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A Green Approach of Zinc Oxide Nanoparticles from *Dodonaea viscosa* and Anti-Inflammatory Properties

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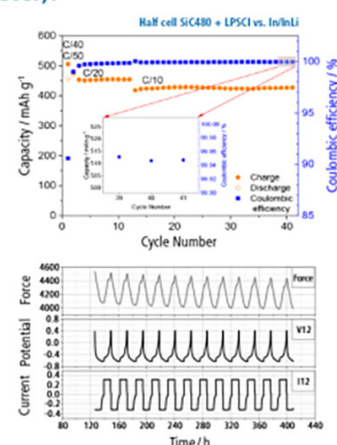
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A Green Approach of Zinc Oxide Nanoparticles from *Dodonaea viscosa* and Anti-Inflammatory Properties

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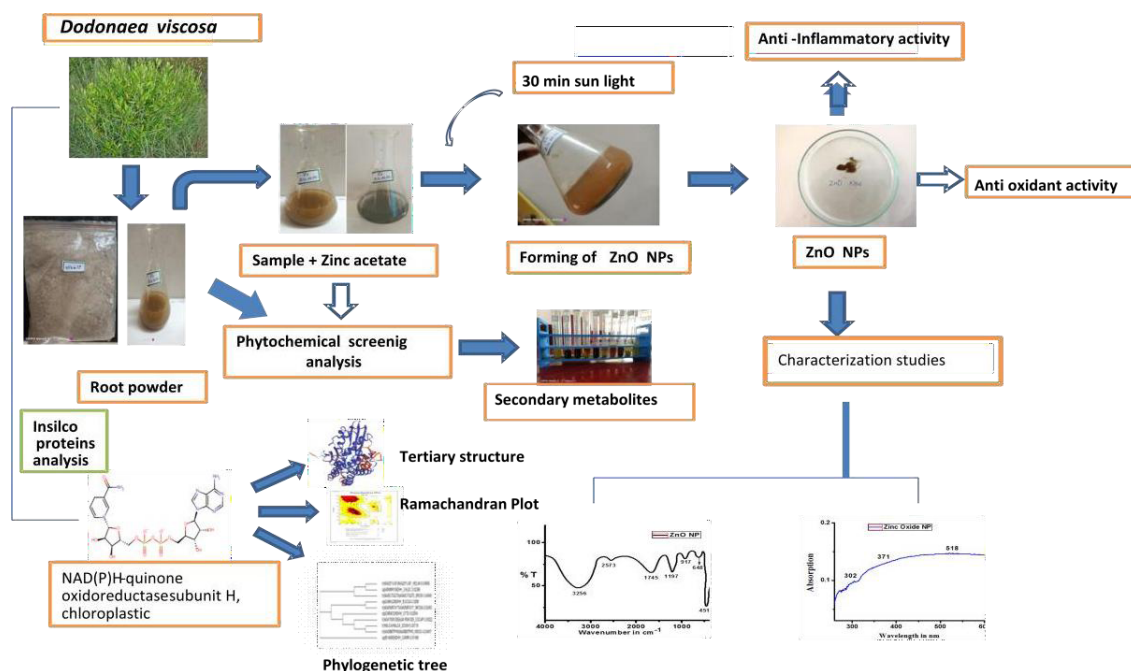
Abstract:

The current study focused on the green synthesis of zinc oxide nanoparticle (ZnO) from the *Dodonaea viscosa* roots and evaluation of its anti-inflammatory properties. The presence of phytochemicals in the root extract of *D. viscosa* root extract was analyzed with different solvents including methanol, chloroform, aqueous and petroleum ether. The methanolic extract had higher activity among the four solvents. UV-Visible and FT-IR spectrophotometry was used to validate the synthesized ZnO. The FT-IR bands revealed the existence of several functional groups, and the UV-Vis spectrum showed the absorption peak of 371 nm for ZnO nanoparticles. The green synthesized ZnO nanoparticles showed an anti-inflammatory activity that was confirmed by albumin denaturation and membrane stabilization. Maximum inhibition of 70% was observed from ZnO nanoparticle when compared with aspirin. IC₅₀ value of plant extract was 36.34 µg/ml and for aspirin was found to be 36.26 µg/ml. The *in vitro* RBCs membrane stabilization study emphasized the anti-inflammatory activity of ZnO nanoparticles. The IC₅₀ value of aspirin was determined to be 21.19, whereas the plant extract was 23.49. At varying doses, the methanolic extract of *D. viscosa* showed efficacy in preventing heat-induced hemolysis. The three-dimensional data of the protein structure is used to predict the structural levels of proteins. Proteins of *D. viscosa* were analyzed using ExPASy (structural prediction), Protparam server (functional characterization), and (3-D structure) SWISS MODEL, and homology modeling was used to compare them with known plant biomaterials. Bioinformation on useful proteins was also analyzed and reported. Thus, the synthesized nanoparticles possess strong anti-inflammatory activity that could be utilized in numerous pharmacological formulations.

Keywords: *Dodonaea viscosa*, Green synthesis, ZnO nanoparticles, Anti-inflammatory activity, protein structure prediction.



GRAPHICAL ABSTRACT



1. Introduction

Worldwide, there are about partly million plants, medical activities of those plants could be decisive in the treatment of present or prospective studies. Most local communities mainly rely on herbal medicine. But most of the plants and their medical activities have not investigated. The medicinal plants have a promising prospective for the drug discovery [1]. The geographic location and climate have an impact on the therapeutic properties and the techniques applied by ethno-medicinalists. The secondary metabolites of the plants are the active principles that substantially make a physiological effect on the mammalian systems. They have important pharmacological activity such as anti-oxidative, anti-carcinogenic, anti-allergic, hypoglycemic and antibiotic. The damage that irregular molecules like free radicals inflict on cells is prevented by these secondary metabolites [2].

A complicated biological reaction involving blood vessels, chemical mediators, immune cells, and other protective elements, inflammation is the body's tissues' defence against oxidative insults, infections, irritants, and damaged cells. Inflammation is the complex biological response of vascular tissue to harmful stimuli that leads to damaged cells or irritants and infections. The body uses inflammation as a defence mechanism to get rid of harmful stimuli, start the healing process, and rebuild structure and function. It is involved in the maintenance of many severe disorders, neurodegenerative diseases, chronic inflammatory bowel diseases, rheumatoid arthritis, diabetes, asthma and cancer [3]. The inflammatory mediators such as serotonin, cytokine, histamine and prostaglandin increase the vascular permeability to all leukocytes cell to act on the inflamed tissue. *Dodonaea viscosa* is a woody perennial shrub is known for various biological responses. The anti-inflammatory action of the plant was attributed to flavones and diterpenes of the labdane type [4].

The primary goal of contemporary material science is the application of nanotechnology in several pharmaceutical domains. Within the nanometer range of 1 to 100 nm, it investigates the synthesis, manipulation, and fabrication of surface atoms. Compared to their bulk counterparts, nanomaterials have better physicochemical features, such as increased electrical, catalytic, optical, and medicinal qualities [5]. Metal oxide nanoparticles play a crucial role in the biomedical sciences, such as medication delivery, biosensor development, and cell imaging. Compared to other metal oxide nanoparticles, a lot of attention was gained by zinc oxide nanoparticles (ZnO NPs) because of its advantageous biological characteristics. Through the regulation of enzymes, hematopoiesis, cell redox balance, and the machinery that produces DNA and proteins, due to their ability to provide synergistic benefits for treatment, ZnO NPs are being considered as a possible drug delivery mechanism for conventional medications [6, 7]. Drug discovery provides new and important leads from important medicinal plants against various diseases and disorders including cancer, cardiovascular diseases, malaria, and neuro developmental disorders [8]. The three structural levels of proteins can be predicted using three-dimensional data containing protein structures. One of the common issues in biology is the functional characterization of protein sequences. The precise 3-D structure of the protein makes this study easier. Secondary structure of protein prediction was exhibited as α -helix, β - turn, random coil. The protein's transmembrane or soluble nature was examined using the SOSUI programme. In order to ascertain the proteins of *D. viscosa* predicted structures and functional characteristics, the Expasy Protparam server was employed, whereas SWISS MODEL was employed to ascertain the 3D structure [9].

In this study, the ZnO nanoparticle from *D. viscosa* is being synthesized sustainably, and its anti-inflammatory properties are being assessed. The research could provide a comprehensive understanding of the plant *D. viscosa* potential for synthesizing functional nanoparticles for medical applications.

2. Materials and Methodology

2.1 Extraction of collected plant

D. viscosa samples were gathered freshly from the surroundings in and around Kolli hills. The authentication certificate number is GRD/2021/410. After being cleaned under running water, the sample materials were allowed to air dry before being homogenised into a fine powder and kept in the refrigerator in airtight bottles.

2.2 Phytochemical analysis

Following conventional procedures outlined by the method of [10] preliminary phytochemical analysis, were done for the four distinct solvents to detect the availability of phenolic groups, glycosides, alkaloids, terpenoids, tannins, saponins, flavonoids, oils and gums.

2.3 ZnO Nanoparticles

2.3.1 Synthesis of Nanoparticles

About 5g of *D. viscosa* root are pounded into a fine powder. ZnO nanoparticles were produced using zinc acetate. In light of this, a 500 ml acetate solution with 1 mm was taken, and 100 ml of *D. viscosa* root extract was added. After that, the mixture was continuously allowed to react at room temperature in a 1000 ml flask. Zinc ion reduction causes the yellow-colored solution to turn brown. After a 15-minute centrifugation at 10,000 rpm, the ZnO NPs pellets are separated and cleaned with distilled water. At last, ZnO Nps were dried at 50°C for 48 hrs.

2.3.2 Characterization studies

2.3.2.1 UV-Vis Analysis:

UV-Vis spectroscopy method was most frequently employed to characterize the optical properties of nanoparticles. Using UV-Vis spectrophotometry (Model 371, Elico India Ltd.), the ZnO Nps maximum absorbance was determined along a 1 cm path length, ranging from 280 to 800 nm [11].

2.3.2.2 FTIR analysis:

The functional groups from *D. viscosa* binding on ZnO Nps were studied using Fourier transform infrared spectroscopy (FTIR) attenuated total reflection mode with 4000–400 cm⁻¹ range at 4 cm⁻¹ resolution.

2.4 In-vitro anti-inflammatory Activity

2.4.1 Protein denaturation

ZnO Nps, which are biosynthesized from *D. viscosa* root, were found to have anti-inflammatory properties. A slightly altered method of albumin denaturation was used to examine this property. For this reaction, the mixture consists of ZnONps test extracts and bovine albumin fraction along with 1N HCl was added in a small amount to adjust different pH. After 20 minutes of incubation at 37°C, the extracts from the samples were heated for 20 minutes at 51°C. The samples were cooled and a UV-Visible Spectrophotometer (Model 371, Elico India Ltd.) was used to measure turbidity at 660 nm. The denaturation of protein inhibition was determined by the formula:

$$\% \text{ Inhibition} = (\text{Abscontrol} - \text{Abssample}) * \frac{100}{\text{Abscontrol}}$$

2.4.2 Evaluation of Membrane Stabilization

2.4.2.1 Preparation of RBCs suspension

The technique described by Anwar [12] is used to prepare the suspension of red blood cells (RBCs). Protein denaturation will be a well-established source of inflammation.

2.5. Insilico Proteins Analysis – Bioinformatics Study

2.5.1 Bioinformatic Tools

In this investigation, several tools, including Self Optimized Prediction Method with Alignment (SOPMA), Uniprot, Clustal OMEGA, Protparam tools, SWISS MODEL and SOSUI, were utilized to evaluate the valuable proteins of the plant. The results are contrasted with the protein sequences that are known.

2.5.2 Retrieval of sequence

The NCBI-hosted Genbank database provided the FASTA sequences for the proteins [13].

2.5.3 Predication of Primary Structure

The primary structural parameters were obtained using the ExpasyProtparam server [13].

2.5.4 Prediction of Secondary Structure

The SOPMA was utilized for predicting secondary structures [14].

2.5.5 Functional Characterization

The SOSUI tool is utilized to determine if a protein is transmembrane or soluble. Interpro protein sequencing and categorization were used to predict the super family and molecular function [15].

2.5.6 Three Dimensional Protein Structure

The study utilized the FASTA sequence to obtain a 3-D structure of biomolecules using Uniprot and SWISS MODEL servers, a fully computerized protein structure homology-modeling service managed via Expasy web server [16].

2.5.7 Sequence Alignment

For pairwise sequence alignment, the NCBI-BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was utilized, and for multiple sequence alignment, EBI-CLUSTAL OMEGA (<http://www.ebi.ac.uk/Tools/msa/clustalo/>) was employed [17].

2.5.8 Phylogenetic Analysis:

PheZophorbidase (PPD) was analyzed using phylogenetic techniques, and Neighbour Joining (NJ) technique using bootstrap was used to create phylogenetic tree [18].

2.5.9 Statistical Analysis

The Sigma Stat statistical tool (Version 3.1) was used to perform statistical analysis on the biochemical parameters under study. The mean $\pm$ SD was used to express the experimental outcomes. The results obtained for the various parameters analyzed in the four major phases and the salient findings made during the study are presented in the next chapter.

3. Result and Discussion

3.1 Phytochemical analysis

The qualitative phytochemical screening of *D. viscosa* root extracts was carried out using petroleum ether, chloroform, aqueous, methanol and Hydro alcohol solvents. The study revealed the existence of bioactive compounds of flavonoids, phenols, saponins and Steroids (Table 1).

Table 1: Phytochemical constituents of various solvent extracts of *D. viscosa*

S.No	Phytochemical Constituents	Solvents			
		Methanol	Chloroform	Aqueous	Petroleum ether
1.	Alkaloids	++	ND	+	+
2.	Flavonoids	+++	+	+++	++
3.	Phenols	+++	ND	++	++
4.	Tannins	++	ND	+	++
5.	Saponins	+++	ND	+++	++
6.	Glycosides	ND	ND	ND	ND
7.	Steroids	+++	++	++	+
8.	Proteins	+	ND	ND	ND
9.	Carbohydrates	+	ND	ND	ND



Figure 1: Preliminary Phytochemical analysis of *D. viscosa*

For anti-inflammatory activity, the methanolic root extracts of *D. viscosa* was taken due to its good efficacy when compared to the other different solvents.

3.2 Characterization studies

3.2.1 UV-VIS Analysis:

The optical absorption spectra of ZnO Nps dissolved in water were recorded using UV-Vis spectroscopy [11]. Following a 24-hour reaction, the reaction mixture absorbance was observed. ZnO Nps UV-Vis absorption spectrum is shown in Fig. 2. At normal temperature, typical excitation absorption at 371 nm was seen.

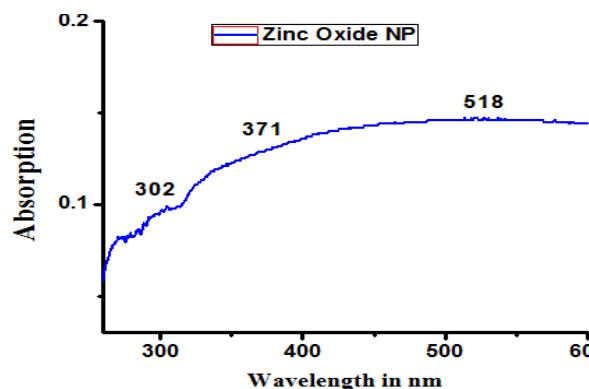


Figure 2: The UV-Vis for ZnO Nps after 24h.

3.2.2 FT IR analysis

The IR spectrum of the sample dried for one hour at 400 °C is displayed in Fig. 3. The bands are seen at 3256, 2573, 1745, 1197, 917, 648 and 451 cm^{-1} , as shown in Fig. 3. ZnO Nps. FTIR spectra were obtained within the 450–4000 cm^{-1} range. Additionally, ZnO stretching vibration is assigned to the band near 648 cm^{-1} . The hydroxyl group (O–H) is shown by the bands at 3256 and 1197 cm^{-1} . The peaks located at 917 and 1745 cm^{-1} can be attributed to the stretching modes of –C–O–C (ether) and –C=O (ketone). Stretching of C=C (alkene) is the cause of the band that emerged at 2573 cm^{-1} . Therefore, by employing this technique, the biomolecules present in plant extracts that are crucial for the reduction and stabilisation stages of the green synthesis of nanoparticles may be found [11].

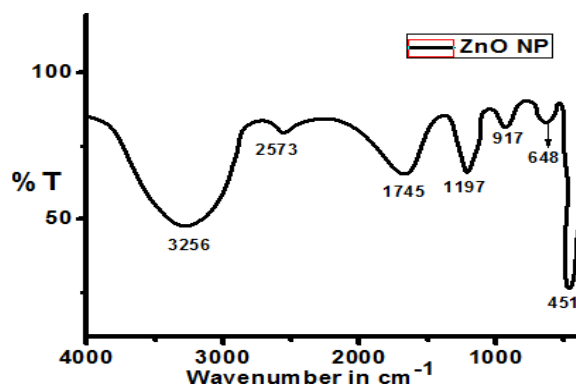


Figure 3: FT-IR analysis of ZnO Nps synthesized using *D. viscosa*.

3.3 Antiinflammatory Activity of ZnO Nps of *D. viscosa*

3.3.1 Protein denaturation by albumin

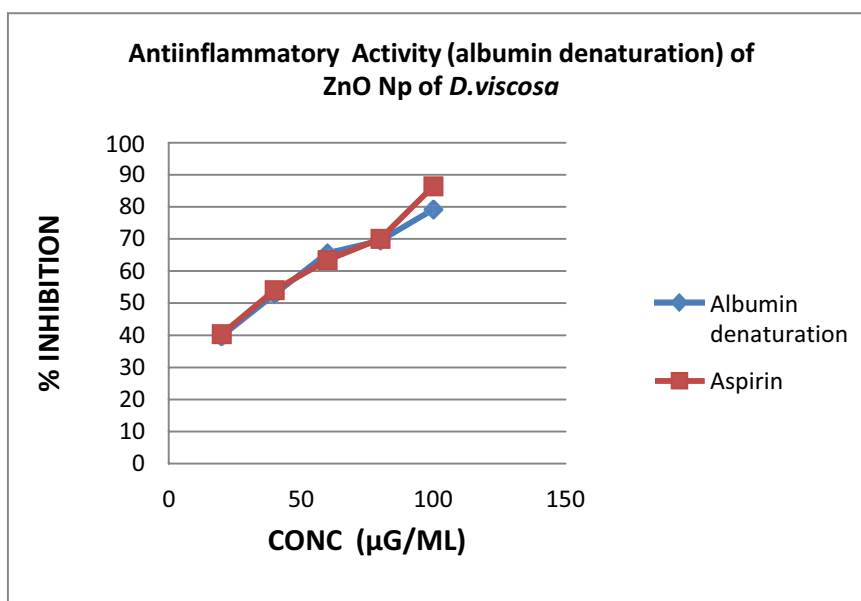
Inflammation is known to be caused by protein denaturation. The study examined the anti-inflammation mechanism of a plant extract by examining its ability to denature proteins using methanol. It was successful in preventing albumin denaturation caused by heat (Table 2 & Figure 4). ZnO Nps demonstrated the highest level of inhibition at 80%, whereas aspirin, a common anti-inflammatory medication, demonstrated the highest levels of inhibition at 67% and 82% at concentrations of 250 and 20 µg/ml. The ZnO Nps of *D. viscosa* were found to have an IC₅₀ value of 36.34 µg. Flavonoids, among other secondary metabolites, may be responsible for the extract's anti-inflammatory qualities. Interestingly, anti-inflammatory properties are widely shown by flavonoids [19].

3.3.2 Evaluation of Membrane Stabilization

The red blood cells membrane stabilization was investigated in order to elucidate the mechanism of ZnO Nps of *D. viscosa* anti-inflammatory effect. At varying doses, they demonstrated efficacy in preventing heat-induced hemolysis. This might stop lysosomal material from being released by neutrophils at the site of inflammation. Bacteriocidal enzymes and protease, which are found in the lysosomal components of neutrophils, induce further tissue inflammation and damage when they are released extracellularly. Table 2 and Figure 4 and 5 demonstrate the different degrees to which the test medication (50-250 µg/ml) reduced the heat-induced hemolysis of RBCs. At 250 µg/ml, it displayed the highest inhibition of 76%. 23.49 µg/ml was found to be the IC₅₀. Standard medication aspirin exhibited the highest level of inhibition, 89%, at 100 µg/ml. At varying doses, the plant leaf extract demonstrated efficacy in preventing heat-induced hemolysis, indicating membrane stabilization as a potential mechanism for its anti-inflammatory properties. The noteworthy and advantageous impact on membrane stabilization activities is attributable to adequate flavonoid concentration of *D. viscosa* root [20].

Table 2: Anti-inflammatory Activity of ZnO Nps of *D. viscosa*

S.No	Name of the Drug	Test Conc (µg/ml)	Albumin denaturation	% Inhibition		
				IC ₅₀ (µg/ml)	Membrane Stabilisation	IC ₅₀ (µg/ml)
1.	ZnONps	100	79.19	36.34	87.30	23.49
		80	69.43		71.60	
		60	65.55		66.46	
		40	52.74		60.40	
		20	39.69		47.36	
2.	Aspirin	100	86.40	36.26	90.00	21.19
		80	70.00		76.53	
		60	63.41		70.06	
		40	54.06		58.40	
		20	40.32		50.07	

**Figure 4:** The graphic representation illustrates the anti-inflammatory activity (Protein denaturation by albumin) of ZnO Nps from *D. viscosa*

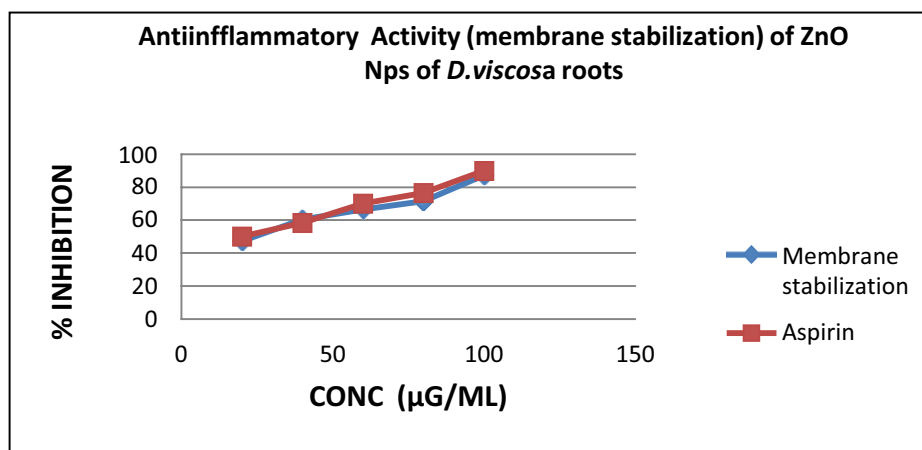


Figure 5: Graphical representation of Anti-Inflammatory activity (Membrane Stabilization) of ZnO Nps of methanol extract of *D. viscosa*

4. Insilico Proteins Analysis – Bioinformatics Study

4.1 Sequence Retrieval

The sequences of the plant proteins from *D. viscosa* are known (Table 3) and will be used in upcoming pharmacological investigations. The proteins were modelled using homology, and the fundamental theoretical characteristics were computed.

Table 3: Accession number of *D. viscosa* proteins

S No	Accession number	Protein	Length
1	A0A291F6H1	Photosystem II protein D1	353
2	A0A291F6R8	Cytochrome f	320
3	A0A291F6R7	Protein Ycf2	2,286
4	C3S9F6	DNA-directedRNA polymerase118	118
5	A0A291F6M0	NAD(P)H-quinone oxido reductase subunit H, chloroplastic	393
6	A0A291F6I9	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta, chloroplastic	502
7	A0A291F6L8	ATP synthase subunit beta, chloroplastic	498
8	A0A291F6G8	Maturase K	514
9	A4KC74	Ribulosebisphosphate carboxylase large chain	465
10	A0A291F6P1	Plastid-encoded RNA polymerase subunit alpha	327

4.2 Primary Structure Prediction

The protparam tool was used for primary structure prediction and physico-chemical properties computation of *D. viscosa* and the values have shown in (Table 4). Using the protparam tool in Expasy, the parameters were calculated, and the results showed, that the Protein TIC 214 has highest molecular weight 172304.64 for chain length 1434 sequence and flavohemoprotein has lowest molecular weight 44750.56 with chain length of 404 sequence. The theoretical PI value ranges from 5.00 to 9.70 for *D. viscosa*. Five proteins of *D. viscosa* have pI values less than 7, indicating that they are acidic, whereas five proteins had pI values larger than 7, revealing their basic characteristics. The proteins in *D. viscosa* are designed for compactness and stability, with seven out of ten having instability index (II) below 40 and an aliphatic index (AI) ranging from 80.00 to 115.00. The calculated extinction coefficients are useful for the quantitative analysis of the interactions between proteins and ligands in solution. It was discovered that the *D. viscosa* proteins GRAVY (Grand Average of Hydropathicity) ranged from -0.718 to 0.514. The likelihood of improved interaction with water is indicated by the lowest GRAVY value.

Table 4: Primary Structure Prediction of *D. viscosa* using expasy's prot param tool

S N O	ACCESIO N NUMBER	PROTEIN	LENG TH	MOL. WT	PI	-R	+ R	EC	II	AI	GRA VY
1	A0A4P9CY U	Photosystem I P700 chlorophyll a apoprotein A2	734	90211.0 7	6.8 6	47	42	2219 80	31.5 8	91.0 4	0.113
2	A0A4P9CZ 09	NAD (P) H- Quinone oxido reductase subunit 5, chloroplastic	746	93198.0 1	8.5 2	51	57	1284 10	32.7 7	114. 08	0.514
3	A0A4V1GP WS	Protein TIC 214	1434	172304. 64	9.6 5	17 5	26 2	2224 85	38.5 3	81.9 2	-0.718
4	A0A4P9D0 Q9	Photosystem II CP47 reaction center protein	508	63862.3 0	6.1 2	52	45	1206 95	36.4 3	80.7 4	0.045
5	A0A4P9D1 95	DNA-directed RNA polymerase subunit beta"	1393	165708	9.2 5	14 3	17 8	1403 85	45.3 9	95.0 2	-0.236
6	A0A6I1QA	Flavohemopr	404	44750.5	5.8	50	40	4183	39.0	92.0	-0.212

	U8	otein		6	8			0	6	3	
7	A0A4P9D1 95	DNA-directed RNA polymerase subunit beta	1071	121017. 08	8.6 7	12 0	12 9	1108 80	42.2 8	95.2 1	-0.273
8	Q85XY8	Maturase K	506	604007. 27	9.5 3	45	69	7956 5	50.9 8	99.1 9	-0.077
9	A0A0M3VI 47	Cystathionine beta-lyase	457	49521.9 0	6.1 1	48	44	3477 5	31.3 2	92.6 0	0.043
10	A0A4P9D2 04	NAD(P)H- Quinone oxido reductase subunit, chloroplastic	510	55796.7 8	5.1 5	60	50	2682 0	35.2 6	103. 31	- 0.0976

Note: Mol. Wt – molecular weight (Daltons), pI – Isoelectric point, -R - Number of negative residues, +R – Number of Positive residues, EC – Extinction Coefficient at 280 nm, II –Instability Index, AI – Aliphatic Index, GRAVY – Grand Average Hydropathicity

4.3 Predicting Secondary Structure

SOPMA's secondary structure predictions of *D. viscosa* proteins showed that the most common structural features were the prolonged random coil, alpha helix, beta turn, and strand. Among the ten proteins of *D. viscosa*, seven proteins have showed dominance in random coil and the remaining three proteins showed the dominance in alpha helix structure (Table 5).

Proteins exist in two forms: transmembrane and soluble proteins [21]. The first explanation of TMHMM, which is based on the hidden Markov model (HMM) method, was published by Tamposis[22]. TMHMM has a sensitivity and specificity of more than 99% for differentiating between membrane and soluble proteins; this high level of precision allows us to predict integral membrane proteins in massive genome collections[23]. TMHMM v.2.0 and SOSUI predicts that three proteins of *D. viscosa* Photosystem II protein D1, Cytochrome f and Protein Ycf2 were transmembrane protein (Table 6,7 & 8; Figure 6,7&8). The basic chain bond length distribution and bond angle dispersion in our data was determined to be within these proteins' tolerances. These numbers from the Ramachandran plot demonstrate the high calibre of the anticipated models (Figure 7). It can determine angles permitted and obtained the insight into the structure of peptides.

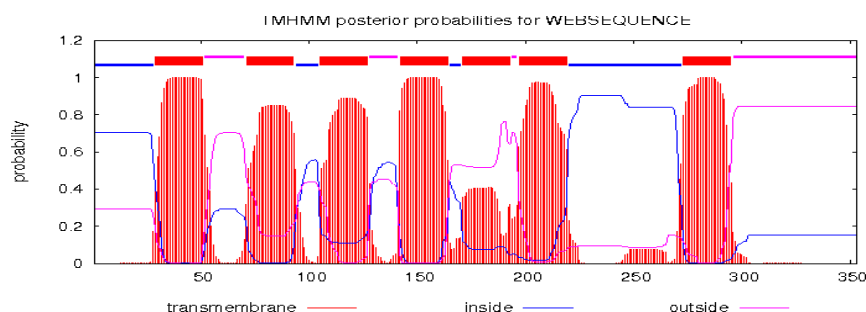
Table 5: Secondary Structure Results of proteins in *D. viscosa*

S.No	Protein	Secondary structure			
		Alpha helix	Extended strand	Beta turn	Random coil
1	Photosystem II protein D1	41.08	21.08	5.10	29.75
2	Cytochrome f	19.69	28.75	5.94	45.62
3	Protein Ycf2	37.71	18.59	5.51	38.19
4	DNA-directed RNA polymerase 118	9.32	32.20	16.95	41.53
5	NAD(P)H-quinone oxidoreductase subunit H, chloroplastic	47.84	16.03	4.33	31.81
6	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta, chloroplastic	36.06	19.92	7.17	36.85
7	ATP synthase subunit beta, chloroplastic	41.57	16.27	7.83	34.34
8	Maturase K	49.61	19.07	4.06	27.24
9	Ribulose biphosphate carboxylase large chain	44.95	15.70	6.24	33.12
10	Plastid-encoded RNA polymerase subunit alpha	42.51	20.18	6.42	30.89

A membrane protein with four transmembrane helices is represented by this amino acid sequence.

Table 6: Transmembrane region of Photosystem II protein D1 predicted by SOSUI server

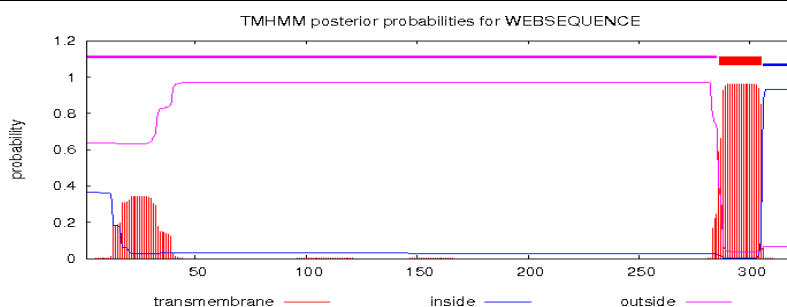
S. No	Amino terminal	Integral membrane region	Carboxyl terminal	Protein type	Length
1	33	FGVLMIPTLLTATSVFIIAFAIAA	55	Primary	23
2	68	SGSLLYGNNIISGAIPTSAAG	90	Secondary	23
3	107	YNGGPYELIVLHFLLG VACYMG	128	Secondary	22
4	144	AVAYSAPVAAATAVFLIYPIGQG	166	Secondary	23

**Figure 6:** TMHMM Result Showing Transmembrane Region of Photosystem II protein D1

A membrane protein with two transmembrane helices is represented by the amino acid sequence.

Table 7: Transmembrane region of Cytochrome F

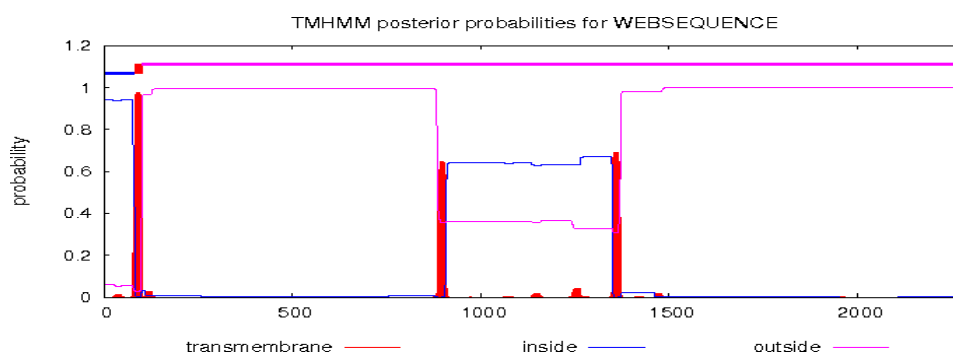
S. No	Amino terminal	Integral membrane region	Carboxyl terminal	Protein type	Length
1	16	RFISISLMIYIITRASISKAYPI	38	Secondary	22
2	284	LRVQGLLFFLASVVLAQIFLVL	305	Primary	23

**Figure 7:** TMHMM Result Showing Transmembrane Region cytochromeF

A membrane protein with three transmembrane helices is represented by the amino acid sequence.

Table 8: Transmembrane region of Protein Ycf2 predicted by SOSUI server

S.No	Amino terminal	Integral membrane region	Carboxyl terminal	Protein type	Length
1	80	NRYFTIKGVVLFVVAILLYRINN	102	Primary	23

**Figure 8:** TMHMM Result Showing Transmembrane Region Of NAD(P)H-quinone oxidoreductase subunit 3, chloroplastic

4.4 Functional Characterization

The homology modelling process is made simpler and more efficient by the fully automated workflows and servers offered by SWISS-MODEL server. Users without specialized computational knowledge can also produce accurate protein models and easily access the modelling results, as well as their visualization and interpretation. The three-dimensional structures of proteins produced by SWISS-MODEL's results offer important insights into their molecular functions and guide a wide range of applications in life science research. protein that has anti-inflammatory properties.

4.4.1 ATP Synthase subunit beta, chloroplastic

The mitochondrial H⁺-ATP synthase, a rotatory engine, uses respiratory chain proton electrochemical potential for ATP synthesis, regulated by ATPase Inhibitory Factor 1, potentially aiding in promoting macrophage class switching for therapeutic purposes [24].

4.4.2. Acetyl-coenzyme a carboxylase carboxyl transferase subunit beta, chloroplastic

Enzymes called acetyl-CoA carboxylases (ACCs) are essential for cells' lipid metabolism. They devote carbon to the production of fatty acids by converting acetyl-CoA to malonyl-CoA. Mammals encode two isoforms of ACC: ACC1, which produces malonyl-CoA for lipogenesis, and ACC2, which controls the β -oxidation of fatty acids by preventing acylcarnitine from entering mitochondria. Nevertheless, its function in controlling inflammation is still unknown [25].

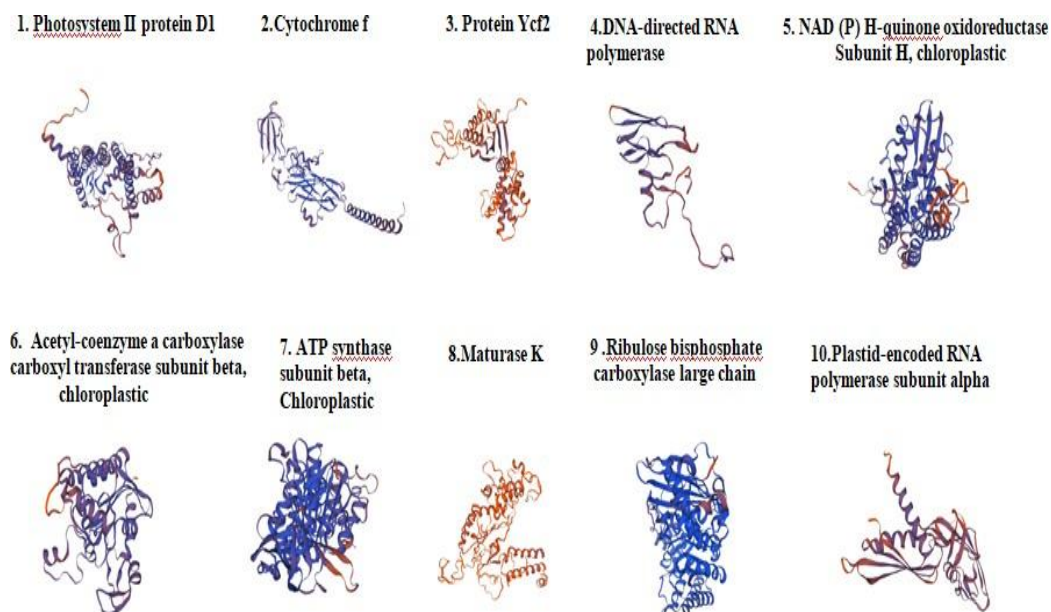


Figure 9: Tertiary structure of protein present in *Dodonaea viscosa*

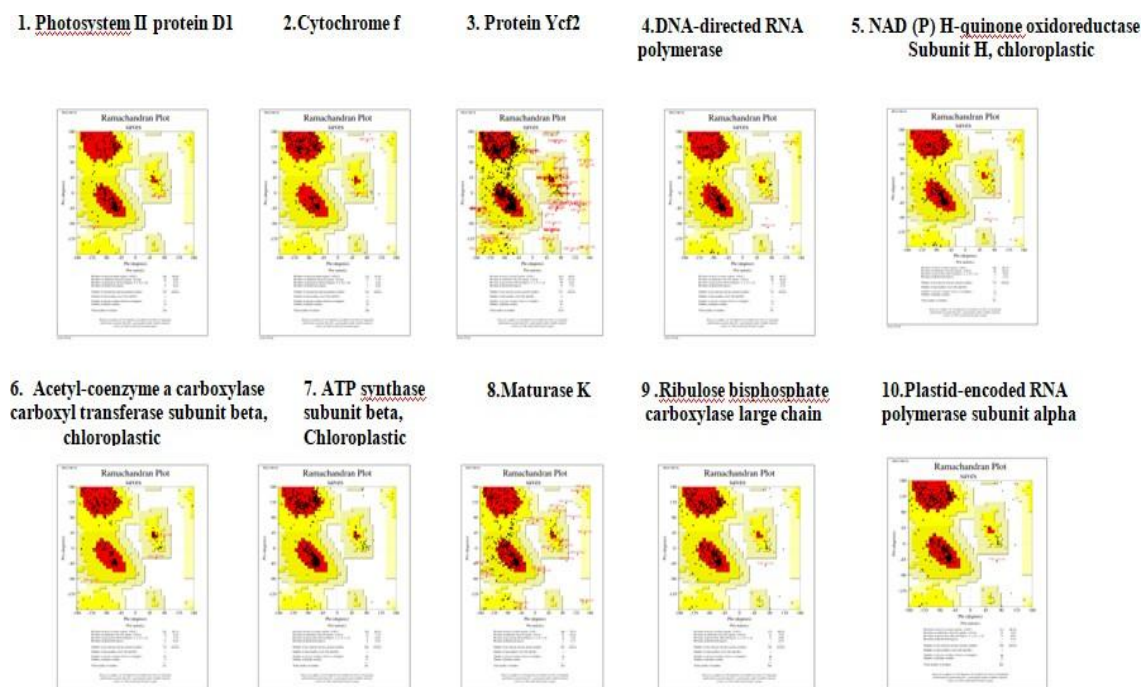


Figure 10: Ramachandran Plot for Proteins of *D. viscosa*

4.5 Phylogenetic Analysis

A phenetic method is typically used in plant systematics to group taxa based on overall resemblance. This leads to the formation of numerous groupings, at least partially, based on shared primitive features. Using phylogenetic systematics techniques, a draught cladogram of land plants is produced. It is demonstrated that assumptions about apparent convergences within main groupings, frequent hybridization, and objections to the application of phylogenetic systematics in botany are false. The best estimate of the natural order of organisms is provided by cladistic analysis, which is why plant systematists should use it to evaluate plant connections.

Table 9: Plant species lists that exhibit 85% or more similarity with the NAD(P)H-quinone oxidoreductase subunit H, chloroplast

S.NO	Species containing NAD(P)H-quinone oxidoreductase subunit H, chloroplast	Family	Accession Number	Identity
1	<i>Carica papaya</i>	<i>Caricaceae</i>	B1A990	93.9%
2	<i>Pterolobium punctatum</i>	<i>Fabaceae</i>	Q2L953	92.4%

3	<i>Acer griseum</i>	<i>Sapindaceae</i>	A0A5C7GQT0	95.8%
4	<i>Firmianacolorata</i>	<i>Malvaceae</i>	A0A5N5FGY7	92.4%
5	<i>Murrayapaniculata</i>	<i>Rutaceae</i>	A0A1R3KVD6	93.7%
6	<i>Gossypium austral</i>	<i>Malvaceae</i>	A0A5B6TPH5	94.0%
7	<i>Sesamum indicum</i>	<i>Pedaliaceae</i>	A6MMH1	92.0%
8	<i>Corchorus capsularis</i>	<i>Malvaceae</i>	A0A251VJ91	90.6%
9	<i>Eucalyptus globulus subsp</i>	<i>Myrtaceae</i>	Q49KU2	91.6%
10	<i>Citrus sinensis</i>	<i>Rutaceae</i>	Q09MC0	93.2%

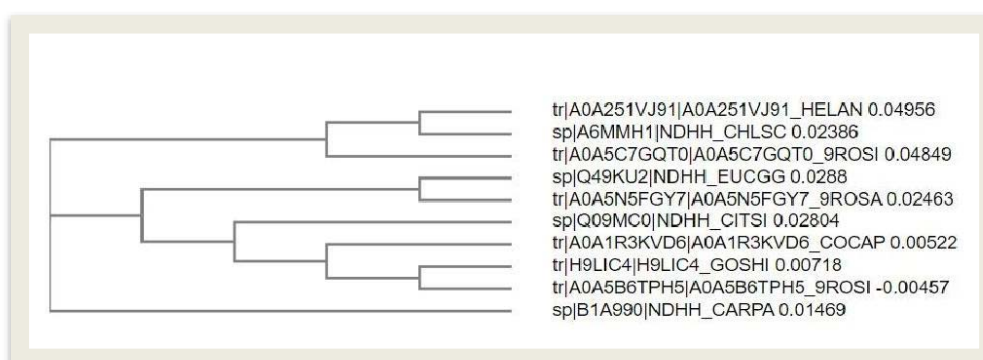


Figure 11: Phylogenetic tree of NAD(P)H-quinone oxidoreductase subunit H, chloroplatic plants

The study examined the evolutionary relationships among plants in Figure 11 Neighbor joining method was used for the phylogenetic analysis of *D. viscosa* protein sequences. Protein sequence groupings that are closely and distantly related can be distinguished using two crucial phylogenetic characteristics: divergence and convergence. The *Corchorus capsularis* showed the least degree of divergence, while the *Acer griseum* showed the largest degree of divergence. *D. viscosa* protein sequences appear to be conserved and descended from a common ancestor, according to this discovery.

5. Conclusion

This study provides an overview of current knowledge on phytochemistry, pharmacological properties, nanoparticle manufacture, and extended applications of *D. viscosa* species. The previous studies showed that a variety of ailments, such as wound healing, inflammation, burns, infections, and tumors, were historically treated using *Dodonaea* species. It has been noted that every plant under study is a member of the same genus. A crucial precursor for the environmentally friendly manufacturing of nanoparticles has been found in *Dodonaea* species. The primary bioactive substances that have been identified from *Dodonaea* include steroids, phenols, flavonoids, and saponins. These substances are widely used in contemporary medicine. The ZnO Nps that were synthesized were examined using FT-IR and UV-Vis spectroscopy methods. An effective tool for achieving anti-inflammatory qualities naturally is provided by

the *in vitro* anti-inflammatory assessment of the *D. viscosa* root samples utilizing membrane stabilization and denaturation by albumin techniques. The *insilico* homology investigations of *D. viscosa* were conducted using several bioinformation techniques, and successful characterization of the species with the proteins that were already known of proven plant medications was achieved. Proteins important physical and chemical characteristics were computed and contrasted with those of known therapeutic plant proteins. NAD(P)H-quinone oxidoreductase subunit H, chloroplastic protein sequences are nearly identical to plant protein sequences. This team used biomaterials to study diverse therapeutic qualities for future drug development.

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