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IN VITRO ANTI-INFLAMMATORY, IN SILICO MOLECULAR DOCKING ANALYSIS, AND PHYLOGENETIC ANALYSIS OF CURCUMA NEILGHERRENSIS

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ABSTRACT

Background: Plants not only supply vital nutrients for people, but also contain physiologically active phytochemicals that are advantageous for human life and the management of several ailments. The herbal plants are regarded as a treasured reservoir of possible anti-inflammatory phytochemicals. **Objective:** The present work was focused at analyzing the in vitro anti-inflammatory properties and in silico analysis of the saponins extracted from the *Curcuma neilgherrensis* roots. **Methodology:** The total saponins were extracted from the root extract of C. neilgherrensis. The anti-inflammatory effectiveness of the total saponins from the roots of C. neilgherrensis was examined by albumin denaturation inhibition analysis and membrane stabilization analysis. In silico studies and phylogenetic studies were done to detect the protein components responsible for the therapeutic effects of C. neilgherrensis, including cytochrome c oxidase subunit 1, ribulose bisphosphate carboxylase large chain, maturase K, and ATP synthase subunit 1. **Results:** The total saponins obtained from the roots of C. neilgherrensis demonstrated an effective anti-inflammatory properties in vitro, which is evidenced by its inhibitory effects on albumin denaturation and HRBC membrane stabilization. The findings of an in silico molecular docking analysis and phylogenetic analysis demonstrated the possible roles of cytochrome c oxidase subunit 1, ribulose bisphosphate carboxylase large chain, maturase K, and ATP synthase subunit 1 protein molecules in the therapeutic effects of the saponins extracted from C. neilgherrensis roots. **Conclusion:** The present study demonstrated that the total saponins extracted from the roots of C. neilgherrensis revealed an encouraging anti-inflammatory potentials. Thus, it was clear that C. neilgherrensis has the capacity to be beneficial in treating diseases related to inflammation in the future.

KEYWORDS: Inflammation, *Curcuma neilgherrensis*, Molecular docking, Maturase K, Membrane stabilization.

INTRODUCTION

The utilization of herbal plants in conventional medicine has a historical background worldwide. In numerous countries, they are commonly employed as conventional medicine for the treatment of numerous inflammatory ailments.[1,2] Plants not only supply vital nutrients for people, but also contain physiologically active phytochemicals that are advantageous for human life and the management of several ailments. They encompass a diverse array of substances such as phytochemicals and pharmaceutics and have found applications in the food, pharmacy, and cosmetics sectors, among others.[3] In emerging nations such as India, the utilization of conventional medicines serves as the basic foundation of the healthcare system. The key factors driving the usage of plants in traditional medical practices include the availability of phytochemical substances. The ubiquitous usage of traditional medicine in India can be accredited to the occurrence of numerous plant species and the socio-economic circumstances of the local population.[4,5] The phytochemicals found in medicinal plants possess a diverse array of antibacterial, anticancer, anti-inflammatory, and antioxidant properties. Medications originating from plants are often formulated from unrefined extracts, which consist of an intricate blend of different phytochemicals and are employed for the treatment of numerous illnesses.[6] Though numerous plant species possess a wide range of phytochemicals, only a few of them have been thoroughly assessed and shown to be a imperative reservoir of bioactive chemicals. The establishment of robust analyzing protocols is crucial in the quest for new compounds and in ensuring quality control. The separation and characterization of these bioactive chemicals have led to the development of targeted drugs with a remarkable level of activity.[7]

Inflammation is an inherent and important physiological reaction of the body to detrimental stimuli, like microbes, pathological cells, or irritants. The mechanism of inflammation is to eradicate the underlying source of cellular damage, remove impaired cells and tissues, and trigger the process of tissue regeneration. A multifaceted process, it encompasses the immune system, blood arteries, and many signaling molecules.[8] The classification of inflammation as either acute or chronic is contingent upon the extent of the infection. An inflammatory response is an intricate and ever-changing reaction to cellular damage, infection, trauma, or toxins, which can persist for a short period of time (acute inflammation) or for an extended period (chronic inflammation). The excessive synthesis of pro-inflammatory mediators together with immunological targets, released by immune cells and macrophages, significantly contribute to the facilitation of inflammatory responses.[9] Furthermore, there exists a strong correlation between inflammation and oxidative stress, since one triggers the activation of the other.[10]

The herbal plants are regarded as a treasured reservoir of possible anti-inflammatory phytochemicals. Insufficient proof exists to substantiate the anti-inflammatory action of these herbal plants, as the majority of them have not undergone chemical, pharmacological, or toxicological investigations to explore their bioactive components.[11] The plant Curcuma neilgherrensis, belonging to the Zingiberaceae family is extensively employed as a conventional medicine by the indigenous communities of Western Ghats region. Furthermore, several tribal populations in India ingest the tuber specifically for its palatability. The leaf is known to be the advantageous element for alleviating the negative effects of diabetes.[12] Although highly regarded, this plant has not yet been subjected to scientific scrutiny, thereby justifying the need of doing a comprehensive analysis of its therapeutic virtues. The current body of evidence about this plant is highly restricted and inadequate. Hence, this work investigates the in vitro anti-inflammatory properties and in silico studies of the saponins extracted from the C. neilgherrensis roots.

MATERIALS AND METHODS

Plant material collection and authentication

The C. neilgherrensis root samples were collected from the Yercaud hill region, Salem district, Tamil Nadu. After that, the collected plant specimen was authenticated (certificate number: GRD/2024/470). The root samples were underwent a 15-day period of shade drying to remove any moisture. The sample is subsequently pulverized using a mechanical grinder. Ultimately, the grinded root specimen was employed for the extraction procedure.

Extraction of saponins from the C. neilgherrensis root

In the first step, fat was removed from the powdered material by the use of petroleum ether and n-hexane. The defatted concentrate was then isolated by methanol extraction. Condensation of the ethanolic extract was achieved by vacuum during rotating evaporation. The desiccated ethanolic extract of C. neilgherrensis root was dissolved in purified water and then homogenized with n-butanol. The resultant liquid was subsequently subjected to diethyl ether in order to induce the precipitation of a crude Saponin combination. 100 g of the sample were added in a conical flask, after the addition of 500 ml of aqueous methanol. The suspension described above is subjected to heating in a water bath for 4 h, while being continuously agitated at 55°C. The suspension undergoes filtration, and the residual solid is once more isolated by further extraction with 200 ml of ethanol at a 20% concentration. The combined extracts are refined to around 40 ml by immersing them in a water bath maintained at 90°C. To prepare the suspension, it is shifted into a 250 ml funnel and combined with 20 ml of diethyl ether. Next, the mixture is vigorously stirred. The aqueous layer is gathered while the layer of organic solvent is disposed. The analogues of n-butanol are introduced and thereafter rinsed with 10 ml of NaCl. The remaining suspension is heated by immersion in a water bath. Subsequent to evaporation, the sample undergoes oven drying until it attains a consistent weight, and the saponin concentration is thereafter measured as a percentage.

In vitro anti-inflammatory activities of the saponins extracted from the C. neilgherrensis

Albumin denaturation inhibition analysis

The anti-inflammatory activity of the saponins extracted from the C. neilgherrensis were assessed following the previously described method.[13] The 0.05 mL of the saponins extracted from the C. neilgherrensis was mixed at doses of 20, 40, 60, 80, and 100 µg/ml to the 0.45 mL of 1% bovine serum albumin. A small quantity of 1N HCl was utilized to neutralize the pH level. Following a 20-minute incubation at 37 °C, the suspension was then heated at 55°C for 30 min. Following the heating procedure, the samples were let to cool, and the absorbance was quantitatively measured at 660 nm. Aspirin was as the reference drug to compare the obtained results.

The albumin denaturation inhibition (%) by the saponins extracted from the C. neilgherrensis was assessed using the equation: Inhibition (%) = (Control absorbance – Sample's absorbance / Control absorbance) × 100.

Human red blood cell (HRBC) membrane stabilization assay

An in vitro assessment of anti-inflammatory activity of the saponins extracted from the C. neilgherrensis was conducted using the HRBC method.[14] An equivalent amount of Alsever's solution was added to the blood taken from healthy volunteers. The blood solution was centrifuged at 3,000 rpm to isolate the packed cells. A 10% v/v solution was prepared by washing the packed cells with saline. The HRBC was utilized to study the anti-inflammatory property of the saponins extracted from the C. neilgherrensis. Varying amounts of saponin sample (20-100 µg/ml), standard drug aspirin, and control was mixed with 1 mL of PBS, 2 mL of hyposaline, and 0.5 mL of hematopoietic stem cell powder suspension. Every assay mixture was subjected to incubation at 37 °C for 30 min and then centrifuged at 3,000 rpm. Decantation of the supernatant liquid was followed by estimation of the hemoglobin concentration using a spectrophotometer at 560 nm. The estimation of the proportion of hemolysis was based on the assumption that the controlled group exhibited 100% hemolysis.

Membrane stabilization (protection %) = 100 - (Sample's OD / Control OD) × 100.

In silico analysis to detect the protein components responsible for the therapeutic effects of _C. neilgherrensis_

Retrieval and structure predictions of sequence

The proteins' FASTA sequence was obtained from Genbank database maintained by the NCBI at http://www.ncbi.nlm.nih.gov. We calculated the theoretical Isoelectric Point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index, and grand average of hydropathy (GRAVY) for physio-chemical characterisation using the Expasy Protparm server available at http://us.expasy.org/tools/protparam.html. Self Optimized Prediction Method with Alignment (SOPMA) was utilized to detect the secondary structure. SWISS MODEL was utilized to predict the tertiary structures.

Functional characterization

The protein was characterized using the SOSUI and TMHMM v.2.0 databases to assess its solubility or transmembrane nature. The InterPro database is a complete collection of protein families, domains, and functional sites. InterPro consolidates the primary protein signature databases into a unified resource. These comprise: PROSITE that utilizes regular expressions and profiles, PRINTS that utilizes Position Specific Scoring Matrix-based (PSSM-based) fingerprints, ProDom that utilizes automatic sequence clustering, and Pfam, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D and PANTHER, all of which utilize hidden Markov models (HMMs). Superfamily and molecular function were predicted using Interpro protein sequencing and categorization platform available at http://www.ebi.ac.uk/interpro/.

Sequence alignment

Pairwise sequence alignment was conducted using the NCBI-BLAST tool available at http://blast.ncbi.nlm.nih.gov/Blast.cgi. Multiple sequence alignment was performed using EBI-CLUSTAL OMEGA program available at http://www.ebi.ac.uk/Tools/msa/clustalo/. Clustal Omega provides robust capabilities for incorporating sequences into and exploring information within pre-existing alignments, leveraging the extensive updated data available in public databases such as Pfam. The primary objective of this study is to identify the areas of sequence similarity, therefore enabling us to derive functional and evolutionary connections among the proteins under investigation in this paper.

Phylogenetic analysis

A phylogenetic study was conducted on ten proteins to ascertain the number of proteins that have shared structure and functional profiles. All sequences in fasta formats were provided as input to Clustal Omega with default predefined settings. Detailed analysis was conducted on the output of sequences aligned for their whole length, scores, alignment, conserved residues, substitutes, and semi-conserved substituted residue patterns. A phylogenetic tree was generated using the bootstrap Neighbour Joining (NJ) technique. Evaluation of the internal nodes' stability was conducted using bootstrap technique with 1000 replicates.

Primary tools for sequence comparison

The principal tools for sequence comparison and assembly have developed in parallel with the growth of the datasets they evaluate. The absence of fundamental local alignment search tool (BLAST) and associated sequence comparison tools would render a significant portion of the data generated by high-throughput sequencing laboratories as mere sequences of letters. BLAST continues to be the most efficient method for identifying particular sequences in extensive datasets and allows for faster annotation of new sequences. While BLAST is widely used for detecting sequence similarities in extensive datasets, there exist a variety of alternatives for constructing sequence datasets. The selection of these alternatives is contingent upon the availability of hardware, the size of the dataset, the data format, the structure, and the genetic composition of the organism.

Statistical analysis

The results of an experimental biochemical parameters were studied statistically using the SigmaStat software (Version 3.1). The data were represented as a mean±SD of triplicates.

RESULTS AND DISCUSSIONS

Effect of the saponins extracted from the C. neilgherrensis on the albumin denaturation inhibition analysis

Inflammation is a degenerative process that leads to the local accumulation of low molecular weight catabolic substances. This accumulation causes an increase in tissue osmotic pressure, which attracts more fluids. The creation of enough heat for tissue temperature elevation may or may not occur during inflammation.[15] The prevailing disease disorders are linked to inflammation, which manifests as tissue swelling, elevated tissue temperature, redness at the inflammatory site, heightened sensitivity to harmful stimuli, and impaired function of the affected organ. The biochemical mechanism of inflammation is a complex process that is triggered by the recognition of a certain molecular pattern associated with injury. A multitude of regulators facilitate the whole mechanism of the inflammatory reactions, which include the control of diverse pro-inflammatory chemicals.[16]

The albumin denaturation inhibition test was employed to assess the anti-inflammatory efficacy of saponins extracted from the C. neilgherrensis roots. Experiments were conducted to evaluate the saponins extracted from the C. neilgherrensis at various doses (20-100 μg/ml) and compare its inhibitory effects with standard drug aspirin. The results indicated that an inhibition percentage of 66.11% was observed at a dose of 100 μg/ml, 54.15% at 80 μg/ml, 37.05% at 60 μg/ml, 29.28% at 40 μg/ml, and 19.03% at 20 μg/ml (Table 1). The obtained results suggest that the saponins extracted from the C. neilgherrensis roots demonstrates substantial anti-inflammatory properties by suppressing the denaturation of albumin. Furthermore, the saponins extracted from the C. neilgherrensis roots exhibited likely similar anti-inflammatory effects to the reference drug aspirin at all the dosages (Table 1 and Figure 1).

Table 1: Antiinflammatory activity of saponins from C. neilgherrensis roots.

S. No.	Sample Name	Concentration (μg/ml)	Inhibition (%)			
			Albumin denaturation	IC50 value	Membrane stabilization	IC50 value
1	Saponins from C. neilgherrensis	100	66.11±0.84	74.22 μg/ml	75.45±1.06	64.68 μg/ml
		80	54.15±2.47		59.61±1.92	
		60	37.05±2.08		47.2±2.83	
		40	29.28±2.36		30.06±2.90	
		20	19.03±2.86		21.26±1.92	
2	Aspirin	100	86.3±3.29	51.39 μg/ml	79.87±2.65	36.39 μg/ml
		80	68.1±2.03		64.14±3.41	
		60	48.83±3.57		41.04±2.67	
		40	38.62±2.56		32.62±3.35	
		20	31.25±2.56		29.48±3.47	

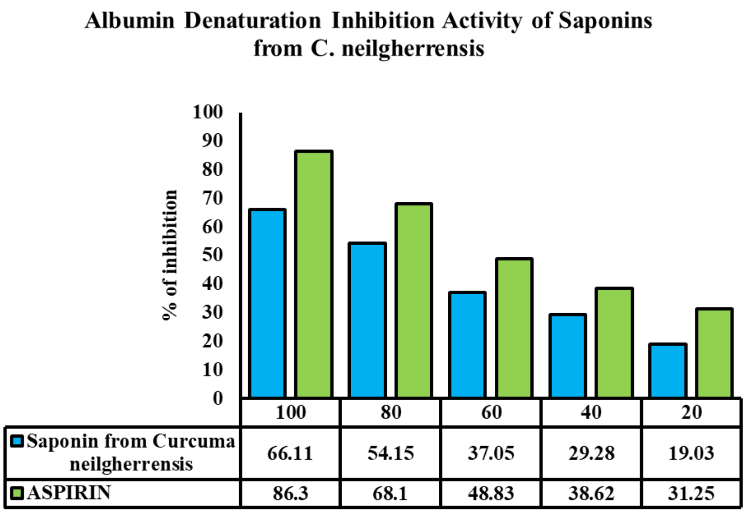


Figure 1: Effect of the saponins extracted from the C. neilgherrensis on the albumin denaturation inhibition analysis.

The results are represented as a mean ± SD of three triplicate measurements. The findings were studied statistically by one-way ANOVA and Tukey's post hoc assay using the Sigma Stat statistical software (Version 3.1).

The in vitro albumin denaturation inhibition assay is a valuable assay in the field of pharmaceutical research, as it provides a reliable method for evaluating the potential of various sample drugs to inhibit the albumin denaturation, a crucial protein in the body. This test has numerous uses, from drug development to the assessment of the therapeutic potential of natural compounds.[17] One of the primary applications of the albumin denaturation inhibition assay is in the screening and examination of promising drug agents. By testing the capability of a compound to inhibit the denaturation of albumin, researchers can gain insights into its potential anti-inflammatory, antioxidant, and protein-stabilizing properties. This information can be used to identify promising lead compounds for further development and optimization, ultimately contributing to the discovery of new therapeutic agents.[18] Another important application of this assay is in the assessment of natural compounds and their potential therapeutic benefits. Many natural products, like plant extracts and isolated phytochemicals, have been revealed to possess the ability to inhibit the albumin denaturation, indicating their capability as anti-inflammatory and antioxidant agents.[19] The present results suggest that the saponins extracted from the C. neilgherrensis roots demonstrates substantial anti-inflammatory properties by suppressing the denaturation of bovine serum albumin.

Effect of the saponins extracted from the C. neilgherrensis on the HRBC membrane stabilization analysis

The HRBC membrane stabilization test is extensively employed method for examining the bioactive potential of various compounds, particularly their anti-inflammatory and membrane-stabilizing properties. This assay provides a simple and efficient method to assess the capability of a sample drugs to inhibit the hemolysis or lysis of RBCs, which can be induced by various chemical or physical stressors.[20]

The anti-inflammatory activities of the saponins from the C. neilgherrensis roots were evaluated by applying the HRBC stabilization assay. The saponins extracted from the C. neilgherrensis underwent testing at various concentrations (20-100 µg/ml) and was subsequently the obtained results are in comparison with aspirin (Table 1). The findings indicated that the inhibition percentage was 75.45% at a dosage of 100 µg/ml, 59.61% at 80 µg/ml, 47.2% at 60 µg/ml, 30.06% at 40 µg/ml, and 21.26% at 20 µg/ml, compared to 79.87%, 64.14%, 41.04%, 32.62%, and 29.48% at the same concentrations of the standard drug aspirin, respectively. In the membrane stabilization test, these outcomes exhibit a notable anti-inflammatory effect of saponins extracted from the C. neilgherrensis roots (Table 1 and Figure 2). One of the primary applications of this assay is in the assessment of anti-inflammatory ability.[21] The in vitro HRBC membrane stabilization assay can be employed to examine the capability of a compound to stabilize the cell membrane and prevent the release of inflammatory mediators, which is a key mechanism of anti-inflammatory action.[22] Additionally, this assay has been employed to evaluate the cytotoxic and hemolytic potential of natural products and their constituents. The assay can provide insights into the potential toxicity of these compounds, which is crucial for their development as therapeutic agents.[23] The present outcomes of this work demonstrated the notable anti-inflammatory property of saponins extracted from the C. neilgherrensis roots.

Table 1: Antiinflammatory activity of saponins from C. neilgherrensis roots.

S. No.	Sample Name	Concentration (µg/ml)	Inhibition (%)			
			Albumin denaturation	IC50 value	Membrane stabilization	IC50 value
1	Saponins from C. neilgherrensis	100 80 60 40 20	66.11±0.84 54.15±2.47 37.05±2.08 29.28±2.36 19.03±2.86	74.22 µg/ml	75.45±1.06 59.61±1.92 47.2±2.83 30.06±2.90 21.26±1.92	64.68 µg/ml
2	Aspirin	100 80 60 40 20	86.3±3.29 68.1±2.03 48.83±3.57 38.62±2.56 31.25±2.56	51.39 µg/ml	79.87±2.65 64.14±3.41 41.04±2.67 32.62±3.35 29.48±3.47	36.39 µg/ml

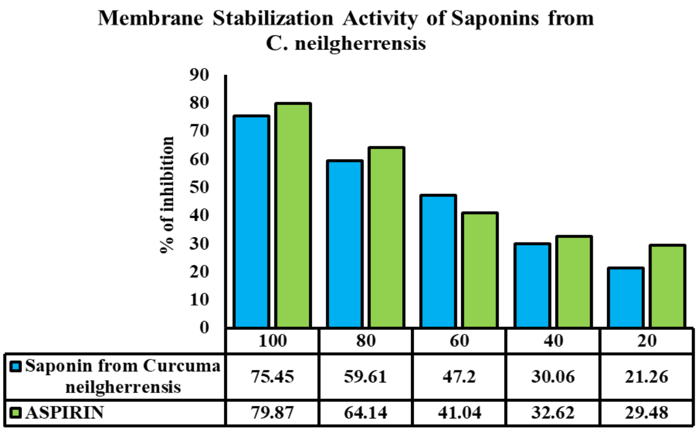


Figure 2: Effect of the saponins extracted from the C. neilgherrensis on the HRBC membrane stabilization analysis.

The results are represented as a mean ± SD of three triplicate measurements. The findings were studied statistically by one-way ANOVA and Tukey's post hoc assay using the Sigma Stat statistical software (Version 3.1).

In silico molecular docking analysis

Historically, medicinal plants have been of great importance in the traditional medical systems of several nations. They are abundant reservoirs of bioactive chemicals and so function as crucial raw materials for pharmaceutical manufacturing. The historical usage of C. neilgherrensis in several traditional medicinal formulations has been documented. Within the post-genomic age, there has been a substantial surge of data originating from several sources. Beginning with genomic analysis, gene sequencing, and protein structures derived from experiments, laboratories worldwide generate vast amounts of data on a daily basis.[24] Data of this nature must undergo processing in order to generate genuinely valuable information. All this information is intended to facilitate the discovery of novel pharmaceuticals and therapeutic materials suitable for human use. Nevertheless, there is still untapped potential for the application of contemporary scientific techniques such as genomics, proteomics, metabolomics, and bioinformatics in this particular plant.[25] Biological informatics will streamline the analysis and integration of data from various interconnected disciplines to identify genes and gene products and clarify the functional connections between genotype and observable phenotype.[26] This study presents a comprehensive summary of the current state-of-the-art bioinformatics investigation of C. neilgherrensis, offering the essential resources to comprehend and facilitate development in this significant area.

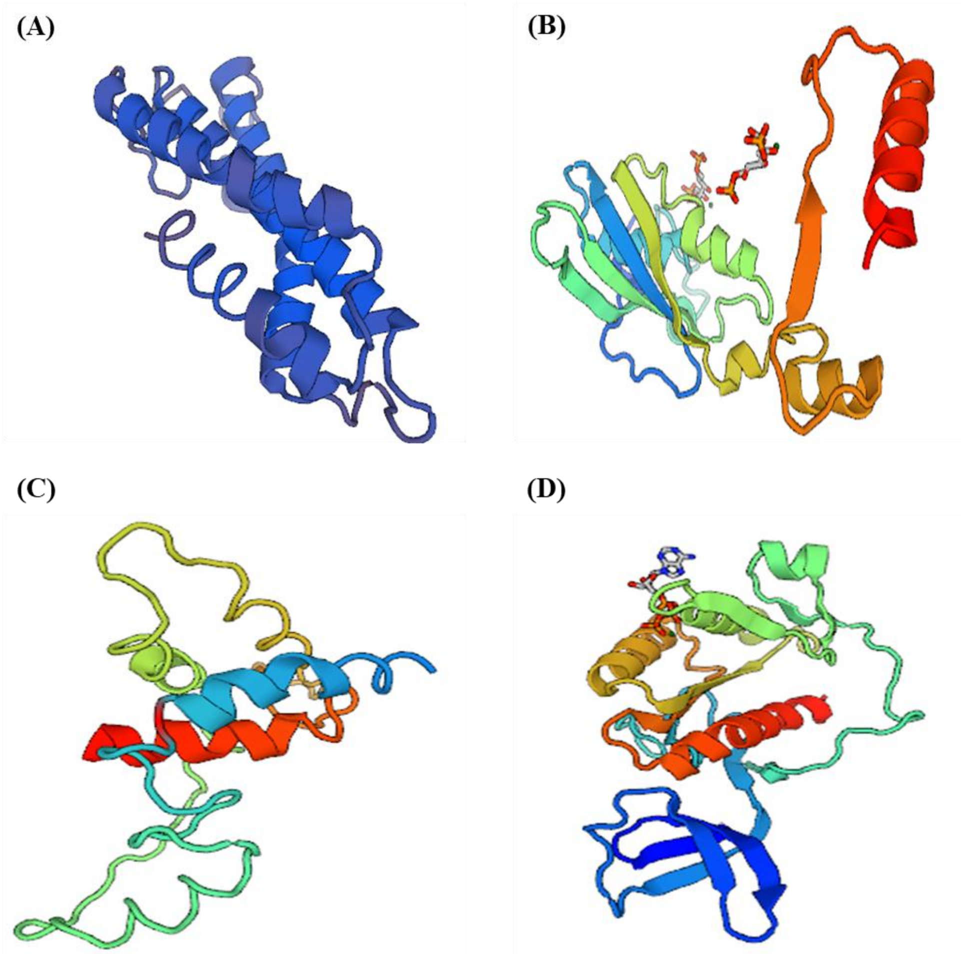
The major structural prediction was performed using the protparam tool (Tables 2 and 3). In order to determine the molecular weights of various proteins, the markers were calculated using Expasy's protparam tool. Iteration 18384.87 Subunit 1 of cytochrome c oxidase (21177.01) Extended chain of ribulose bisphosphate carboxylase (16681.40) Maturase K(5149.98) Alpha-subunit 1 of ATP synthase (964.09) Protein K of photosystem II (Table 2). Two proteins had a pI value below 7, indicating acidity, while two proteins had a pI value over 7, determining basicity. The proteins are observed to be densely packed and stable at their pI values; see Table 2. Among the five proteins, eight had an instability value below 40, suggesting that the proteins are stable.[27] Aliphatic index values for the proteins varied from 61.25 to 120.88 (Table 3). In solution, the calculated extinction coefficients facilitate the quantitative investigation of protein–protein and protein–ligand interactions (Figure 3).

Table 2: Primary structures of cytochrome c oxidase subunit 1, ribulose bisphosphate carboxylase large chain, maturase K, and ATP synthase subunit 1 computed using expasy's.

S.No	Accession number	Protein	Length	Mol.Wt	PI	-R	+R	EC	II	AI	GRAVY
1	AQM55920.1	Cytochrome c oxidase subunit 1	171	18384.87	5.98	6	4	24980	32.32	120.88	0.930
2	AGS45035.1	Ribulose bisphosphate carboxylase large chain	192	21177.01	7.58	22	23	30370	24.68	78.23	-0.328
3	AFW97708.1	Maturase K	515	16681.40	9.45	39	51	100160	42.43	95.73	0.028
4	AQM55890.1	ATP synthase subunit 1	47	5149.98	6.50	2	2	9970	11.57	109.79	0.606

Table 3: Secondary structures of cytochrome c oxidase subunit 1, ribulose bisphosphate carboxylase large chain, maturase K, and ATP synthase subunit 1 computed using expasy's.

S. No	Secondary sutures	Proteins			
		AQM55920.1	AGS45035.1	AFW97708.1	AQM55890.1
1	Alpha helix	33.33	34.38	50.49	21.28
2	3_{10} helix	0.00	0.00	0.00	0.00
3	Pi helix	0.00	0.00	0.00	0.00
4	Beta bridge	0.00	0.00	0.00	0.00
5	Extended strand	26.32	20.83	19.22	40.43
6	Bend region	0.00	0.00	0.00	0.00
7	Beta region	5.85	6.77	4.08	12.77
8	Random coil	34.50	38.02	26.21	25.53
9	Ambiguous states	0.00	0.00	0.00	0.00
10	Others	0.00	0.00	0.00	0.00

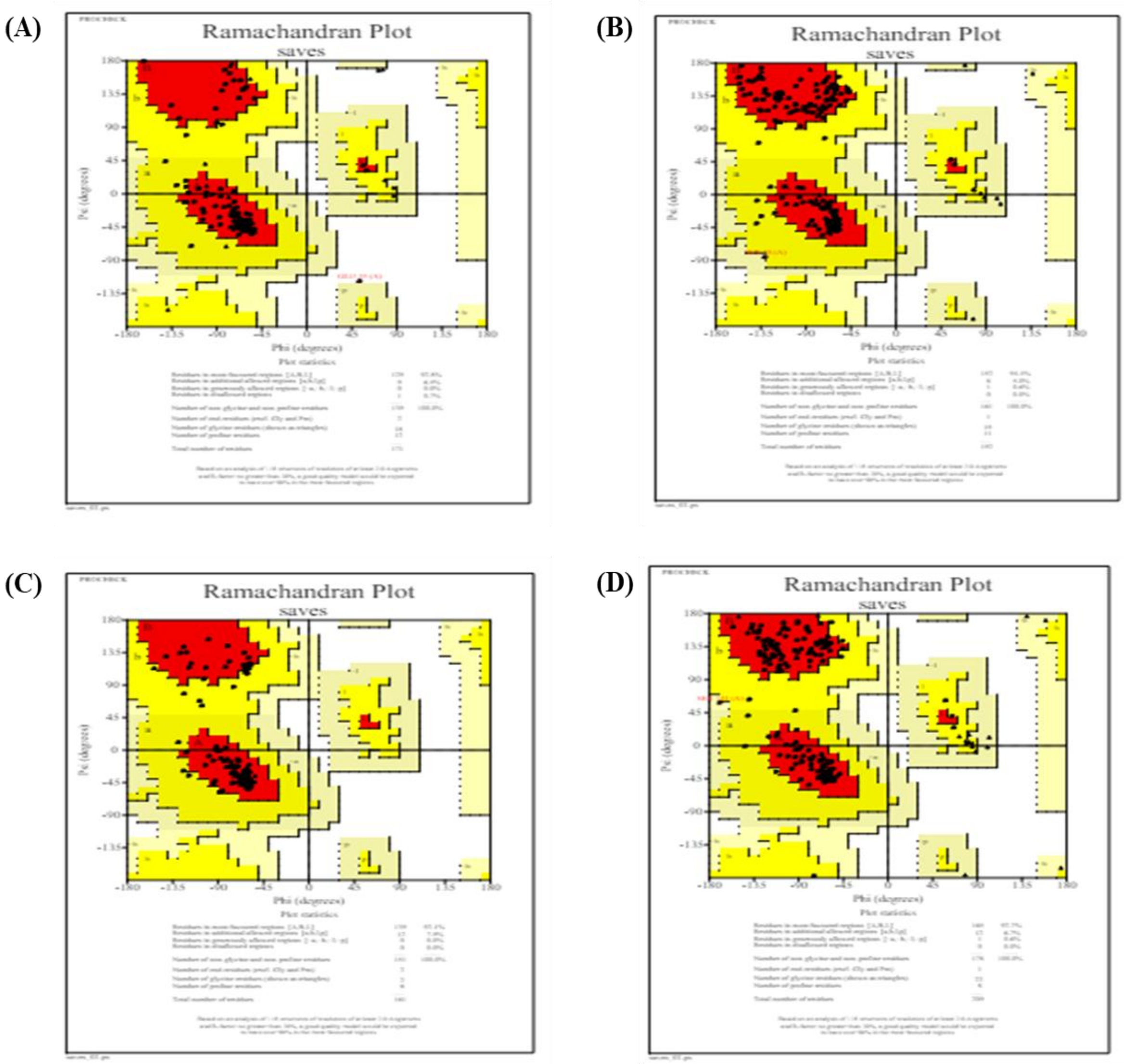


Note: (A): Cytochrome c oxidase subunit 1; (B): Ribulose bisphosphate carboxylase large chain; (C): Maturase K; (D): ATP synthase subunit 1 proteins.

Figure 3: Tertiary structures of cytochrome c oxidase subunit 1, ribulose bisphosphate carboxylase large chain, maturase K, and ATP synthase subunit 1 proteins.

Ramachandran plot analysis

The secondary conformations of polypeptides in proteins are determined by hydrogen bonding connections between the negatively charged carbonyl oxygen atoms and the positively charged amide hydrogen atoms in the molecule's backbone chain. These hydrogen-bonding interactions can establish the structural foundation that provides stability to the secondary structure. Although other secondary structures with favorable hydrogen bonding networks could be suggested, we observe only a limited number of potential options in polypeptides consisting of L-amino acids (proteins). The configuration of the backbone of each amino acid residue imposes restrictions on the feasibility of most potential secondary structures. Gaining insight into these constraints will facilitate comprehension of the secondary configurations presented by proteins. It is possible to adjust the angle ψ within the range of -180° to 180°, which corresponds to 360° of rotation for each angle. However, many combinations of these angles are rarely observed, whereas others are highly prevalent in proteins. Consider the plot of the values of ψ against the values of φ for a globular protein (Figure 4). Our objective is to acquire a dataset containing the spatial coordinates of every atom. Such data can be obtained from one of the many repositories of protein structural data. We shall adopt XRD data, as it offers the highest level of precision. Despite its lack of perfect accuracy, crystal packing pressures often cause small distortions in proteins. Molecular magnetic resonance (NMR) data for proteins in solution lack precision but are regarded to be more accurate due to the protein being in its natural environment. We may analyze this data to identify the amino acid residues (almost automatically done for us as the XRD ".PDB" data format categorizes all atoms and assigns them to certain residues). Following that, we utilize a computer to calculate the dihedral angles that establish the values of ψ and φ. A computer software may analyze a ".PDB" file and provide the recorded values of ψ and φ angles for every individual residue. First, let us graph these values for the yeast protein hexokinase. The plot of ψ against φ is referred to as a Ramachandran plot (Figure 4).



Note: (A): Cytochrome c oxidase subunit 1; (B): Ribulose bisphosphate carboxylase large chain; (C): Maturase K; (D): ATP synthase subunit 1 proteins.

Figure 4: Ramachandran plot analysis of cytochrome c oxidase subunit 1, ribulose bisphosphate carboxylase large chain, maturase K, and ATP synthase subunit 1 proteins.

Phylogenetic analysis of *C. neilgherrensis*

Phylogeny is the chronological record of the lineage of a set of taxa, like species, from their shared progenitors, comprising the sequence of branching and occasionally the dates of divergence. Molecular phylogeny is the examination of the connections between animals or genes by the comparison of DNA or protein sequences. Discrepancies among the sequences suggest genetic divergence caused by molecular evolution over time. Shortly, while the traditional phylogenetic method focuses on the physical features of an organism, the molecular methods rely on the nucleotide sequences of RNA and DNA, as well as the sequences of amino acids in a protein, which are identified using contemporary technology.[28] Evolutionary time refers to the duration required for the diversification of a set of proteins or DNA from a shared progenitor. These phylogenetic analysis techniques can discern the number of changes taking place in evolution. It quantifies the number of change, namely the number of mutations, in the protein sequence. Multiple sequence alignment is the initial step in this process. Thus, it relies on distance values calculated from sequence similarity scores.[29]

The present results found the plant species demonstrating similarity of 90% and above with the Cytochrome c oxidase subunit 1 was Curcuma bhatii (Zingiberaceae) was 100%, Sagittaria latifolia (Alismataceae) was 97.7%, Ranalisma humile (Mesangiospermae) was 97.1%, Setaria italica (Poaceae) was 96.5%, Hypseocharis pimpinellifolia (Geraniaceae) was 97.6%, Utricularia triloba (Lentibulariaceae) was 95.3%, Plantago rugelii (Plantaginaceae) was 97.0%, Lagarosiphon major (Hydrocharitaceae) was 97.7%, Physaria ludoviciana (Brassicaceae) was 98.5%, and Ophioglossum engelmannii (Ophioglossaceae) was 95.9% (Table 4).

Table 4: Lists of plant species showing similarity of 90% and above with the Cytochrome c oxidase subunit 1.

S.No	Plant species containing Cytochrome c oxidase subunit 1	Family Name	Accession Number	Identity (%)
1.	Curcuma bhatii	Zingiberaceae	AQM55903.1	100
2.	Sagittaria latifolia	Alismataceae	ANY30535.1	97.7
3.	Ranalisma humile	Mesangiospermae	ANY30531.1	97.1
4.	Setaria italica	Poaceae	RCU61537.1	96.5
5.	Hypseocharis pimpinellifolia	Geraniaceae	AAF77693.1	97.6
6.	Utricularia triloba	Lentibulariaceae	AAM22168.2	95.3
7.	Plantago rugelii	Plantaginaceae	ACD44445.1	97.0
8.	Lagarosiphon major	Hydrocharitaceae,	ANY30550.1	97.7
9.	Physaria ludoviciana	Brassicaceae	AMC32810.1	98.5
10.	Ophioglossum engelmannii	Ophioglossaceae	AAD01666.1	95.9

The findings of the phylogenetic analysis was found the list of plant species exhibiting the similarity of 90% and above with the Ribulose bisphosphate carboxylase large chain are Curcuma neilgherrensis (Zingiberaceae) was 100%, Tropidia polystachya (Orchidaceae) was 97.9%, Heliconia irrasa (Heliconiaceae) was 98.4%, Dioscorea aspersa (Dioscoreaceae) was 97.4%, Smilax glaucochina (Smilacaceae) was 98.8%, Rhizophora apiculata (Rhizophoraceae) was 97.4%, Fritillaria Montana (Liliaceae) was 96.9%, Cypripedium calceolus (Orchidaceae) was 96.9%, and Dendrobium aphyllum (Orchidaceae) was 98.5% (Table 5).

Table 5: Lists of plant species showing similarity of 90% and above with the Ribulose bisphosphate carboxylase large chain.

S.No	Plant species containing Ribulose bisphosphate carboxylase large chain	Family Name	Accession Number	Identity (%)
1.	Curcuma neilgherrensis	Zingiberaceae	AGS45035.1	100
2.	Tropidia polystachya	Orchidaceae	ANA76490.1,	97.9
3.	Musa ABB Group	Musaceae	AKG26181.1	99.0
4.	Heliconia irrasa	Heliconiaceae	AGB56362.1	98.4
5.	Dioscorea aspersa	Dioscoreaceae	ADU33626.1	97.4
6.	Smilax glaucochina	Smilacaceae	APG29210.1,	98.8
7.	Rhizophora apiculata	Rhizophoraceae	AKL78739.1	97.4
8.	Fritillaria Montana	Liliaceae	AXG21080.1	96.9
9.	Cypripedium calceolus	Orchidaceae	AXG21056.1	96.9
10.	Dendrobium aphyllum	Orchidaceae	AGZ83938.1	98.5

The findings of the phylogenetic analysis was found the list of plant species exhibiting the similarity of 90% and above with the Maturase K are Curcuma neilgherrensis (Zingiberaceae) was 100%, Curcuma bicolor (Zingiberaceae) was 99.1%, Curcuma aeruginosa (Zingiberaceae) was 98.9%, Boesenbergia sp (Zingiberaceae) was 98.1%, Pyrgophyllum yunnanense (Zingiberaceae) was 97.5%, Kaempferia parviflora (Zingiberaceae) was 96.9%, Scaphochlamys kunstleri (Zingiberaceae) was 96.3%, Meistera masticatorum (Zingiberaceae) was 97.4%, Meistera oligantha (Zingiberaceae) was 94.5%, and Globba ophioglossa (Zingiberaceae) was 93.0% (Table 6).

Table 6: lists of plant species showing similarity of 90% and above with the Maturase K.

S.No	Plant species containing Maturase K	Family Name	Accession Number	Identity (%)
1.	Curcuma neilgherrensis	Zingiberaceae	ANY59765.1	100
2.	Curcuma bicolor	Zingiberaceae	AAN63194.1	99.1
3.	Curcuma aeruginosa	Zingiberaceae	ANY59753.1	98.9
4.	Boesenbergia sp	Zingiberaceae	AWO67563.1	98.1
5.	Pyrgophyllum yunnanense	Zingiberaceae,	AAN63235.1	97.5
6.	Kaempferia parviflora	Zingiberaceae	ABD72951.1	96.9
7.	Scaphochlamys kunstleri,	Zingiberaceae	BAW33276.1	96.3
8.	Meistera masticatorum	Zingiberaceae,	AVK93970.1	97.4
9.	Meistera oligantha	Zingiberaceae	AFD04767.1	94.5
10.	Globba ophioglossa.	Zingiberaceae,	AVK93971.1	93.0

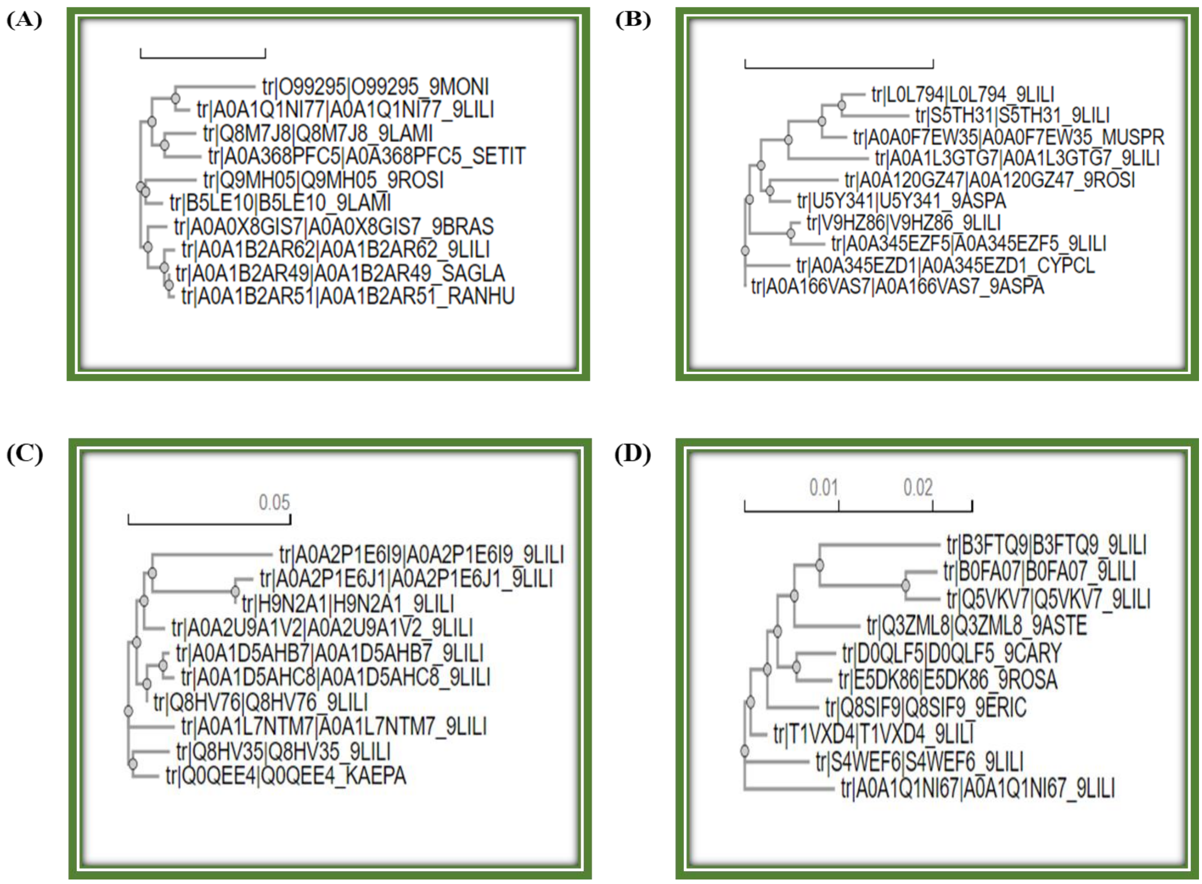
The findings of the phylogenetic analysis was found the list of plant species demonstrating the similarity of 90% and above with the ATP synthase subunit 1 are Curcuma neilgherrensis (Zingiberaceae) was 100%, Pandanus pacificus (Pandanaceae) was 98.6%, Asplundia rigida (Cyclanthaceae) was 98.2%, Canna indica (Cannaceae) was 98.1%, Clethra arborea (Clethraceae) was 94.2%, Tapeinochilos sp (Costaceae) was 96.2%, Silene hookeri (Caryophyllaceae) was 96.2%, Burmannia alba (Burmanniaceae) was 95.3%, Elaeagnus sp (Elaeagnaceae) was 96.7%, and Cornus sericea (Cornaceae) was 97.8% (Table 7).

Table 7: lists of plant species showing similarity of 90% and above with the ATP synthase subunit 1.

S.No	Plant species containing ATP synthase subunit 1	Family Name	Accession Number	Identity (%)
1.	Curcuma neilgherrensis	Zingiberaceae	AQM55890.1	100
2.	Pandanus pacificus	Pandanaceae	AGT95775.1	98.6
3.	Asplundia rigida	Cyclanthaceae	AGO90026.1	98.2
4.	Canna indica	Cannaceae	ABY66736.1	98.1
5.	Clethra arborea,	Clethraceae	AAM12431.1	94.2
6.	Tapeinochilos sp	Costaceae	AAQ74610.1	96.7
7.	Silene hookeri	Caryophyllaceae,	ACU30207.1	96.2
8.	Burmannia alba	Burmanniaceae	ACD71503.1	95.3
9.	Elaeagnus sp	Elaeagnaceae	ADL63204.1	96.7
10.	Cornus sericea	Cornaceae	AAW57436.1	97.8

In the ever-evolving world of drug discovery, the integration of molecular docking analysis and phylogenetic analysis has emerged as a powerful approach for exploring the medicinal potential of plant species. Molecular docking, a computational method, allows researchers to predict the binding interactions between ligands and their target proteins, providing insights into the therapeutic applications of various compounds.[30] Simultaneously, phylogenetic analysis, which examines the evolutionary relationships among organisms, can offer crucial information about the shared genetic characteristics and medicinal properties of different plant species.[31] The advancements in molecular docking strategies have revolutionized the drug discovery process, enabling researchers to examine the vast number of drug candidates in silico before committing to expensive and time-consuming experimental studies.[32] By predicting the binding affinities and orientations of ligands within target receptor sites, molecular docking can help identify promising lead compounds and optimize their pharmacological properties.[33] This approach has been particularly valuable in the exploration of medicinal plants, as it allows researchers to rapidly screen a wide range of plant-derived compounds for their potential therapeutic applications.

Phylogenetic analysis, on the other hand, can provide a comprehensive understanding of the evolutionary relationships among herbal plants, providing insights on their shared biochemical pathways and the underlying mechanisms responsible for their medicinal properties.[34] By analyzing the genetic similarities and differences among plant species, researchers can identify closely related species that may possess similar medicinal compounds, facilitating the discovery of novel therapeutic agents.[35] The integration of molecular docking and phylogenetic analysis has the capacity to significantly accelerate the discovery of new plant-based medicines. By combining these complementary approaches, researchers can gain a more understanding of the pharmacological properties of medicinal plants, identify promising lead compounds, and explore the evolutionary origins of their therapeutic potential.[36] In this work, the findings of the molecular analysis and phylogenetic analyses are revealed the significant role of Cytochrome c oxidase subunit 1, Ribulose bisphosphate carboxylase large chain, Maturase K, and ATP synthase subunit 1 proteins in the therapeutic effects of the *C. neilgherrensis* roots (Figure 5).



Note: (A): Cytochrome c oxidase subunit 1; (B): Ribulose bisphosphate carboxylase large chain; (C): Maturase K; (D): ATP synthase subunit 1 proteins.

Figure 5: Phylogenetic analysis of plant species with cytochrome c oxidase subunit 1, ribulose bisphosphate carboxylase large chain, maturase K, and ATP synthase subunit 1 proteins.

CONCLUSION

The current study has provided evidence of the anti-inflammatory activities of the saponins from C. neilgherrensis roots. The present results of the in silico studies has revealed the pivotal role of Cytochrome c oxidase subunit 1, Ribulose bisphosphate carboxylase large chain, Maturase K, and ATP synthase subunit 1 proteins in the therapeutic effects of the C. neilgherrensis roots. Moreover, further investigation is necessary to determine the phytochemical profile and precise underlying molecular mechanisms by which C. neilgherrensis exhibits therapeutic properties.

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