th to 7th minute in a 10-minute cycle. The difference in retention time between standard thymoquinone and the hydroalcoholic sample was 0.37% (<5%). The difference in retention time between conventional thymoquinone and separated thymoquinone

was 1.2% (<5%). Thymoquinone content in standard solution (SND) was 1 mg/ml, whereas N. sativa seed powder concentration in methanol extract (SMP) was 250 mg/ml. So, we used the following formulas to calculate the concentration of thymoquinone in the produced extract:

$$\frac{\text{Peak area of SMP}}{\text{peak area of SND}} \times \frac{\text{concentration of SND}}{\text{concentration of SND}} \times 100 \quad \text{---(1)}$$

$$\text{Concentration of sample} - \frac{\text{peak area of sample}}{\text{Response factor}} \text{-------(2)}$$

Protein Preparation and receptor grid generation –

This work used well-established computational techniques to assess the actions of particular protein inhibitor complexes. The protein complexed with dye-decolorizing peroxide (DyP) and (2R)-2-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxy-2H-furan-5-one (PDB ID:3VXI) was analyzed for antioxidant activity and its crystal structure was obtained from the Protein Data Bank. The crystal structure of the androgen receptor ligand binding domain attached to 3-hydrox-2-imino-6-piperidin-1-ylpyrimidin-4-amine (PDB ID: 4K7A) was used to analyze the anti-alopecia activity. The crystal with the structure of cytochrome P450 EryK co-crystallized with the inhibitor 1-[92-chlorophenyl)-diphenylmethyl]imidazole (PDB ID: 2XFH) was chosen for the antifungal activity. The Protein Preparation Wizard was used to meticulously build the protein structures, guaranteeing their superior quality and suitability for additional computer investigation. A grid was created around the active site of each protein using the receptor grid generation tool in Glide v9.1. These preparation steps enabled a comprehensive investigation of the proteins interaction with their respective inhibitors, providing insight into their roles in driving the targeted biological activities.

Ligand Preparation –

The molecules were initially drawn using a 2D sketching tool to establish their basic structures. The precision of these structures was subsequently increased by optimizing them using geometric minimization with the OPLS 5 force field. LigPrep was utilized to further purify the compounds, guaranteeing that the molecules were ready for the subsequent analytical steps. This meticulous planning was essential to enhancing the computational studies dependability and delivering a precise depiction of the characteristics and interactions of the molecules. This careful preparation was crucial for improving the reliability of the computational studies and providing an accurate representation of the molecules properties and interactions.

Ligand Docking with Glide –

A molecular docking study was conducted using the Glide application, and either Standard Precision (SP) or Extra Precision (XP) was chosen as the preferred docking strategy. Even while XP is more accurate, it requires more computational resources. Configure the docking parameters, modifying the posture sampling, scoring, and ligand flexibility options as necessary. The docking process begins once everything is configured. After docking was finished, the score was analyzed to evaluate the binding affinities and interactions.

MM-GBSA –

The relative binding energies of a few chosen ligands were assessed using the Prime/MM-GBSA technique. The input was the pv.maegz file produced by XP docking. In order to provide a comprehensive assessment of the ligand-protein interactions while taking the active site's flexibility into account, the protein's active site was arranged to permit the ligand to move within a 5 Å range. This method provided insightful information about the energetics of ligand binding. Since they suggested more robust possible interactions with the target protein, ligands with lower total binding free energy were given priority for additional research.

ADME Analysis –

The compound was further analyzed using the SwissADME system, accessible at http://www.swissadme.ch. the chemical was further examined. First, ChemDraw 2D software20 was used to transform the compounds into their respective SMILES Ids. After that, these IDs were sent to the SwissADME portal for review. Numerous physicochemical and pharmacokinetic properties were predicted by the server, including interactions with P-glycoprotein, adherence to Lipinski's Rule of Five (a metric for drug likeness), the quantity of hydrogen bond donors and acceptors, the possibility of blood-brain barrier penetration, and gastrointestinal absorption. This comprehensive research provided insightful information about the compounds' pharmacological potential and drug-like properties.

Toxicity Analysis –

The toxicity profiles of the compounds were evaluated using the ProTox-II platform, available at http://tox.charite.de/protox_II. To begin, the SMILES Ids o the compound, generated with ChemDraw 2D software, were uploaded to the server for analysis. The ProTox-II platform then provided comprehensive toxicity predictions, including the LD50 values (median lethal dose), toxicity classifications, and potential organ-specific adverse effects[21-23]. The analysis also covered eye toxicity parameters such as hepatotoxicity, mutagenicity, carcinogenicity and cytotoxicity. This in-depth evaluation helped identify safety concerns and guided the selection of compound with more favourable toxicity profiles for further research.

3. RESULT–

Phytochemical screening -

The phytochemical screening results give information on the qualitative and quantitative analysis's composition for the ethanolic seeds extract. The existence of alkaloids, flavanoids, saponins, tannins, and phenols is described in table no. 1. The ethanolic extract also contained steroids, terpenoids, glycosides, and resins. The quantitative analysis (Table No. 2) gives information on the yield or concentration of each phytochemical as determined by a variety of methods. The percentage yield of phytochemical constituents in *Nigella sativa* varies across different compounds. Alkaloids yield between 0.4% and 1.8%, typically low in this plant. Flavonoids range from 1.2% to 3.5%, with positive results indicating a yellow to red color change, Saponins show a yield between 1.4%, measured by foam stability and Tannins 3.2%, with precipitation indicating higher concentrations. Terpenoids 1.8%, with a blue to green color change. Phenolic compounds yield 2.6%, indicated by blue color formation. Steroids with 0% yield. Coumarins yield 1.3%, identified under UV light. Proteins range from 2.6%, with violet color formation indicating their presence. Carbohydrates show a yield 2.1%, indicated by yellow color formation. Lipids/fats yield 2.2% determined by Soxhlet extraction.

Table No – 1 Phytochemical Screening of Nigella sativa Seed Ethanol Extract

Phytochemical Test	Test Method	Result	Observation
Alkaloids	Dragendorff's reagent (orange precipitate)	Positive	Orange-brown precipitate indicating alkaloids.
	Mayer's reagent (cream-colored precipitate)		Cream-colored precipitate indicating alkaloids.
Flavonoids	Shinoda test (with magnesium and hydrochloric acid)	Positive	Formation of a red or orange colour.
	Alkaline test (with sodium hydroxide solution)		Yellow colour in solution.
Saponins	Foam test (shake test)	Positive	Formation of persistent foam.

Tannins	Ferric chloride test (blue or greenish-blue colour)	Positive	Formation of a blue or greenish-blue color.
	Gelatine test (formation of precipitate)		Formation of a precipitate.
Terpenoids	Salkowski test (add concentrated H2SO4 and shake)	Positive	Red to blue or greenish colour.
	Liebermann-Burchard test (add acetic anhydride and H2SO4)		Formation of a blue to green colour.
Phenolic Compounds	Folin-Ciocalteu method (blue colour formation)	Positive	Blue colour formation indicating phenol compounds.
Glycosides	Borntrager's test (with dilute sulfuric acid and ammonia)	Negative	No Pink or red colour formation.
	Kedde's test (reaction with sodium nitroprusside)		Purple colour formation.
Steroids	Salkowski test (with concentrated H2SO4)	Negative	Red or blue colour.
	Liebermann-Burchard test (add acetic anhydride and H2SO4)		Blue or green colour.
Coumarins	UV fluorescence test (reaction under UV light)	Positive	Greenish fluorescence under UV light.
	Sodium hydroxide test (yellow fluorescence)		
Proteins	Biuret test (add copper sulfate and sodium hydroxide)	Positive	Violet colour
Carbohydrates	Molisch's test (add alpha-naphthol and sulfuric acid)	Positive	Violet ring at interface.
	Benedict's test (add Benedict’s reagent and heat)		Formation of a red/orange precipitate.
Lipids/Fats	Grease spot test (on filter paper)	Positive	Transparent spot on filter paper.
	Sudan III test (add Sudan III dye)		Red-orange coloration in the extract.
Volatile Oils	Distillation (GC-MS)	Positive	Presence of characteristic essential oils like thymoquinone.

Table No – 2 Phytochemical Screening of Nigella sativa Seed Extract in Ethanol (Quantitative Analysis)

Phytochemical Name	Test Method	Result (% Yield)	Observation
Alkaloids	Titrimetric method (e.g., using acid-base titration)	1.4%	Alkaloids yield is calculated based on titration with an acid. Alkaloid content is often low in Nigella sativa, hence lower yields.
	Colorimetric method (e.g., Dragendorff's reagent)		Orange-brown precipitate indicates alkaloid presence.
Flavonoids	Spectrophotometric method (e.g., AlCl3 reagent at 415 nm)	2.2%	Flavonoid yield is measured by absorbance. Positive results show a yellow to red colour.
	Colorimetric method (e.g., Shinoda test)		Red or orange colour formation indicates flavonoids presence.
Saponins	Foam index method (measuring foam stability after shaking)	2.8%	Foam formation and stability indicate saponin content. The foam index method provides a quantitative measure.
Tannins	Gravimetric method (precipitation with lead acetate)	3.2%	Precipitation yields tannins. Higher yields indicate a higher concentration of tannins in the extract.
	Spectrophotometric method (Folin-Ciocalteu reagent at 765 nm)	1.8%	Blue colour formation indicates phenolic compounds (tannins).
Terpenoids	Salkowski method (with concentrated H2SO4)		Blue to green colour formation indicates terpenoid presence.
	GC-MS or HPLC (for volatile terpenoids such as thymoquinone)		Thymoquinone can be quantified using GC-MS or HPLC based on retention time or peak area.
Phenolic Compounds	Folin-Ciocalteu method (spectrophotometer at 765 nm)	2.6%	Blue colour formation corresponds to the phenolic content. Higher absorbance indicates higher phenolic concentration.
Glycosides	Borntrager's test (reaction with dilute sulfuric acid)	–	Pink or red colour formation indicates glycoside presence.
	Kedde's test (reaction with sodium nitroprusside)		Purple colour formation indicates glycoside content.
Steroids	Liebermann-Burchard method (colour change with acetic anhydride)	–	Blue or green colour after treatment with acetic anhydride indicates steroids.
	HPLC or GC-MS (for quantification of steroids)		HPLC or GC-MS can quantify specific steroids by their retention times or peak areas.
Coumarins	UV fluorescence method (under UV light)	1.3%	Greenish fluorescence under UV light indicates the presence

			of coumarins.
	Sodium hydroxide test (yellow fluorescence under UV light)		Yellow fluorescence indicates the presence of coumarins.
Proteins	Lowry method or Biuret method (protein quantification)	2.6%	Violet colour formation indicates protein presence. The yield depends on protein content in the extract.
Carbohydrates	Phenol-sulfuric acid method (absorbance at 490 nm)	2.1%	Yellow colour formation, measured at 490 nm, indicates carbohydrate presence.
	Molisch's test (formation of violet ring)		Violet ring at the interface indicates carbohydrates.
Lipids/Fats	Soxhlet extraction method (lipid quantification)	2.2%	Lipid content is determined by the weight of extracted fat.
	Grease spot test (on filter paper)		Transparent spot on filter paper indicates lipids.
Volatile Oils	Hydro distillation or GC-MS (quantification of essential oils)	0.4%	Presence of essential oils such as thymoquinone can be quantified by GC-MS based on peak area.

The qualitative screening of water extract of flower revealed the presence of several bioactive compound. The test (Table No.- 3) confirmed the presence of various phytochemicals. Specifically alkaloids and flavonoids were showed the presence by the formation of orange and yellow precipitates, respectively, while saponins, phenols, tannins, glycosides, resins, carbohydrates and proteins. In terms of quantitative analysis (Table No. - 4), the percentage yield of various phytochemicals in the water extract was also determined. The phytochemical content in Nigella sativa varies across different compounds, with each showing distinct yield percentages. Alkaloids are present in low amounts (0.7%) with detection confirmed by an orange-brown precipitate. Other phytochemicals as Flavonoids (2.0%), Saponins (3.5%), Tannins, (3.5%), Terpenoids (0.9%), and Phenolic compounds (2.4%), Glycosides are (0.9%), Steroids (0.8%), Coumarins (0.7%), Proteins (1%), Carbohydrates (4.0%), Lipids/fats (1.2%) were found in various % yields.

Table No.- 3 Phytochemical Screening of Nigella sativa Seed Extract in Water

Phytochemical Test	**Test Method**	**Result**	**Observation**
Alkaloids	Dragendorff's reagent (orange precipitate)	Positive	Orange-brown precipitate indicating alkaloids.
	Mayer's reagent (cream-colored precipitate)		Cream-colored precipitate indicating alkaloids.
Flavonoids	Shinoda test (with magnesium and hydrochloric acid)	Positive	Formation of a red or orange colour.
	Alkaline test (with sodium hydroxide solution)		Yellow colour in solution.
Saponins	Foam test (shake test)	Positive	Formation of persistent foam.
Tannins	Ferric chloride test (blue or greenish-blue color)	Positive	Formation of a blue or greenish-blue colour.
Terpenoids	Salkowski test (add	Positive	Red to blue or greenish

	concentrated H2SO4 and shake)		colour.
	Liebermann-Burchard test (add acetic anhydride and H2SO4)		Formation of a blue to green colour.
Phenolic Compounds	Folin-Ciocalteu method (blue color formation)	Positive	Blue colour formation indicating phenolic compounds.
Glycosides	Borntrager's test (with dilute sulfuric acid and ammonia)	Positive	Pink or red colour formation.
	Kedde's test (reaction with sodium nitroprusside)		Positive: Purple colour formation. Negative: No colour change.
Steroids	Salkowski test (with concentrated H2SO4)	Positive	Red or blue colour.
	Liebermann-Burchard test (add acetic anhydride and H2SO4)		Positive: Blue or green colour. Negative: No colour change.
Coumarins	UV fluorescence test (reaction under UV light)	Positive	Greenish fluorescence under UV light.
	Sodium hydroxide test (yellow fluorescence)		Yellow colour formation under UV light.
Proteins	Biuret test (add copper sulfate and sodium hydroxide)	Positive	Violet colour.
Carbohydrates	Molisch's test (add alpha-naphthol and sulfuric acid)	Positive	Violet ring at interface.
	Benedict's test (add Benedict’s reagent and heat)		Formation of a red/orange precipitate.
Lipids/Fats	Grease spot test (on filter paper)	Positive	Transparent spot on filter paper.
	Sudan III test (add Sudan III dye)		Red-orange coloration in the extract.
Volatile Oils	Distillation	Negative	Absence of characteristic essential oils like thymoquinone.

Table No- 4 Phytochemical Screening of Nigella sativa Seed Extract in Water (Quantitative Analysis)

Phytochemical Name	**Test Method**	**Result (% Yield)**	**Observation**
Alkaloids	Titrimetric method (e.g., acid-base titration)	0.7%	Alkaloid content in water extract tends to be lower. The yield can be calculated based on titration.
	Dragendorff's test (colour change with Dragendorff's reagent)		Orange-brown precipitate indicates alkaloids.
Flavonoids	Spectrophotometric method (e.g., AlCl3 reagent at 415 nm)	4.0%	Yellow or red colour indicates flavonoids presence. Yield is quantified by absorbance.
	Shinoda test (reaction		Red colour formation indicates

	with magnesium and HCl)		flavonoids.
Saponins	Foam index method (measuring foam stability after shaking)	3.5%	Foam formation and stability indicate saponin content. Foam index determines the yield.
Tannins	Gravimetric method (precipitation with lead acetate)	3.5%	Precipitation with lead acetate gives the yield of tannins. Higher yields indicate greater tannin concentration.
	Folin-Ciocalteu reagent (spectrophotometry at 765 nm)		Blue colour indicates phenolic compounds, which include tannins.
Terpenoids	Salkowski test (colour change with concentrated H2SO4)	0.9%	Blue or green colour indicates terpenoids.
Phenolic Compounds	Folin-Ciocalteu method (spectrophotometry at 765 nm)	2.4%	Blue colour formation indicates phenolic content. Absorbance is measured to quantify yield.
Glycosides	Borntrager's test (reaction with dilute sulfuric acid)	0.9%	Pink or red colour formation indicates glycosides. Yield depends on the colour intensity.
Steroids	Liebermann-Burchard method (colour change with acetic anhydride)	0.8%	Blue or green colour after treatment with acetic anhydride indicates steroids.
Coumarins	UV fluorescence method (under UV light)	0.7%	Greenish fluorescence under UV light indicates coumarin presence.
Proteins	Lowry method or Biuret method (protein quantification)	1.0%	Violet colour formation indicates protein presence. Protein yield is based on colour intensity.
Carbohydrates	Phenol-sulfuric acid method (absorbance at 490 nm)	4.1%	Yellow colour formation indicates carbohydrate presence. Absorbance at 490 nm is used to quantify.
Lipids/Fats	Soxhlet extraction method (lipid quantification)	1.2%	Lipids are usually present in lower amounts in water extracts. Quantification is based on weight.
Volatile Oils	Hydrodistillation or GC-MS (quantification of essential oils)	_	Presence of essential oils such as thymoquinone can be quantified by GC-MS.

The Hydro-alcoholic flower extract was analyzed for a range of phytochemicals using both qualitative and quantitative (Table No.-5&6) methods. Qualitative test confirmed the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, steroids, glycosides, antraquinones, carbohydrates, proteins and resins. The orange precipitate shows the presence of alkaloids, yellow precipitate shows the presence of flavonoids, saponins produced stable froth and tannins, phenols changes color to green-black and deep blue, respectively. Terpenoids,steroids and glycosides were indicated by reddish brown precipitates and a bluish green ring. Thequalitative analysis revealed that the table provides an overview of various phytochemicals and their respective test methods, yield results, and observations. Alkaloids yielding 0.7%, with positive results showing an orange-brown precipitate. Flavonoids yielding 2.0%, Saponins yield 2.7%, while tannins are with a 3.1% yield. Terpenoids yield 1.6%. Phenolic compounds are measured by spectrophotometry, yielding 2.7%. Glycosides 1.5% yield, indicated by color changes. Steroids yielding 1.7%, Coumarins show

a 1.2% yield, Proteins yield 2.0%, Carbohydrates with a 3.5% yield and Lipids/fats yielding 2.4%, and volatile oils yield 1.5%. Each test method provides a specific indicator to identify and quantify the presence of these compounds in plant extracts.

Table No- 5 Phytochemical Screening of *Nigella sativa* Seed Extract in Hydroalcoholic Solution

Phytochemical Test	Test Method	Result	Observation
Alkaloids	Dragendorff's reagent (orange precipitate)	Positive	Orange-brown precipitate.
	Mayer's reagent (cream-colored precipitate)		Cream-colored precipitate.
Flavonoids	Shinoda test (with magnesium and hydrochloric acid)	Positive	Formation of a red or orange colour.
	Alkaline test (with sodium hydroxide solution)		Yellow colour in solution.
Saponins	Foam test (shake test)	Positive	Formation of persistent foam.
Tannins	Ferric chloride test (blue or greenish-blue color)	Positive	Blue/greenish colour formation.
	Gelatin test (formation of precipitate)		Formation of a precipitate.
Terpenoids	Salkowski test (add concentrated H2SO4 and shake)	Positive	Red to blue or greenish colour.
	Liebermann-Burchard test (add acetic anhydride and H2SO4)		Blue to green colour.
Phenolic Compounds	Folin-Ciocalteu method (blue colour formation)	Positive	Blue colour indicating phenolics.
Glycosides	Borntrager's test (with dilute sulfuric acid and ammonia)	Positive	Pink or red colour formation
	Kedde's test (reaction with sodium nitroprusside)		Positive: Purple colour formation.
Steroids	Salkowski test (with concentrated H2SO4)	Positive	Red or blue colour.
	Liebermann-Burchard test (add acetic anhydride and H2SO4)		Positive: Blue or green colour.
Coumarins	UV fluorescence test (reaction under UV light)	Positive	Greenish fluorescence under UV light.
	Sodium hydroxide test (yellow fluorescence)		Yellow colour formation under UV light.
Proteins	Biuret test (add copper sulfate and sodium hydroxide)	Positive	Violet colour.
Carbohydrates	Molisch's test (add alpha-naphthol and sulfuric acid)	Positive	Violet ring at interface.
	Benedict's test (add Benedict’s reagent and heat)		Red/orange precipitate.
Lipids/Fats	Grease spot test (on filter paper)	Positive	Transparent spot on filter paper.
	Sudan III test (add Sudan III dye)		Red-orange coloration in extract.

Volatile Oils	Distillation (GC-MS)	Positive	Presence of essential oils like thymoquinone.

Table No- 6 Phytochemical Screening of *Nigella sativa* Seed Extract in Hydroalcoholic Solution (Quantitative Analysis)

Phytochemical Name	Test Method	Result (% Yield)	Observation
Alkaloids	Titrimetric method	0.7%	Alkaloid yield is calculated based on titration. The result depends on the extraction process and the alkaloid content.
	Colorimetric method		Positive result gives an orange-brown precipitate when alkaloids are present.
Flavonoids	Spectrophotometric method	2.0%	Flavonoids yield can be measured by the absorbance at 415 nm after reaction with AlCl3.
	Colorimetric method		Positive result gives a red or orange color when flavonoids are present.
Saponins	Foam index method (measuring foam stability)	2.7%	Foam formation indicates saponin content. The yield is determined by the foam index.
Tannins	Gravimetric method (using precipitation with lead acetate)	3.1%	Tannins yield is calculated based on precipitate formation.
	Spectrophotometric method		Blue color formation indicates phenolic compounds, including tannins.
Terpenoids	Salkowski method (absorption spectrum or color formation)	1.6%	Positive result shows a blue or greenish color, confirming the presence of terpenoids.
	GC-MS analysis (for volatile terpenes like thymoquinone)		GC-MS can quantify terpenoids based on peak area. Thymoquinone is a major component.
Phenolic Compounds	Folin-Ciocalteu method (spectrophotometry at 765 nm)	2.7%	Blue color intensity is measured to determine phenolic content.
Glycosides	Borntrager's test (with dilute sulfuric acid)	1.5%	Positive result indicates the presence of anthraquinone glycosides. The yield is determined by color intensity.
	Kedde's test (reaction with sodium nitroprusside)		Glycosides yield can also be quantified based on the color change to purple.
Steroids	Liebermann-Burchard method (color change)	1.7%	Blue/green color formation after treatment with acetic anhydride indicates steroid presence.
	GC-MS or HPLC method		Steroid quantification based on

			retention time or peak area in chromatograms.
Coumarins	UV fluorescence method (under UV light)	1.2%	Positive result gives greenish fluorescence under UV light, indicating coumarins.
	Sodium hydroxide test (yellow fluorescence)		Yellow color under UV light can be used to estimate coumarin content.
Proteins	Lowry method or Biuret method (protein quantification)	2.0%	Violet color formation after adding copper sulfate and alkali indicates protein presence.
Carbohydrates	Phenol-sulfuric acid method (absorbance at 490 nm)	3.5%	Carbohydrate yield is quantified based on the absorbance at 490 nm after phenol and sulfuric acid reaction.
	Molisch's test (formation of violet ring)		Positive result yields a violet ring at the interface, indicating carbohydrate presence.
Lipids/Fats	Soxhlet extraction method	2.4%	Lipids are extracted and weighed to determine their percentage yield.
	Grease spot test (qualitative analysis)		Transparent grease spot on filter paper confirms lipid presence.
Volatile Oils	Hydro distillation or GC-MS	1.5%	GC-MS provides the most accurate quantification of essential oils like thymoquinone.

The pytochemical screening of *Nigella Sativa* seeds extracts in ethanol, water and hydro-alcoholic solvents revealed various type of bioactive compounds, which may contribute to its therapeutic potential. The qualitative and quantitative analysis of these extracts demonstrated significant variations in the % yield of various phytochemicals with different solvent used.

The quantitative data presented in Table 2 (ethanol extract), Table 5 (water extract), and Table 6 (hydro-alcoholic extract) demonstrate variations in the yield of phytochemicals. The comparative analysis of phytochemical yields from ethanol, water, and hydroalcoholic extracts of Nigella sativa seeds shows varied results across different compounds. Ethanol extracts generally yield moderate amounts of alkaloids (1.4%) and flavonoids (2.2%), while water extracts excel in flavonoids (4.0%) and saponins (3.5%). Hydroalcoholic extracts often show slightly higher yields for compounds like saponins (3.7%) and terpenoids (1.6%) and flavonoids (3%) compared to ethanol and water extracts. Water and hydroalcoholic extracts have relatively similar yields for tannins, with water showing a marginally higher yield (3.5%). Glycosides and steroids, however, are more effectively extracted in hydroalcoholic solvents (1.5% and 1.7%, respectively), compared to water and ethanol. Overall, water is particularly effective for saponin and tannins, while hydroalcoholic extracts provide a balanced yield across various compounds, showing better extraction for flavonoids, terpenoids and steroids.

Table No- 7 Comparison of % yield for ethanol, water, and hydroalcoholic extracts

Phytochemical	Ethanol Extract (% Yield)	Water Extract (% Yield)	Hydroalcoholic Extract (% Yield)
Alkaloids	1.4%	0.7%	1.7%
Flavonoids	2.2%	4.0%	3.0%
Saponins	2.8%	3.5%	3.7%
Tannins	3.2%	3.5%	3.4%
Terpenoids	0.9%	0.9%	1.6%
Phenolic Compounds	2.6%	2.4%	2.7%
Glycosides	—	0.9%	1.5%
Steroids	—	0.8%	1.7%
Coumarins	1.3%	0.7%	1.4%
Proteins	2.6%	1.0%	2.4%

Thymoquinone analysis –

The hydroalcoholic extract was solvent extracted with chloroform in a separating funnel. Exhaustive extraction (EE) uses increasing polarity solvents to extract as many active components as feasible with the highest biological activity. This caused the extract to be separated into two layers and was separated. The lower organic layer, which had a milky white appearance, was allowed to pass through a silica column, producing colorless fractions that were collected after a minute interval. The solvents utilized were hexane and dichloromethane (7:3).

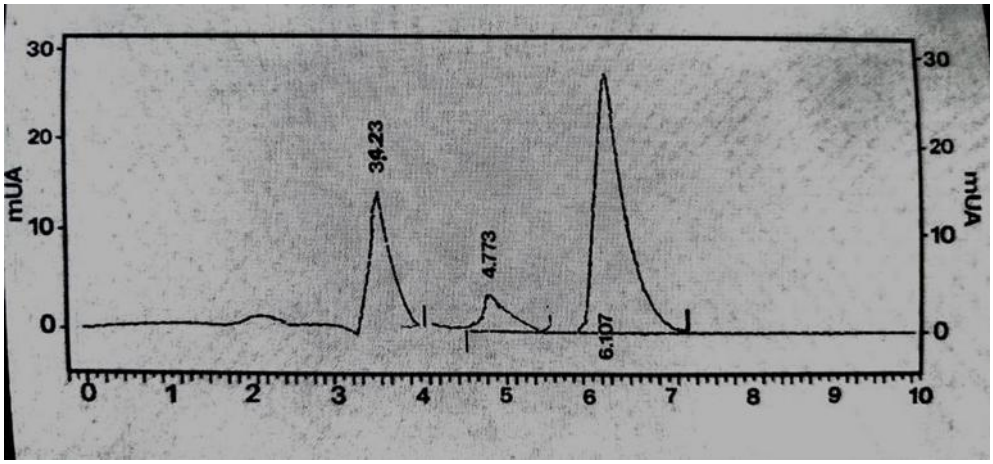
Thin layer chromatography –

The original 80% methanolic extract, the upper and lower organic layers acquired by solvent extraction, and six randomly selected colorless fractions obtained by column chromatography of the methanolic extract were all submitted to TLC. TLC was done using hexane and dichloromethane (1:1) as the mobile phase. Figures 1 show the marked spots obtained under the UV lamp. The Rf value of the fractions was discovered to be 0. 3.

Quantification of thymoquinone –

HPLC chromatograms for standard thymoquinone, hydroalcoholic Nigella Sativa seed extract, and isolated thymoquinone were obtained and are shown in Figures 2–4.

Figure No.- 2 HPLC chromatogram of standard thymoquinone solution.



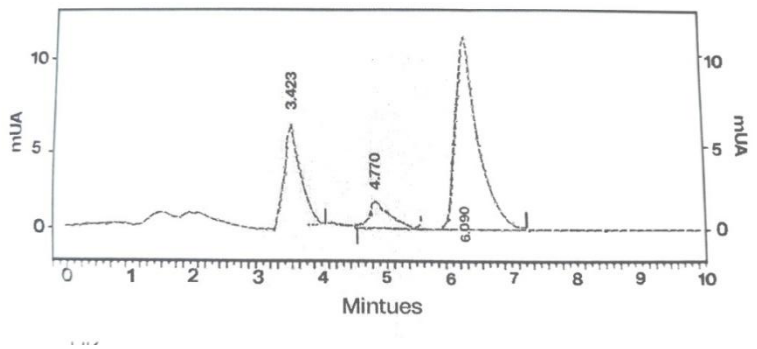
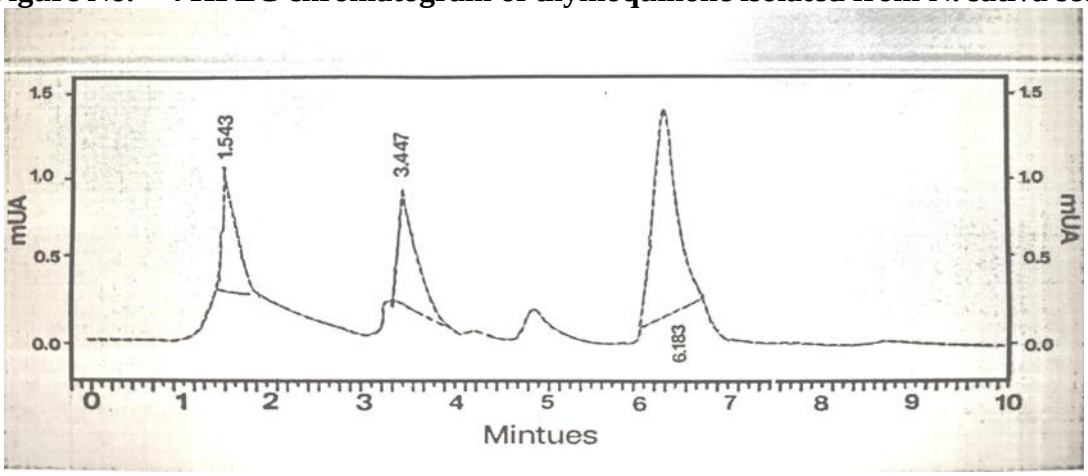


Figure No.- 3 HPLC chromatogram of hydroalcoholic extract of N. sativa seeds.

Figure No. – 4 HPLC chromatogram of thymoquinone isolated from N. sativa seeds.



Thymoquinone is widely recognized as the main bioactive component in N. sativa seeds. Previously, reported values of thymoquinone were 5 µg/mL or less. The HPLC-UV technique detected 327.3 µg/mL of thymoquinone in hydroalcohol solvent and 32.94 µg/ml in separated thymoquinone. These are amazing figures when compared to existing data. This is due to the care exercised in preparing, storing, and analyzing samples, which were maintained in reagent bottles covered with aluminum foil and refrigerated after each use.

Previously, 3.19 ± 0.43 µg/mL thymoquinone was found in methanolic extract, however the level in our study is much higher. HPLC has not been used to quantify thymoquinone from whole N. sativa seeds in hydroalcoholic extract. Thus, swallowing N. sativa whole seed is still significantly beneficial because simple hydroalcoholic extraction and subsequent thymoquinone extraction from N. sativa whole seed show a large quantity of bioactive in them, as opposed to purchasing expensive oil in contrast to seed. The determined proportion of TQ in the seed extract was 1.3%, which is more than the 1% reported in earlier research.

This quantity was significantly higher than the tested TQ. Oil, despite its biological power as an antibacterial agent, lagged behind seed extracts. The TQ fraction with higher antibacterial activity is still not very outstanding given the effort necessary to obtain it. As a consequence of this investigation, water: Ethanol was found to be the most effective solvent for phytochemical extraction.

Docking study -

The Docking study were performed on all the molecules with the PdB IDs (3VXI, 4K7A, 2XFH) and there results are arranged as per the dock score is on the top.Based on their docking scores and binding energies, which are similar to those of the reference molecules, the compounds shows potential as Anti-oxidant, Anti-Alopecia and Anti-fungal activity agents. Their strong binding affinity indicates they may effectively interact with the receptors or pathways involved in hypertension. However, further testing through in vitro and in vivo studies is needed to evaluate their effectiveness, safety, and the specific ways they work to treat healthy cell growth, enhanced hair growth and prevention of microbial growth.

The Docking study was performed on the chemical constituents of Hibiscus rosa-sinensis for three biological activity likewise Antioxidant, Anti-Alopecia and Anti-fungal activity. The result of the docking analysis, based on the obtained score (kcal/mol) and the dG binding energies (kcal/mol), along with ADME predictions, are summarized below for each activity.

Anti-oxidant activity (PdBID : 3VXI) -

The obtain result (Table No.-8) determined scores and binding energies for the anti-oxidantactivity. The compound Thymoquinone exhibited the highest docking scores (-3.994 kcal/mol) and the strongest binding energy (-44.72 kcal/mol), significantly outperforming the standard reference, Ascorbic acid, which had a docking score of -6.023 kcal/mol and a dG binding of -10.7 kcal/mol. The interactions of Thymoquinone with key amino acids (HIE326, ASN313) and the formation of several hydrogen bonds (with distances between 1.89,2.28) suggests a strong binding affinity (Table No.-13). This denote that Thymoquinone may act as an effective anti-oxidant agent by interacting with critical receptors or enzymes involved in oxidative stress pathways(Table No.-11).

Table No – 8 Docking score and dG binding of *Nigella Sativa* seeds constituents for Anti-oxidant Activity (PdB id:3VXI) -

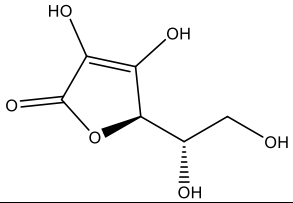
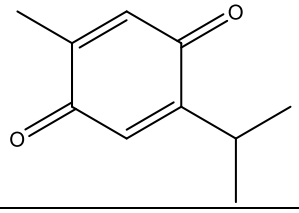
COMPOUND NAME	2D STRUCTURE	DOCK SCORE (kcal/mol)	dG BIND (kcal/mol)
Standard Reference Ascorbic Acid		-5.607	-17.77
Thymoquinone		-3.994	-44.72

Table No. – 9 Docking score and dG binding of *Nigella Sativa* seeds constituents for Anti-Alopecia Activity (PdB id:4K7A) –

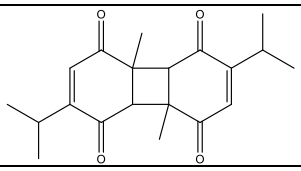
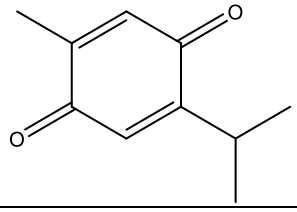
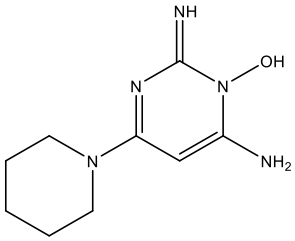
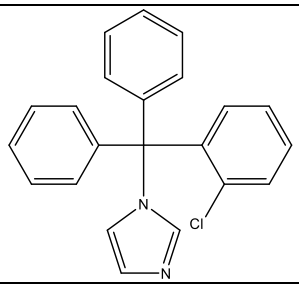
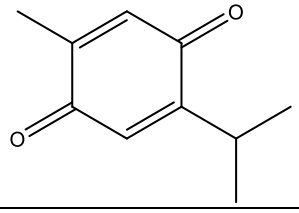
COMPOUND NAME	2D STRUCTURE	DOCK SCORE (kcal/mol)	dG BIND (kcal/mol)
Nigellone		NO DOCK SCORE	NO DG BIND
Thymoquinone		-1.800	-24.69
Standard Reference Minoxidil		-4.925	-25.91

Table No.-10 Docking score and dG binding of *Nigella Sativa* seeds constituents for Anti-Fungal Activity (PdB id:2XFH) -

COMPOUND NAME	2D STRUCTURE	DOCK SCORE (kcal/mol)	dG BIND (kcal/mol)
Standard Reference Clotrimazole		-6.064	-73.26
Thymoquinone		-4.380	-47.20

ADME prediction (Table No.-16) for Thymoquinone indicates a molecular weight (164.20g/mol) and relatively low bioavailability (Score 0.55), with highgastrointestinal absorption. The compound also violates Lipinski's rule (Score 0), indicating challenges in drug like properties, despite its strong docking performance. The toxicity prediction (Table No – 19) for antioxidant activity was assessed using the ProTox-II platform for two compounds: Thymoquinone and Ascorbic Acid. Both compounds demonstrate low toxicity risk in most categories, with Thymoquinone showing a slightly higher risk for liver toxicity compared to Ascorbic Acid.

Anti-Alopecia Activity (PdBID : 4K7A) –

In the anti-alopecia docking study (Table No.-9), Thymoquinone showed the best docking score (-1.800 kcal/mol) and dG binding energy (-24.69 kcal/mol), indicating a strong interaction with the target protein. The reference drug, minoxidil, had a lower docking score (-4.925 kcal/mol) and dG binding energy (-25.91 kcal/mol), suggesting that Thymoquinone may be more promising candidate for treating alopecia. The binding interactions involve hydrogen bonds with residues such as GLN858 (Table No.-14) with distance 1.82 Å, indicating effective ligand-receptor binding(Table No.-12).

Table No -11 2D and 3D images of Docked complex of *Nigella Sativa* seeds Chemical Constituents for Anti-oxidant activity

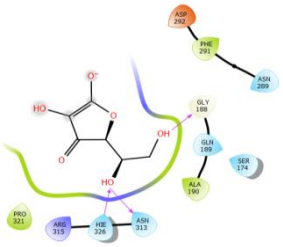
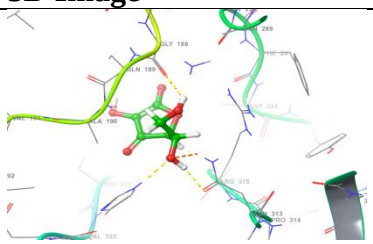
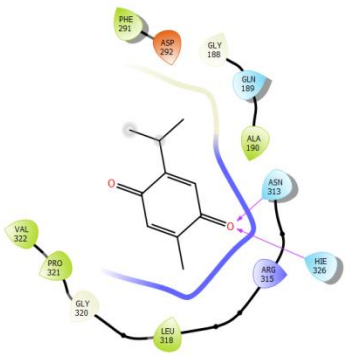
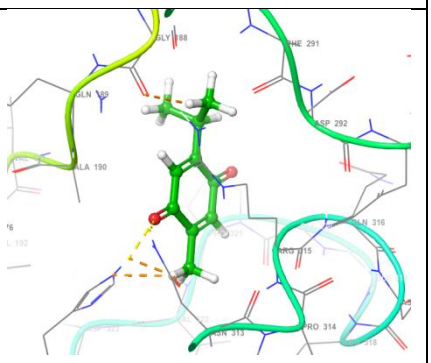
Compound name	2D Image	3D Image
Standard Reference Ascorbic Acid		
Thymoquinone		

Table No – 12 2D and 3D images of Docked complex of *Nigella Sativa* seeds Chemical Constituents for Anti-Alopacia activity

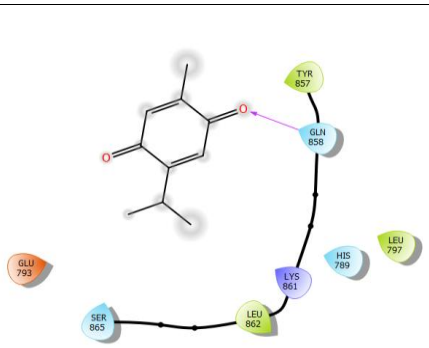
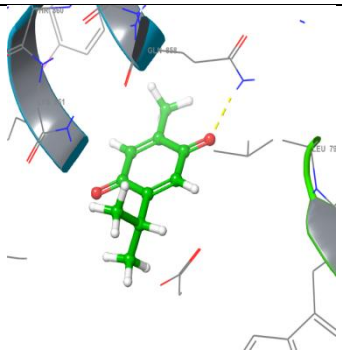
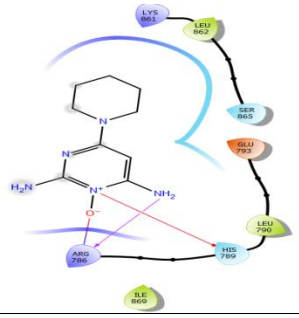
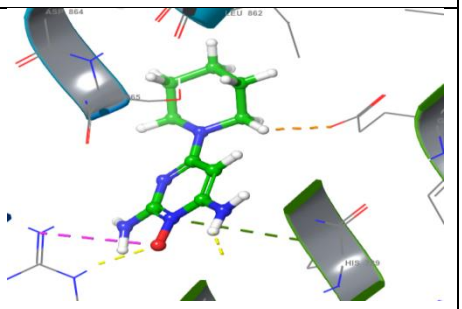
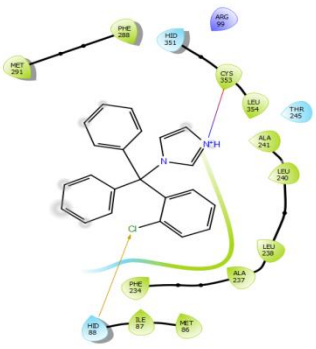
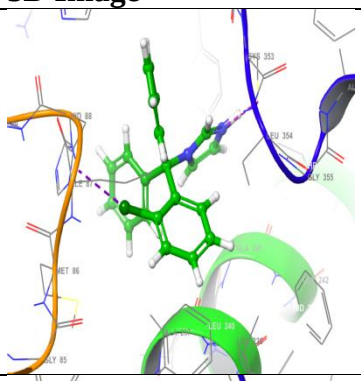
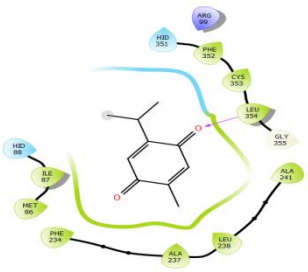
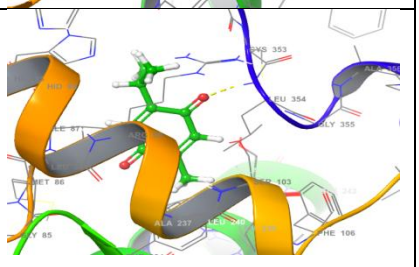
Compound name	2D Image	3D Image
Nigellone	NO IMAGE	NO IMAGE
Thymoquinone		
Standard Reference Minoxidil		

Table No – 13 2D and 3D images of Docked complex of *Nigella Sativa* seeds Chemical Constituents for Anti-Fungal activity

Compound name	2D Image	3D Image
Standard Reference Clotrimazole		
Thymoquinone		

For ADME prediction (Table No. 17), Thymoquinone has a molecular weight of 164.20 g/mol, with low bioavailability (0.55) and low GI absorption, indicating a possible challenge for systemic absorption. The compound don'tviolates Lipinski's rule (Score 0), suggesting the need for modification to improve its drug like properties. The toxicity prediction (Table No-20) for compounds related to anti-alopecia activity was evaluated using the ProTox-II platform.

Thymoquinone showed less inactive for carcinogenicity and immunotoxicity, and inactive for mutagenicity and cytotoxicity, suggesting minimal or no risk in these categories. Minoxidil(the standard reference) was classified as less inactive for carcinogenicity, less active for immunotoxicity, and less active for mutagenicity, with less inactive for cytotoxicity, suggesting a low but slightly higher risk for immunotoxicity and mutagenicity compared to the other two compounds.

Anti-fungal Activity (PdBID : 2XFH) –

The docking results for anti-fungal activity, summarized (Table No.-10), show that Thymoquinone had the highest docking score (-4.380 kcal/mol) and the lowest dG binding energy (-47.20 kcal/mol), significantly outperforming the other compounds in this study.The reference drug clotrimazole had a docking score of -6.064kcal/mol and a dG binding of -73.26 kcal/mol, positioning it as less effective compared to the studied compounds.Thymoquinone interacted with amino acid residues like LEU354 with distances (Table No.-15) of 1.80 Å, indicating significant interactions that could lead to inhibition of fungal growth.

The ADME prediction (Table No. – 18) results for Thymoquinone and Clotrimazole show their molecular characteristics and potential pharmacokinetic properties. Thymoquinone, with a molecular weight (MW) of 164.20 g/mol, exhibits two hydrogen bond acceptors, no hydrogen bond donors, a Log P value of 1.99, and high gastrointestinal (GI) absorption. It also crosses the blood-brain barrier (BBB) and has a bioavailability score of 0.55 with no violations of Lipinski's rule, indicating favorable oral bioavailability. Its synthetic accessibility is 2.83, suggesting moderate ease of synthesis. On the other hand, Clotrimazole, a standard reference compound, has a higher MW of 344.84 g/mol, with one hydrogen bond acceptor and no donors, a Log P value of 3.07, high GI absorption, and a similar bioavailability score of 0.55. It violates one of Lipinski's rules, implying slightly reduced oral bioavailability compared to Thymoquinone.

Table No. – 14 2D Interactions and Distance from Amino acids used in Docked complex of *Nigella Sativa* seeds Chemical Constituents for Anti-Oxidant activity

Compound name	**2D Interaction**	**Distance(A^0)**
Standard Reference Ascorbic Acid	H-Bond:HIE326,GLY188,ASN313	1.87,2.03,2.12
Thymoquinone	H-Bond:HIE326, ASN313	1.89,2.28

Table No. – 15 2D Interactions and Distance from Amino acids used in Docked complex of *Nigella Sativa* seeds Chemical Constituents for Anti-Alopecia activity

Compound name	**2D Interaction**	**Distance(A^0)**
Nigellone	NO INTERACTION	NO DISTANCE
Thymoquinone	H-Bond:GLN858	1.82
Standard Reference Minoxidil	H-Bond:ARG786 Pi-Cation:HIS789 Salt-Bridge:ARG786	1.90 4.98 3.78

Table No. – 16 2D Interactions and Distance from Amino acids used in Docked complex of *Nigella Sativa* seeds Chemical Constituents for Anti-Fungal activity

Compound name	2D Interaction	Distance(A^0)
Standard Reference Clotrimazole	Salt-Bridge:CYS353 Halogen Bond:HID88	3.11 3.09
Thymoquinone	H-Bond:LEU354	1.80

The toxicity prediction (Table No- 21) forThymoquinone and Clotrimazolein the context of anti-fungal activity reveals their safety profiles across various toxicological endpoints. Thymoquinone is classified under ProTox-II class 5, indicating a relatively low risk, with Less Inactive results for hepatotoxicity and immunotoxicity, and Inactive for carcinogenicity, mutagenicity, and cytotoxicity. Clotrimazoleis classified under ProTox-II class 4, slightly indicating a higher risk compared to Thymoquinone, but still showing Less Inactive for hepatotoxicity and immunotoxicity, and Inactive for mutagenicity and cytotoxicity. These predictions suggest that both compounds exhibit a favorable safety profile for anti-fungal activity, with minimal concerns regarding toxicity.

Table No-17 : ADME prediction of all reported molecules for Anti-Oxidant Activity
ADME prediction (http://www.swissadme.ch/index.php)

Sr. no.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1.	Thymoquinone	164.20 g/mol	2	0	1.99	High	Yes	0.55	0	2.83
2.	Standard Reference Ascorbic Acid	176.12 g/mol	6	4	-0.31	High	No	0.56	0	3.47

Table No-18 :ADME prediction of all reported molecules for Anti-Alopecia Activity
ADME prediction (http://www.swissadme.ch/index.php)

Sr.no.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1.	Thymoquinone	164.20 g/mol	2	0	1.99	High	Yes	0.55	0	2.83
2.	Nigellone	328.40 g/mol	4	0	2.46	High	Yes	0.55	0	4.65
3.	Standard Reference Minoxidil	209.25 g/mol	3	3	1.23	High	No	0.55	0	2.51

Table No-19 : ADME prediction of all reported molecules for Anti-Fungal Activity

ADME prediction (http://www.swissadme.ch/index.php)

Sr. no.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility
1.	Thymoquinone	164.20 g/mol	2	0	1.99	High	Yes	0.55	0	2.83
2.	Standard Reference Clotrimazole	344.84 g/mol	1	0	3.07	High	Yes	0.55	1	2.25

Table No – 20 Toxicity prediction for Anti-oxidant activity

(https://tox.charite.de/protox3/index.php?site=compound_input)

Comp.Name	ProTox-II Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Thymoquinone	5	Less Inactive	Less Inactive	Inactive	Inactive	Inactive
Standard Reference Ascorbic Acid	5	Inactive	Inactive	Inactive	Inactive	Less Inactive

Table N0 – 21 Toxicity prediction for Anti-Alopecia Activity

(https://tox.charite.de/protox3/index.php?site=compound_input)

Comp.Name	ProTox-II Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Thymoquinone	5	Less Inactive	Less Inactive	Inactive	Inactive	Inactive
Nigellone	5	Less Inactive	Less Inactive	Inactive	Less Inactive	Inactive
Standard Reference Minoxidil	4	Less Inactive	Less Active	Inactive	Less Active	Less Inactive

Table No – 22 Toxicity predictionfor Anti-Fungal Activity

(https://tox.charite.de/protox3/index.php?site=compound_input)

Comp.Name	ProTox-II Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Thymoquinone	5	Less Inactive	Less Inactive	Inactive	Inactive	Inactive
Standard Reference Clotrimazole	4	Less Inactive	Less Inactive	Inactive	Less Inactive	Inactive

4. DISCUSSION –

The phytochemical screening indicate that water is the most effective solvent for extracting saponins and tannins, but hydroalcoholic extracts produce a more balanced yield of phytochemicals, particularly flavonoids, terpenoids, and steroids. When compared to the other two solvents, ethanol is only moderately successful in terms of extracting any given chemical. This study emphasizes the relevance of solvent selection in maximizing the yield of bioactive chemicals from NS seeds, which may have consequences for their medical and pharmacological applications. Compared to earlier

findings of 3.19 ± 0.43 µg/mL TQ in methanolic extracts, our results indicate a substantially higher concentration. Notably, this is the first time hydroalcoholic extract on HPLC has been used to quantify TQ from whole *N. sativa* seeds. This suggests that consuming whole seeds remains highly beneficial, as simple hydroalcoholic extraction yields a substantial amount of bioactive TQ, providing a cost-effective alternative to expensive *N. sativa* oil. This study underscores the advantages of whole seed consumption and hydroalcoholic extraction as viable methods for maximizing TQ yield, offering a more accessible alternative to costly oil extracts.**Comparison of Docking scores and binding energies –**

Anti-oxidant activity – Thymoquinone showed the strongest binding affinity, significantly surpassing Ascorbic acid.

Anti-alopecia activity – Thymoquinone outperformed Minoxidil, indicating its potential as a better agent for alopecia treatment.

Anti-fungal activity – Thymoquione again showed the highest docking score, surpassing Clotrimazole in binding affinity.

5. CONCLUSION –

The comparative analysis of the phytochemical composition of *Nigella Sativa* seed extracts in ethanol, water, and hydro-alcoholic solvents reveals that the extraction efficiency varies depending on the solvent used. Ethanol and hydro-alcoholic extracts yield higher quantities of alkaloids, flavonoids, and carbohydrates, while water extracts are more efficient in extracting phenolic compounds. The diversity and concentration of these bioactive compounds across different extracts suggest that *Nigella Sativa* seeds have considerable pharmacological potential, which could be optimized depending on the solvent used for extraction. Further studies could focus on the synergistic effects of these compounds, which may enhance the therapeutic efficacy of seeds extracts.Based on the docking scores and binding energies, Thymoquinoneemerged as the most potent compound for anti-oxidant and anti-fungal activities and showed promise for anti-alopecia activity. However, the compounds showed some limitations in terms of ADME properties, which could hinder their oral bioavailability. Further studies, including in vitro and in vivo testing, are required to validate the pharmacological potential of these compounds and to address their bioavailability challenges.

6. REFERENCE –

1. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA, Anwar F. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pac J Trop Biomed. 2013 May;3(5):337-52. doi: 10.1016/S2221-1691(13)60075-1. PMID: 23646296; PMCID: PMC3642442.
2. Hannan MA, Rahman MA, Sohag AAM, Uddin MJ, Dash R, Sikder MH, Rahman MS, Timalsina B, Munni YA, Sarker PP, Alam M, Mohibbullah M, Haque MN, Jahan I, Hossain MT, Afrin T, Rahman MM, Tahjib-Ul-Arif M, Mitra S, Oktaviani DF, Khan MK, Choi HJ, Moon IS, Kim B. Black Cumin (*Nigella sativa* L.): A Comprehensive Review on Phytochemistry, Health Benefits, Molecular Pharmacology, and Safety. Nutrients. 2021 May 24;13(6):1784. doi: 10.3390/nu13061784. PMID: 34073784; PMCID: PMC8225153.
3. Alberts, A., Moldoveanu, E.-T., Niculescu, A.-G., & Grumezescu, A. M. (2024). *Nigella sativa*: A Comprehensive Review of Its Therapeutic Potential, Pharmacological Properties, and Clinical Applications. *International Journal of Molecular Sciences, 25*(24), 13410. https://doi.org/10.3390/ijms252413410
4. Fidan, Hafize& Stankov, Stanko &Daraba, Aura &doğan, Hülya &Alexieva, Iordanka & Stoyanova, Albena &Ercisli, Sezai. (2019). Phytochemical composition of black cumin (Nigella sativa L.) seeds from Turkey as an unconventional source for the food industry. 1-9.
5. Adam, S. H., Abu, I. F., Kamal, D. A. M., Febriza, A., Kashim, M. I. A. M., & Mokhtar, M. H. (2023). A Review of the Potential Health Benefits of *Nigella sativa* on Obesity and Its Associated Complications. *Plants, 12*(18), 3210. https://doi.org/10.3390/plants12183210

6. George A. Burdock, Assessment of black cumin (Nigella sativa L.) as a food ingredient and putative therapeutic agent, Regulatory Toxicology and Pharmacology, Volume 128, 2022, 105088, ISSN 0273-2300
7. Ansary J, Giampieri F, Forbes-Hernandez TY, Regolo L, Quinzi D, Gracia Villar S, Garcia Villena E, Tutusaus Pifarre K, Alvarez-Suarez JM, Battino M, Cianciosi D. Nutritional Value and Preventive Role of *Nigella sativa* L. and Its Main Component Thymoquinone in Cancer: An Evidenced-Based Review of Preclinical and Clinical Studies. Molecules. 2021 Apr 7;26(8):2108. doi: 10.3390/molecules26082108. PMID: 33916916; PMCID: PMC8067617.
8. Ferizi R, Ramadan MF, Maxhuni Q. Black Seeds (*Nigella sativa*) Medical Application and Pharmaceutical Perspectives. J Pharm Bioallied Sci. 2023 Apr-Jun;15(2):63-67. doi: 10.4103/jpbs.jpbs_364_22. Epub 2023 Jun 8. PMID: 37469646; PMCID: PMC10353664.
9. Azar Hosseini, Hossein Hosseinzadeh, Chapter 23 - Effect of Nigella sativa on Blood Diseases: A Review, Editor(s): Victor R. Preedy, Ronald Ross Watson, Nuts and Seeds in Health and Disease Prevention (Second Edition), Academic Press, 2020, Pages 315-328.
10. Derosa, G., D'Angelo, A., Maffioli, P., Cucinella, L., & Nappi, R. E. (2024). The Use of *Nigella sativa* in Cardiometabolic Diseases. *Biomedicines*, *12*(2), 405. https://doi.org/10.3390/biomedicines12020405
11. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. J Exp Pharmacol. 2023 Feb 8;15:51-62. doi: 10.2147/JEP.S379805. PMID: 36789235; PMCID: PMC9922502.
12. Simhadri Vsdna N, Muniappan M, Kannan I, Viswanathan S. Phytochemical analysis and docking study of compounds present in a polyherbal preparation used in the treatment of dermatophytosis. Curr Med Mycol. 2017 Dec;3(4):6-14. doi: 10.29252/cmm.3.4.6. Erratum in: Curr Med Mycol. 2018 Mar;4(1):34. doi: 10.18502/cmm.4.1.33. PMID: 29707673; PMCID: PMC5917095.
13. S.K. Gabr, R.O. Bakr, E.S. Mostafa, A.M. El-Fishawy, T.S. El-Alfy, Antioxidant activity and molecular docking study of Erythrina neillii polyphenolics, South African Journal of Botany, Volume 121, 2019, Pages 470-477, ISSN 0254-6299.
14. Bhat, S., Rather, M., Gani, S. et al. Identification of plant based potential antifungal compounds against BMK-1 protein of Bipolaris oryzae using molecular docking approach. Sci Rep **14**, 15665 (2024).
15. Sharma S, Sharma A, Gupta U (2021) Molecular Docking studies on the Anti-fungal activity of Allium sativum (Garlic) against Mucormycosis (black fungus) by BIOVIA discovery studio visualizer 21.1.0.0. Ann Antivir Antiretrovir 5(1): 028-032. DOI: 10.17352/aaa.000013.
16. Chikowe I, Bwaila KD, Ugbaja SC, Abouzied AS. GC-MS analysis, molecular docking, and pharmacokinetic studies of Multidentiacrassa extracts' compounds for analgesic and anti-inflammatory activities in dentistry. Sci Rep. 2024 Jan 22;14(1):1876. doi: 10.1038/s41598-023-47737-x. PMID: 38253619; PMCID: PMC10803350.
17. Selvaraj N., Ekambaram G., Malathi S., Rengarajan M., Jayaprakash J., Palanisamy P., Kuppusamy S., Molecular docking based screening dynamics for plant based identified potential compounds of PDE12 inhibitors, Current Research in Green and Sustainable Chemistry, Volume 4, 2021, 100122, ISSN 2666-0865.
18. Ralte, L., Khiangte, L., Thangjam, N.M. et al. GC–MS and molecular docking analyses of phytochemicals from the underutilized plant, Parkia timoriana revealed candidate anti-cancerous and anti-inflammatory agents. Sci Rep **12**, 3395 (2022). https://doi.org/10.1038/s41598-022-07320-2.
19. Vidhya Rekha U, Varadhachariyar R, Rajagopal P, Priya PH, Shazia Fathima JH, Govindan R, Palanisamy CP, Veeraraghavan VP, Jayaraman S. Molecular docking analysis of bioactive compounds from Plectranthusamboinicus with glucokinase. Bioinformation. 2022 Mar 31;18(3):261-264.

20. Srivastava V., Yadav A., Sarkar P., Molecular docking and ADMET study of bioactive compounds of Glycyrrhiza glabra against main protease of SARS-CoV2, Materials Today: Proceedings, Volume 49, Part 8, 2022, Pages 2999-3007, ISSN 2214-7853.
21. Ningsih, A. W., Syahrani, A., Septama, A. W., & Sukardiman. (2024). Predicting toxicity and conducting molecular docking analysis of compounds from unripe kayu banana fruit (Musa paradisiaca L. var. Kayu) against 3mzd and 2q85 proteins in Escherichia coli for antibacterial activity . Pharmacy Education, 24(3), p. 329–335.
22. Sultana, S., Hossain, M. A., Islam, M. M., & Kawsar, S. M. A. (2024). Antifungal potential of mannopyranoside derivatives through computational and molecular docking studies against Candida albicans 1IYL and 1AI9 proteins. Current Chemistry Letters, 13(1), 1–14.
23. A. Rakić, D. Dimić, J. D. Marković, D. Milenković and Z. Marković, "Toxicity, structural analysis, and molecular docking studies of selected isonicotinohydrazide analogs," 2021 IEEE 21st International Conference on Bioinformatics and Bioengineering (BIBE), Kragujevac, Serbia, 2021, pp. 1-6.