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EXPLORING THE ANTIOXIDANT POTENTIAL OF FLAVANONES IN *STROBILANTHES HAMILTONIANA* (STEUD.) BOSSER & HEINE: A COMBINED QUANTUM MECHANICAL, MOLECULAR DOCKING, AND BIOCHEMICAL ASSAY APPROACH

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Keywords:

Strobilanthes hamiltoniana, Antioxidant activity, Anti-inflammatory activity, Molecular docking, Density functional theory

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ABSTRACT: *Strobilanthes hamiltoniana* (Paalkurinji), a perennial herb of the Acanthaceae family, has traditional use in treating ulcers, diabetes, arthritis, and wounds. *S. hamiltoniana* leaves were extracted using ethanol, chloroform, and water, and screened for phytochemicals using standard protocols. LC-MS analysis was used to identify flavonoids. Antioxidant activity was evaluated via DPPH, HRS, and FRAP assays, while anti-inflammatory activity was assessed using the NO scavenging assay. Additionally, DFT (B3LYP/6-311G(d,p)) calculations and molecular docking were performed to investigate interactions with antioxidant-related proteins: monoamine oxidase-B, catalase, cytochrome P450, and NFE2-related factor 2. Based on the DPPH assay, aqueous and chloroform extracts showed 25.74% and 45.94% inhibition, while ethanol and methanol extracts exhibited higher inhibition at 89.10% and 86%. HRS ranged from 25.5% to 86.4%, with ethanol extract showing the highest activity (86.4%) at 300 µg/ml and an IC_{50} of 25.54 µg/ml. All extracts were effective in the FRAP assay, with ethanol showing the highest activity (79.41%, IC_{50} = 28.24 µg/ml). In the NO scavenging assay, ethanol again showed the strongest activity (80.41%, IC_{50} = 26.26 µg/ml). Molecular docking revealed catechin had the strongest affinity for Monoamine Oxidase (−9.869), and also bound well to Cytochrome P450 (−6.399) and Catalase (−6.691). Epigallocatechin also showed strong binding to Monoamine Oxidase (−9.567) and Cytochrome P450 (−8.650). The plant extracts, especially the ethanolic one, showed strong antioxidant and anti-inflammatory activities with favorable IC_{50} values. These dual properties highlight their potential as therapeutic agents against arthritis, where oxidative stress and inflammation are key factors.

INTRODUCTION: *Strobilanthes hamiltoniana* is a perennial shrub belonging to the *Acanthaceae* family, commonly referred to by names such as India blue bell, Chinese rain bell, Assam indigo, and pink strobilanthes.

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Native to the subtropical regions of the Himalayas and parts of Southeast Asia, this plant has become widely naturalized across various global regions.

Its distinct characteristics dark green leaves with rib-like veins and delicate purplish bell-shaped flowers make it easily recognizable. Historically, *S. hamiltoniana* has played a significant role in traditional medicine, attributed to its diverse array of bioactive compounds, including flavonoids, terpenoids, and phenolic compounds [1]. The traditional medicinal applications of *S. hamiltoniana* reveal its strong anti-arthritic

properties, which aid in reducing inflammation and alleviating symptoms associated with rheumatoid arthritis. Furthermore, its notable antioxidant activity helps combat free radicals and offers protection against oxidative stress, a factor linked to chronic diseases such as cancer, diabetes, and neurodegenerative disorders [2]. The leaves of this plant contain various phytochemicals with significant biological activities, including antimicrobial, antihelminthic, and anti-inflammatory properties, positioning it as an effective remedy against conditions like helminthiasis and spider bite poisoning [2, 3].

Phytochemical composition of *S. hamiltoniana*, a plant native to the Indian subcontinent, has been traditionally used for various medicinal purposes. The therapeutic potential of this plant is largely attributed to its phytochemical constituents. The plant is known as a garden plant, and medicinal properties of this plant are not yet reported. Most of the works were done in other species of *Strobilanthes*. Baby *et al.,* provided the first detailed insight into the bioactivity of *S. hamiltoniana* leaves. Ethanol extracts of the plant showed significant antimicrobial, anthelmintic, and antioxidant properties of *S. hamiltoniana* leaves in a dose-dependent manner, suggesting its potential for developing antimicrobial agents. Additionally, the plant's strong antioxidant activity indicates its capacity to neutralize oxidative stress, making it a valuable candidate for treating inflammatory and chronic diseases [4].

In-vitro studies by Shameer *et al.,* demonstrated the successful use of various explants, including nodes, internodes, petioles, shoot buds, and leaf laminae, for tissue culture of *S. hamiltoniana*. These studies pave the way for large-scale propagation and further biotechnological research, although specific details on the phytochemical influences on growth were not elaborated. Gangwar *et al.,* developed an environmentally friendly and cost-effective method for synthesizing zinc oxide nanoparticles (ZnONPs) using leaf extracts of *S. hamiltoniana*. The plant's phytochemicals, likely including flavonoids and tannins, acted as natural reducing and capping agents. This finding highlights the plant's potential in green chemistry and nanotechnology [5]. A mixoploid karyotype study revealed novel chromosome numbers in *S. hamiltoniana*, with a symmetric karyotype, considered primitive. The discovery of a hypoploid variant alongside the normal chromosome complement contributes to a better understanding of the plant's genetic diversity and evolutionary significance [6]. These findings establish *S. hamiltoniana* as a plant of medicinal interest, warranting further research to explore its phytochemistry, Antioxidant property and therapeutic applications, particularly through advanced molecular docking and quantum mechanical studies.

Advanced analytical techniques, such as Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS), have been employed to identify the phytochemicals present in *S. hamiltoniana*. LC-MS analysis has revealed key compounds such as β,3,4-trihydroxybenzenepropanoic acid, hexadecanoic acid, catechin, and epigallocatechin, all known for their diverse biological activities [7]. Meanwhile, GC-MS analysis has highlighted the presence of volatile compounds like hexadecanoic acid and phytol. These phytochemicals are effective in neutralizing free radicals, reducing oxidative stress, and enhancing the activity of endogenous antioxidant enzymes like superoxide dismutase and catalase [8]. By targeting and inhibiting oxidative stress, these bioactive compounds could alleviate symptoms and slow disease progression [9].

In addition to experimental methods, computational techniques such as density functional theory (DFT) calculations and molecular docking studies have been utilized to further explore the potential of these phytochemicals for drug development [10]. DFT, a quantum mechanical modeling approach, aids in understanding the electronic structure of molecules, helping to determine important molecular properties like energy levels, charge distribution, and molecular stability. This is essential for evaluating the reactivity and potential therapeutic applications of the compounds derived from *S. hamiltoniana*.

Molecular docking serves as a valuable computational technique to predict the interactions between the bioactive compounds and specific biological targets, such as enzymes or receptors involved in disease pathways. By simulating the

binding of these phytochemicals to protein targets, molecular docking offers insights into their potential therapeutic effects [11, 12]. This approach, combined with DFT calculations, facilitates the identification and optimization of promising drug candidates, enhancing their efficacy and reducing toxicity. To comprehensively evaluate the antioxidant properties of *S. hamiltoniana*, it is crucial to examine the effects of different extraction solvents on phytochemical yield. The choice of solvent can significantly impact the efficiency of extracting various bioactive compounds, including phenolics and flavonoids [13]. This study aims to systematically explore the antioxidant potential of *S. hamiltoniana* through targeted extraction and analysis, providing valuable insights into its bioactive compounds and paving the way for the development of natural antioxidant products for health and industrial applications.

MATERIALS AND METHODS: For the study, *S. hamiltoniana* leaves were collected from kurumpanmoozhy, Pathanamthitta district. The identification of the collected plant material was accomplished with the use of dichotomous keys; published plant descriptions, illustrations and photographs and comparison with properly identified herbarium specimens. The plant material is recorded with Master ID: 1187 and Species ID: 2480. The voucher specimen PKB1124, collected from her private property and identified by Bindu Palaghat Krishnamoorthy, Assistant Professor, Department of Botany, Sanatana Dharma College, Alappuzha, has been deposited in the herbarium of Sanatana Dharma College, Alapuzha, Kerala, India.

Sample Collection: The plant material was collected from the private property of Bindu Palaghat Krishnamoorthy, Assistant Professor, Department of Botany, Sanatana Dharma College, Alappuzha. The leaves of *S. hamiltoniana* were washed thoroughly with distilled water. Leaves showing signs of browning, curling or any other signs of damage were discarded. The selected samples were then shade dried for 5 days, and then powered using a mechanical grinder and sieved through a nylon sieve (100 mm). The larger particles were again ground well and sieved to obtain the products with uniform particle size. The powder obtained was stored in a sterilized airtight container for further use.

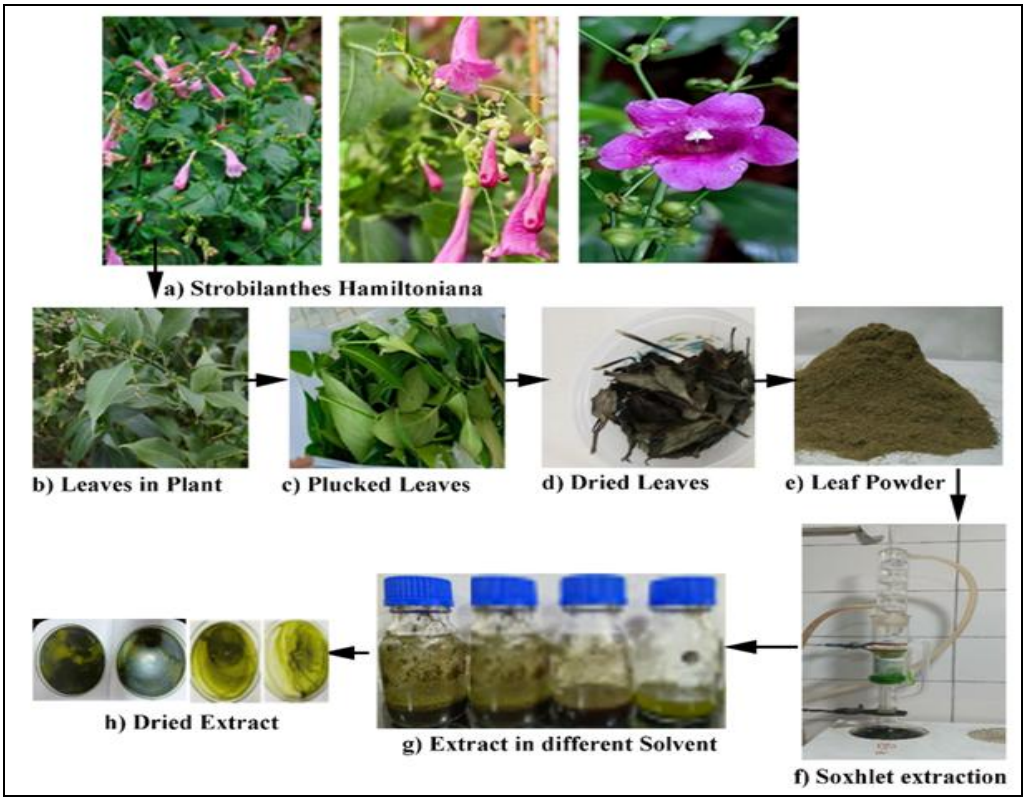


FIG. 1: PICTURES OF A) *S. HAMILTONIANA*, B) LEAVES IN PLANT, C) PLUCKED LEAVES, D) DRIED LEAVES, E) LEAF POWDER, F) EXTRACTION USING SOXHLET APARATUS, G) EXTRACTS IN DIFFERENT SOLVENTS LIKE WATER, CHLOROFORM, ETHANOL AND METHANOL AND H) DRIED EXTRACT

Extraction Method: To extract phytochemicals from *S. hamiltoniana*, 50 grams of powdered plant material were subjected to Soxhlet extraction using four solvents: water, methanol, ethanol, and chloroform as depicted in **Fig. 1**.

In each case, the plant powder was placed in a porous thimble within the Soxhlet apparatus, and the solvent was heated to generate vapors that condensed and repeatedly extracted compounds from the plant. Aqueous extraction targeted hydrophilic compounds (e.g., tannins, glycosides), while methanol isolated polar compounds (e.g., alkaloids, flavonoids).

Ethanol, with intermediate polarity, extracted a broad range of polar and non-polar compounds (e.g., phenolics, terpenoids), and chloroform focused on non-polar compounds (e.g., fats, oils). Each solvent effectively isolated specific phytochemicals, contributing to a comprehensive analysis of the plant's medicinal properties [14, 15].

Chemical Profiling:

Phytochemical Analysis: Each extract was subjected to standard phytochemical screening protocols to detect a wide range of constituents like alkaloids, flavonoids, terpenoids, saponins, tannins, glycosides, and more. This multi-solvent approach ensured the comprehensive extraction of different classes of phytochemicals based on their polarity and solubility in various solvents [16, 17].

Alkaloids (Mayer's Test): Yellow precipitate indicates the presence of alkaloids.

Saponins (Froth Test): Persistent foam formation (1 cm layer) signifies the presence of saponins.

Phenols (Ferric Chloride Test): bluish-black color shows the presence of phenols.

Flavonoids (Alkaline Reagent Test): Intense yellow color that becomes colorless with acid indicates flavonoids.

Glycosides: Bluish-black color in the acetic acid layer confirms glycosides.

Terpenoids (Salkowski's Test): Reddish-brown coloration at the interface reveals terpenoids.

Tannins: Brownish-green or blue-black color confirms the presence of tannins [18].

Liquid Chromatography-mass Spectrometry (LCMS): The LC-MS/MS experiments were conducted using an Agilent 6520 accurate mass MS Q-TOF coupled with an Agilent LC 1200. The MS analysis was performed with a dual AJS ESI ion source in both positive and negative modes. Mass spectral data analysis was carried out using Agilent's molecular ion extraction algorithm. The general conditions for mass spectrometry were as follows: drying gas (nitrogen) flow at 13 L/min; nebulizer pressure at 35 psig; drying gas temperature at 250°C; capillary voltage at 3500V; fragmentor volt at 750V; and Oct RF Vpp. For ESI ionization mode, a gradient of 95% water and 5% acetonitrile was used as the mobile phase at a constant flow rate of 0.3 ml/min additionally, a mobile phase consisting of a gradient of acidified methanol (A) and water (B) system was employed for ESI ionization mode. Gradient elution was carried out at a constant flow rate of 0.9 ml/min and 1200.00 bar pressure [7].

***In-vitro* Anti-oxidant Assays:**

DPPH Radical Scavenging Assay: The DPPH scavenging activity was evaluated with little modification. Prepare DPPH (0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 16 100ml of ethanol). Different volumes (2 - 20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % of radical scavenging activity of the plant extracts was calculated using the following formula, DPPH scavenging effect

$$\% \text{ of RSA} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Where, RSA is the radical scavenging activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + plant extract. A standard graph is plotted with the percentage of free radical scavenging at Y-axis and concentration of ascorbic acid standard at X- axis. Ascorbic acid was used as a standard compound and IC_{50} values were calculated.

Hydroxyl Radical Scavenging Assay: The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with

Thiobarbituric acid (TBA). Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction). The reaction mixture contained, in a final volume of 1 ml, 2-deoxy-2-ribose (2.8mM); KH_2PO_4-KOH buffer (20mM, pH 7.4); $FeCl_3$ (100µM); EDTA (100µM); H_2O_2 (1.0mM); ascorbic acid (100µM) and various concentrations (0–200µg/ml) of the test sample or reference compound. After incubation for 1 h at 37°C, 0.5 ml of the reaction mixture was added to 1 ml 2.8% Trichloroacetic acid (TCA), then 1 ml 1% aqueous TBA was added and the mixture was incubated at 90°C for 15 min to develop the colour. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. All tests were performed six times. Mannitol, a classical OH scavenger, was used as a positive control. Percentage inhibition was evaluated by comparing the test and blank solutions [19].

Ferric-Reducing Power Assay: Different concentrations of the extract were mixed with 1.25 mL of 0.2 mol/L, pH 6.6 sodium phosphate buffers and 1.25 mL of potassium ferricyanide (1%). The mixture was incubated at 50°C for 20 min. After incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%) and centrifuged at 3, 000 rpm/min for 10 min. Finally, 0.5 mL of freshly prepared $FeCl_3$ (0.1%) was added to this solution, and the absorbance was measured at 700 nm. Ascorbic acid at various concentrations was used as standard [20].

***In-vitro* Anti-inflammatory Assays:**

Nitric Oxide Scavenging Activity: Nitric oxide (NO) can react with other free radicals to produce highly toxic peroxynitrite radicals. The capacity of a sample to scavenge NO can be measured. The test relies on the decomposing of sodium nitroprusside to NO (at physiological pH) which can react with O_2 to 17 produce a stable compound, which can be further determined by the Griess reagent. In the presence of NO scavenging molecules, the production of nitrite ions is reduced and so the absorbance. For this assay, different concentration of the sample (lg/ml) (standard, ascorbic acid (AA)) is mixed with 10 mM sodium nitroprusside solution (prepared in PBS/pH = 7.4) and the whole mixture is incubated for 2 h at 30 0C. Then, 0.5 ml of Griess reagent containing 2% H_3PO_4, 1% sulfanilamide, and 0.1% N-(1-Naphthyl) ethylenediamine is added to all reaction tubes, and the produced colour is read at 550 nm. The NO scavenging ability is calculated as a percentage of inhibition from the standard curve [21].

***In-silco* Studies:**

Density Functional Theory (DFT): Density functional theory (DFT) plays a significant role in predicting the structural and electronic properties of phytochemicals, which are bioactive compounds found in plants. DFT provides a reliable quantum mechanical framework to analyze the molecular geometry, electron density distribution, and reactivity of these natural compounds. By modeling the electronic structure, DFT can predict how phytochemicals interact with biological targets, their stability, and their potential efficacy as drugs. This method allows researchers to explore molecular properties such as binding affinities, ionization potential, and dipole moments, which are crucial for understanding the mechanisms of action of phytochemicals. Additionally, DFT helps in predicting spectroscopic properties, facilitating the identification and characterization of phytochemical structures from experimental data.

In this study, quantum chemical calculations were performed using density functional theory [22–26] combining Becke's exchange functional and Lee-Yang-Parr's correlation functional [27, 28] with the 6-311++G (d,p) basis set using Gaussian 09 software which enabled tasks such as geometry optimization and energy minimization with GaussView 6.0 as the graphical interface, for the visualization of molecular structures and facilitating result analysis. These computational tools were essential for predicting molecular properties with precision and accuracy.

Molecular Docking: Molecular docking studies were conducted to explore the interaction between protein targets related to anti-oxidant and anti-rheumatoid arthritis activities and selected phytocompounds [17] using Schrödinger's Maestro. Protein structures were obtained from the Protein Data Bank (PDB) and prepared using Schrödinger's Maestro Protein Preparation Wizard optimizing protonation states and hydrogen bonding networks. Docking simulations were carried out with Glide and grid generation was performed around the ligand-binding pocket with a

10 Å radius. Phyto-compounds were docked into the active site of the proteins, and their binding modes and affinities were evaluated using Glide's scoring functions. Docking results were analysed based on binding modes, docking scores, and interactions with key residues. The best docking poses were visually inspected in Maestro and validated against experimental data. Top-scoring compounds were selected for further analysis. Phyto compound properties and chemical structures were sourced from PubChem [29, 30], while 3D protein structures were retrieved from the PDB. Additional tools like SiteMapwere used to identify binding pockets, and Maestro provided an interface for the entire docking and analysis workflow.

RESULTS AND DISCUSSION: Fresh, mature leaves of *S. hamiltoniana* were collected from Pathanamthitta district, Kerala, and identified by experts. The shrub, 1.5–2 m tall, had elliptic leaves with acuminate tips, acute bases, serrated margins, and a dark green upper surface. The leaves were glabrous with prominent veins. The open panicle inflorescence showed no distinction between leaves and bracts. The bright violet corolla featured a narrowly cylindrical tube that abruptly widened. Four stamens with ellipsoid, ribbed pollen and four-seeded, pilose fruit were also observed. These characteristics aid in species identification and further phytochemical analysis.

Phytochemical Analysis: The phytochemical analysis shows that alkaloids and glycosides are present in all four solvent extracts. Terpenoids were detected in all solvents except ethanol, suggesting that they are partially soluble in less polar solvents. Phenols and tannins were consistently found across all solvents. Flavonoids were present in the water and ethanol extracts but not in the chloroform and methanol extracts, showed on **Table 1.** Saponins were not detected in any of the solvents, indicating their absence or presence below the detection limit.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF PLANT EXTRACT

Sl. no.	Tests	Water	Chloroform	Methanol	Ethanol
1.	Test For Alkaloids	Positive	Positive	Positive	Positive
2.	Test For Glycosides	Positive	Positive	Positive	Positive
3.	Test For Saponin	Negative	Negative	Negative	Negative
4.	Test For Terpenoids	Positive	Positive	Positive	Negative
5.	Test For Phenol	Positive	Positive	Positive	Positive
6.	Test For Tannin	Positive	Positive	Positive	Positive
7.	Test For Flavanoids	Positive	Negative	Negative	Positive

LCMS Analysis: Table 2 shows the phytochemicals that come from the leaf extracts of *S.hamiltoniana*, which were obtained using LCMS methods. The following phytochemicals were found. The phytochemical analysis identified several bioactive compounds in the extracts, each associated with various biological activities. The phytochemical analysis identified several bioactive compounds in the extracts, each associated with various biological activities.

Notably, β,3,4-trihydroxybenzenepropanoic acid exhibited anti-microbial and anti-oxidant properties, while hexadecanoate (palmitic acid) demonstrated neuroprotective, anti-inflammatory, and analgesic effects. Additionally, tri-hydroxy flavonoids and catechin were recognized for their anti-bacterial, anti-fungal, and anti-cancer activities. Other compounds such as dicoumaroglycerol and hydroxybenzoic acid also showed significant anti-microbial properties. Among the distinguished compounds, four selected ligands from the LC-MS analysis epigallocatechin, tri-hydroxy flavanone, catechin, and tricin are all flavonoids known for their strong antioxidant and antimicrobial properties. Epigallocatechin, found in green tea and cocoa, exhibits antimicrobial, antioxidant, and anti-inflammatory effects, and can inhibit *Plasmodium falciparum*.

Tri-hydroxy flavanone shows antibacterial, antifungal, and antiviral activities, while catechin, a potent antioxidant, also offers anti-inflammatory and anticancer benefits, particularly useful in treating cancer and cardiovascular diseases.

Tricin, sourced from plants like rice and wheat, possesses antiviral, anti-inflammatory, antioxidant, and anti-tubercular properties, making it a promising compound for various therapeutic applications.

TABLE 2: LCMS PROFILE OF PHYTOCHEMICALS

S. no.	RT	Parent ion	Compound	Biological activity
1	3.274	197.1179(M+)	B,3,4,trihydroxyBenzenepropanoic acid	Anti-microbial, anti- oxidant
2	3.109	244.1699(M+)	Hexadeconate or palmitic acid	Neuroprotein, anti-infalammtory, analgeic
3	7.367	279.2320(M+)	Trihydroxy flavanoid	Anti-bacterial, anti -fungal, antiviral
4	4.676	291.1964(M+)	Catechin	Anti-oxidant, anti-inflammatory, anticancer
5	5.312	387.1931(M+)	Dicoumaroglycerol	Anti-fungal, anti-bacterial
6	3.657	137.0240(M-)	Hydroxy benzoic acid	Anti-microbial
7	4.972	305.1754(M-)	Epigallocatechin	Anti-microbial
8	4.676	307.1911(M-)	Methoxydihydrofuscin	Anti-microbial
9	4.581	309.2078(M-)	Dihydroxyoctadecadienoic acid	Anti-bacterial
10	4.068	327.2169(M-)	Trihydroxy octadecadienoic acid	Anti-microbial, anti-bacterial, anti-fungal
11	4.242	329.2321(M-)	Tricin	Anti-viral, anti-inflammatory, antioxidant

In this work, we focus on the antioxidant properties of the identified compounds; hence, we selected catechin, epigallocatechin, tri-hydroxy flavanone, Trihydroxy octadecadienoic acid, trihydroxy-benzoic acid, hexadecdienoic acid, dodecanal, phytoland tricin for further quantum mechanical and molecular docking studies. These compounds have shown significant potential in exhibiting strong antioxidant effects, making them suitable candidates for in-depth analysis of their interactions with biological targets.

Antioxidant Activity: The antioxidant activity of various solvent extracts of *S. hamiltoniana* was evaluated using three different assays namely, DPPH, HRS and FRAP. **Fig. 2** displays the percentage of radical scavenging activity for the various solvent extracts of *S. hamiltoniana*. In the DPPH assay, all extracts demonstrated significant antioxidant activity, with aqueous and chloroform extracts showing percentage inhibitions of 25.74% and 45.94%, respectively. In contrast, ethanol and methanol extracts exhibited higher percentage inhibitions of 89.10% and 86%, closely approaching the positive control. A higher percentage of inhibition indicates a greater ability to neutralize the DPPH radical, signifying stronger radical scavenging activity; thus, methanol and ethanol extracts showed superior radical scavenging compared to chloroform and aqueous extracts. Additionally, the HRS potential of the extracts ranged from 25.5% to 86.4% at specified concentrations, with ethanol extract achieving the highest scavenging activity at 86.4%. The FRAP assay revealed a concentration-dependent increase in reducing activity up to 300 µg/ml for all extracts, with ethanol extract showing the highest scavenging potency at 79.41%. Overall, these results highlight the strong antioxidant potential of *S. hamiltoniana* extracts, particularly the ethanol extract.

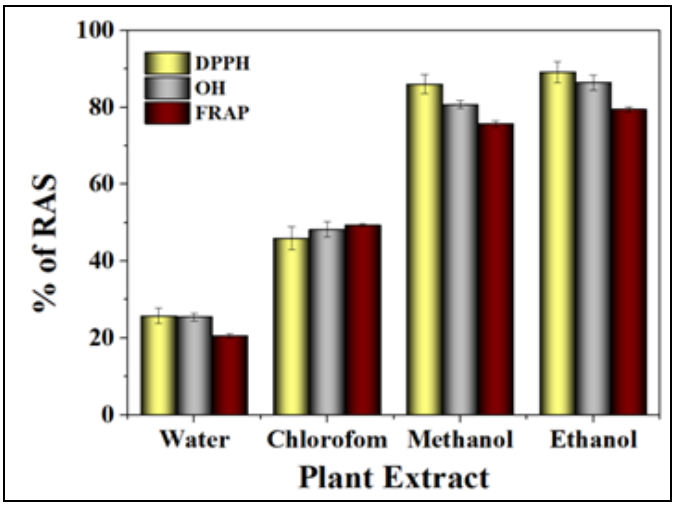


FIG. 2: FREE RADICAL SCAVENGING ACTIVITY BY DPPH, HRS AND FRAP ON DIFFERENT SOLVENT EXTRACT OF *S. HAMILTONIANA* PLANT

Hence, the IC_{50} values for the ethanol extract of *S. hamiltoniana*, which demonstrated the highest antioxidant activity, were determined across different assays as depicted in **Fig. 3**. In the DPPH assay, the IC_{50} value was found to be 31.08 µg/ml. In the HRS assay, the IC_{50} value was 25.54 µg/ml. Additionally, the IC_{50} value in the FRAP assay was calculated to be 28.24 µg/ml. These findings underscore the strong antioxidant capacity of the ethanol extract in various free radical scavenging scenarios. Ethanol, being a polar solvent, appears to extract a broader range of phytochemicals that contribute to scavenging activity compared to the less polar chloroform and water. The strong antioxidant potential of the ethanol extract, as shown in the **Fig. 3**, highlights its possible application in pharmaceutical formulations aimed at combating oxidative stress and related diseases. Further investigations are warranted to elucidate

the specific bioactive compounds responsible for these effects and their mechanisms of action.

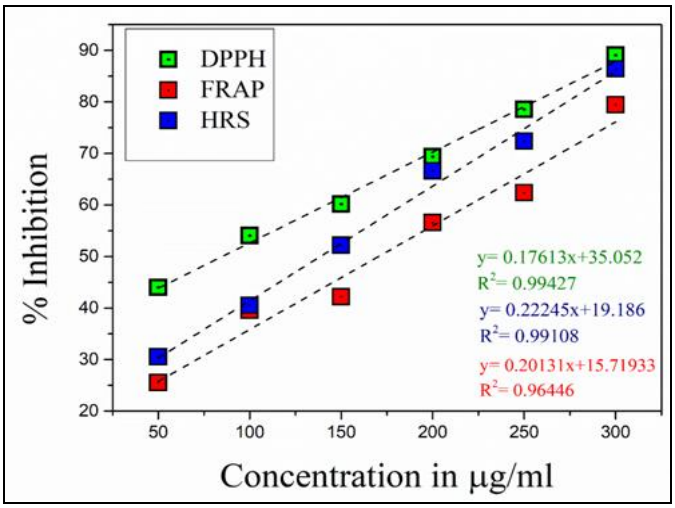


FIG. 3: CONCENTRATION DEPENDENT BEHAVIOUR OF ETHANOL EXTRACT OF *S. HAMILTONIANA* WITH DPPH, FRAP AND HR ASSAYS

Anti-inflammatory Activity: The **Fig. 4** illustrates the percentage of radical scavenging activity (RAS) of various extracts from *S. hamiltoniana* (water, chloroform, methanol, and ethanol) in neutralizing nitric oxide (NO). The ethanol extract shows the highest radical scavenging activity, achieving nearly 90% inhibition of NO.

This indicates that the ethanol extract has significant antioxidant properties, effectively neutralizing NO radicals. The methanol extract also exhibits substantial scavenging activity, closely following the ethanol extract. Chloroform and water extracts display lower scavenging activities compared to the ethanol and methanol extracts, with the water extract showing the least efficacy.

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through the intermediates NO_2, N_2O_4 and N_3O_4. It is estimated by using the Griess reagent.

All the extracts showed scavenging activity and there was a decrease in the amount of nitrous acid. The extent of decrease reflects the extent of scavenging and ethanolic extract showed the highest scavenging activity. While scavenging hydroxyl radical, the ability of ethanol extract (80.41%) was found to be higher than other sample extracts.

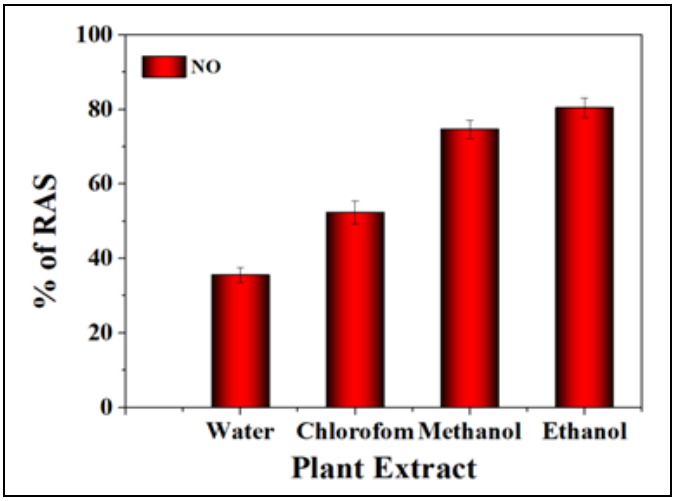


FIG. 4: NITRIC OXIDE SCAVENGING OF *S. HAMILTONIANA* IN DIFFERENT SOLVENT EXTRACTS

The IC_{50} value was calculated for ethanol extract(300µg/ml) that showed highest activity and it was found to be 26. 26µg/ml as depicted in **Fig. 5.** The significant increase in RAS from water to ethanol suggests that the solvent polarity influences the extraction efficiency of bioactive compounds responsible for anti-inflamatory activity. Ethanol, being a polar solvent, appears to extract a broader range of phytochemicals that contribute to scavenging activity compared to the less polar chloroform and water.Thestrong anti-inflammatory potential of the ethanol extract, as indicated by its ability to scavenge NO, underscores its possible therapeutic applications in treating inflammatory conditions. High levels of NO are implicated in various inflammatory diseases, and its neutralization can help mitigate inflammation.

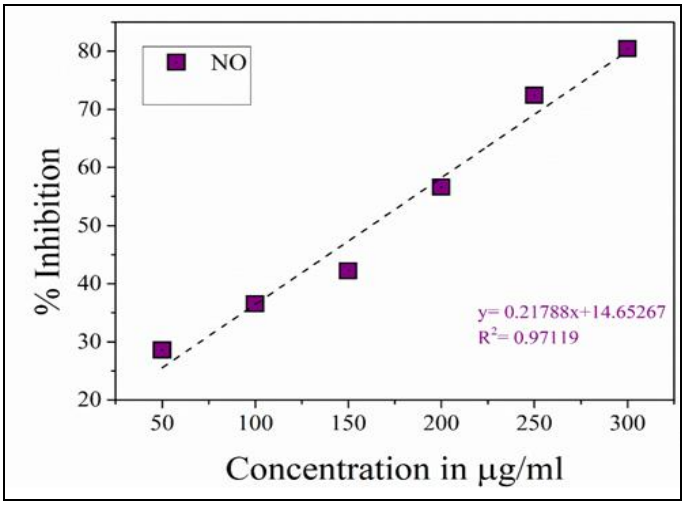


FIG. 5: CONCENTRATION DEPENDENT BEHAVIOUR OF ETHANOL EXTRACT OF *S. HAMILTONIANA* IN NITIC OXIDE SCAVENGING ASSAY

Molecular Docking: Molecular docking is a critical tool in this study, used to predict and analyze the interaction between bioactive compounds and specific protein targets related to antioxidant and anti-inflammatory pathways. By simulating the binding affinities and interactions of the selected phytochemicals, such as epigallocatechin, tryhydroxy flavanone, catechin, and tricin, molecular docking helps in identifying how these compounds may inhibit or activate target proteins such as Monoamine Oxidase B (MAO-B), catalase, cytochrome P450 (CYP2C9), and Nuclear factor erythroid-2-related factor 2 (NF-E2 Related Factor 2 or Nrf2). MAO-B is involved in the metabolism of neurotransmitters, and its inhibition can reduce oxidative damage associated with neurodegenerative diseases. Catalase is crucial in breaking down hydrogen peroxide, a reactive oxygen species, into water and oxygen, thereby protecting cells from oxidative damage. CYP2C9, a member of the cytochrome P450 family, plays a vital role in drug metabolism and oxidative stress regulation, while Nrf2 is a key transcription factor that activates antioxidant defense mechanisms in response to oxidative stress. Through molecular docking, the study reveals potential ligand-protein interactions that could modulate these proteins' activity, enhancing their antioxidant response. Strong binding affinities with these proteins indicate that the selected phytochemicals may help in reducing oxidative damage, thus offering potential therapeutic applications in diseases linked to oxidative stress, such as cancer, cardiovascular disorders, and neurodegenerative diseases. These findings lay the foundation for further exploration of the antioxidant efficacy of these compounds at a molecular level [19].

MAO-B: A dimeric, mitochondrial enzyme that metabolizes neurotransmitters like dopamine. This process generates reactive oxygen species (ROS), contributing to oxidative stress in neurodegenerative disorders such as Parkinson's disease [31].

Catalase: A tetrameric enzyme that converts hydrogen peroxide (H_2O_2) into water and oxygen, preventing oxidative damage and maintaining cellular health by neutralizing ROS [32].

Cytochrome P450 (CYP2C9): A haemoprotein involved in the metabolism of drugs and xenobiotics. By detoxifying harmful substances, it helps maintain cellular balance and reduce oxidative stress.

Nrf2: A transcription factor that activates antioxidant response genes. It protects cells from oxidative damage and is a key in preventing diseases like cancer and arthritis by enhancing cellular defences.

These antioxidant target proteins are central to the regulation of oxidative stress, and docking studies with bioactive ligands will provide deeper insights into potential therapeutic interventions to reduce oxidative damage and associated diseases. The 3D structure of the target proteins are shown in the **Fig. 6.**

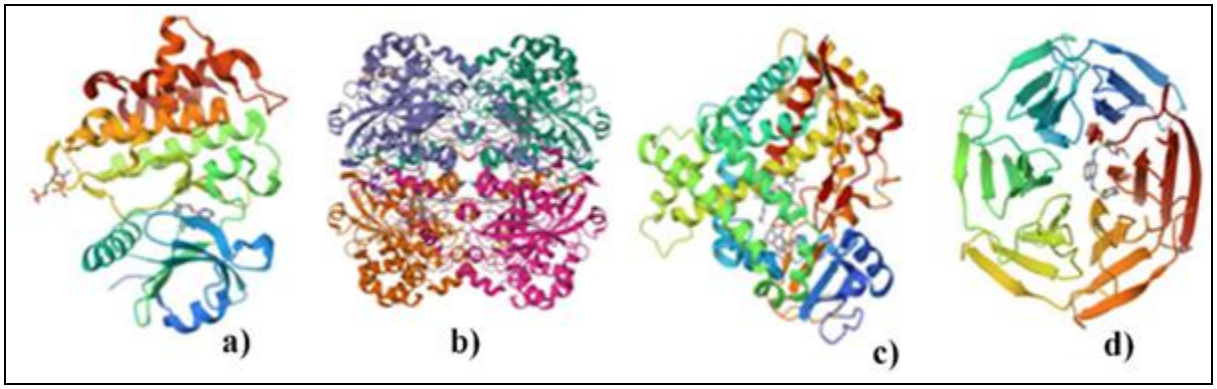


FIG. 6: 3D STRUCTURE OF SELECTED ANTIOXIDANT TARGET PROTEINS, A) MONOAMINE OXIDASE B, B) CATALASE C) CYTOCHROME P450 D) NRF2 RELATED FACTOR 2

Molecular Dockingwith MOA-B: The docking studies of selected phytochemicals, including catechin, epigallocatechin, dodecanal, hexadecane, hexadecadienoic acid, trihydroxy octadecadienoic acid, 1,2-benzene dicarboxylic acid, and trihydroxybenzoic acid, with MAO-B revealed that

several hydrogen bonds and other molecular interactions play a crucial role in stabilizing these ligands within the enzyme's active site. This stabilization directly influences their binding affinity and potential inhibitory activity against MAO-B. **Fig. 7** depicts the 3D images of docking results of the selected phytochemicals along with the inbuilt ligands. The docking scores and binding affinities of MAO-B for selected ligands were tabulated in **Table 3** Catechin shows a strong binding affinity with a docking score -9.869 and binding affinity -78.052 kcal/mol, indicating favorable interactions within the enzyme's active site. Epigallocatechin (EGC) follows closely with a score of -9.567 and binding energy -46.290 kcal/mol, suggesting a high binding affinity and potential for effective inhibition of monoamine oxidase. Tricin has a score of -7.77 wind binding energy -62.76 kcal/mol, demonstrating significant binding but slightly weaker affinity than catechin and EGC. Phytol displays a score of -6.05, indicating relatively moderate binding interactions. Dodecanal, with a score of -1.83, shows weak binding affinity, suggesting minimal interaction with the enzyme. Hydroxybenzoic acid exhibits stable binding with a docking score of -7.7 and binding energy -56.7 kcal/mol, while hexadecane shows a weak affinity with a score of -1.28. Hexadecenoic acid has a score of -4.3, further indicating weak binding. 1,2-benzene dicarboxylic acid demonstrates stable binding with a score of -7.739 with binding energy -57.905 kcal/mol, while trihydroxy octadecadienoic acid shows moderate binding with a score of -5.327. These results suggest that catechin, and epigallocatechin may serve as effective inhibitors of MOA-B due to their strong binding affinities, while other ligands like dodecanal and hexadecane exhibit weaker interactions. **Fig. 8** represents the 2D interaction picture of monoamine oxidase with selected ligands.

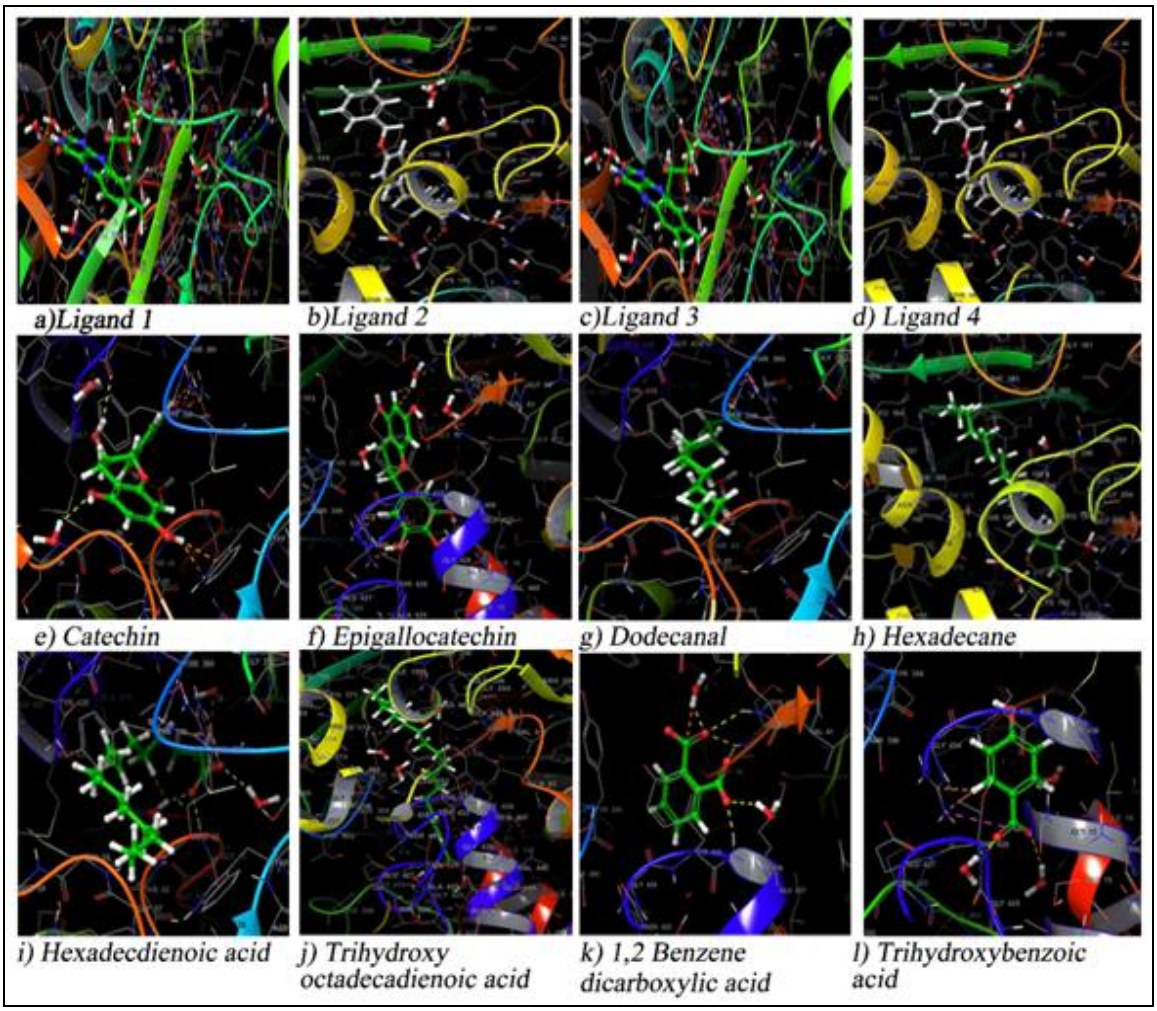


FIG. 7: 3D IMAGE OF SELECTED PHYTOCHEMICALS DOCKING WITH MOA-B (DOTTED LINE INDICATES HYDROGEN BONDING OF LIGAND WITH AMINO ACIDS)

The docking of ligands 1, 2, 3, and 4 with monoamine oxidase B (MAO-B) reveals varying levels of interaction complexity. Ligand 1 forms 16 hydrogen bonds, with water molecules and residues like GLY N65, ALA B263, TYR B393, and LEU B268 playing key roles in stabilization, including a p-p interaction. The ligand donates protons to residues such as TYR B398, QU B34, GLY B40, VAL B235, and GLY B434. LYS B296 donates a proton to a water molecule, while LYS B296, THR B426, and VAL B235 also donate protons to water molecules within the protein structure. A p-p interaction between TYR B393 and the ligand further stabilizes the binding, indicating a complex and extensive interaction network. Ligand 2 forms five hydrogen bonds, with simpler water-mediated interactions involving residues like ILE14 and LYS296. The amino acid ILE14 and the ligand each donate a proton to the same water molecule in the protein, while GLY434 donates a proton to the ligand. Additionally, both the ligand and LYS296 donate protons to another shared water molecule within the protein structure. Ligand 3 exhibits a highly intricate network with 20 hydrogen bonds, water-mediated proton exchanges, and a p-p interaction with GLN B65, ALA B263, TYR B393, LEU B263, and GLY B40. Four water molecules each donate a proton to the ligand. Other residues like MET B436, TYR B426, and VAL B235 also contribute to the proton donation process. A p-p interaction between TYR B393 and the ligand indicates a strong binding, supported by both direct and water-mediated interactions. Ligand 4 forms five hydrogen bonds, indicating moderate interaction via proton exchanges with water molecules and key residues like GLY434 and LYS296.

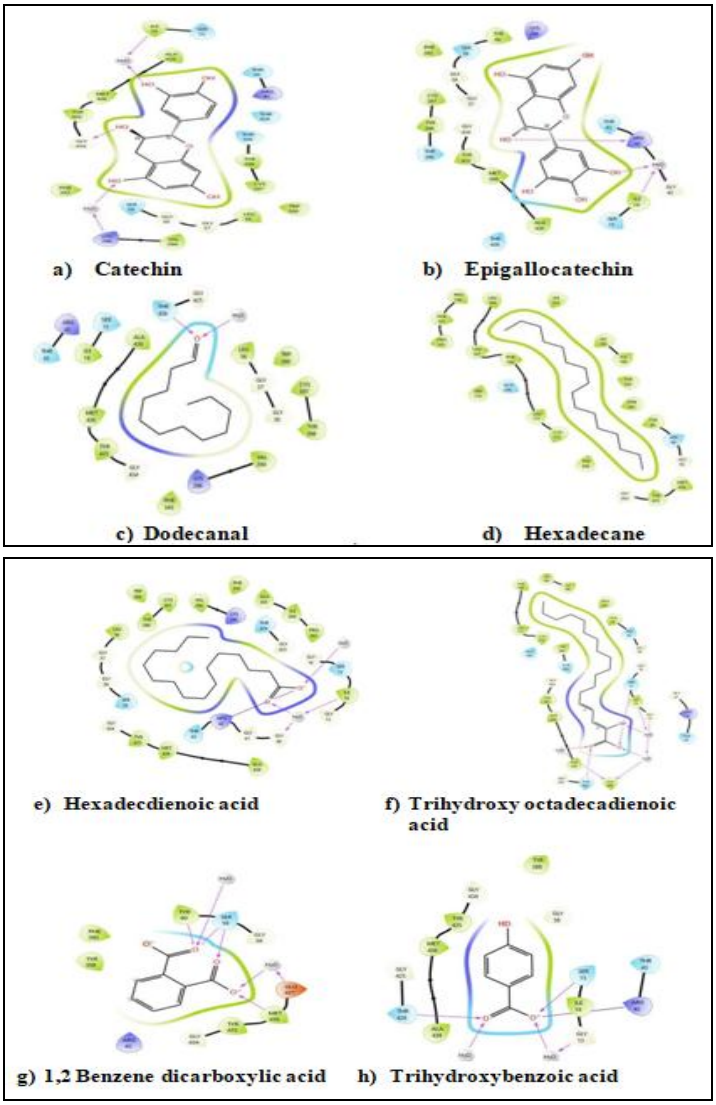


FIG. 8: 2D IMAGE OF SELECTED PHYTOCHEMICALS DOCKING WITH MONOAMINE OXIDASE B

A detailed investigation of the bonding interactions can be conducted using the 2D interaction diagram, as illustrated in the **Fig. 8.** Catechin **Fig. 8A** forms five hydrogen bonds with MAO-B, indicating strong interactions at the enzyme's active site. LYS296 and ILE14 play significant roles by donating protons to a water molecule within the binding site, facilitating hydrogen bonding. Catechin itself actively participates by both donating and accepting protons from the water molecule, strengthening its position in the binding pocket. These interactions suggest that catechin may effectively inhibit MAO-B. Epigallocatechin **Fig. 8B** exhibits three hydrogen bonds in its docking with MAO-B. One key interaction involves ARG42, which accepts a proton from epigallocatechin, while another hydrogen bond is mediated by a water molecule. ILE14 donates a proton to the water molecule, contributing to a stable network of hydrogen bonds. These interactions stabilize the ligand within the active site, supporting its potential as a MAO-B inhibitor. Dodecanal **Fig. 8F** forms two hydrogen bonds, with one mediated by a water molecule and the other involving direct interaction with THR426. The water molecule plays a crucial role in bridging the ligand and the enzyme, ensuring proper positioning within the active site. The limited number of hydrogen bonds suggests a relatively weaker interaction compared to other ligands. Each ligand presents a unique binding mode with MAO-B, characterized by varying numbers of hydrogen bonds, water-mediated interactions, and, in some cases, p-p interactions. These interactions are critical for understanding the potential of these ligands as inhibitors of MAO-B, with varying degrees of stability and binding strength.

Hence, Catechin, with its five hydrogen bonds, shows strong interactions at the enzyme's active site, supported by key residues like LYS296 and ILE14, suggesting its potential as an effective MAO-B inhibitor. Epigallocatechin, with three hydrogen bonds, also demonstrates promising inhibitory potential through interactions with ARG42 and water-mediated bonding. Dodecanal, forming only two hydrogen bonds, exhibits relatively weaker interactions. Each ligand's unique binding mode, characterized by hydrogen bonding and water-mediated interactions, highlights the variability in binding strength and inhibitory potential, offering critical insights into their effectiveness as MAO-B inhibitors.

Molecular Docking with Catalase: In the molecular docking study of various ligands with catalase, key insights into the interactions between the ligands and the enzyme's active site were revealed, particularly in terms of hydrogen bonding and hydrophobic contacts. For catalase, the binding affinities of the selected ligands, and their binding energy were tabulated in **Table 3** Catechin exhibits the strongest interaction with docking score -6.7 and binding affinity -54.04 kcal/mol, indicating stable interactions with the enzyme. Epigallocatechin follows with a slightly weaker binding affinity, having a score of -5.704 and binding energy -52.8 kcal/mol. Tricin shows moderate binding with docking score -5.0 and binding affinity -48.31 kcal/mol, suggesting some interaction but less stability compared to catechin. Phytol, with docking score -1.15 and binding affinity -33.197 kcal/mol, exhibits relatively weak binding, indicating minimal interaction. Dodecanal, interestingly, shows a positive docking score of 0.579 with binding energy 25.171, implying very poor or unfavorable binding with catalase. Hydroxybenzoic acid shows moderate binding with a score of -5.571, while hexadecane, with a score of 0.674, exhibits extremely weak binding. Hexadecenoic acid, with a score of -5.246, displays moderate affinity, while 1,2-benzene dicarboxylic acid shows strong binding with a score of -6.707. Lastly, trihydroxy octadecadienoic acid, with a score of -1.844, shows weak binding. This suggests that catechin and 1,2-benzene dicarboxylic acid have the most stable interactions with catalase, potentially making them effective in binding to this enzyme.

Fig. 9 depicts the 3D images of docking results of the selected phytochemicals along with the inbuilt ligands. For the inbuilt ligand within catalase, four critical hydrogen bonds were observed, involving glutamine (GLN442), lysine (LYS306), and asparagine (ASN213). These residues stabilize the ligand within the enzyme’s active site, ensuring the proper orientation necessary for catalase's functionality. The ligand itself also donates a proton to GLN442, forming a reciprocal hydrogen bond that further strengthens the enzyme-ligand complex.

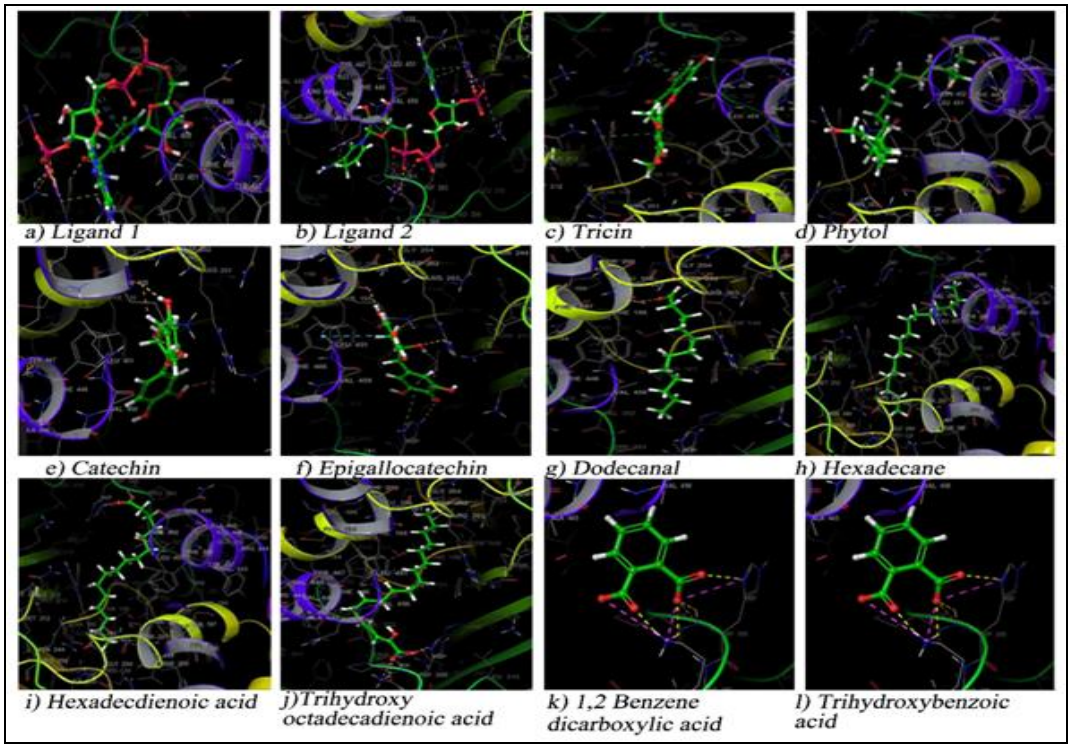
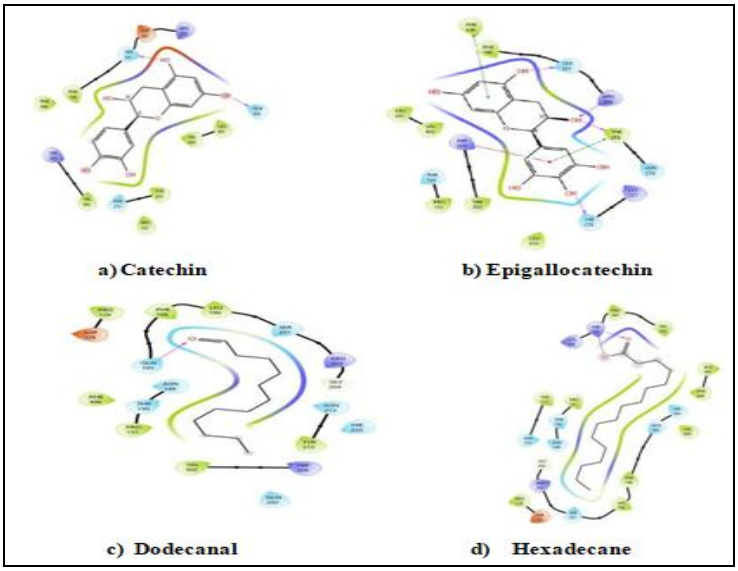


FIG. 9: 3D IMAGE OF SELECTED PHYTOCHEMICALS DOCKING WITH CATALYSE (DOTTED LINE INDICATES HYDROGEN BONDING OF LIGAND WITH AMINO ACIDS)

Fig. 10 depicts the 2D interaction picture of all the selected ligands with the amine groups in the binding pockets. Catechin **Fig. 10A** docking with catalase revealed two hydrogen bonds with GLN455 and SER201, indicating stability within the active site and potential for modulating catalase activity. Epigallocatechin **Fig. 10B** showed multiple hydrogen bonds with histidine, serine, and arginine, suggesting a strong binding affinity and possible regulation of the enzyme's activity. Tricin displayed similar hydrogen bonding and hydrophobic interactions, hinting at its influence on catalase function. Dodecanal **Fig. 10C**, hexadecanoic acid, and trihydroxy octadecadienoic acid **Fig. 10.F** also formed significant interactions, with dodecanal binding to GLN795 and hexadecanoic acid to HID305, suggesting potential inhibitory effects. Benzene dicarboxylic aci **Fig. 10G** and hydroxybenzoic acid **Fig. 10H** also exhibited strong binding due to key hydrogen bonds with LYS305 and HIS305, potentially modulating catalase's role in decomposing hydrogen peroxide.



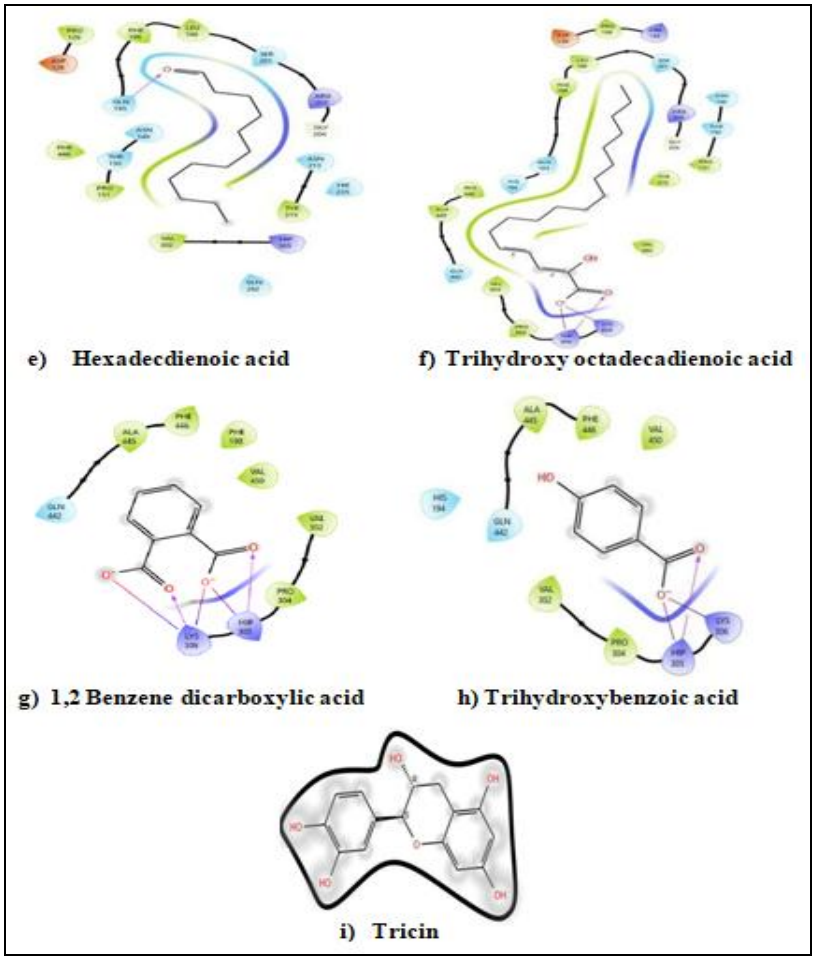


FIG. 10: 2D IMAGE OF SELECTED PHYTOCHEMICALS DOCKING WITH CATALASE

Molecular Docking with Cytochrome P450: Cytochrome P450 enzymes play a crucial role in neutralizing reactive oxygen species (ROS) and act as part of the body's antioxidant defence system. This includes their ability to metabolize harmful compounds, preventing oxidative damage.

Cytochrome c, as mentioned, further supports this antioxidant activity by reducing superoxide back to oxygen (O_2), thus preventing the accumulation of harmful superoxide radicals. The ability of cytochrome c to facilitate electron transfer without being damaged makes it a unique player in cellular defence against oxidative stress.

The docking scores and binding affinities of cytochrome P450 for selected ligands were tabulated in **Table 3** Catechin shows a strong binding affinity with a docking score 6.399 and binding affinity -49.293 kcal/mol, indicating favorable interactions within the enzyme's active site. Epigallocatechin follows closely with a score of -8.650 and binding energy -53.132 kcal/mol, suggesting a high binding affinity and potential for effective inhibition of cytochrome P450. Tricin has a score of -7.076 wind binding energy -55.9 kcal/mol, demonstrating significant binding but slightly weaker affinity than catechin and EGC.

Phytol displays a score of -2.732, indicating relatively weak binding interactions. Dodecanal, with a score of 0.376, shows poor binding affinity, suggesting minimal interaction with the enzyme. Hydroxybenzoic acid exhibits moderate binding with a docking score of -6.358 and binding energy -32.33 kcal/mol, while hexadecane shows a weak affinity with a score of 0.445.

Hexadecenoic acid has a score of -1.819, further indicating weak binding. 1,2-Benzene dicarboxylic acid (BDA) demonstrates strong binding with a score of -6.707 with binding energy -49.5 kcal/mol, while trihydroxy octadecadienoic acid shows weak binding with a score of -1.098. These results suggest that catechin, EGC, and BDA may serve as effective inhibitors of cytochrome P450 due to their

strong binding affinities, while other ligands like dodecanal and hexadecane exhibit weaker interactions. **Fig. 11** represents the 3D interaction picture and **Fig. 12** represents the 2D interaction picture of Cytochrome P450 with selected ligands.

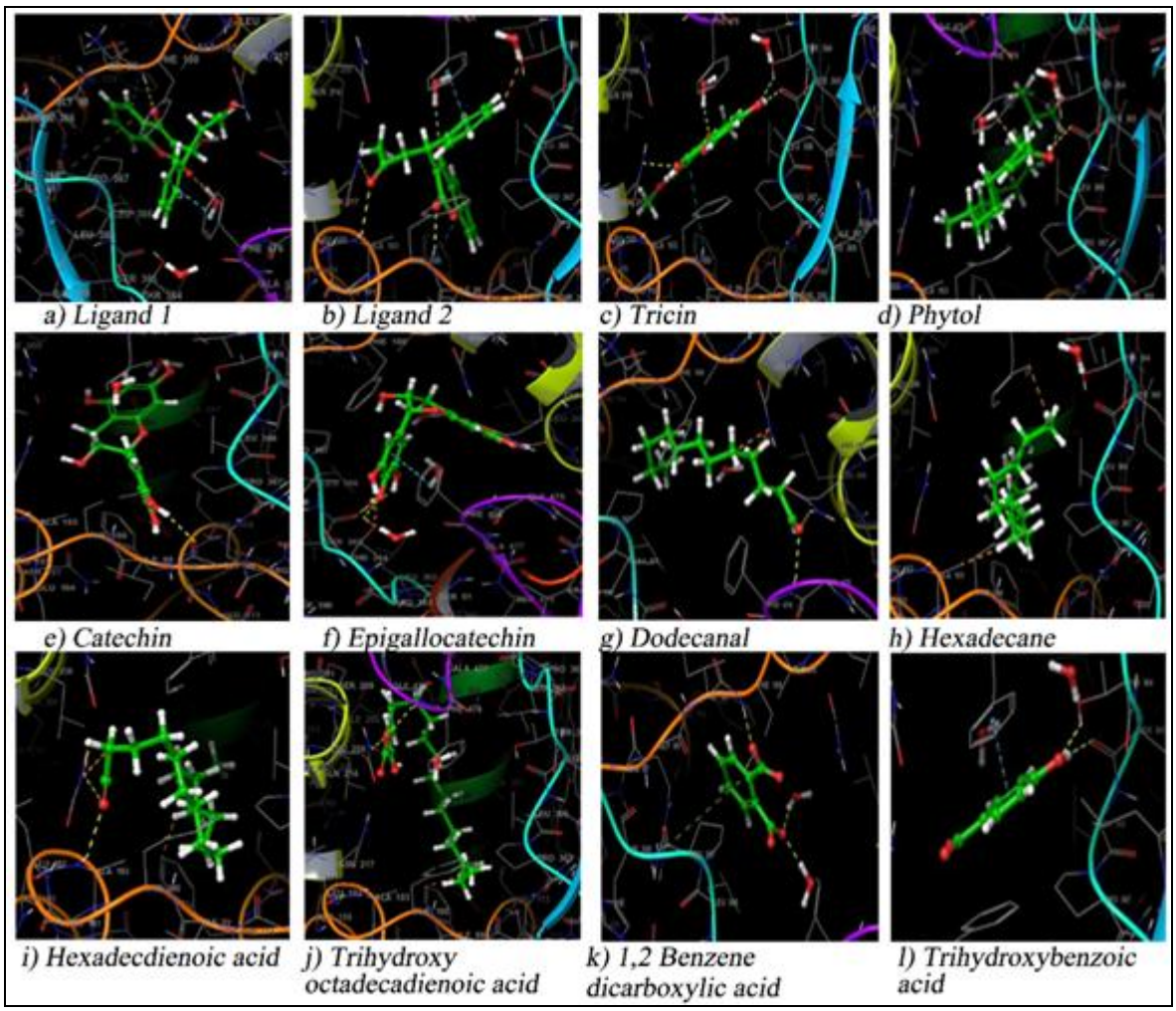


FIG. 11: 3D IMAGE OF LIGANDS DOCKING WITH CYTOCHROME P450

For Ligand 1, two hydrogen bonds are formed with asparagine (ASN217) and a water molecule, both of which donate protons to the ligand. Additionally, a pi-pi interaction between phenylalanine (PHE476) and the ligand contributes to stabilization through aromatic ring interactions. Ligand 2 exhibits three hydrogen bonds involving water, PHE100, and ASN217, each donating a proton to the ligand. A similar pi-pi interaction with PHE476 may enhance the stability of the ligand within the binding site.

In the case of catechin **Fig. 12A**, three hydrogen bonds are established, with threonine (THR301) donating a proton to a water molecule that subsequently donates a proton to alanine (ALA297). The ligand also donates a proton to glycine (GLY98), indicating multiple interaction points with surrounding molecules. Epigallocatechin **Fig. 12B** demonstrates five hydrogen bonds, where the ligand donates two protons to serine (SER365), while threonine (THR354), leucine (LEU208), and glutamine (GLN214) each donate protons to the ligand. This interaction is further reinforced by a π- π interaction with PHE476, suggesting a robust interaction network that enhances binding affinity.

For tricin **Fig. 12H**, four hydrogen bonds are observed, with the ligand donating a proton to SER365, while THR365 and ASN217 donate protons to the ligand. A π- π interaction between the ligand and DHE114 indicates a strong interaction profile. Phytol forms a single hydrogen bond by donating a proton to SER365, suggesting simpler interactions relative to other ligands.

Dodecanal **Fig. 12C** exhibits two hydrogen bonds, with glutamine (GLN214) donating a proton to the ligand, indicating moderate interaction potential. Hexadecanoic acid **Fig. 12E** also forms two hydrogen bonds, with ASN217 and alanine (ALA103) each donating a proton to the ligand, reflecting straightforward interactions. For 1,2-benzene dicarboxylic acid **Fig. 12G,** multiple hydrogen bonds are present; PHE100 donates a proton, while water contributes two protons. Additionally, a π-π interaction with arginine (ARG97) may stabilize its position within the binding site. Trihydroxyoctadecadienoic acid **Fig. 12F** forms two hydrogen bonds, with PHE476 and GLN214 each donating protons to the ligand, highlighting its binding potential.

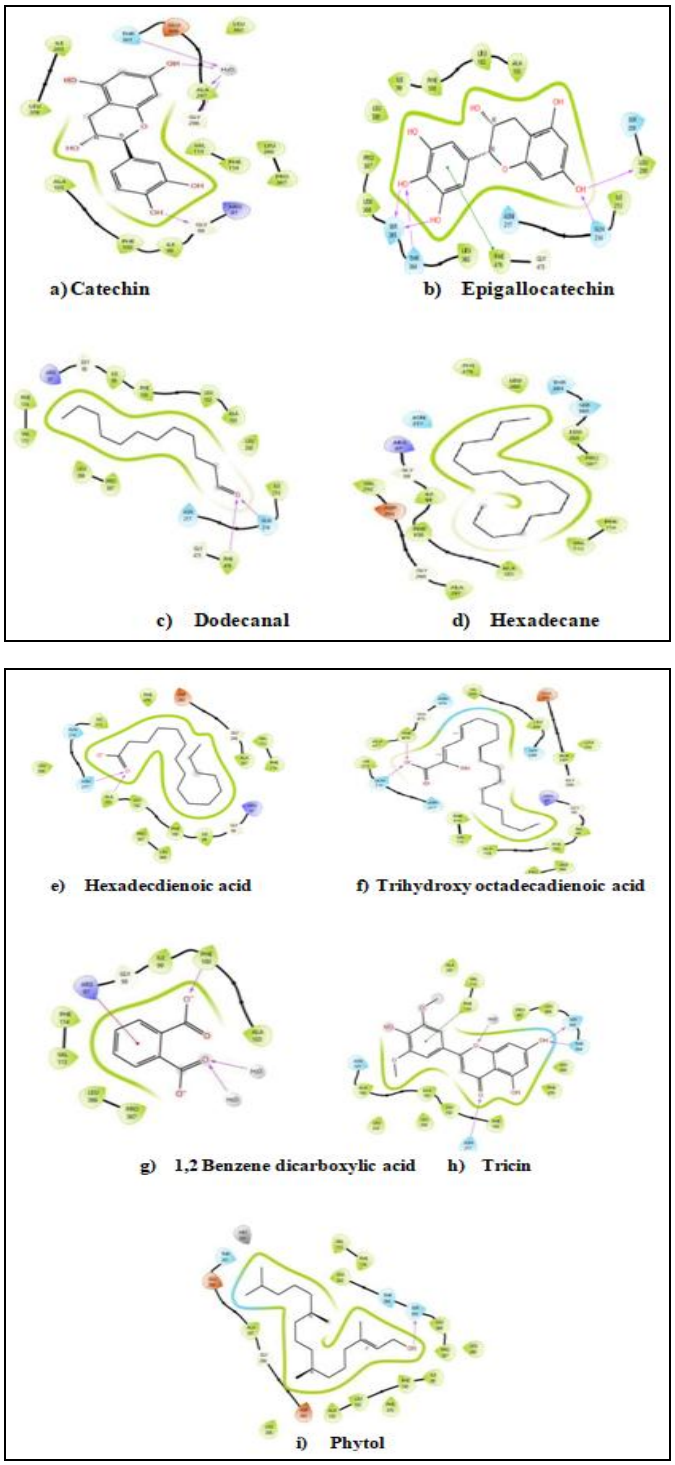


FIG. 12: 2D IMAGE OF LIGANDS DOCKING WITH CYTOCHROME P450

Docking Studies with Nrf2: Nrf2provide insights into how different ligands interact with this key regulator of the antioxidant defence system. Nrf2 plays a crucial role in controlling the expression of genes that protect cells from oxidative stress.

The docking analysis of ligands with Nrf2 reveals varying binding affinities are tabulated in **Table 3** For catechin, the docking score of -6.027 suggests a moderate binding affinity, with strong interactions contributing to its potential role in modulating the activity of Nrf2. Epigallocatechin exhibits a slightly higher docking score of -5.602, indicating comparable binding potential, likely enhanced by multiple hydrogen bonds and interactions with key residues.

Tricin shows a docking score of -5.071, suggesting a relatively weaker affinity, but still highlights its potential to influence the function of Nrf2. Phytol presents a docking score of -5.071, indicating similar binding characteristics to tricin, suggesting a straightforward interaction profile. Dodecanal demonstrates a docking score of -0.582, reflecting a notably weaker interaction with the protein, which may limit its effectiveness as a modulator. Hexadecanoic acid, with a docking score of -5.11, suggests moderate binding affinity, supported by hydrogen bonding interactions. The ligand 1,2-benzenedicarboxylic acid exhibits a docking score of -1.098, indicating a relatively weak binding affinity, potentially due to fewer significant interactions within the binding pocket. Lastly, trihydroxyoctadecadienoic acid has a docking score of -4.46, highlighting its modest interaction potential. Hydroxybenzoic acid, with a docking score of -5.71, demonstrates a favorable binding profile, suggesting its capacity to interact effectively with Nrf2. **Fig. 13** represents the 3D interaction picture and **Fig. 14** represents the 2D interaction picture of Nrf2 with selected ligands.

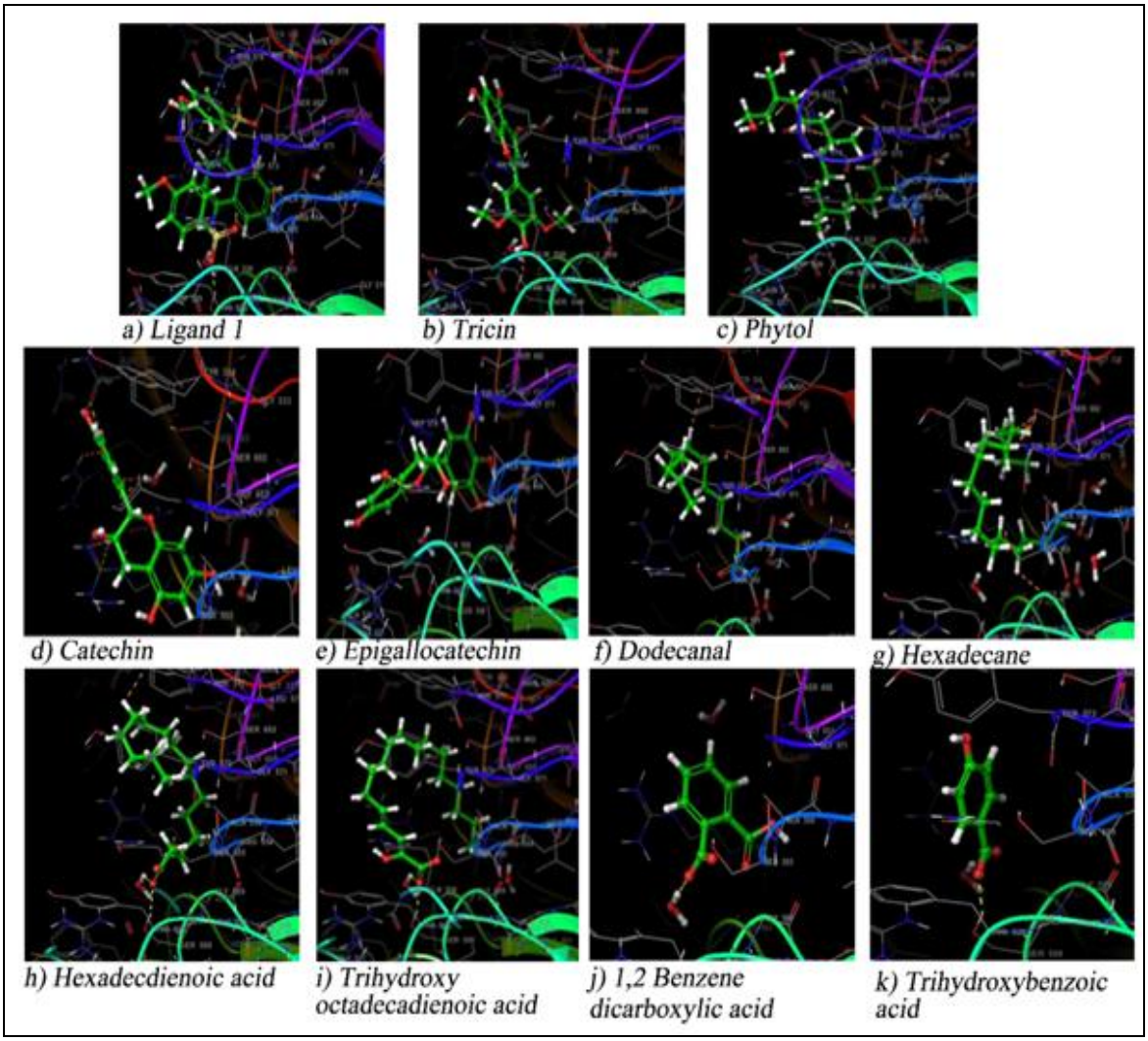


FIG. 13: 3D IMAGE OF LIGANDS DOCKING WITH NRF2

Ligand 1 forms five hydrogen bonds, with water donating protons to both SER365 and the ligand. SER602, SER55, and SER508 also donate protons to the ligand, with SER508 contributing an additional proton. A π–π interaction with PHE577 and ARG415 further strengthens the binding, indicating strong affinity. Catechin **Fig. 14A** establishes two hydrogen bonds, where the ligand donates protons to ASN382, suggesting moderate binding affinity with Nrf2. Epigallocatechin **Fig. 14B** also forms two hydrogen bonds, receiving protons from GLN530 and SER555, demonstrating stable interaction with Nrf2.

Hexadecanoic acid **Fig. 14E** forms two hydrogen bonds with contributions from water and SER508, resulting in a moderate interaction. Tricin **Fig. 14I**, with three hydrogen bonds from SER508, water, and SER555, shows a robust interaction with Nrf2. Phytol, on the other hand, forms only one hydrogen bond, with TYR572 donating a proton, indicating weaker binding. Trihydroxyoctadecadienoic acid **Fig. 14F** forms three hydrogen bonds involving SER508, water, and SER555, highlighting strong binding potential. 1,2-Benzenedicarboxylic acid forms a single hydrogen bond with water, suggesting minimal affinity.

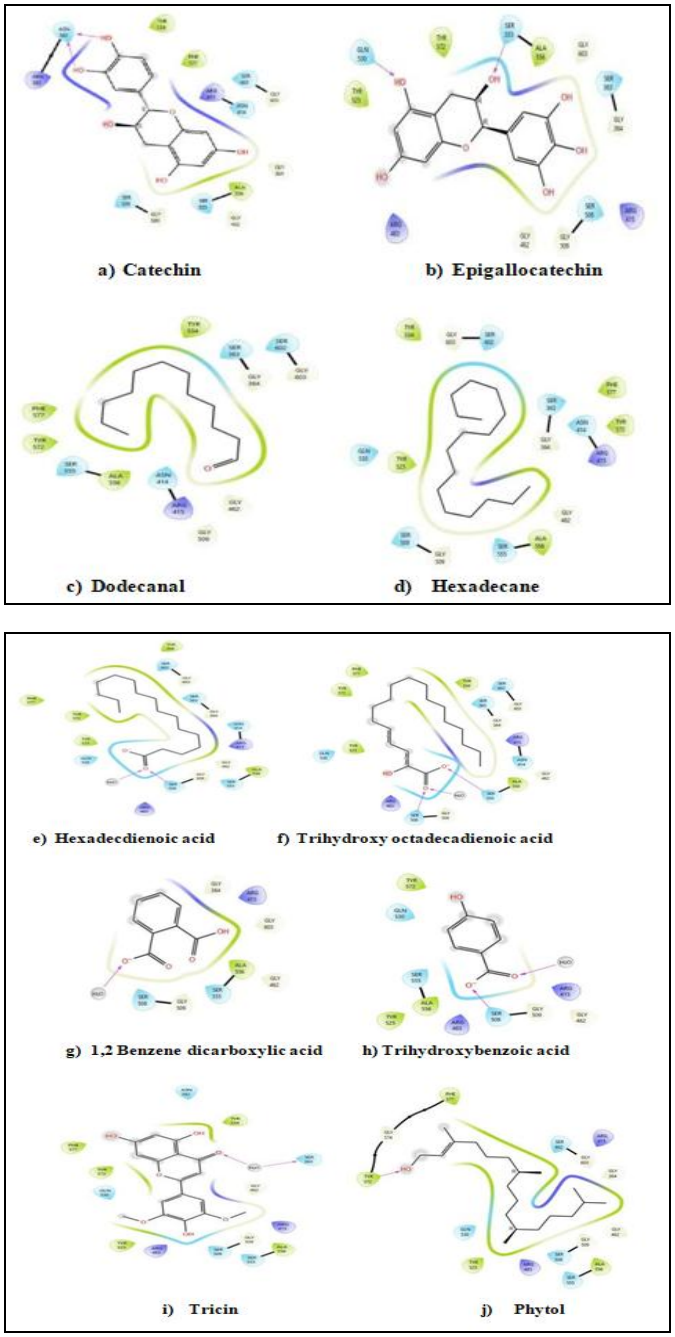


FIG. 14: 2D IMAGE OF LIGANDS DOCKING WITH NRF2

For better insight, combine the docking results and calculate the docking score and binding affinity of selected phytocompounds with four targeted proteins. The results are shown in the **Table 3.**

TABLE 3: DOCKING OF PHYTOCOMPOUNDS WITH TARGET PROTEINS

Ligand	Target Protein															
	Monoamine Oxidase (2V5Z)				Catalase (1DGF)				Cytochrome P450 (1OG5)				NFE-2 Related Factor 2 (41QK)			
	Site	Docking Score	Glide e Model	Glide g Score	Site	Docking Score	Glide e Model	Glide g Score	Site	Docking Score	Glide e Model	Glide g Score	Site	Docking Score	Glide e Model	Glide g Score
Catechin	3	-9.869	-9.869	-78.052	2	-6.691	-6.691	-54.041	2	-6.399	-6.399	-49.293	1	-6.027	-6.027	-50.443
Epigallocatechin	1	-9.567	-9.567	-46.290	2	-5.695	-5.704	-52.817	1	-8.650	-8.659	-53.132	1	-5.602	-5.611	-48.122
Tricin	4	-7.773	-7.821	-62.769	1	-4.953	-5.001	-48.307	2	-7.076	-7.124	-55.981	1	-5.071	-5.611	-48.122
Phytol	4	-6.055	-6.055	-54.567	1	-1.149	-1.149	-33.197	1	-2.732	-2.732	-33.356	1	-5.071	-5.119	-47.711
Dodecanal	3	-1.834	-1.834	-35.885	2	0.579	0.579	-25.171	1	0.376	0.376	-21.800	1	-0.819	-0.819	-19.103
Hydroxybenzoic acid	1	-7.705	-7.705	-56.747	2	-5.571	-5.571	-42.633	2	-6.358	-6.358	-32.338	1	-4.4866	-4.866	-31.436
Hexadecane	2	-1.280	-1.280	-31.778	1	0.647	0.674	-25.457	2	0.445	0.445	-21.800	1	2.163	2.163	-16.108
Hexadecanoic acid	3	-4.377	-4.382	-66.444	1	-5.246	-5.246	-36.750	2	-0.521	-0.525	-25.096	1	-0.582	-0.587	-27.965
1,2-Benzenedicarboxylic acid	1	-7.739	-7.753	-57.905	2	-6.694	-6.707	-61.391	1	-7.382	-7.396	-49.509	1	-5.164	-5.177	-39.138
Trihydroxycatadecadienoic acid	1	-5.327	-5.327	-73.817	2	-1.844	-1.844	-40.921	1	-1.098	-1.098	-31.341	1	-4.66	-4.66	-30.909
Inbuilt ligand 1	3	-19.824	-19.824	-315.562	1	-8.610	-8.610	-174.917	1	-9.208	-9.208	-63.280	1	-8.164	-8.164	-93.694
Inbuilt ligand 2	2	-12.465	-12.465	-106.758	2	-9.474	-9.474	-173.233	2	-9.271	-9.271	-68.521	-	-	-	-
Inbuilt ligand 3	3	-19.824	-19.824	-315.562	-	-	-	-	-	-	-	-	-	-	-	-
Inbuilt ligand 4	2	-12.465	-12.465	-106.758	-	-	-	-	-	-	-	-	-	-	-	-

Upon comparing the docking scores and binding affinities (Glide e model) across the four target proteins—Monoamine Oxidase (2V5Z), Catalase (1DGF), Cytochrome P450 (1OG5), and NFE-2 Related Factor 2 (41QK). Catechin, Epigallocatechin, and Tricin display the highest overall binding affinities, especially with Monoamine Oxidase and Cytochrome P450, indicating strong interactions. Phytol, Hydroxy-benzoic Acid, and 1,2-Benzenedicarboxylic Acid show moderate binding, while Dodecanal, Hexadecane, and Hexadecanoic Acid demonstrate weaker interactions. Trihydroxyoctadecadienoic Acid exhibits strong binding particularly with Monoamine Oxidase. These results suggest that ligands such as catechin, epigallocatechin, and tricin may have the highest potential for therapeutic applications involving these proteins.

CONCLUSION: This study provides the first comprehensive investigation into the biological activity of *S. hamiltoniana*, a plant with a rich history in traditional medicine. Through phytochemical analysis, antioxidant assays, and anti-inflammatory evaluations, the bioactive compounds present in various extracts have been shown to exhibit strong therapeutic potential. The use of advanced techniques such as LC-MS for flavonoid identification. Phytochemical analysis revealed that alkaloids and glycosides were present in all solvent extracts. Terpenoids were found in all except ethanol, indicating partial solubility in less polar solvents. Phenols and tannins were consistently detected across all extracts, while flavonoids appeared only in water and ethanol. Saponins were absent or below the detection limit in all solvents. Based on the DPPH assay, the aqueous extract showed 25.74% inhibition, while the chloroform extract showed 45.94%. The ethanol and methanol extracts exhibited higher inhibition, at 89.10% and 86%, respectively. Hydroxyl radical scavenging (HRS) ranged from 25.5% to 86.4%, with the ethanol extract showing the highest activity (86.4%) at a concentration of 300 µg/ml. The IC_{50} value for ethanol extract was 25.54 µg/ml. All extracts were effective in the FRAP assay, with the ethanol extract showing the highest activity (79.41%) and an IC_{50} of 28.24 µg/ml.

All extracts exhibited anti-inflammatory activity by scavenging nitrous acid, with the ethanolic extract showing the highest scavenging ability (80.41%) and an IC_{50} value of 26.26 µg/ml. Upon comparing the docking scores and binding affinities (Glide e model) across the four target proteins MAO-B, Catalase, Cytochrome P450, and Nrf2-several patterns emerge. Catechin and Epigallocatechin show consistently strong binding, with Catechin displaying the highest affinity for Monoamine Oxidase (-78.052) and significant interactions with Cytochrome P450 and Nrf2. Epigallocatechin also exhibits strong binding, particularly with Cytochrome P450 (-53.132).

Tricin shows moderate to strong affinities, especially towards MAO-B(-62.769) and Cytochrome P450, while Phytol demonstrates moderate interactions. Dodecanal and Hydroxybenzoic Acid display weaker interactions across targets, with minimal binding to Nrf2 and moderate interactions with MAO-B. Hexadecane shows the lowest affinities, while Hexadecanoic Acid and 1,2-Benzenedicarboxylic Acid show moderate to strong binding with Monoamine Oxidase. Trihydroxyoctadecadienoic Acid exhibits strong binding to Monoamine Oxidase (-73.817) with moderate interactions with other proteins.

Given these findings, *S. hamiltoniana* shows promise as a source of natural therapeutic agents. Moving forward, further investigation into its antiarthritic potential is warranted, given its significant antioxidant and anti-inflammatory activities, which could contribute to the development of treatments for arthritis and related inflammatory conditions.

Declaration: Human Ethics and Consent to Participate declarations: not applicable. This study does not involve human participants, human data, or human tissue.

Ethics Approval and Consent to Participate: The plant material was collected from the private property of Bindu Palaghat Krishnamoorthy, Assistant Professor, Department of Botany, Sanatana Dharma College, Alappuzha. As the first author of the manuscript, no additional license was required, consent is taken and the consent letter is attached.

Data Availability Statement: The voucher specimen PKB1124, collected from her private property and identified by Bindu Palaghat Krishnamoorthy, Assistant Professor, Department of Botany, Sanatana Dharma College, Alappuzha, has been deposited in the herbarium of Sanatana Dharma College, Alapuzha, Kerala, India.

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