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Original Article

Computational and *In Vitro* Exploration of Antioxidant and Anti-inflammatory Potential of Clitoria ternatea White-Flower Leaf Extract

Sumayya Khan[1,*], Imran Khan Pathan[2], Ruqaiya Jamal[3] and Sharwan Hudda[3]

[1]*Faculty of Pharmacy, Department of Pharmacology, Maulana Azad University, Jodhpur, Rajasthan, India*
[2]*Faculty of Pharmacy, Department of Pharmacognosy, Maulana Azad University, Jodhpur, Rajasthan*
[3]*Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Maulana Azad University, Jodhpur, Rajasthan, India*

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ABSTRACT

Aim: This study evaluates the therapeutic potential of Clitoria ternatea (White flower plant) ethanolic leaves extract using an in-silico approach, focusing on its antioxidant and Anti-Inflammatory Properties.
Materials and Methods: Ethanolic extraction was performed using the Soxhlet method, followed by qualitative analysis, including phytochemical, FT-IR, and GC-MS analyses, as well as total flavonoid, total phenolic, and terpenoid content analyses. Predict the therapeutic potentials of the extract with PDB ID: 3VLN (Antioxidant), PDB ID: 2AZ5 (Anti-inflammatory), and validate the in-silico study by performing the respective in vitro study.
Results: Flavonoids, anthocyanins, saponins, phenolic compounds, and terpenoids are bioactive compounds in the extract, and their functional groups are reported by phytochemical tests and FTIR, respectively. GC-MS identifies 43 different molecules. Total flavonoid content is 0.0153 mg of Quercetin QUE /gm extract, total phenolic 0.0182 of GAE/gm extract, and terpenoid content is 96.5%. Molecular docking study predicted 14 molecules for antioxidant activity, 14 molecules for anti-inflammatory activity, and 21 molecules for antimicrobial activity. Extract and ascorbic acid % Radical Scavenging Assay at 300 µg/mL are 57.16±0.48 and 39.15±0.62%, respectively. The IC_{50} values for the extract and ascorbic acid are 171.35 µg/mL and 120.81 µg/mL, respectively. The HRBC membrane stabilization assay showed % Protection at concentrations of 1000 µg/ml of extract (53.12±0.82%) and Aspirin (85.13±0.87%).
Conclusion: The present research focuses on the therapeutic properties of Clitoria ternatea (white flower) ethanolic leaf extract, which is abundant in flavonoids, phenolics, and terpenoids. GC-MS, molecular docking, ADMET, and in vitro studies indicate its antioxidant and anti-inflammatory properties, indicating potential use in phytopharmaceuticals and functional food development.

Introduction

For centuries, people have utilized herbal remedies to treat illnesses and preserve their health. Herbal formulations, which are rich in bioactive chemicals, help to control chronic conditions of diseases and are a rapidly expanding area of study, are an essential component of healthcare systems around the globe since they support sustainability and holistic health [1,2].

Clitoria ternatea (C. ternatea) Butterfly pea is a perennial herbaceous legume belonging to the Fabaceae family. Its distinctive ternatin anthocyanin pigments give flowers their distinctively bright blue hue. Apart from these unique pigments, C. ternatea is abundant in many metabolites that have great potential for use in both medicine and agriculture [3].

Furthermore, cyclotides, which are ultra-stable, cyclic plant defence peptides, are present in C. ternatea extract and add to its environmentally favourable pesticide qualities [4]. Several studies have demonstrated C. ternatea's pharmacological potential, which includes its antimicrobial, analgesic, antipyretic, diuretic, antioxidant, anti-inflammatory, and antidiabetic properties [5-8]. Several other bioactive chemicals, including flavanols [9], cyclotides [10], and delphinidin [11], are essential contributors to the herb's therapeutic advantages. Ethanolic extract has been reported to have Anticancer properties [12]. Extracts of this plant have reported polyphenolic compounds, including anthocyanins, which have antioxidant potentials and are used in cancer treatment [13,14]. The extract of the flower and leaf has antimicrobial and antibiofilm therapeutic potentials reported [15].

Drug discovery and herbal medicine are significantly impacted by research using computational chemistry to find bioactive molecules in herbal extracts. Scientists can effectively identify potential phytochemicals that cause pharmacological activity by utilizing techniques like molecular docking, molecular dynamics, QSAR, and virtual screening. The effort and money involved in using conventional trial-and-error isolation and characterisation techniques are significantly decreased by this technique [16,17].

*Corresponding author: Sumayya Khan, PhD Scholar, Faculty of Pharmacy, Department of Pharmacology, Maulana Azad University, Jodhpur, Rajasthan, India.

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Additionally, computational research offers a structural understanding of compound-target interactions, enabling logical lead compound optimization. Additionally, they facilitate the conversion of herbal metabolites into safe, efficient, and standardized medicinal medicines by assisting in the knowledge of toxicity profiles, mechanistic pathways, and ADMET properties [18,19].

This method improves the accuracy, effectiveness, and long-term viability of drug discovery based on natural products, encouraging the creation of novel therapies using conventional medicinal resources.

Several researchers have studied plant extracts in vitro with an in-silico approach, like C. ternatea leaf extract molecular docking study in receptor acbR protein (5ENR) associated with antibiotic resistance in E. coli [15], Antioxidant and Antifungal activities of leaf extracts of Ocimum basilicum by in silico in receptor human peroxiredoxin five enzyme (PDB ID: 1HD2) and secreted aspartic proteinase (PDB ID: 2QZX) [20]. Active phenolic compounds found in H. digitata extract have antioxidant and anti-inflammatory properties. Good binding and stability results with PDB IDs 4O1Z, 5F1A, and 3V92 have been demonstrated by isolated molecules [21]. Cinnamaldehyde dimethyl acetal, cinnamaldehyde, and α-copaene, the main compounds of Cinnamomum verum, show antioxidant, antibacterial, and antifungal activity, as performed in an in silico and in vitro study [22].

Existing literature outcomes are beneficial to design the present research work. Ethanol extracts of C.ternatea have been used for the study of phytochemical profiling with different analytical techniques like UV-Spectrometer, FT-IR, and GC-MS. The identified compounds of the extract by GC-MS have been further used for Molecular docking and ADMET study. Three different receptors have been used for three different pharmacological activities, which are antioxidants and anti-inflammatories. The in-silico studies' outcome has been validated by conducting in-vitro studies.

Reducing NF-κB activity decreases inflammation by lowering the expression of pro-inflammatory genes, while COX inhibition prevents prostaglandin production. These two mechanisms are linked: NF-κB can trigger COX-2 activity, and blocking COX-2 can also impact NF-κB activation. Together, these pathways reduce the production of inflammatory mediators like cytokines, chemokines, and prostaglandins, ultimately weakening the inflammatory response.

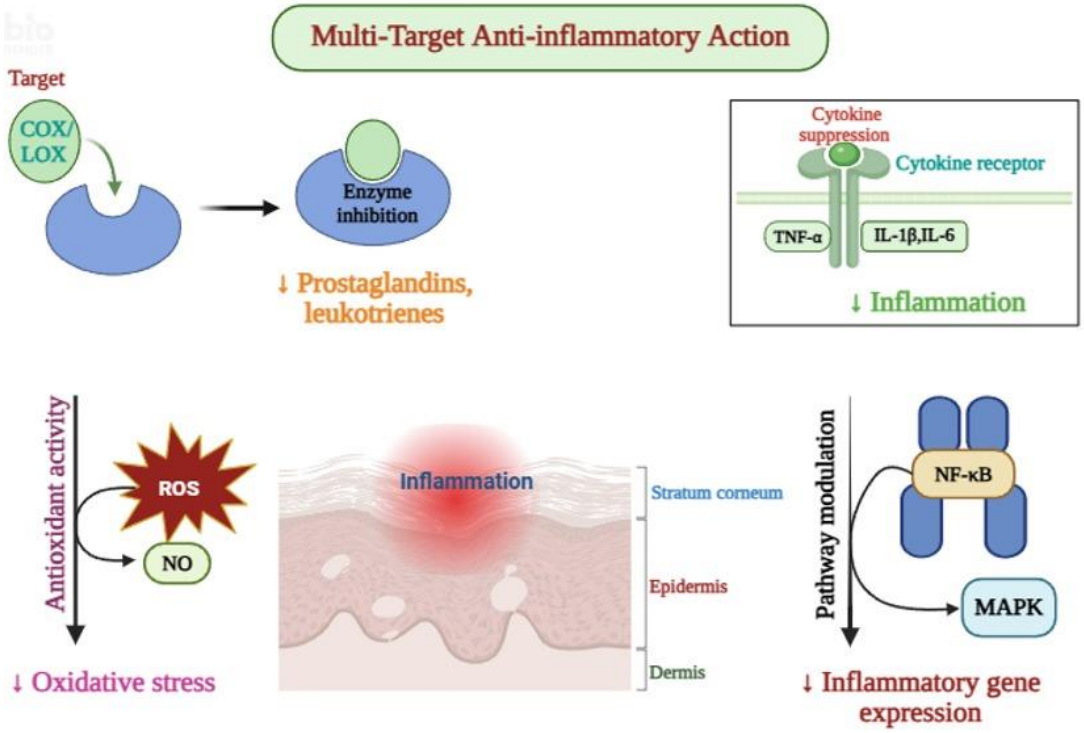


Figure 1: Anti-inflammatory action of *Clitorea ternatea.*

Materials and Methods

Extraction of dried leaves of *C. ternatea*

The Botanical Survey of India Arid Zone Regional Centre authenticated *C. ternatea* with registration number A.12012/Tech./2023-24(PI.I.) 540. Following this identification, the extraction process was initiated, commencing with preparing the plant material. The dried leaves of *C. ternatea* were subjected to grinding, resulting in a coarse powder.

Subsequently, 150 grams of the powdered material was placed in a Soxhlet apparatus, which was combined with 500 mL of ethanol as the solvent. The extraction procedure involved continuous hot percolation, comprising 9 to 10 cycles, for 24 hours at a controlled temperature ranging from 50 to 60 degrees Celsius. The successful completion of the extraction was indicated by a change in color from light green to a dark, resinous substance [23].

Qualitative analysis:

Chemical Tests: Phytochemical tests of ethanolic extract have been performed to identify the class of bioactive molecules [24].

GC-MS analysis of ethanolic extract of *C. ternatea* (White flower plant):

The instruments used are Shimadzu GC-MS (QP2010) gas chromatograph-mass spectrometers (Shimadzu, Milan, Italy) outfitted with Shimadzu auto-injector AOC-20, with solution software used to acquire the data.

Chromatographic conditions: A modified chromatographic condition was followed [25]. The injector was operated at 260 °C, and the oven temperature was programmed as follows: 60°C for 2 minutes, increasing the temperature at 10°C/min to 300°C, and then holding for 6 minutes. The column used Supelco wax (Merck, Darmstadt, Germany) with dimensions (30 mm x 250 µm i.d., 0.25 µm film thickness).

Forty-three different bioactive compounds were identified by GC-MS **(Figure 1).**

FT-IR: In an analysis involving the ethanolic extract of C. ternatea, the FT-IR spectra were examined within the infrared wavelength range of 400-4000 cm-1. The different functional groups are identified, which indicate the presence of other classes of compounds [26].

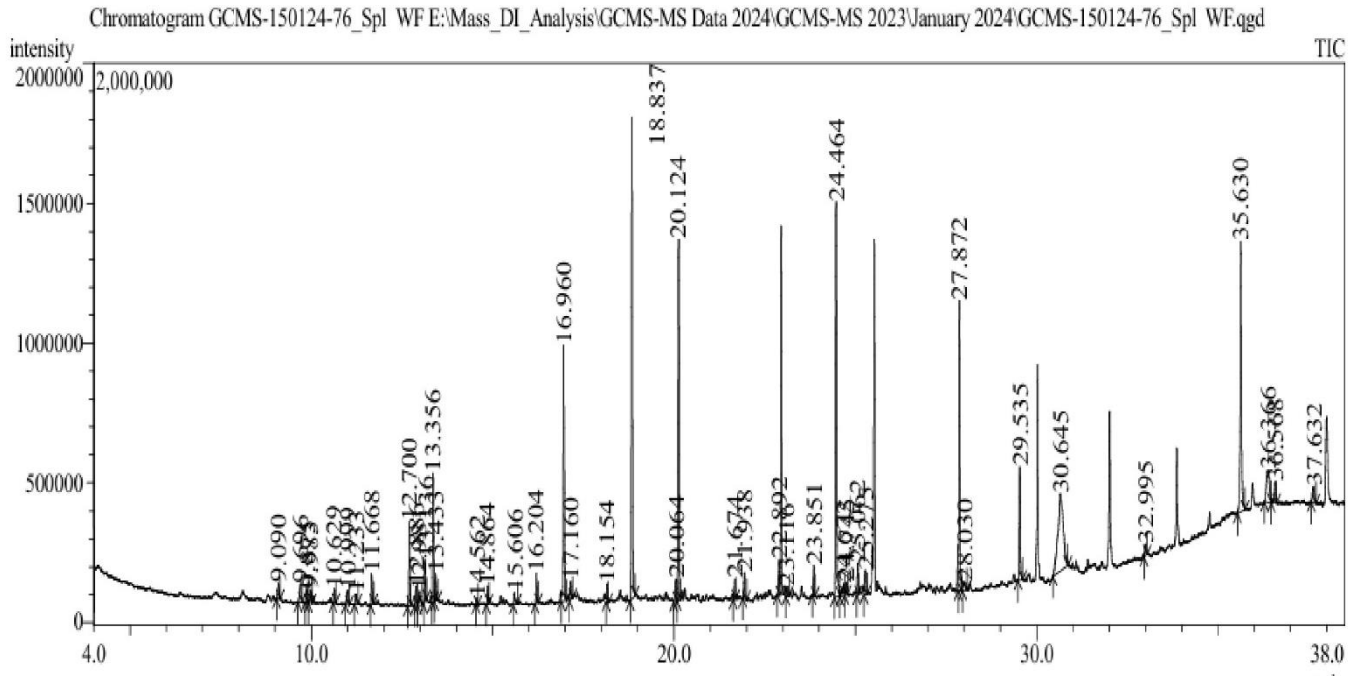


Figure 2: GC-MS spectra of *C. Ternatea* leaves extract.

Quantitative analysis [27,28]

Total Flavonoid content: The total flavonoid content was estimated using a colorimetric assay with quercetin as the standard (100–500 µg/ml) (n=3). A stock solution (100 µg/ml) was prepared, and 0.5 ml of each standard was mixed with 1.5 ml of methanol (95%), 0.2 ml of $AlCl_3$ (10%), 0.2 ml of potassium acetate (1 M), and 2.8 ml of distilled water. After 30 min incubation at room temperature, absorbance was measured at 420 nm. The blank contained water instead of $AlCl_3$.

For sample analysis, 200 mg of methanolic *C. ternatea* leaf extract was diluted in 25 mL of methanol. A 1 ml aliquot was processed as per the standard method, and absorbance was recorded at 420 nm after 30 min incubation. All analyses were performed in triplicate, and results were expressed as mg quercetin equivalents per g dry weight (mg QE/g DW).

Total phenolic content: The methanolic extract's total phenolic content was assessed using the Folin-Ciocalteu technique, with ferulic acid as a reference (4-32 µg/ml) (n=3). One milliliter of each standard or sample was combined with 10 ml of distilled water and 1.5 ml of Folin-Ciocalteu reagent, incubated for 5 minutes, and then 4 ml of 20% sodium carbonate was added, diluted to 25 ml, and left for 30 minutes. The absorbance was measured at 760 nm. For each sample, 100 mg of extract was dissolved in methanol and treated identically. The results were represented as mg ferulic acid equivalents (FAE) per 100 g dry weight, with all measurements taken in triplicate.

Determination of Terpenoids: 2 g of *C. ternatea* leaf extract were soaked in 25 ml methanol for 24 h, filtered, and partitioned with 10 ml petroleum ether in a separating funnel. The ether layer was collected in pre-weighed vials, dried completely, and the total terpenoid yield (%) was calculated from initial (wi) and final (wf) weights.

Total Terpenoid Content (%) = wi – wf / wi × 100

The methanolic extracts obtained from *C. ternatea leaves* were evaluated for the total terpenoid content.
Weight of dry extract = 0.07gm
Terpenoid content (%) = Initial weight of powdered flower−Wt. of the dry extract Initial /
wt. of powdered flower ×100
2.4 Molecular docking and ADMET:

Further, an in-silico study has been performed of the extract's GC-MS spectra for antioxidant and anti-inflammatory activites. Four significant steps were followed:

Protein Preparation and Receptor Grid Generation: Ascorbic acid drug binds with human serum albumin [PDB ID:3VLN], 6,7-Dimethyl-3-[(Methyl{2-[Methyl({1-[3-(Trifluoromethyl) Phenyl]-1h-Indol-3-Yl [PDB ID: 2AZ5], that were retrieved from Protein Data Bank (PDB).

Ligand Preparation: LigPrep produced it by utilizing the OPLS 5 force field for geometric minimization.

Ligand-Based Docking: The Ligand Docking Module in GLIDE was used for this.

MM-GBSA: To determine the relative binding energy of particular ligands, Prime/MM-GBSA was utilized [29,25].

The in silico ADME screening and drug-likeness assessment were conducted using the publicly available web application Swiss ADME (www.swissadme.ch), which the Swiss Institute of Bioinformatics created. Only the compounds with the highest binding energy scores were subjected to this screening stage. Atom counts, polar surface area (PSA), molecular weight (MW), and molecular refractivity (MR) were among the essential physicochemical characteristics that were computed. Additionally, the drug-likeness of the chosen compounds was evaluated using Lipinski's Rule of Five (RO5) [30]. The toxicity of bioactive molecules has been predicted by the freely available link Protox=3.0 Toxicity Prediction (https://tox.charite.de/protox3/index.php?site=compound_input)

***In vitro* study**

Antioxidant

Materials Required: DPPH solution (0.135 mM in methanol), Ascorbic acid (standard), methanol, and a double-beam UV-Visible spectrophotometer.

Preparation of Standard and Sample: Ascorbic acid solutions (50, 100, 150, 200, 250, and 300 µg/mL) and extract solutions at the same concentrations were prepared in methanol (n=3).

Assay Procedure: To each standard or extract solution, add 1mL of DPPH solution. The mixes were blended thoroughly before being incubated in the dark at room temperature for 30 minutes. A UV-Vis spectrophotometer was used to detect absorbance at 517 nm wavelength. The control included 1 mL of methanol and 1 mL of DPPH solution [30].

Calculation of Scavenging Activity (%):

$$\text{Inhibition (\%)} = (Ac\text{-}At/Ac) \times 100.$$

Where Ac is the absorbance of the control and At is the absorbance of the test or standard.

All tests were performed in triplicate. The IC_{50} value (concentration at which 50% inhibition occurs) was determined from the plot of % inhibition versus concentration.

Anti-inflammatory

Human red blood cell (HRBC) membrane stabilization method:

Sample Collection and Preparation: Blood was drawn from healthy individuals who hadn't used NSAIDs in at least two weeks. The mixture was combined with an equivalent volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, 0.42% NaCl in water) and centrifuged at 3000 rpm. The packed cells were washed three times with isosaline (0.85%, pH 7.4), and a 10% v/v HRBC solution was made.

Assay Procedure: The assay mixture (n=3) included 0.5 mL of extract or standard drug (Aspirin) at different doses (62.5, 125, 250, 500, and 1000 µg/mL), 1 mL phosphate buffer (0.15 M, pH 7.4), 2 mL hypotonic saline (0.36%), and 0.5 mL of HRBC suspension. In the control group, 2 mL of pure water was used instead of hypotonic saline solution [31].

All mixtures were incubated at 37 °C for 30 minutes and centrifuged. The haemoglobin content in the supernatant was measured at 560 nm using a UV-Vis spectrophotometer.

Calculations: % Haemolysis was calculated by considering the absorbance of the control (distilled water) as 100%. % Membrane Stabilization was determined using the formula

Percentage protection =

1- (Optical density of test/Optical density of control) x 100

All tests were performed in triplicate.

Statistical Analysis: Statistical analysis was performed using GraphPad Prism. Data are presented as mean ± standard error of the mean (SEM) and analyzed by one-way ANOVA, followed by the Newman–Keuls post hoc test.

a b c

Figure 3: (a) Dried leaves, (b) Powder, (c) Extract of *C. Ternatea* in the extraction step

Results

Extraction: In Figure 2, the leaves. Powder and extract are presented. Extraction has been successfully performed.

Qualitative analysis

Phytochemical tests: By performing chemical tests of the extract, the different classes of bioactive compounds are reported: Alkaloids (-), Carbohydrates (+), Flavonoids (+), Resin (+), Phenol (+), Glycosides (+), and Protein (+).

GC-MS analysis: In Table 1, identified bioactive molecules are presented with their chemical name, 2D structure, retention time, and % area. The highest % area (12.76) of 2,4-Di-tert-butylphenol at retention time 18.837 min., followed by (% 11.34) 7,9-ditert-butyl-1-oxaspiro [4.5] deca-6,9-diene-2,8-dione, at 24.464 min., (%11.16) 3- (tert. -Butyl dimethyl silyl) oxy-7,2',4'-trimethoxyflavone at 30.645 min., and (% 10.73) 3,6,13,16-tetraoxatricyclo [16.2.2.2(8,11)] tetracosa-8,10,18,20,21,23-hexaene-2,7,12,17-tetrone at 35.630 min., and then all remaining thirty-nine compounds are below ten % at different retention time over run time of 38 minutes are observed.

FT-IR: Spectrum of FT-IR (Figure 3), the of extract confirm the functional group by observed wave number, 3361.93, 3348.42 cm^{-1} (Alcohol O-H stretch), 1357.89 cm^{-1} (Alcohol O↓H bend), 1153.43,1044.34,926.13 cm^{-1} (Alcohol C-O(H) stretch), 2929.87 cm^{-1} (Alkanes C-H stretch), 1627.92 cm^{-1} (Alkenes C=C stretch), 1409. 96 cm^{-1} (Alkenes CH_2-(C=C) stretch). The presence of these functional groups confirms the class of compounds like carbohydrates, flavonoids, resin, phenol, glycosides, and protein.

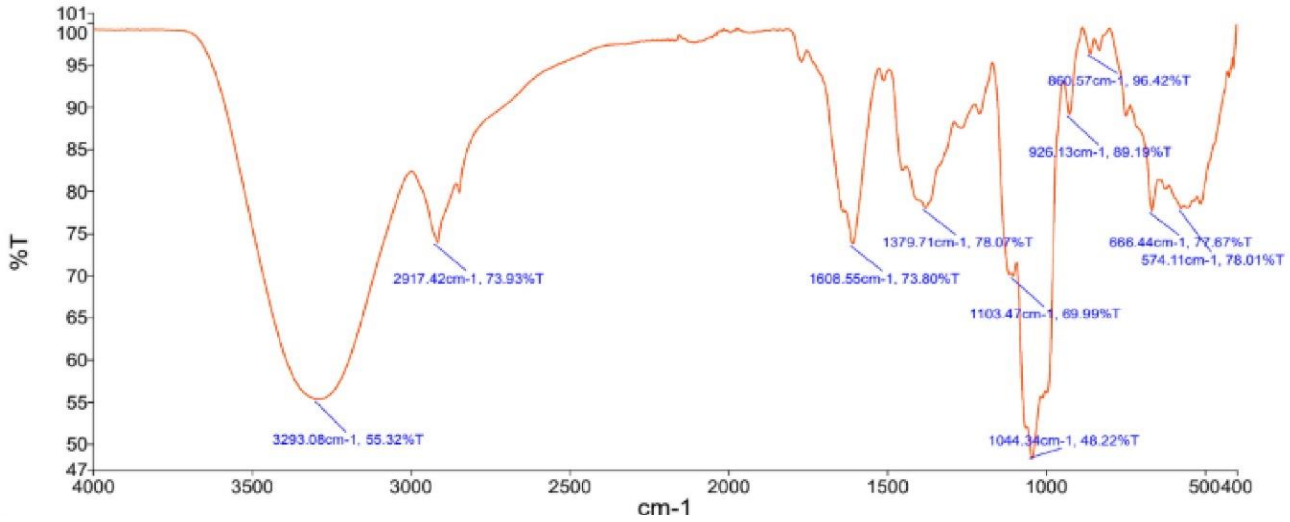
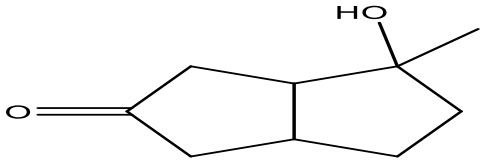
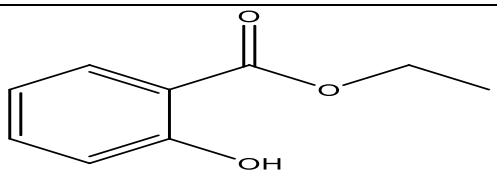
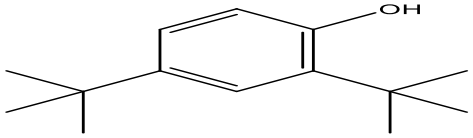
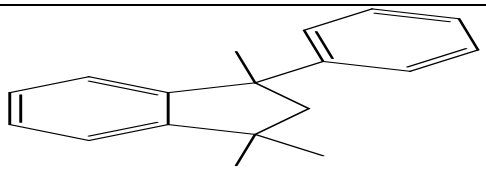
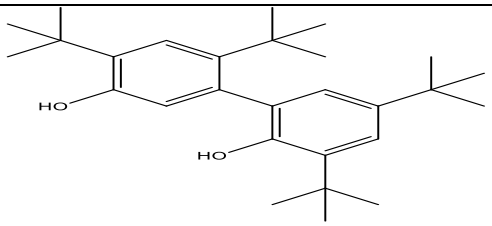
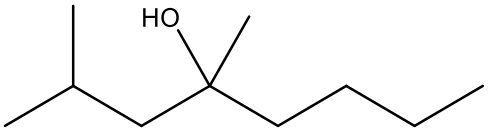


Figure 4: FT-IR of *C. Ternatea* leaves extract.

Table 1: Identified bioactive compounds chemical name, 2D structure, retention time and % arear by GCMS.

S. No.	Compound Name	2D Structure	Retention Time	% Area
1	Bicyclo[3.3.0]octan-3-one, 6-hydroxy-6-methyl-		14.562	0.13
2	Benzoic acid, 2-hydroxy-, ethyl ester		14.864	0.49

3	2,5-Furandione, dihydro-3-tetradecyl-		17.160	0.47
4	2,4-Di-tert-butylphenol		18.837	12.76
5	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-		21.938	0.71
6	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-		29.535	3.08
7	4-Octanol, 2,4-dimethyl-		11.668	0.85

Quantitative analysis

Flavonoid content: It has been calculated as Y=0.001427*X+0.02970, R2=0.9823. methanolic leaves extract of *C. ternatea* 0.0153 mg of Quercetin QUE /gm extract. (Figure 4 A)

Phenol content: It has been calculated as Y=0.002631*X+0.001730, R^2=0.9714. The results were expressed as Gallic acid equivalents (FAE), milligrams per 100 g of dry weight (dw) of methanolic leaves extract of C. ternatea, with a concentration of 0.0182 GAE/gm extract. (Figure 5 B).

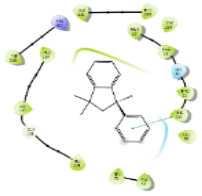
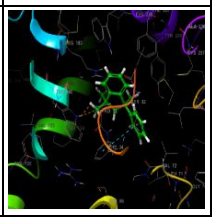
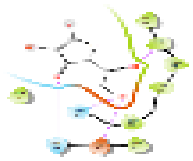
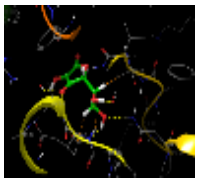
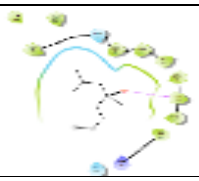
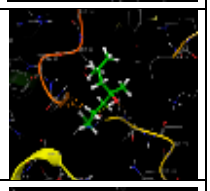
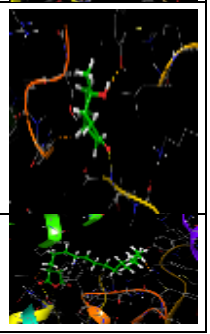
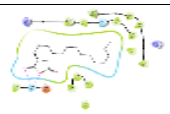
Determination of Terpenoids: The total terpenoid content in the methanolic leaf extract of ***C. ternatea*** was found to be 96.5%.

Molecular docking and ADMET:

Antioxidant: The identified compounds' antioxidant activity has been predicted with PDB ID: 3VLN. The dock score, dG bonding, and hydrogen bond interaction have been expected, and these values have been compared with those of ascorbic acid as a reference. Out of 43 molecules of *C. ternatea* leaves extract, 13 molecules have given dock scores, dG binding, 2D and 3D images, and hydrogen bonding interaction values (Table 2). All 13 molecules are collectively responsible for antioxidant activity. The ascorbic acid has good antioxidant potential, as predicted with this receptor. All 13 bioactive molecules, to varying degrees, exhibit antioxidant potential, similar to ascorbic acid.

Figure 5: Calibration curve for (a)Total Flavonoids Content (TFC) using Quercetin at different concentrations, (b) Total Phenolic Content (TPC), *Gallic acid at* different concentrations.

Table 2: Molecular docking and Hydrogen bond interaction of *C. ternatea* ethanolic leaves extract molecules with receptor PDB ID:3VLN

S. No.	Compound Name	Dock Score	dG Bind	2D Interaction	Distance (A^0)	2D Image	3D image
1	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	-7.213	-53.32	Pi-Pi Stacking: PHE34	5.43		
2	Reference Ascorbic Acid	-7.164	-30.71	H-bond:PRO73, ASN67, GLU85, SER86	2.05, 2.04,1.81, 1.83		
3	4-Octanol, 2,4-dimethyl-	-5.487	-45.10	H-bond: VAL72	1.88		
4	Bicyclo[3.3.0] octan-3-one, 6-hydroxy-6-methyl-	-5.371	-40.74	H-bond: TYR229, VAL72	1.82,2.14		
5	2,5-Furandione, dihydro-3-tetradecyl-	-4.277	-74.22	H-bond: SER86, SER86	1.99,2.07		

Anti-inflammatory: PDB ID: 2AZ5 has been used to predict the anti-inflammatory properties of the identified molecules in the extract. Predicted values for the hydrogen bond interaction, dG bonding, and dock score have been compared to the compound 6,7-Dimethyl-3-[(Methyl{2-[Methyl({1-[3-(Trifluoromethyl) Phenyl]-1h-Indol-3-Yl} Methyl) Amino] Ethyl} Amino) Methyl]-4h-Chromen-4-One as a reference. Thirteen of the forty-three molecules of *C. ternatea* leaf extract have provided dock scores, hydrogen bonding interaction values, dG binding, and two and three-dimensional pictures (Table 3). Together, the 13 molecules are responsible for the anti-inflammatory properties. As expected with this receptor, ascorbic acid has good anti-inflammatory activity. All 13 bioactive compounds can reduce inflammation.

In vitro study

Antioxidant: The extract exhibited a concentration-dependent increase in DPPH radical scavenging activity. The maximum inhibition observed at the highest concentration tested (300 µg/mL) was 39.15±0.62% (Figure 5). The IC_{50} value was calculated to be 171.35 µg/mL, indicating the potency of the antioxidant activity of the extract and ascorbic acid, IC_{50} = 120.81 µg/mL (Figure 6).

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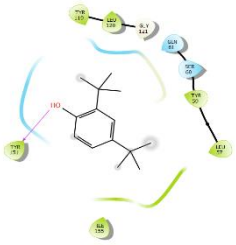
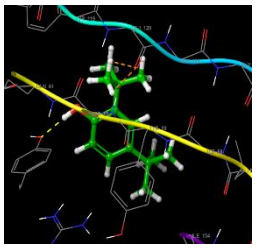
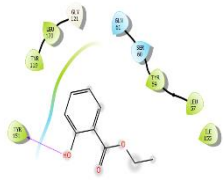
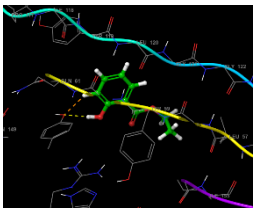
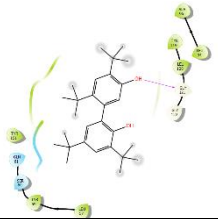
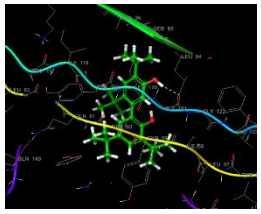
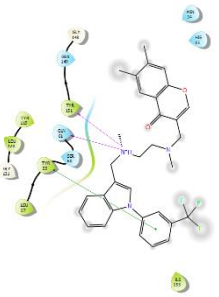
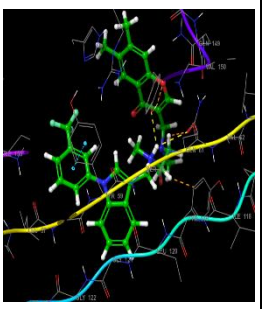
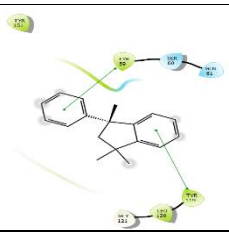
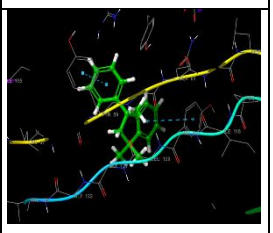
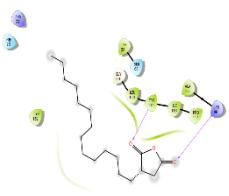
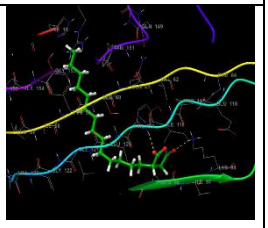
Table 3: Molecular docking and Hydrogen bond interaction of *C. ternatea* ethanolic leaves extract molecules with receptor PDB ID: 2AZ5.

S. No.	Compound name	Dock score	dG binding	2D Interaction	Distance (A^0)	2D Images	3D Images
1	2,4-Di-tert-butylphenol	-3.688	-42.88	H-bond: TYR151	1.86		
2	Benzoic acid, 2-hydroxy-, ethyl ester	-3.577	-34.12	H-bond: TYR151	2.00		
3	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	-3.478	-52.95	H-bond: GLY121	2.06		
4	Reference 6,7-Dimethyl-3-[(Methyl{2-[Methyl({1-[3-(Trifluoromethyl) Phenyl]-1h-Indol-3-Yl} Methyl) Amino] Ethyl} Amino)Methyl]-4h-Chromen-4-One	-3.184	-42.97	H-bond: TYR151, GLN61 Pi-Pi Stacking: TYR59	2.24,2.77 4.27		
5	1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-	-3.173	-36.34	Pi-Pi Stacking: TYR59, TYR119	4.39,4.26		
6	2,5-Furandione, dihydro-3-tetradecyl-	-3.040	-46.70	H-bond: TYR119, LYS98	2.14,2.26		

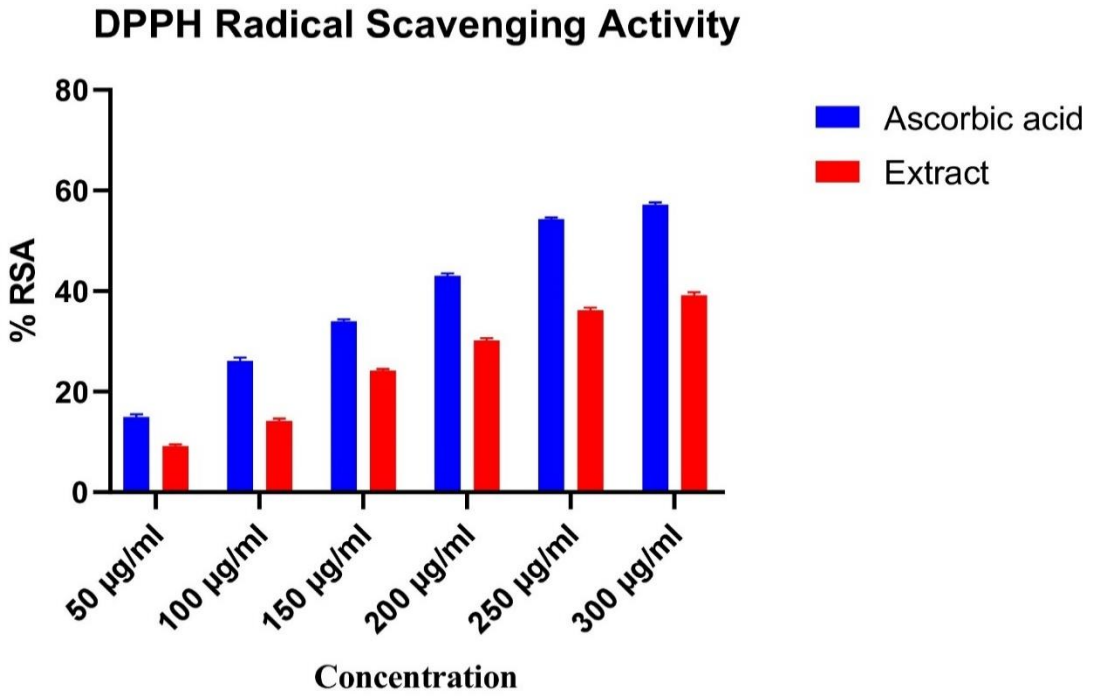


Figure 6: DPPH radical scavenging assay of extract, % RSA vs concentration of ascorbic acid and *C. Ternatea* leaves extract over concentration range (50-300 µg/ml).

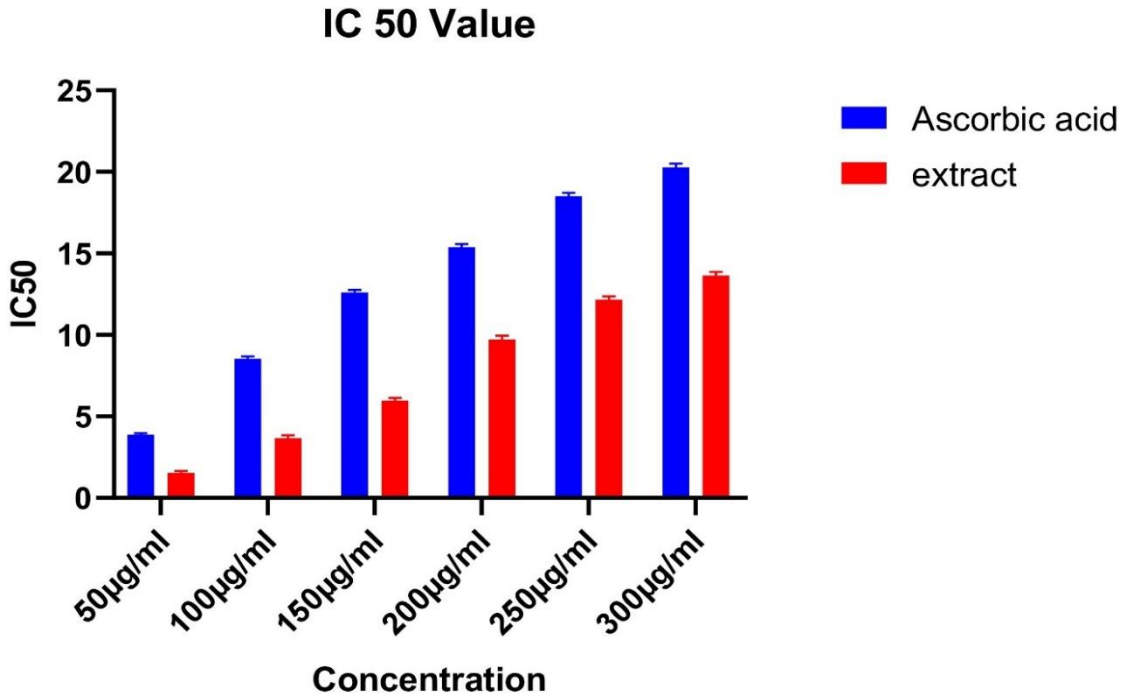


Figure 7: The IC_{50} value of *C. Ternatea* leaves extract is 171.35 µg/mL, and ascorbic acid, IC_{50} = 120.81 µg/mL.

Anti-inflammatory: The HRBC membrane stabilization assay results show that the extract has considerable anti-inflammatory properties in a dose-dependent manner. The membrane-protective activity confirms the extract's use in inflammatory diseases. % Protection at concentration 1000 µg/ml of extract (53.12±0.82 %) and, Aspirin (85.13±0.87%), Figure 8.

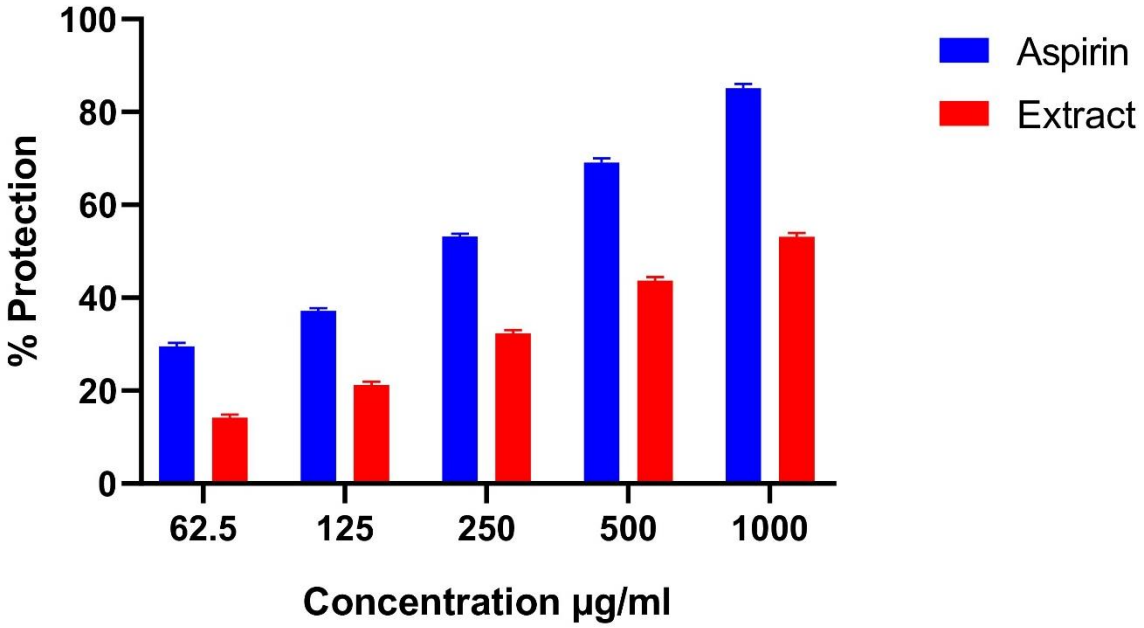


Figure 8: Comparative anti-inflammatory potency of *C. Ternatea* leaves extract and Aspirin by HRBC membrane stabilization assay.

Discussion

The present research work has been designed with existing literature to examine the antioxidant, anti-inflammatory, and antimicrobial therapeutic potentials with the approach of computational and in vitro study. Predicted therapeutic potentials have been validated with in vitro study results.

Antioxidant: ***C. ternatea*** ethanolic leaves extract has 43 molecules identified by GC-MS. Different classes of 43 molecules are present in the extract, but with Human Glutathione Transferase O1-1 **(**PDB ID:3VLN). 13 molecules are showing antioxidant potentials. Antioxidant activity is predicated on their capacity to neutralize free radicals, chelate metal ions, and prevent oxidative reactions. These requirements mostly pertain to the functional groups and molecular structure that have the redox behaviour of the molecule. In this extract, such a class of compounds is present. Chemical molecule 1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-in extract exhibits the highest dock score value (-7.213) & dG binding energy (-53.32), but the hydrogen bond distance is not favorable. The chemical structure of this molecule has affinity with the receptor but does not have a hydrogen bond interaction. 4-Octanol, 2,4-dimethyl- [32] dock score (-5.487) & dG binding (-53.32) has reported antioxidant potential; the presence of an alcoholic group is crucial for antioxidant activity. The hydrogen bond distance is 1.88 A^0 with amino acid residue VAL72. Bicyclo [3.3.0] octane derivatives have antioxidant potentials, as reported [33]. 2,5-Furandione, dihydro-3-tetradecyl, has shown promising results as an antioxidant in this study. Methyl salicylate [34,35], Benzoic acid, 2-hydroxy-, ethyl ester [36,37],3,3,4,4,4-Pentachlorobutan-2-one [38,39], l-Menthone [40,41], 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) [42,43], 1-Decanol, 2-hexyl-[44], 1-Dodecanol [45],1-Hexadecanol [46], Ethyl N-cyclohexylcarbamate[47].

The molecules identified in the extract exhibit antioxidant activity; either these bioactive compounds or their derivatives have been reported in earlier research. Almost all the molecules in the molecular docking study have predicted good dock score (-7.213 to -1.072), dG binding value (-74.22 to -35.43), hydrogen bond interaction with amino acid residue (PHE34, PRO73, ASN67, GLU85, SER86, VAL72, TYR229, ARG37, and GLU85) with more and less (2 A^0) hydrogen bond distance (Table 2). These predicted results are validated with the existing literature, as well.

Anti-inflammatory: Important chemical and structural parameters are evaluated to ascertain the anti-inflammatory properties of bioactive compounds found in plant extracts. The presence of specific functional groups, such as phenolic -OH, methoxy -OCH_3, carbonyl C=O, carboxylic -COOH, and amine -NH_2, helps reduce inflammatory mediators, including COX, LOX, cytokines, and ROS. Many different kinds of phytochemicals, including flavonoids, phenolic acids, alkaloids, terpenoids, saponins, tannins, and coumarins, are well known for their anti-inflammatory qualities. These drugs function by changing key signalling pathways (including NF-κB, COX-2, and iNOS), and their level of action is influenced by structure-activity relationships (SAR). Properties like conjugation,

hydroxylation, and methylation increase the efficacy of these compounds. [48,49]

C. ternatea ethanolic leaves extract has thirteen molecules that are collectively responsible for the anti-inflammatory activity as predicted in a molecular docking study. All molecules (Table 3) with PDBID: 2AZ5 have dock score in the range (-3.688 to -1.530), dG binding (-52.95 to -22.20), hydrogen bond interaction with amino acid residue (TYR151, GLY121, GLN61, TYR59, TYR119, LYS98, SER60, LEU120, GLN149, TYR59) with bond more or less 2 **A^0.** Less than 2 A^0 is favourable for the stability receptor.

2,4-Di-tert-butylphenol [50,51], Benzoic acid, 2-hydroxy-, ethyl ester [52], 2,5-Furandione, dihydro-3-tetradecyl- [53], Methyl salicylate[54], 4-Octanol, 2,4-dimethyl-[56], Ethyl N-cyclohexylcarbamate [57],3,3,4,4,4-Pentachlorobutan-2-one, l-Menthone[58], Phthalic acid, butyl undecyl ester [59], Dibutyl phthalate [60], and 1,2-Benzenedicarboxylic acid, butyl decyl ester [61], earlier researchers have reported ant inflammatory activity of these molecules in their literatures. The remaining molecules in Table 3, because of their functional group and structure, confirm the anti-inflammatory activity. The predicted anti-inflammatory activity by the molecular docking study could be validated with existing literature.

Conclusion

The current research effectively clarifies the medicinal value of *Clitoria ternatea* (white flower) ethanolic leaf extract study, combining phytochemical, computational, and in vitro techniques. The extract is rich in phytoconstituent profile. Quantitative tests found significant quantities of total flavonoids (reported as quercetin equivalents), total phenolics (as ferulic acid equivalents), and total terpenoids, demonstrating the plant's antioxidative potential.

GC-MS profiling revealed various pharmacologically active compounds, including fatty acids, terpenes, and phytosterols, many of which are known antioxidants, anti-inflammatories, and antibacterial agents. We conducted an in-silico study on target proteins related to oxidative stress (e.g., SOD, catalase), inflammation (e.g., COX-2, TNF-α), and microbial enzymes (e.g., DNA gyrase, β-lactamase).

Several phytochemicals had high binding affinities, indicating a function in influencing disease-related pathways. Additionally, ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling revealed excellent pharmacokinetic and safety profiles for the key bioactive.

The extract demonstrated strong in vitro antioxidant activity (DPPH scavenging), anti-inflammatory effects (HRBC membrane stability), and antibacterial effectiveness over selected bacterial strains, confirming the computational expectations. Together, these data indicate *Clitoria ternatea* ethanolic leaf extract's medicinal potential as a herbal source of multifunctional compounds. Further research, including formulation development and in vivo validation, is needed to investigate its use in phytopharmaceuticals or functional foods.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethical Approvals

This study does not involve experiments on animals or human subjects.

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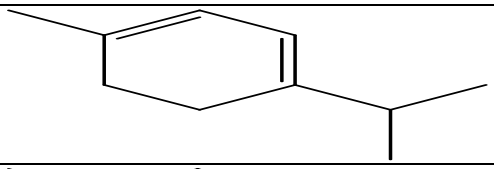
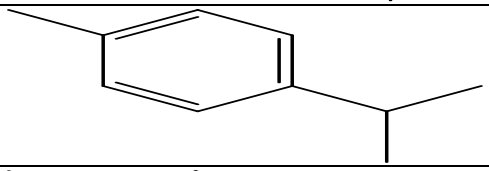
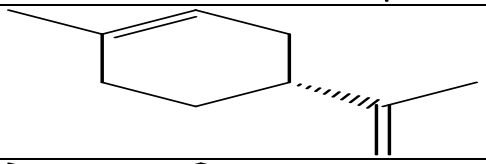
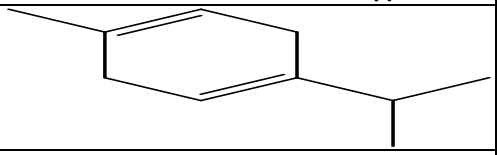
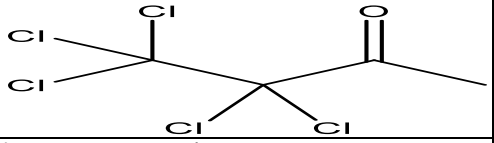
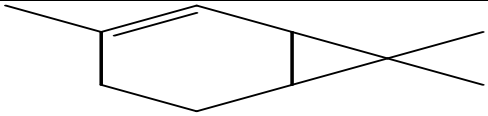
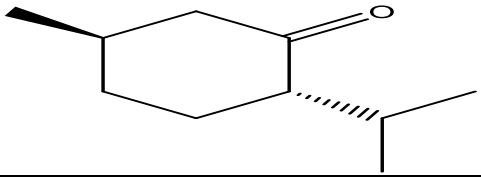
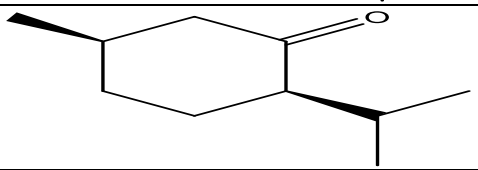
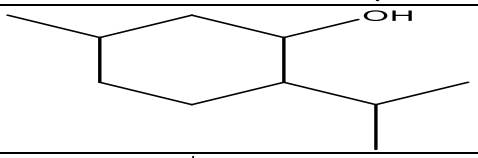
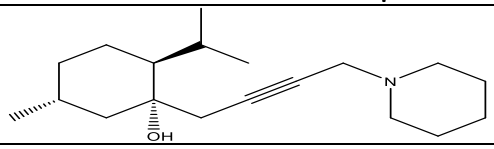
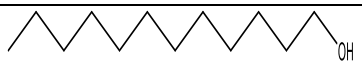
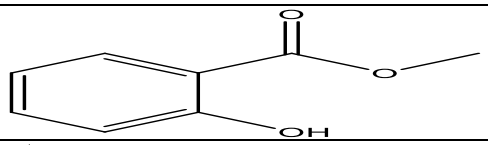
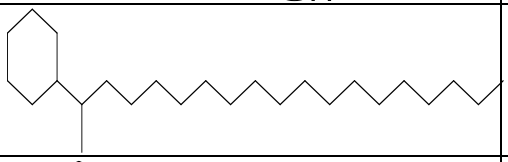
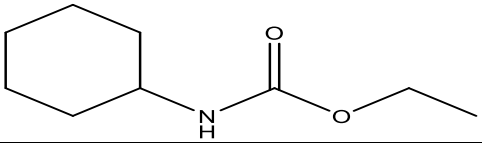
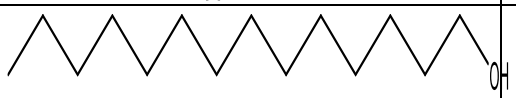
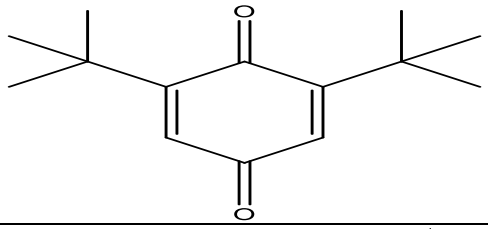
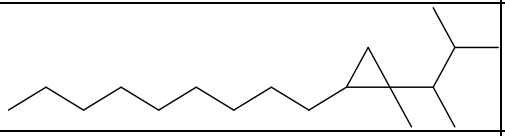
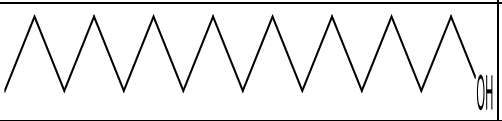
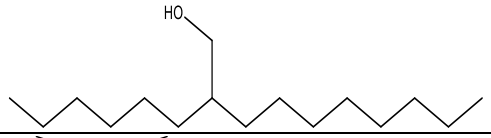
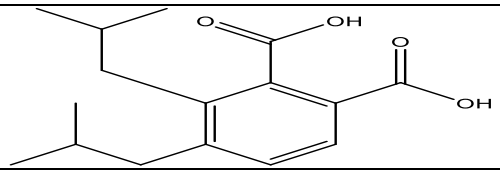
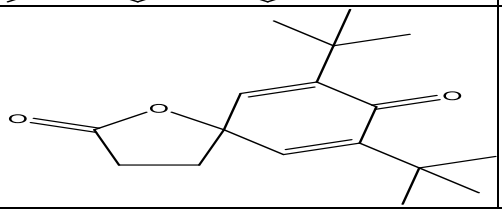
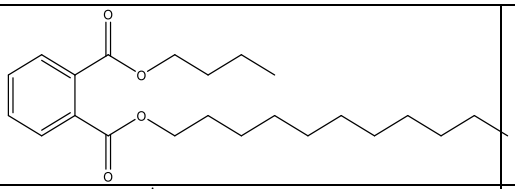
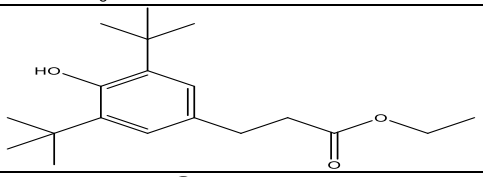
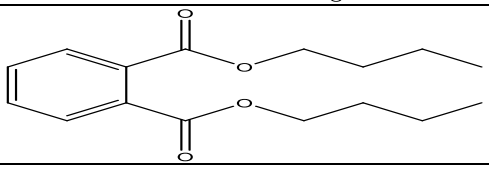
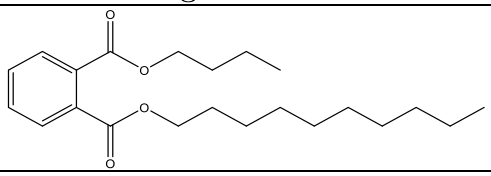
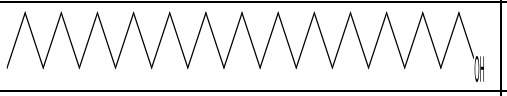
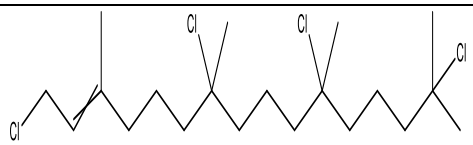
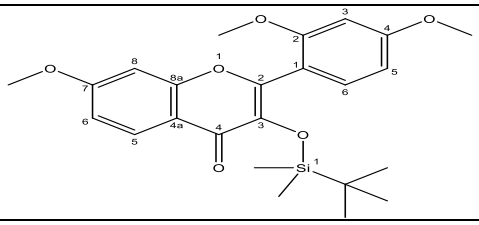
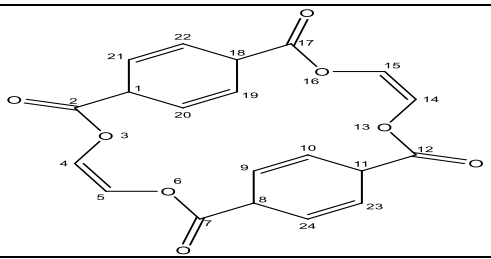
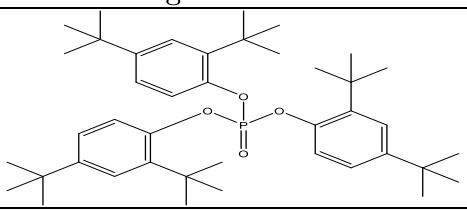
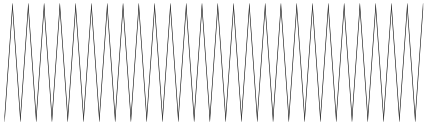
Supplementary Data

Table 1: Identified bioactive compounds chemical name, 2D structure, retention time and % arear by GCMS.

S.No.	Compound Name	2D Structure	Retention Time	% Area
1	1-Decene		9.090	0.67
2	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-		9.696	0.82
3	p-Cymene		9.875	0.51
4	D-Limonene		9.983	0.54
5	.gamma.-Terpinene		10.629	0.45
6	3,3,4,4,4-Pentachlorobutan-2-one		10.999	0.56
7	2-Carene		11.233	0.30
8	l-Menthone		12.700	2.11
9	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)-		12.886	0.46
10	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.alpha.,5.beta.)]-		12.981	0.59
11	Cyclohexanol, 5-methyl-2-(1-methylethyl)-1-(4-(1-piperidinyl)-2-butynyl)-, (1-alpha,2-alpha,5-beta)-		13.136	1.12

12	1-Dodecanol		13.356	3.17
13	Methyl salicylate		13.433	0.79
14	2-Cyclohexylnonadecane		15.606	0.33
15	Ethyl N-cyclohexylcarbamate		16.204	0.81
16	1-Tetradecanol		16.960	6.46
17	2,5-Cyclohexadien-1,4-dione, 2,6-bis(1,1-dimethylethyl)-		18.154	0.47
18	Cyclopropane, 1-(1,2-dimethylpropyl)-1-methyl-2-nonyl-		20.064	1.00
19	1-Hexadecanol		20.124	9.03
20	Eicosane		21.674	0.58
21	1-Nonadecene		22.892	1.06
22	1-Decanol, 2-hexyl-		23.116	0.38
23	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)		23.851	0.74
24	7,9-ditert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione		24.464	11.34

25	Phthalic acid, butyl undecyl ester		24.672	0.58
26	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, ethyl ester		24.745	0.37
27	Dibutyl phthalate		25.062	0.82
28	1,2-Benzenedicarboxylic acid, butyl decyl ester		25.275	0.92
29	1-Hexacosanol		27.872	7.74
30	3,7,11,15-Tetramethylhexadec-2-ene		28.030	0.45
31	3-(tert.-Butyldimethylsilyl)oxy-7,2',4'-trimethoxyflavone		30.645	11.16
32	Tetrapentacontane		32.995	0.36
33	3,6,13,16-tetraoxatricyclo[16.2.2.2(8,11)]tetracosa-8,10,18,20,21,23-hexaene-2,7,12,17-tetrone		35.630	10.73
34	Tris(2,4-di-tert-butylphenyl) phosphate		36.366	2.45
35	Tetrapentacontane		36.568	0.80

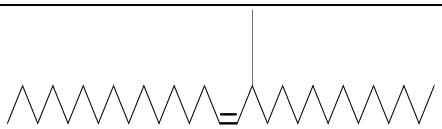
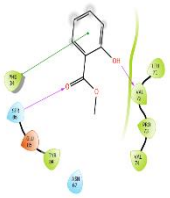
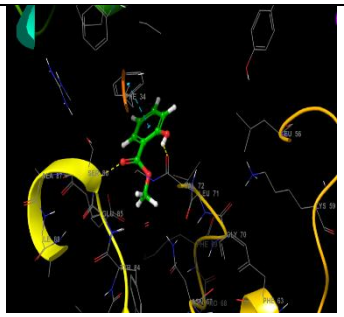
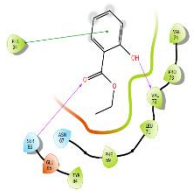
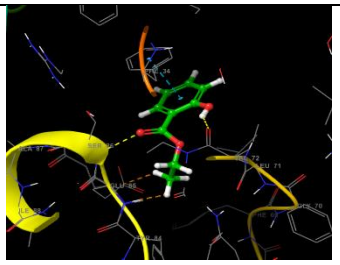
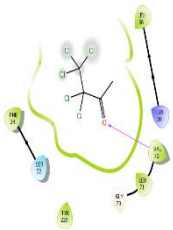
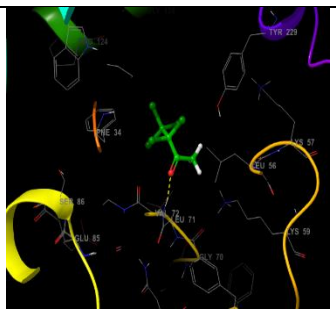
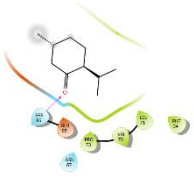
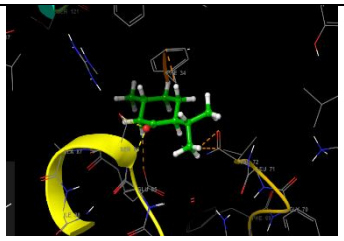
36	13-Methyl-Z-14-nonacosene		37.632	0.83

Table 2: Molecular docking and Hydrogen bond interaction of *C. ternatea* ethanolic leaves extract molecules with receptor PDB ID:3VLN.

S. No.	Compound Name	Dock Score	dG Bind	2D Interaction	Distance (A^0)	2D Image	3D image
1	Methyl salicylate	-4.119	-35.43	H-bond: SER86, VAL72 Pi-Pi Stacking: PHE34	2.15,1.98 3.75		
2	Benzoic acid, 2-hydroxy-, ethyl ester	-4.030	-39.77	H-bond: SER86, VAL72 Pi-Pi Stacking: PHE34	2.19,2.01 3.64		
3	3,3,4,4,4-Pentachlorobutan-2-one	-3.943	-44.54	H-bond: VAL72	2.08		
4	l-Menthone	-3.872	-44.32	H-bond: SER86	1.96		

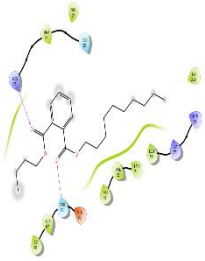
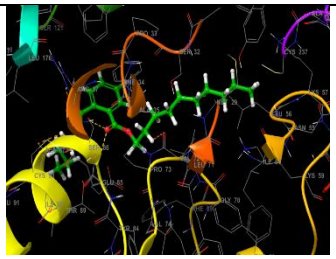
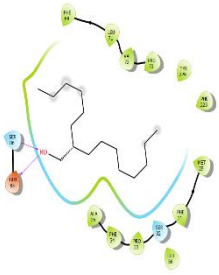
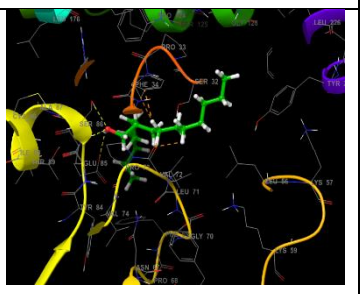
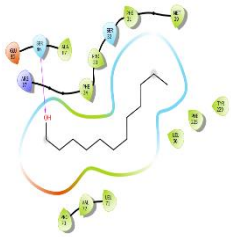
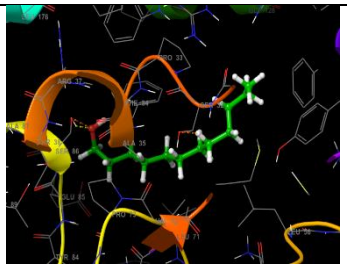
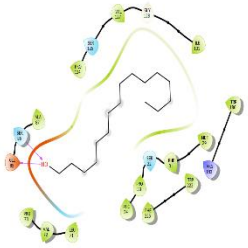
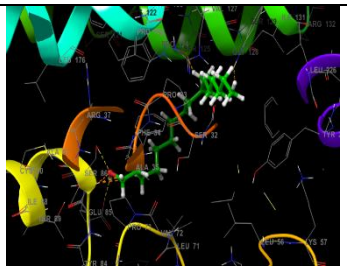
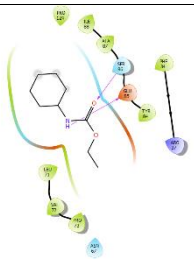
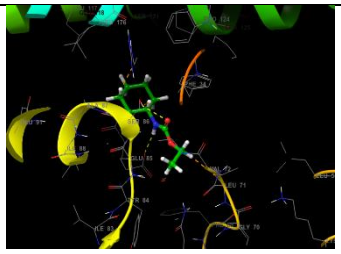
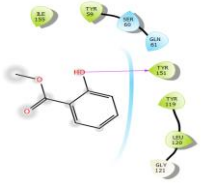
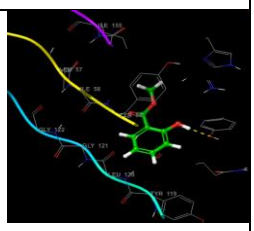
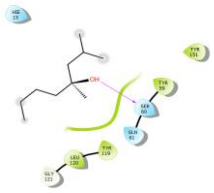
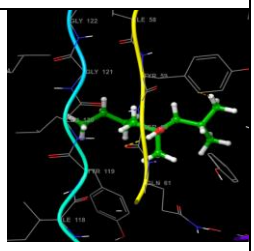
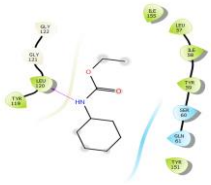
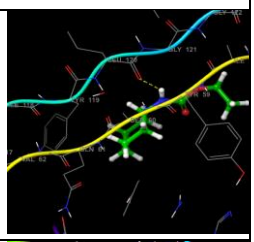
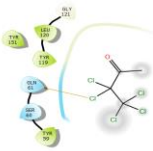
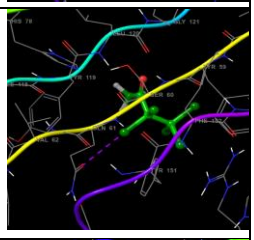
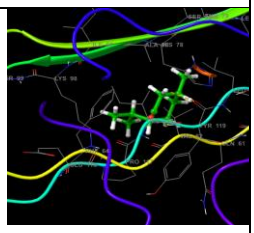
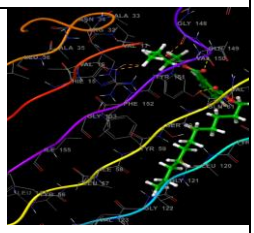
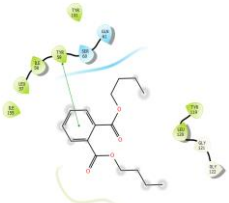
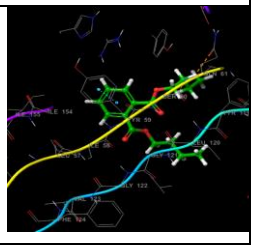
5	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)	-2.842	-48.49	H-bond: SER86, ARG37	2.22,1.99		
6	1-Decanol, 2-hexyl-	-2.797	-65.96	H-bond: SER86, GLU85	1.87,2.73		
7	1-Dodecanol	-2.299	-67.00	H-bond: SER86	1.80		
8	1-Hexadecanol	-1.977	-70.03	H-bond: SER86, GLU85	2.37,2.28		
9	Ethyl N-cyclohexylcarbamate	-1.072	-43.06	H-bond: SER86, GLU85	2.41,1.86		

Table 3: Molecular docking and Hydrogen bond interaction of *C. ternatea* ethanolic leaves extract molecules with receptor PDB ID: 2AZ5.

S. No.	Compound name	Dock score	dG binding	2D Interaction	Distance (A^0)	2 D Images	3D Images
1	Methyl salicylate	-3.021	-27.90	H-bond: TYR151	2.00		
2	4-Octanol, 2,4-dimethyl-	-2.570	-36.77	H-bond: SER60	2.07		
3	Ethyl N-cyclohexylcarbamate	-2.062	-37.05	H-bond: LEU120	2.17		
4	3,3,4,4,4-Pentachlorobutan-2-one	-1.735	-34.30	Halogen bond: GLN61	3.36		
5	l-Menthone	-1.635	-22.20	H-bond: TYR119	1.97		
6	Phthalic acid, butyl undecyl ester	-1.542	-51.15	H-bond: TYR151, GLN149	1.99,2.05		
7	Dibutyl phthalate	-1.530	-40.94	Pi-Pi Stacking: TYR59	4.35		

8	1,2-Benzenedicarboxylic acid, butyl decyl ester	-0.579	-34.34	H-bond: TYR151, GLN149	2.52,2.00