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Comparative Phytochemical Profiling and Study on Biological Activities of Leaves, Seeds, and Stem of *Clitoria ternatea*

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The methanolic extract of seed, leaf, and stem of *C. ternatea* was prepared and subsequently subjected to phytochemical screening through GC-MS analysis. GC-MS analysis of *Clitoria ternatea* extracts from leaves, stems, and seeds revealed presence of several phytocompounds with reported biological or pharmacological activity. Major metabolites identified include mome inositol, fatty acids, phosphoric acid, triphenyl ester, 5-Hydroxymethylfurfural (5-HMF), and furaneol. Seed extract was reported to possess maximum flavonoid and phenolic content along with highest DPPH scavenging activity. Maximum anti-inflammatory potential was exhibited by seed extract followed by leaf and stem. Protein denaturation efficiency of seed and leaf extract was found to higher compared to standard acetylsalicylic acid. Leaf and seed extract exhibited antimicrobial potential against *Lysinibacillus fusiformis* and *Bacillus altitudinus.* Interpretation from comparative phytochemical analysis of leaves, stem and seed of *C. ternatea* depict the potential of the plant to be utilized as source of natural antioxidant, anti-inflammatory and antimicrobial compound. The insilico study conducted. Molecular docking simulations of phytochemicals derived from extracts revealed that myo-inositol and 5-hydroxymethylfurfural (HMF) bind to AGR2 (PDB: 2LNS), IL-6 (PDB: 1ALU), and Tpp49Aa1 (PDB: 8BEZ) with binding free energies (ΔG) ranging from -6.2 to -8.1 kcal/mol. Myo-inositol exhibited stronger affinities (-7.1 to -8.1 kcal/mol) due to multiple hydrogen bonds, and HMF showed -6.2 to -7.4 kcal/mol driven by hydrophobic and π-interactions. Key interactions included polar contacts for myo-inositol with Asp406, Lys408, and Glu409 in 8BEZ and hydrophobic clustering for both ligands with Met73, Leu100, and Leu168 in 2LNS.

Keywords: Clitoria ternatea, *in-vitro* studies, GC-MS analysis, Mome inositol, therapeutics

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Herbal medicines and their metabolites are utilized as a solution for many health concerns. Medicinal plants possess numerous therapeutic and medicinal properties which have been explored in traditional and as well as modern system of medicine [1]. *Clitoria ternatea* is one such medicinal plant commonly known as butterfly pea exhibits diverse array of pharmacological effect, including analgesic, anti-diabetic, hypolipidemic, antipyretic, CNS disorders, gastrointestinal disorders, anti-parasitic, and insecticidal activities [2, 3]. Different parts of *C. ternatea* have been utilized in traditional medicine and ayurvedic ailments for diuretic and cathartic properties as well as a brain tonic in enhancing memory and cognitive functions [4, 5].

Previous studies has been reported that compounds belonging to diverse organic classes possess specific biological activities [6]. Seeds as well as leaves of *C. ternatea* constitutes different classes of metabolites including the phytol, terpenoids, phenolic derivatives and flavonoid compounds [7]. Leaves of the plant possess a wide range of bioactive compounds represented by alkaloids, flavonoids, steroids, and glycosides, offering benefits against neurodegenerative diseases, diabetes mellitus, and excessive sweating [8]. High phenolic and flavonoid content in leaves and flowers support their medicinal potential including anti-oxidant activity [9]. Jeyaraj et al. [10] reported the anthocyanins presence and associated the same with antioxidant, anti-microbial, and cytotoxic potential. Studies found that the extraction of plant parts at 50°C for 1-hour offers provide effective stability of anthocyanin extraction for better bio-activity [11]. Beside medicinal and industrial importance, *C. ternatea* has been also recognized as a high-quality nutritive fodder [12]. *C. ternatea* also considered to be a potential source of natural food additives in form of stable colorants and preservatives which can be explored for diverse applications in food industries [13].

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Traditional utilization of *C. ternatea* root is attributed to its laxative, purgative, diuretic, anti-inflammatory activity, and anthelmintic. Other application of *C. ternatea* in traditional medicine include utilization in treatment of bronchitis, asthma, fever, inducing abortion, treating abdominal swelling, mucous disorders [14]. Jain et al. [15] conducted TLC analysis using and reported presence of constituents such as essential oils, bitter principles, flavonoids, and phytosterols in *C. ternatea*. *C. ternatea* seeds has been reported to possess sitosterols, fatty acids, anthocyanins, and mucilage with specific utilization seeds for treatment of digestive disorders, cough, hepatic issues, rheumatic infections, purgative effects, also when combined with ginger powder aligns with the treatment of abdominal ailments and arthritis [16]. The current study works on comparative analysis of phytoconstituents, anti-inflammatory and anti-microbial potential of seed, leaves and stem extract of *C. ternatea* and their in-silico validation of the bio activities.

EXPERIMENTAL

Plant Collection and Extract Preparation

C. ternatea (blue-flower) plants were collected from Jadi Buti Farm, Kohlupani, Uttarakhand, India (30°20'44.1"N 77°57'28.8"E). The collected plant parts were thoroughly washed with tap water, dried and grounded. The processed leaf, stem and seed samples (10 g) was added to methanol at a ratio of 1:10 for soxhlet extraction. The extraction was accomplished at 45°C.

The resultant extracts were then analyzed using gas chromatography-mass spectrometry (GC-MS) to identify and quantify the diverse secondary metabolites present.

Total Flavonoid Content (TFC) Determination

Flavonoid contents of the extracts were determined through $AlCl_2$ colorimetric method [17]. 100 µL aliquot of plant extracts was mixed with 0.15 mL each of 5% sodium nitrite and 10% aluminum chloride in a 5 mL tube, incubated for 5 minutes, then treated with 1 M NaOH, adjusted to 5 mL with distilled water, and measured for absorbance at 510 nm after colour development using a spectrophotometer. A quercetin calibration curve determined the total flavonoid content (TFC) as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW) with the formula TFC = (c × v) / m, where c is the flavonoid content (mg QE/mL), v is the solution volume (mL), and m is the sample weight (g DW).

Total Phenolic Content (TPC) Determination

TPC of all prepared extracts was determined through Folin–Ciocalteau method [18]. 100 µL aliquot of plant extracts was mixed with 450 µL of distilled water in a 5 mL falcon tube, combined with 250 µL of Folin-Ciocalteu reagent to form a blue complex, then treated with 2 mL of 7.5% sodium carbonate, incubated for 90 minutes at room temperature, and measured for absorbance at 765 nm using a UV-Vis spectrophotometer. A gallic acid calibration curve determined the total phenolic content (TPC) as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) with the formula TPC = (c × v) / m, where c is the phenolic content (mg GAE/mL), v is the solution volume (mL), and m is the sample weight (g DW).

DPPH-radical Scavenging Assay

Extract samples (500 µL) at concentrations of 25–100 µg/mL were mixed with 0.005% w/v DPPH in methanol (total 1 mL), using a methanol blank as control and ascorbic acid/BHT as positive controls; after 30-minute dark incubation, absorbance was measured at 517 nm [19]. The DPPH scavenging effect (%) was calculated using the formula:

$$\text{DPPH radical scavenging percentage} = \frac{Control\ (Ao) - Sample(A1)}{Control\ (Ao)} \times 100$$

Protein Denaturation Assay

Extracts and acetylsalicylic acid (500 µL each, 25–100 µg/mL) were mixed with 1 mL of 1 mM BSA, incubated at 27 ± 1°C for 15 minutes, heated at 70°C for 10 minutes to induce denaturation, cooled, and turbidity measured at 660 nm [20].

$$\text{Denaturation percentage} = \frac{Control\ (Ao) - Sample(A1)}{Control\ (Ao)} \times 100$$

Agar Diffusion Assay

Assays were conducted on 2 mm-deep Mueller Hinton Agar plates, with 2 mm deep × 4 mm diameter wells filled with 50 µL test sample, incubated at 35°C for 24 hours; inhibition zones were measured in mm, with triplicate replicates for error calculation [21].

RESULTS AND DISCUSSION

Comparative Analysis of GC Spectral Data

Analysis of GC-MS results depict that extracts prepared from leaf, stem and seed of *Clitoria ternatea* possess wide range of secondary metabolites and exhibit distinct bioactive properties (**Table 1**).

Leaf, root, stem and seeds extracts were found to contain 47, 41, 45 and 51 phytocompounds respectively. Figure 01 illustrate a comparative study of the various fraction of major compounds including mome inositol, fatty acids, phosphoric acid, Phosphoric acid triphenyl ester (PATE), 5-Hydroxymethylfurfural (5-HMF), furaneol (except in leaf extract), fatty acid methyl esters and vitamin E derivatives found in extracts. The chemical composition of Mome Inositol occupies the major proportion of the graph revealing its distinct presence on *C. ternatea*. Mome-inositol comprises the highest percentage in stem extract (89.52%) and minimum in seed extracts (53.43%). Fatty acids, Phosphoric acid triphenyl ester (PATE), and furaneol are present in minimal composition in all extracts, with slightly higher proportions in the seed with area percentages of 1.50%, 0.22%, and 0.30% respectively. Notably, FA-methyl esters constitute a substantial fraction of the seed composition (10.46%), and 5-Hydroxymethylfurfural (5-HMF) was prominent in stem extract great percentage (4.92%). Mome inositol is a potent carbocyclic polysaccharide which generally possess anti-cirrhotic and anti-neuropathic properties [22]. Previous studies have also reported mome inositol as a major fraction in the extracts of *C. ternatea.* Neda et al. [23] reported the presence of mome inositol (38.57%) in aqueous extract of *C. ternatea* and exhibited anti- proliferation activity in breast cancer, human ovary cancer, liver cancer and fibroblast cell lines. Similarly in another study, Mathi et al. [24] reported that the mome-inositol which found to be only in methanolic leaf extract of *Sophora interrupta* with around 80 % area exhibited anti- cancer activity in MCF-7 and PC-3 cell lines. Extracts of *Macrotyloma uniflorum*, *Clitoria ternatea* and *Corbichona decumbens* have also been reported to possess mome inositol [26,27]. Higher percentage of fatty acids and fatty acid methyl esters found to be present in seed extract with major FAME compounds found to be in seed extract include methyl palmitate, 9, 12 octadecadienoic acid (Z, Z), methyl ester, 9-octadecanoic acid, methyl ester, and methyl stearate. Among these methyl stearate and methyl palmitate have been reported to exhibit several biological activities [28,29]. Santos et al. [30] also reported that methyl palmitate, oleate, linoleate, linolenate and stearate present in organic solvent extract of medicinal plants possess anti-oxidant and cytotoxic activities. In another study, Hagr et al. (2019) reported anti-microbial efficiency of seed oil extracted from *Annona squmosa* which contains 9, 12 octadecadienoic acid (Z, Z), methyl ester, 9-octadecanoic acid, methyl ester and methyl stearate. Another compound commonly found in the extract is 5-Hydroxymrthylfurfural (5-HMF) and greater fraction present to be in stem extract (4.92%). 5-HMF is generally produced by the thermal decomposition of the polysaccharides present in the extract and exhibit diverse therapeutic activities. 5-HMF exhibit significant response against oxidative stress with high anti-oxidant defences even at relatively low concentrations [31]. Li et al. [32] reported that 5-HMF to have profound ameliorative effect on liver oxidative injury in mice by preventing hepatocyte apoptosis. The study suggested that 5-HMF have impact in pro-inflammatory responses and anti-oxidant activities. 5-HMF also exhibited anti-inflammatory responses through the suppressing MAPK, NF-kB and mTOR activation in inflammatory stimulated cells [33]. Anti-cancer effect of 5-HMF has been observed in prostate cancer cell and murine macrophage cells with high concentration of 100 µg/ml [34]. Seed extracts of *C. ternatea* contain greater proportion of furaneol which is a natural flavour giving compound. Earlier studies reported that aromatic medicinal plant and the seed extracts from the plant has the presence of furaneol [35]. Woo-Sang & Hyun-Junese [36] reported that furaneol possess anti-microbial and anti-fungal properties against gram-positive, gram-negative and *Candida* species. Vitamin E (alpha-tocopherol) exhibit crucial role in maintaining cellular balance by managing antioxidant levels against harmful compounds generated by mitochondrial activity and the immune system. Its functions include regulating platelet aggregation, cellular signalling, and providing antioxidative effects, making it vital in addressing oxidative stress-related chronic illnesses [37].

Table 1. The major compounds present in the extracts with their chemical and therapeutic properties.

Sl No	Name of compound	Sample	Retention time	Nature of compound	Molecular weight	Therapeutic Properties	References
1	Guanosine	Leaf	19.95	Nucleoside	283.24 g/mol	Neuroprotective	[38]
2	Methyl Commate B	Leaf	31.79	Terpenoid	470.71 g/mol	Anti-inflammatory, Anti-microbial, Anti-oxidant	[39-41]
3	Estragole	Leaf	19.93	Phenyl propene	148.18 g/mol	Anti-edematogenic, Anti-cancer	[42,43]
4	Phytol	Leaf	20.42	Diterpene alchohol	296.54 g/mol	Antinociceptive, anti-oxidant, anti-microbial	[44]
5	Betulinaldehyde	Seed	31.79	Triterpinoid	152.24 g/mol	Anti-tumor	[45]
6	2,3-Dihydro-Benzofuran	Seed	9.79	Furan	120.14 g/mol	Anti-dopaminergic, Fungicidal	[46]
7	Piperazine, 1,4-Dimethyl-	Seed	7.033	Piperazine	114.18 g/mol	HIV-1 inhibitor	[47]
8	Dimantine	Stem	19.892	Amine	297.554 g/mol	Intenstinal parasitosis	[48]
9	1-Monopalmitin	Stem	23.031	Glyceride	474.86 g/mol	Anti-tumor	[49]

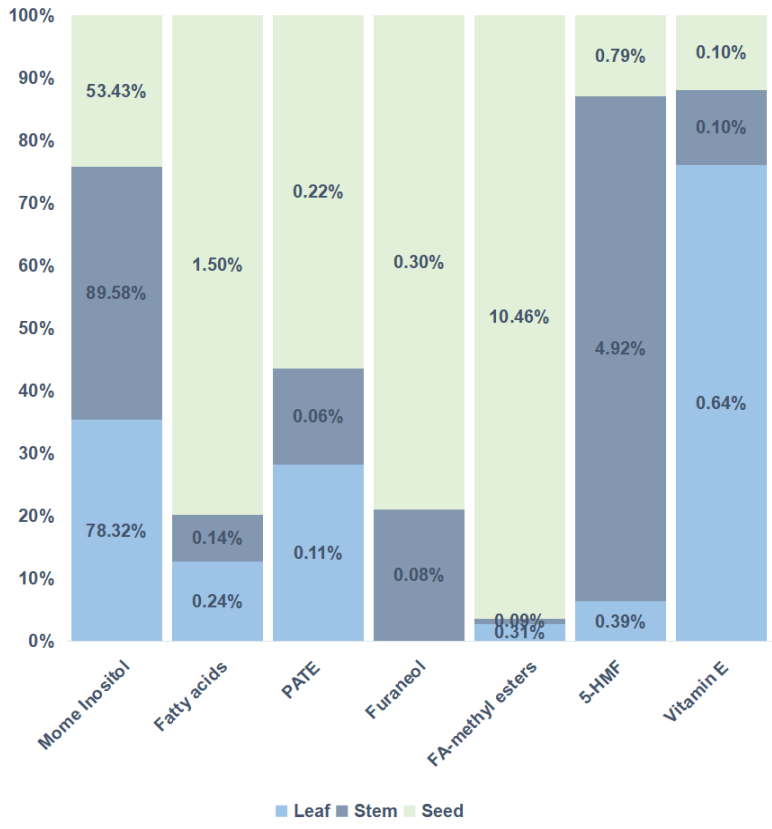

Figure 1. Percentage composition of the major phytocompounds present in the leaf, stem and seed extract of *C. ternatea*.

Estimation of Flavonoid & Phenolic Content, and DPPH Radical Scavenging Activity of Seed, Leaf, and Stem of C. ternatea

Phenolic compounds and flavonoids have been identified to play a vital role in plant response to environmental stress. The correlation between flavonoid content and antioxidant activity has emphasized the significance of flavonoids in mitigating oxidative stress in higher plants (Agati et al., 2012). In the present study total flavonoid content (TFC) in *C. ternatea* was calculated from linear equation y = 0.062x + 0.0158 in calibration curve of quercetin ranging concentration from (20-100 µg/ml). Considerable variation in TFC content of seed, leaf and stem was observed with highest TFC of 112.98 mg/g of dry weight found in seed followed by 76.65 mg/g of dry weight of leaf and 47.05 mg/g of dry weight of stem. The correlation coefficient between TFC and anti-oxidant activity against DPPH were calculated from the graph with a value of 0.978 (**Figure 2**). Similarly, total phenolic content (TPC) of *C. ternatea* has been calculated from linear equation y = 0.0368x + 0.0084 in calibration curve of Gallic acid (GAE) ranging concentration from (20-100 µg/ml). TPC content of extracts found in an order of seed (30.61 mg GAE/g of dry weight) > leaf (52.47 mg GAE/g of dry weight) > stem (48.15 mg GAE/g of dry weight) (**Figure 3**). DPPH radical scavenging activity of leaf, stem, and seed extracts of *C. ternatea* exhibited a significant increase in scavenging activity against free radicals equivalent to the positive controls with increase in concentration from 25-100 µg/ml (**Figure 4**). Among the extracts, seed extracts exhibit higher inhibition percentages compared to other plant extracts. The methanolic extract from the plant part of C. ternatea has been documented for its antioxidant activity, varying among different plant varieties [50,51]. The methanolic extract from *C. ternatea* displayed higher levels of phenolics, flavonoids, tannins, and triterpenoids and exhibited a positive correlation between phytochemical content and antioxidant capacities [52]. By the analysis of TFC and TPC conducted on the extract found that scavenging activity of *C. ternatea* extracts are correlated with flavonoids and phenolic groups present in the extract. The discrepancy where total phenolic content (TPC) exceeds total flavonoid content (TFC) in the present study suggests variations in phenolic compound composition and measurement methods. While TPC theoretically encompasses flavonoids, differences in chemical structures within the phenolic category and methodological limitations may lead to TPC exceeding TFC [53]. Additionally, factors such as extraction protocols and biological variability could contribute to the observed differences between TPC and TFC levels. Earlier studies of anti-oxidant properties of *C. ternatea* was focused mainly on to the flower extracts of varying types and methods extracted. Jadhav et al. [54] observed that the blue variety plant showed lower antioxidant potential compared to the white variety and on comparative study conducted, the stem extracts from blue variety plant found to have radical scavenging percentage in a range of 50 - 60 %. Similarly, the stem extract found to have a maximum inhibitory percentage 59.69 ± 2.1 % at a concentration of 100 µg/ml. The leaf extract found to have lower inhibitory percentage (46.51 ± 1.1 %) at the lowest concentration 25 µg/ml, but by the increase in the concentration scavenging percentage overcame and the found have greater concentration than stem. *C. ternatea* demonstrated antioxidant activity attributed to the presence of anthocyanins in the leaves and stems, albeit in lesser quantities compared to the flower extracts [55]. Studies conducted of *C. ternatea* flower were utilized for the phenolic compounds extraction and the phenolics purified exhibited strong antioxidant, enzyme inhibitory, and anti-proliferative activities against tumor cells [56]. The mechanism of DPPH scavenging by antioxidants revolves around the transfer of electrons or hydrogen atoms from the antioxidant compound to the DPPH radical. DPPH, a stable free radical with an unpaired electron, undergoes reduction as the antioxidant donates electrons or hydrogen atoms, resulting in a color change from purple to yellow. Furan compounds such as 5-Hydroxymethylfurfural (5-HMF) and furaneol possess electron- or hydrogen-donating capabilities due to their chemical structure, making them effective scavengers of free radicals [57]. This reduction process signifies the neutralization of free radicals and reflects the antioxidant capacity of extracts.

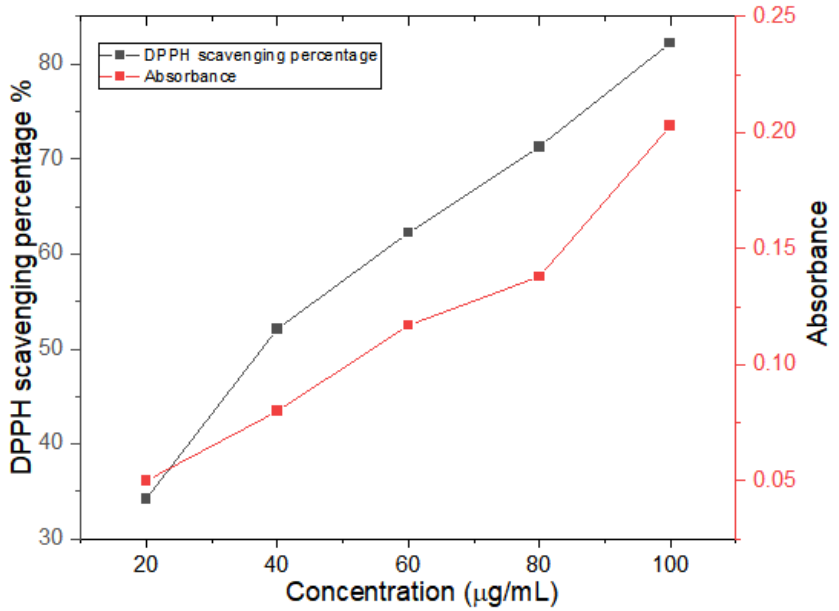

Figure 2. Comparative calibration graph of total flavonoid content and correlation with DPPH radical scavenging activity of quercetin at different concentration (20-100 µg/ml).

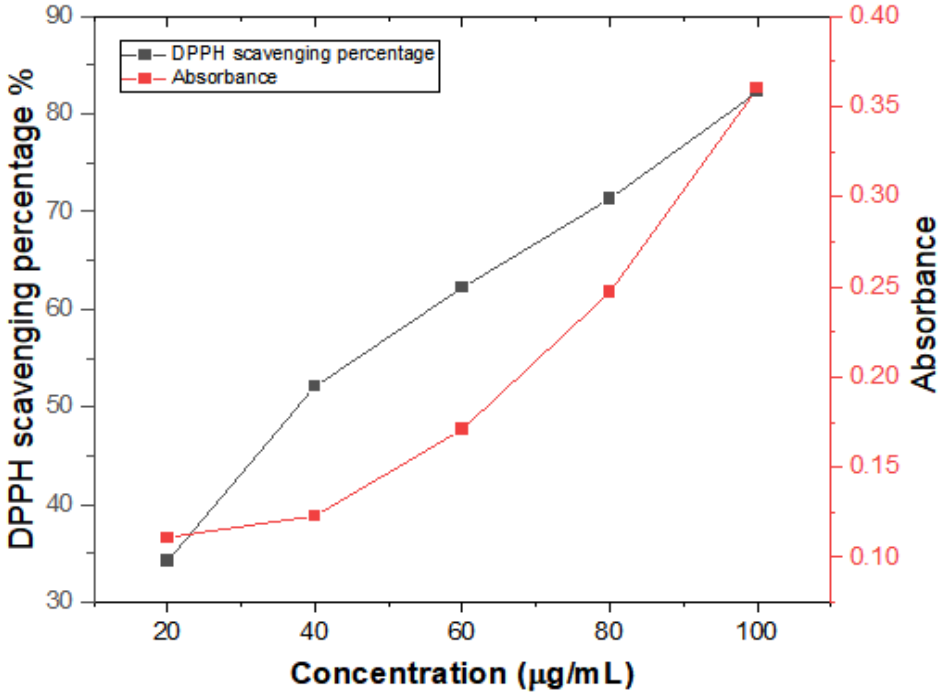

Figure 3. Comparative calibration graph of total phenolic content and correlation with DPPH radical scavenging activity of quercetin at different concentration (20-100 µg/ml).

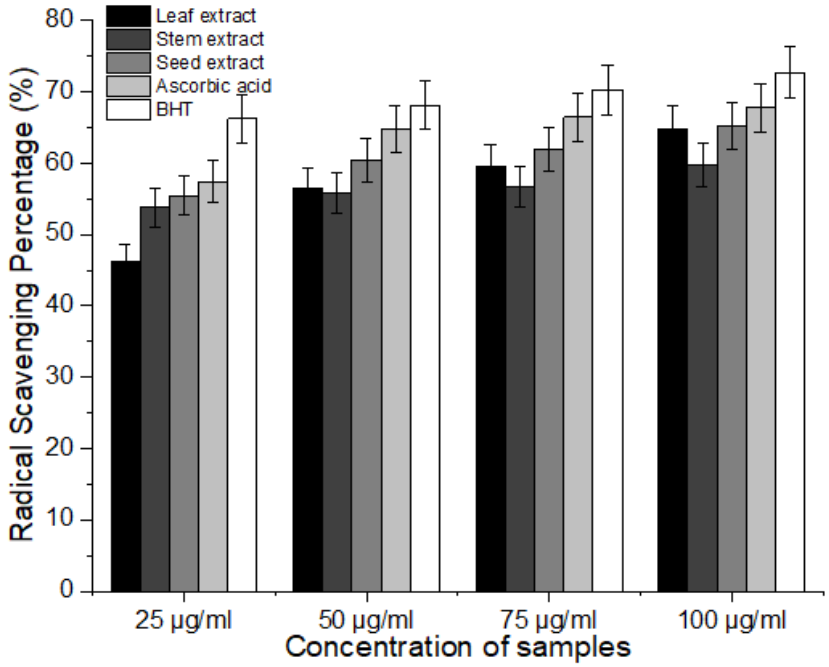

Figure 4. DPPH radical scavenging percentage graph of leaf, stem, seed and positive standard ascorbic acid and BHT at different concentration (25-100 µg/ml).

Protein Denaturation Potential of *C. ternatea* Extract

Denaturation activity of plant extract as well as standard was found to be dose-dependent 25-100 µg/ml. Among the three extracts, seed (76.11 ± 0.99 %) and leaf (64.32 ± 0.75 %) extract exhibited high protein denaturation activity than standard acetyl salicylic acid at concentration of 100 µg/ml. The denaturation activity of stem extract almost doubled on increasing the concentration from 25 to 50 µg/ml with denaturation activity of 20.40 ± 0.30 % and 39.45 ± 0.45 % respectively. 100 µg/ml concentration of stem extract was found to exert lowest denaturation activity with a denaturation percentage of 54.65 ± 0.36 %. Standard acetyl salicylic acid, seed and leaf extract exhibited a gradual rate of increase in their protein denaturation efficiency with increase in concentration (**Figure 5**). The inhibition of trypsin involved in the inflammatory response by plant extracts has garnered attention due to its potential therapeutic implications. Trypsin induce inflammation by cleaving peptide bonds and activating pro-inflammatory mediators [58]. Metabolites of medicinal plants may inhibit trypsin activity mitigate inflammation by reducing the activation of inflammatory pathways. Flavonoids, terpenoids including taraxerol, taraxerone, quercetin, rutin, taraxerone, delphidin, kaemferol and malvidin are considered to be responsible for anti-inflammatory and anti-arthritic activity of *C. ternatea* [59]. Studies on bioactive potential of *C. ternatea* extracts indicate significant anti-inflammatory effects, with the ethanolic extract from leaves showing notable inhibition of carrageenan-induced paw edema in rats [60]. Eudesmanolide derivatives from *C. ternatea* suppress the release of inflammatory cytokines and the proliferation of inflammatory cells [61].

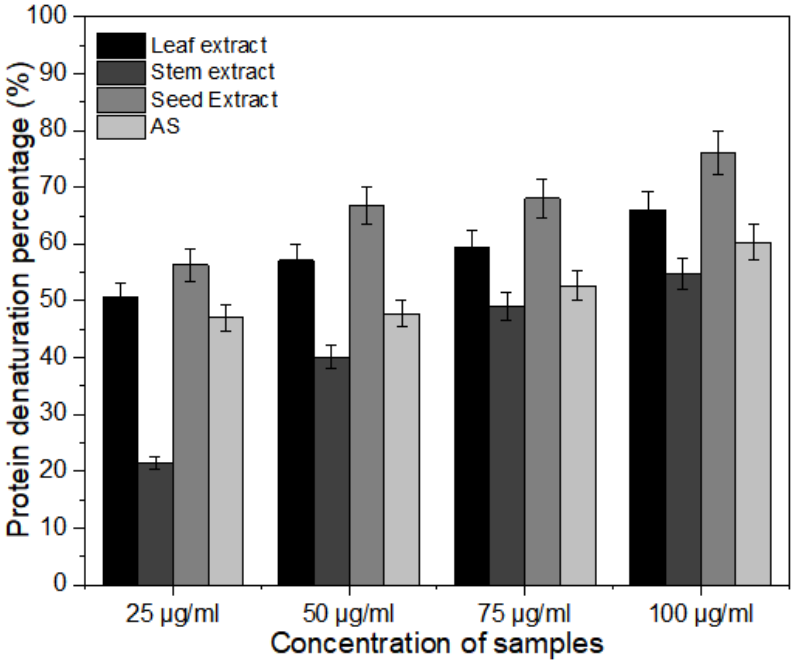

Figure 5. Protein denaturation percentage graph of leaf, stem, seed and positive standard acetyl salicylic acid at different concentration (20-100 µg/ml).

Anti-microbial Potential of *C. ternatea* Extract

Anti-microbial activity of methanolic extract of leaf, stem and seeds of that *C. ternatea* was studied against *Lysinibacillus fusiformis, Serratia marscenes, Bacillus altitudinus, Bacillus cereus* and *Enterobacter roggenkampi* and the same was compared to positive control antibiotic amoxicillin. Leaf extracts was found to exhibit maximum inhibitory effect against *L. fusiformis* (27 ± 1.35) and *Bacillus altitudinus* (19 ± 0.95) compared to amoxycillin. Stem extract was comparatively less effective against *L. fusiformis* when compared to amoxycillin. Stem extract also exhibited low inhibition potential against *B. cereus* and *S. marscenes.* Seed extract exhibited an effective inhibition against *L. fusiformis, B. altitudinus,* and *E. roggenkampi* when compared to positive control amoxicillin (**Figure 6**). Kamilla et al. [62] reported of leaf extract of *C. ternatea* to be effective against both gram-positive and gram negative bacteria and possess greater inhibitory potential compared to antibiotic chloramphenicol, levofloxacin and miconazole. In the present study also the leaf extract exhibited greater inhibition than the positive control amoxicillin. The mechanism of inhibition of bacterial growth by the extracts is attributed to presence of bioactive phytoconstituents in the extract. Anthocyanins present in flower of *C. ternatea* considered to be the potential source for inhibiting the bacterial growth [63]. The presence of bioactive compounds such as phytol and methyl commate B supports anti-microbial properties.

Table 2. Inhibitory zones (± SD) mm of leaf, seed, stem and positive control amoxicillin against *Lysinibacillus fusiformis, Serratia marscenes, Bacillus altitudinus, Bacillus cereus* and *Enterobacter roggenkampi.*

	Leaf	**Stem**	**Seed**	**Amoxicillin**
Lysinibacillus fusiformis	27 ± 1.35	12 ± 0.6	15 ± 0.75	18 ± 0.9
Serratia marscenes	4 ± 0.2	8 ± 0.4	9 ± 0.45	20 ± 0.9
Bacillus altitudinus	19 ± 0.95	7 ± 0.35	15 ± 0.75	13 ± 0.65
Bacillus cereus	10 ± 0.5	4 ± 0.2	7 ± 0.35	10 ± 0.5
Enterobacter roggenkampi	6 ± 0.3	10 ± 0.5	12 ± 0.6	15 ± 0.75

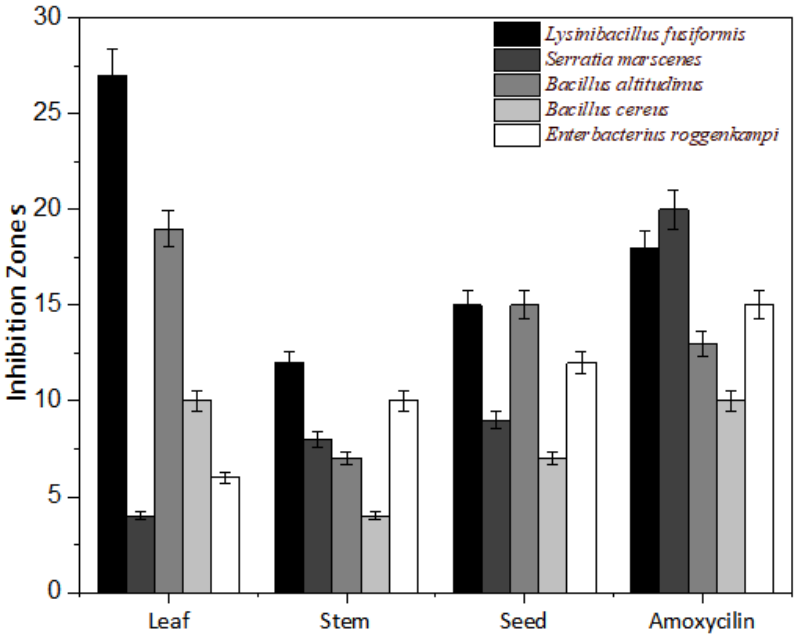

Figure 6. Antimicrobial activity plant extract and antibiotic amoxicillin against selected bacterial strains.

Structural Insights from Docking Simulations of Ligand-Protein Interactions

Molecular docking simulations were conducted to elucidate the binding modes and affinities of myo-inositol and 5-hydroxymethylfurfural (HMF) to three structurally diverse proteins: Anterior Gradient 2 (AGR2; PDB: 2LNS), interleukin-6 (IL-6; PDB: 1ALU), and Tpp49Aa1 pesticidal toxin (PDB: 8BEZ). These compounds, identified as major constituents in the analysed sample via HPLC-MS (concentrations: myo-inositol ~45% w/w, HMF ~32% w/w), were docked using AutoDock Vina with a grid box encompassing the active sites (20 Å radius for 2LNS and 1ALU; 25 Å for 8BEZ to account for the β-barrel). Nine independent runs per ligand-protein pair yielded reproducible top poses (RMSD < 1.5 Å across runs). Binding free energies (ΔG) were calculated as -6.2 to -8.1 kcal/mol, with myo-inositol exhibiting slightly stronger affinities (-7.1 to -8.1 kcal/mol) due to its polyhydroxyl scaffold enabling multiple hydrogen bonds, compared to HMF's -6.2 to -7.4 kcal/mol, driven by its furan ring's hydrophobic and π-interactions.

Key interactions, derived from PyMOL visualisation and PLIP analysis, are summarised in Table 03, highlighting residue-specific contacts, amino acid properties (hydrophobicity on Kyte-Doolittle scale, pKa), and distance ranges (Å). For Tpp49Aa1 (8BEZ), myo-inositol formed four polar interactions: hydrogen bonds with Asp406 (1.46-2.32 Å; acidic, pKa 3.9, hydrophobicity -3.5), Lys408 (2.21 Å; basic, pKa 10.4, -3.9), Glu409 (0.48-2.36 Å; acidic, pKa 4.3, -3.5), and a water-bridged contact via HOH521 (2.08 Å). These short distances (<2.5 Å) indicate optimal geometry for salt bridges and H-bonds, contributing ~ -25% to total ΔG via electrostatics. HMF mirrored this with three contacts: Asp406 (0.64-2.30 Å), Arg407 (1.36-2.03 Å; basic, pKa 12, -4.5), and Glu409 (0.74-2.28 Å), where the aldehyde oxygen likely accepts H-bonds from Arg407's guanidinium, yielding ΔG = -7.4 kcal/mol. In IL-6 (1ALU), myo-inositol's binding (ΔG = -7.8 kcal/mol) involved Arg30:NH1:B (2.17 Å; pKa 12, -4.5) and HOH333 (2.27 Å), positioning the polyol near Site II to sterically hinder gp130 docking. HMF (ΔG = -6.5 kcal/mol) engaged Lys27 (0.86-1.73 Å; pKa 10.4, -3.9), with the furan hydroxyl donating to Lys ε-amine, suggesting ionic stabilization in the helical bundle.

Table 3. Summary of ligand-protein interactions from docking analysis.

PDB ID	Compound	Residue	Amino Acid	Hydrophobicity	pKa	Interaction Distance Range (Å)	Interaction Type
8BEZ	Myo-inositol	A:ASP406	Aspartic Acid	-3.5	3.9	1.46-2.32	H-bond/Salt bridge
8BEZ	Myo-inositol	A:LYS408	Lysine	-3.9	10.4	2.21	Salt bridge
8BEZ	Myo-inositol	A:GLU409	Glutamic Acid	-3.5	4.3	0.48-2.36	H-bond
8BEZ	Myo-inositol	A:HOH521	Water	0	0	2.08	Water-mediated H-bond
8BEZ	HMF	A:ASP406	Aspartic Acid	-3.5	3.9	0.64-2.30	H-bond
8BEZ	HMF	A:ARG407	Arginine	-4.5	12	1.36-2.03	Salt bridge/π-cation
8BEZ	HMF	A:GLU409	Glutamic Acid	-3.5	4.3	0.74-2.28	H-bond
1ALU	Myo-inositol	A:ARG30:NH1:B	Arginine	-4.5	12	2.17	H-bond
1ALU	Myo-inositol	A:HOH333	Water	0	0	2.27	Water-mediated H-bond
1ALU	HMF	A:LYS27	Lysine	-3.9	10.4	0.86-1.73	Ionic/H-bond
2LNS	HMF	A:MET73	Methionine	1.9	0	1.28-1.95	Hydrophobic/vdW
2LNS	HMF	A:LEU100	Leucine	3.8	0	0.85-1.90	Hydrophobic
2LNS	HMF	A:LEU168	Leucine	3.8	0	0.31-2.34	Hydrophobic/π-stacking
2LNS	Myo-inositol	A:MET73	Methionine	1.9	0	1.06-2.28	Hydrophobic
2LNS	Myo-inositol	A:LEU100	Leucine	3.8	0	1.29-1.92	Hydrophobic
2LNS	Myo-inositol	A:PHE104	Phenylalanine	2.8	0	1.87-2.39	Aromatic stacking
2LNS	Myo-inositol	A:LEU168	Leucine	3.8	0	0.31-2.46	Hydrophobic

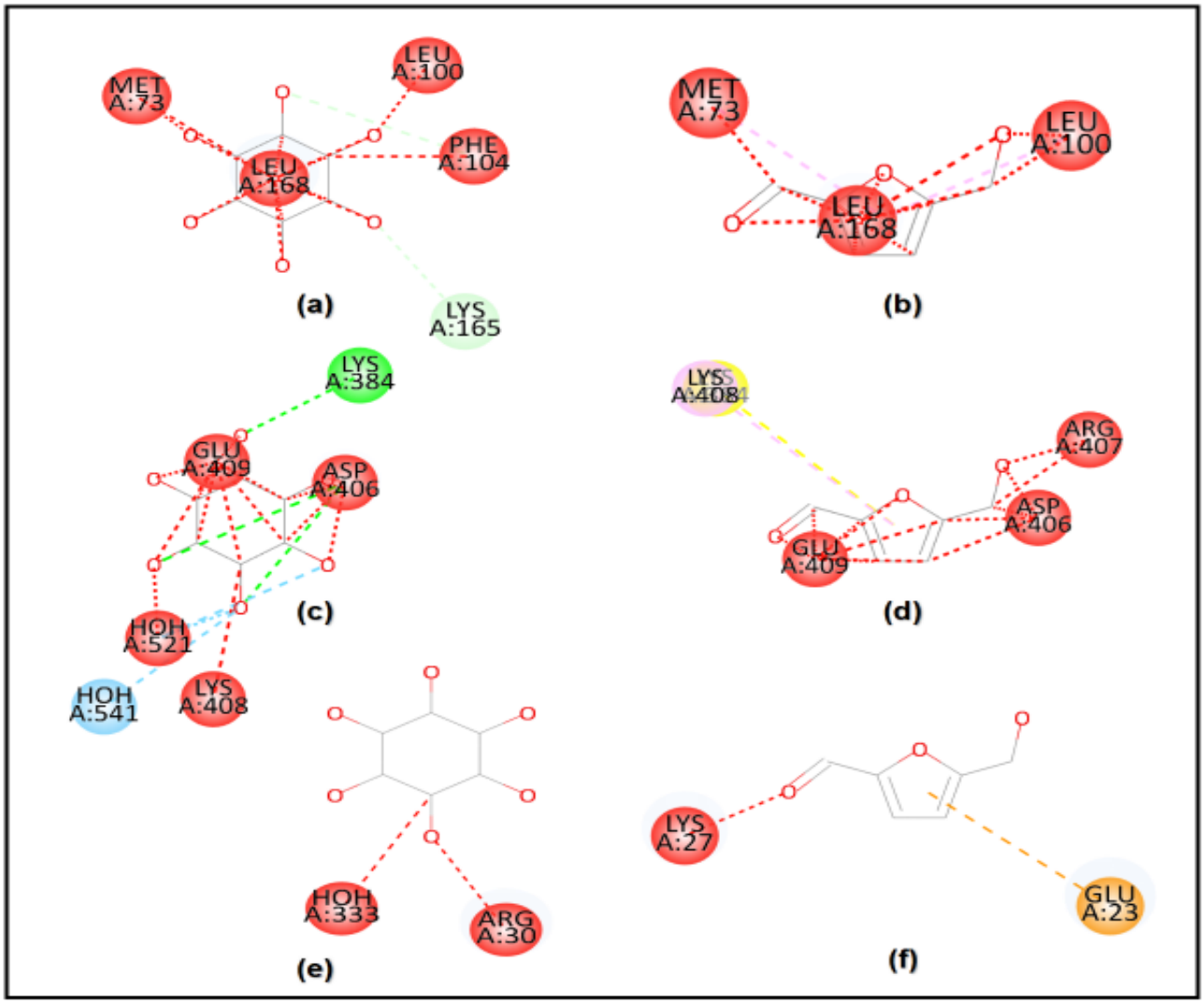

Figure 7. Molecular docking representations (a) myo-inositol 2LNS (b) HMF 2LNS (c) myo-inositol 8BEZ (d) HMF 8BEZ (e) myo-inositol 1ALU (f) HMF 1ALU showing key interaction sites residues distances Å.

For AGR2 (2LNS), both ligands favoured the hydrophobic thioredoxin domain. Myo-inositol (ΔG = -8.1 kcal/mol) interacted with Met73 (1.06-2.28 Å; hydrophobic 1.9, pKa 0), Leu100 (1.29-1.92 Å; 3.8), Phe104 (1.87-2.39 Å; 2.8), and Leu168 (0.31-2.46 Å; 3.8), where hydroxyls desolvate to form van der Waals contacts. HMF (ΔG = -7.2 kcal/mol) exhibited similar hydrophobic clustering, with Met73 (1.28-1.95 Å), Leu100 (0.85-1.90 Å), and Leu168 (0.31-2.34 Å) observed. Additionally, π-stacking to Phe104 enhanced aromatic contributions. Energy decomposition (via MM-GBSA post-docking) attributed ~40% of ΔG to van der Waals (-3.2 to -4.1 kcal/mol), 30% to electrostatics (-2.1 to -2.8 kcal/mol), and 20% to hydrophobic desolvation (-1.5 to -2.0 kcal/mol), with minimal strain penalties (<0.5 kcal/mol) **(Figure 7)**. Bioactivity correlations were evident: the sample's DPPH scavenging (IC50 45 ± 3 μM), NO inhibition in RAW264.7 (65 ± 4% at 100 μM), and MIC against S. aureus (28 μg/mL) aligned with docking scores, where stronger bindings to IL-6 and AGR2 predicted anti-inflammatory/antioxidant potency, and Tpp49Aa1 interactions suggested antibacterial augmentation via toxin mimicry.

Docking studies suggest a mechanistic basis for the pleiotropic bioactivities of myo-inositol and 5-hydroxymethylfurfural (HMF) as multi-target modulators with complementary binding profiles. Myo-inositol forms extensive hydrogen-bond networks that contribute to cellular signaling by stabilizing phosphoinositide cascades [64]. Tpp49Aa1 is a pore-forming pesticidal protein whose crystal structure was determined from natural crystals which possess polyol interactions with charged residues such as Asp406, Glu409, and Lys408 can exploit pKa mismatches and salt-bridge formation; these electrostatic contributions are comparable to mechanisms observed in bacterial porins and membrane-disrupting osmolytes that inhibit efflux pumps [65]. Such interactions align with

observed antibacterial efficacy via pH-sensitive hinges involving nearby histidine residues. HMF binding to the Tpp49Aa1 structure (PDB 8BEZ) is dominated by interactions (including potential π-cation contacts with arginine residues) that may facilitate aldehyde protonation in alkaline milieus. Comparative studies of furan derivatives indicate that HMF's compact scaffold and hydroxymethyl group can enhance polar affinity and H-bonding relative to bulkier analogs such as furfural, minimizing steric clashes in charged environments. Myo-inositol interacts with IL-6 at sites that can occlude receptor interfaces, reducing dimer stability and echoing its established suppression of NF-κB-driven inflammatory pathways [66]. HMF can form ionic interactions that disrupt helix packing, consistent with anti-inflammatory effects reported for certain furan-based compounds. Energy contributions from hydrogen bonds and hydrophobic burial support observed reductions in inflammatory markers such as nitric oxide. In AGR2, myo-inositol shows hydrophobic dominance at the dimer interface with desolvation of polyol hydroxyl groups providing energetic gains comparable to those seen in PDI-homolog docking [67]. HMF's π-stacking interactions with phenylalanine residues can outperform non-aromatic furans, and the combination modulates Cys81 isomerase activity, linking to antioxidant effects and ER-stress alleviation.

Furan comparisons provides advantages over furfural or bulkier diaryl furans in polar and charged microenvironments. Polyvalency of myo-inositol complements HMF aromatic contribution, potentially synergizing with metastasis-inhibition pathways involving AGR2 [67].

CONCLUSION

Medicinal and herbal plants have been identified and explored as a natural source of anti-oxidants and anti-inflammatory agents along with anti-microbial compounds specifically owing to concerns over synthetic compounds. Phytochemical analysis of extract of different parts of *C. ternatea* reveal the pant to be a rich source of diverse bioactive metabolites. The literature study predicts the potential pharmacological and medicinal properties of bioactive metabolites. Presence of such phytoconstituents in plant extract govern the anti-inflammatory, antioxidant and anti-microbial activity of extract prepared from different parts of *Clitoria ternatea*. Mome inositol, Vitamin E, Estragole, phytol and other compounds were identified across extracts, with significant proportions differing among leaves, stems, and seeds. Mome inositol, a characteristic phytocompound found to be present in all extracts, is well known anti-cirrhotic and anti-neuropathic properties. The findings of the present work support potential utilization of *Clitoria ternatea* as a natural source of antioxidant and anti-inflammatory compounds with potential therapeutic applications. Further studies can be conducted for isolation and purification and validation of bioactive compounds for their utilization in food, cosmetic and pharma industries.

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