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In vitro ASSESSMENT OF PHARMACOLOGICAL ACTIVITIES OF *Jacaranda mimosifolia* Mart. SEED EXTRACT AND *in silico* APPROACHES FOR PROTEIN ANALYSIS

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ABSTRACT

The genus *Jacaranda* is an important representative of tribe Tecomeae in family Bignoniaceae, with the species native to South America and widely distributed across the tropical regions. This study was aimed to comprehensively screen the phytochemicals of crude extract of *Jacaranda mimosifolia* and evaluate its antioxidant, anti-inflammatory, and antimicrobial activities. Phytochemical analysis revealed the presence of various bioactive compounds, including glycosides, carbohydrates, tannins, proteins, amino acids, terpenoids, fats, and oils. Antioxidant activity was assessed using ABTS and DPPH scavenging assays, which yielded IC_{50} values of 144.231 and 12.957 µg µL^{-1}, respectively. The extract showed significant antimicrobial activity and effectively inhibited *Pseudomonas aeruginosa, Salmonella typhi, Streptococcus mutans* and *Escherichia coli* at various concentrations. The anti-inflammatory potential was evaluated through bovine serum albumin denaturation assay, which showed an IC_{50} value of 213.85 µg µL^{-1}. Additionally, bioinformatics analysis of ribosomal protein L23 in *J. mimosifolia* revealed structural and physiochemical characteristics associated with its antioxidant properties. The study suggests that *J. mimosifolia* seed extract could be a promising natural alternative to synthetic drugs for antioxidant, antimicrobial, and anti-inflammatory applications.

Keywords: Anti-inflammatory, antimicrobial, antioxidant, *in silico* analysis, *Jacaranda mimosifolia*, phytochemicals

INTRODUCTION

The natural compounds derived from medicinal plants significantly contribute to the development of novel pharmacological agents, with ethnopharmacological data playing a pivotal role in this progress (Bonito *et al*., 2011; Maione *et al.*, 2013). Medicinal plants are widely used across the globe, and offer an alternative to the chemical drugs that often have severe side effects. The low cost, accessibility, and availability of plant-based remedies make them a cornerstone of healthcare systems, particularly in the regions with limited access to modern medicine. Approximately 80% of global population relies on plant-based medicines for treatment of various diseases (Sen and Chakraborty, 2017). The WHO's Traditional Medicine Strategy 2014-2023 has emphasized on the integration of traditional and modern therapies to enhance healthcare outcomes, highlighting the growing importance of herbal practices in providing safe, reliable, and cost-effective healthcare solutions (WHO, 2013).

Among the vast array of medicinal plants, *Jacaranda* species, belonging to the Bignoniaceae family, have garnered attention for their numerous pharmacological properties. This genus includes 49 species, with *Jacaranda mimosifolia* Mart. being particularly well-known for its ornamental value and is widely distributed in tropical and subtropical regions, including Argentina, Bolivia, and Brazil

(Bravo *et al.*, 2020). The tree is used in floriculture due to its striking purple flowers. *J. mimosifolia* has been studied for its antioxidant, anti-inflammatory, anticancer, and antimicrobial activities (Santos *et al.*, 2012; Mostafa *et al.*, 2016; Yuan *et al.*, 2018). Among the various proteins identified in this plant, ribosomal protein L23, located in the chloroplast, has shown antioxidant properties and potential regulation of cellular apoptosis (Qi *et al.*, 2017). Despite these promising findings, most research on *Jacaranda* has focused on its leaves or flowers, leaving a significant gap in the studies related to its seeds. The seed extracts, though rich in bioactive compounds, have not extensively been studied for their pharmacological effects, particularly for their antibacterial, anti-inflammatory, and antioxidant properties. Furthermore, the molecular mechanisms, including the structural characteristics of ribosomal protein L23, remain underexplored. The present study was aimed to bridge these gaps by studying the methanolic extracts of *J. mimosifolia* seeds for their phytochemical composition and pharmacological activities. Specifically, the study evaluated the extract's antibacterial, anti-inflammatory, and antioxidant properties. Additionally, the study employed *in silico* approaches to predict the structural and phylogenetic features of ribosomal protein L23, which may elucidate the molecular basis of plant's therapeutic effects. To our knowledge, no previous research has comprehensively evaluated the pharmacological activities of *J. mimosifolia* seed extracts, and this study provides novel insights that could unfold the plant's medicinal potential.

MATERIALS AND METHODS

Collection of plant materials

Seeds of *Jacaranda mimosifolia* Mart. were collected from healthy plants in growing in Pathanamthitta, Kerala (India) during the flowering season, ensuring that no noticeable physiological or biological damage was present on the plant material.

Seed extract preparation

The collected seeds were cleaned, air-dried, and finely powdered. A 1:10 ratio (w/v) of powdered seed material to methanol (10 mL methanol g^{-1} plant material) was used for extraction. The crushed plant material was first mixed with methanol and placed in a conical flask. The mixture was then incubated at 25°C overnight in a shaking incubator to prepare the cold methanol extract. In addition, a crude extract was prepared using Soxhlet extraction method (Redfern *et al.*, 2014) to ensure a comprehensive extraction of bioactive compounds. The extract was filtered and concentrated under reduced pressure using a rotary evaporator.

Phytochemical screening

Phytochemical screening of both extracts [cold methanol extract and Soxhlet extract] was carried out by employing standard techniques suggested by Ugochukwu *et al.* (2013).

Antioxidant activity

The antioxidant activity of *J. mimosifolia* seed extracts was evaluated using two common radical scavenging assays:

ABTS radical scavenging assay: The ABTS solution was prepared by adding 7 mM ABTS to 2.45 mM potassium persulfate and allowing the solution to stand in dark at room temperature for 12-16 h. Different concentrations of plant extract were mixed with ABTS solution, and the absorbance measured at 734 nm on a Chemito UV2100 spectrophotometer (Re *et al.*, 1999).

DPPH radical scavenging assay: DPPH solution (0.1 mM) was prepared in methanol. The extract was mixed with DPPH solution at final concentrations ranging from 50 to 500 µg mL^{-1}. The reaction mixture was incubated for 30 min, and the absorbance measured at 517 nm as per Blois (1958). The IC_{50} were calculated in terms of µg mL^{-1}, representing the concentration at which 50% of the radicals were scavenged.

Antimicrobial activity

The antimicrobial activity of extracts was evaluated using agar well diffusion assay (Hudzicki, 2009). The bacterial strains tested included *Pseudomonas aeruginosa*, *Salmonella typhi*, *Streptococcus mutans*, and *Escherichia coli*. The bacterial cultures were prepared to a final concentration of [specific concentration] CFU mL^{-1}, and the extracts were tested at concentrations of [specific concentrations, e.g., 10, 50, 100, 200 µg mL^{-1}]. The inhibition zone diameters were measured to determine the antimicrobial efficacy of extracts.

Anti-inflammatory activity

The anti-inflammatory potential of extract was assessed by using albumin denaturation assay (Mizushima and Kobayashi, 1968; Sakat *et al.*, 2010). The reaction mixture contained 1 mL of bovine serum albumin (BSA) solution (1 mg mL^{-1} in phosphate-buffered saline), 1 mL plant extract at varying concentrations, and 1 mL distilled water. The mixture was heated at 70°C for 10 min. Diclofenac sodium was used as a positive control. The inhibition percentage for BSA denaturation was calculated using the following formula:

100 – [(Absorbance of sample - Absorbance of control)/ Absorbance of positive control) x 100]

In silico analysis

For sequence retrieval and prediction of protein structure, FASTA sequence of ribosomal protein L23 (chloroplast) was obtained from NCBI GenBank database. The physical and chemical characteristics of protein, including theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index (Ii), aliphatic index (Ai), and grand average of hydropathy (GRAVY), were calculated using the Expasy ProtParam server (Perrière *et al.*, 1996). The secondary structure of protein was predicted using the Self Optimized Prediction Method with Alignment (SOPMA) (Combet *et al.*, 2000). For tertiary structure prediction, 3D structure of protein was predicted using the Swiss Model tool (Arnold *et al.*, 2006).

The protein's solubility and potential membrane-associated features were analysed using SOSUI and TMHMM v.2.0 (Gomi *et al.*, 2004). The stereo-chemical quality of predicted tertiary structure was evaluated with Ramachandran plot analysis (Sasisekharan *et al.*, 1963) and verified by PROCHECK (Laskowski *et al.*, 1996) and ProSA-web servers (Sippl, 1993; Wiederstein *et al.*, 2007). For phylogenetic analysis, a multiple sequence alignment of ribosomal protein L23 from different species was performed using SMART-BLAST (Sarkar *et al.*, 2007), and a phylogenetic tree was constructed to determine the evolutionary relationships.

RESULTS AND DISCUSSION

Phytochemical tests

Phytochemical screening is essential to identify the bioactive compounds present in plants possessing medicinal properties. The phytochemical analysis of *Jacaranda mimosifolia* seed extracts (cold methanol and Soxhlet) revealed the presence of varied bioactive compounds. Both extracts showed the presence of carbohydrates, proteins, amino acids and terpenoids. Soxhlet extract additionally showed the presence of cardiac glycosides, tannins, and fats & oils which were absent in cold methanol extract. Both the extracts were negative for alkaloids, sterols, phenols, flavonoids, phlobatannins, quinines, saponins and oxalates. These variations indicate that extraction methods affect the yield of phytochemical profiles, which is crucial for understanding the bioactive potential of a plant.

Antioxidant activity

Antioxidant activity was assessed by DPPH and ABTS assays. Both assays confirmed the high antioxidant potential of *J. mimosifolia* seeds as compared to its standard antioxidant (gallic acid). DPPH assay revealed an IC_{50} value of 12.957 µg mL^{-1} for seed extract, which was significantly lower

than the IC_{50} value of gallic acid (79.7 µg mL^{-1}), indicating that the extract has stronger antioxidant property (Fig. 1). Similarly, ABTS assay showed an IC_{50} value of 144.231 µg mL^{-1} for seed extract, again lower than gallic acid (174.813 µg mL^{-1}), confirming its superior antioxidant potential (Fig. 2). The presence of antioxidants in secondary metabolites such as tannins and terpenoids might be responsible for this activity (Ranabhat *et al.*, 2022; Upadhyay *et[al.*, 2010).

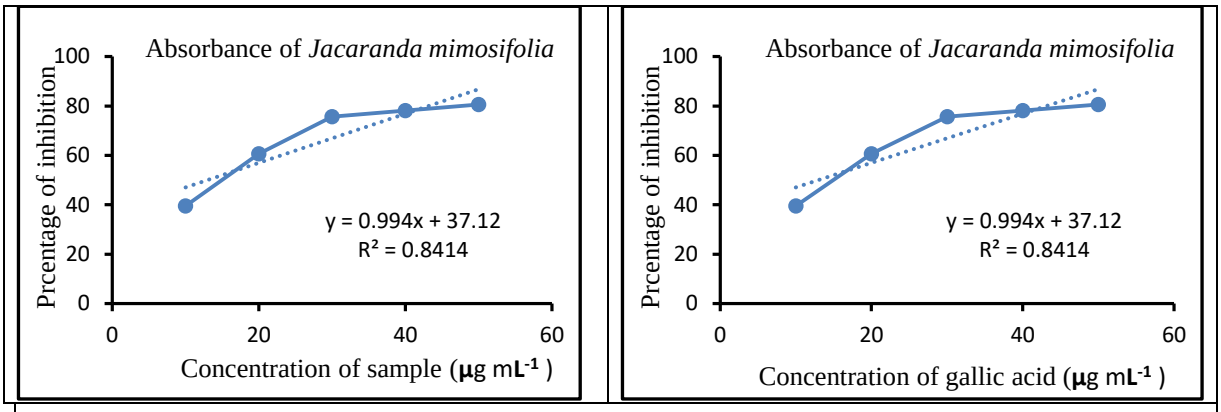

Fig. 1: **DPPH assay showing absorbance of a) *Jacaranda mimosifolia* extract (left); b) gallic acid (right)**

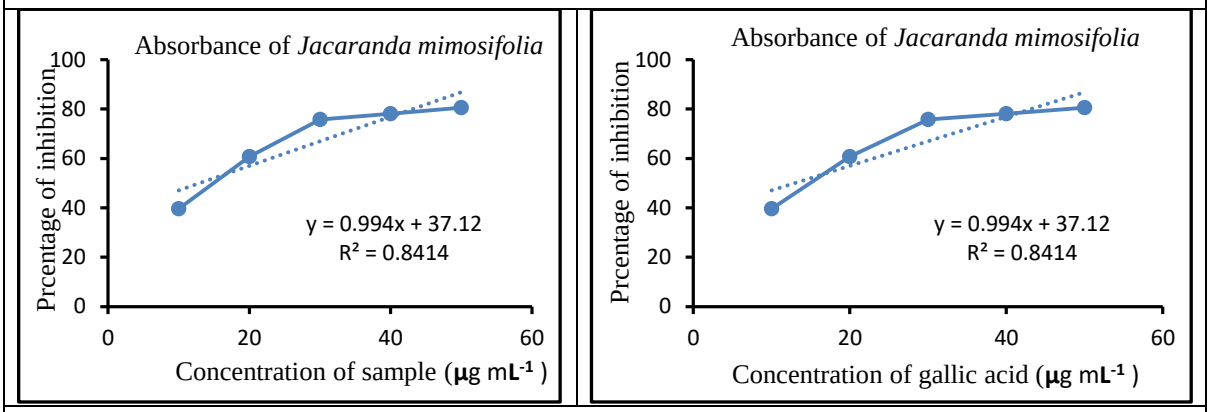

Fig. 2: **ABTS assay showing absorbance of a) *Jacaranda mimosifolia* extract (left); b) gallic acid (right)**

Antimicrobial activity

The antimicrobial activity of *J. mimosifolia* seed extract was evaluated against both Gram-negative (*P. aeruginosa*, *S. typhi*, and *E. coli*) and Gram-positive bacteria (*S. mutans*, and *B. cereus*). The extract exhibited significant antibacterial activity against *S. typhi*, *E. coli*, *S. mutans*, and *P. aeruginosa*, with inhibition zones greater than 10 mm. However, no activity was observed against *B. cereus* and *S. aureus* (Fig. 3). In contrast, no antifungal activity was detected against *A. niger* and *C. albicans***.** The antibacterial properties are likely attributed to the presence of tannins, terpenoids, and other polyphenolic compounds, which have been linked to antibacterial effects (Hong *et al.*, 2011). These findings suggest that *J. mimosifolia* has the potential to be developed as a natural antimicrobial agent, especially for use in food preservation and pharmaceutical applications.

Anti-inflammatory activity

To assess the anti-inflammatory properties of seed extract, the inhibition of protein denaturation was evaluated using bovine serum albumin (BSA) denaturation assay. The Soxhlet extract significantly suppressed BSA denaturation, with an IC_{50} value of 213.85 mg mL^{-1} though diclofenac sodium also showed a stronger effect with an IC_{50} value of 158.3051 mg mL^{-1} (Fig. 4). This suggests that the anti-inflammatory potential of seed extract is moderate, but still notable. The anti-inflammatory activity may be attributed to the compounds such as tannins and terpenoids, which have been reported to possess anti-inflammatory properties (Sultana and Saify, 2012).

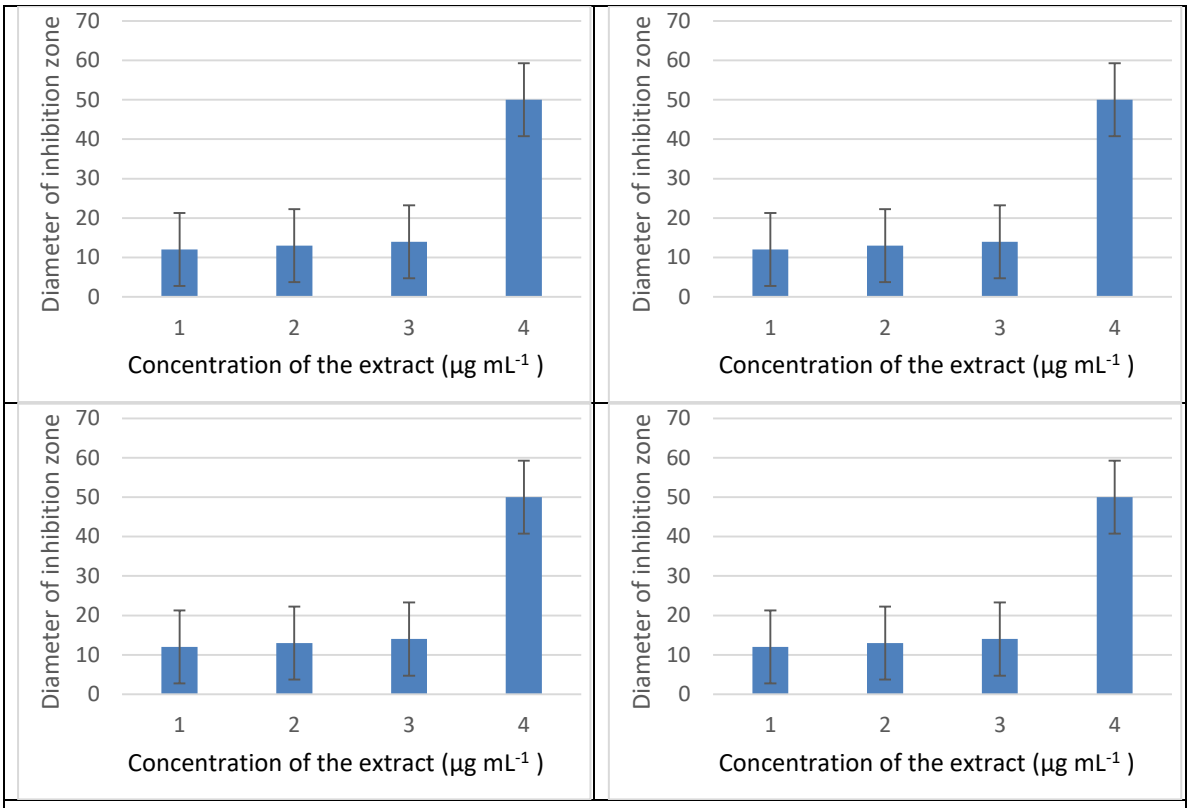

Fig. 3: Antibacterial activity of *Jacaranda mimosifolia* seed extract (left) against *E. coli* (above left side); *P. aeruginosa* (above right side), *S. mutans* (below left side), and *S. typhi* (below right side)

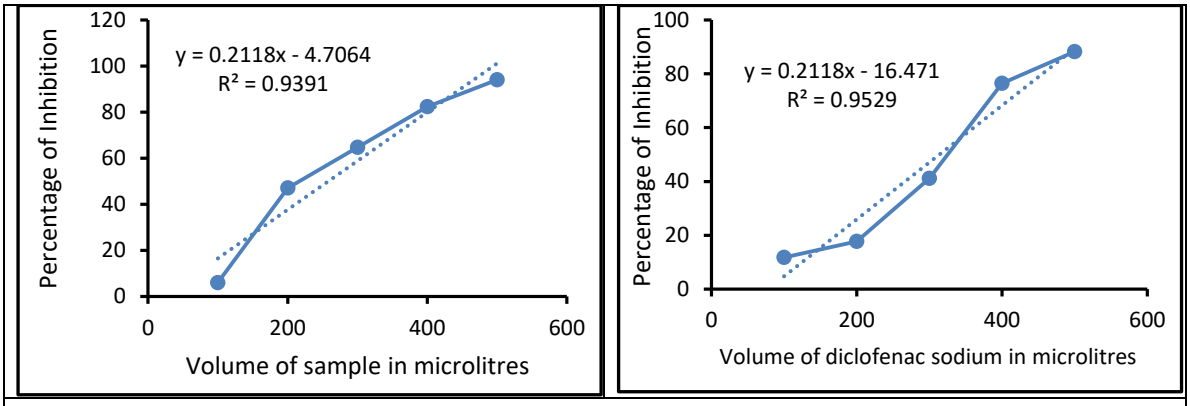

Fig. 4: **BSA denaturation assay showing turbidity of *Jacaranda mimosifolia* extract (left); b) diclofenac sodium(right)**

Bioinformatics and phylogenetic analysis of ribosomal protein L23

Bioinformatics tools were used to analyze the structural and functional properties of ribosomal protein L23 (chloroplast). The primary structure predictions revealed a molecular weight of 10732.67 Da and an isoelectric point (pI) of 10.82**,** which suggests that the protein is stable (Table 1). The protein's high aliphatic index (76.45) suggests enhanced thermostability, which is useful in optimizing protein purification techniques in future studies.

In present study, Expasy's ProtParam tool enumerated that the molecular weight of ribosomal protein L23 as 10732.67 Da with chain length of 93 sequence and the N-terminal of sequence considered was M (Met). The estimated pI value of the protein is 10.82, which ensures the stability of this

Table 1: Primary structural prediction of ribosomal protein L23

Accession No.	Protein	Length	Mol. wt.	PI	-R	+R	EC	II	AI	GRAVY
QQN90328	Ribosomal protein L23 (chloroplast)	93	10732.7	10.82	5	17	11460	39.86	76.45	-0.477

[Note: Mol. Wt – molecular weight (Da), pI – Isoelectric point, -R – No. of negative residues, +R – No. of positive residues, EC – Extinction coefficient at 280 nm, II – Instability index, AI – Aliphatic index, GRAVY – Grand average hydropathy, * - No Trp, Tyr or Cys residue (should not be visible by UV spectrophotometry)]

protein after purification, and an aliphatic index of 76.45. The instability index was computed to be 39.86, classifies the protein as stable. And also this protein has total count of 5 negatively charged residues [-R (Asp + Glu)] and 17 positively charged residues [+R (Arg + Lys)]. The number negatively charged residues was less than that of positively charged residues, which indicated the intercellular protein-protein interactions. From Table 1, it is evident that extinction coefficient, measured in water at wavelength 280 nm, illustrates the quantity of light a protein absorbs at this particular wavelength. This estimation may help in the optimization of protein purification techniques in future studies, which exhibited the value of 11460 M^{-1} cm^{-1}. The aliphatic index which explains the relative volume allocated for aliphatic side chains of Val, Leu, Ala and Ile, was 76.45, and its high value indicated the enhanced thermostability of protein. GRAVY value, the sum of hydropathy scores for each amino acid divided by the total quantity of amino acids, in the ordered sequence was -0.477.

Table 2: Secondary structural prediction of ribosomal protein L23

Protein	α-helix (Hh)	β-turn (Tt)	Extended strand (Ee)	Random coil (Cc)
Ribosomal protein L23 (chloroplast)	24.73%	7.53%	25.81%	41.94%

The secondary structural prediction of ribosomal protein L23 was done by using bioinformatic tool SOPMA. Table 2 illustrates the percentages of secondary elements of structure, comprising of α helices, β turns, extended strands and random coils. Various default parameters for estimating the secondary structure include window width: 17, similarity threshold: 8 and number of states: 4. Also, the tools named SOSUI and TMHMM v.2.0 were employed to confirm the solubility of ribosomal protein L23 and was found to be a soluble protein with 0 transmembrane helices (Fig. 5).

SWISS-MODEL was used to predict the three-dimensional protein structure (Fig. 6). Also, to verify the structure, a Ramachandran plot was generated by PROCHECK programme, and Phi and PSi distribution of Ramachandran map of protein was also summarised using the plot (Fig. 7). Depending on the arrangement of non-Gly residues in the forbidden regions, the plot depicts the core configuration of protein. In the plot, the residues were organised based upon their quadrangle regions. In graph, the yellow regions depicted the allowed zones while the red regions illustrate the most allowed regions. The plot demonstrated that total number of non-glycine and non-proline residues were 77, the number of glycine residues were 9 (shown as triangles), and number of proline residues were 5. Further, 94.8% residues were in most favoured regions, which indicated the better quality of projected model. The bio-informatics tool, ProSA-web was used to examine the modelled protein structure, which provide z-score of protein that denotes its degree of native-ness (Wiederstein and Sippl, 2007).

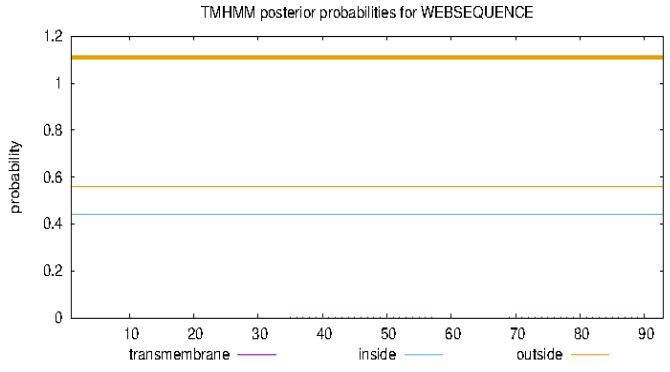

Fig. 5: TMHMM of ribosomal protein L23

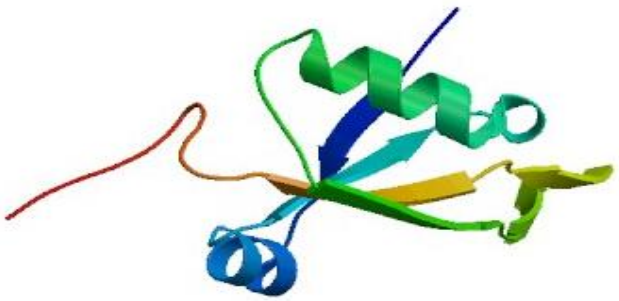

Fig. 6: 3D structure of ribosomal protein L23; Modeling server SWISS-MODEL was employed to predict the three-dimensional protein structure

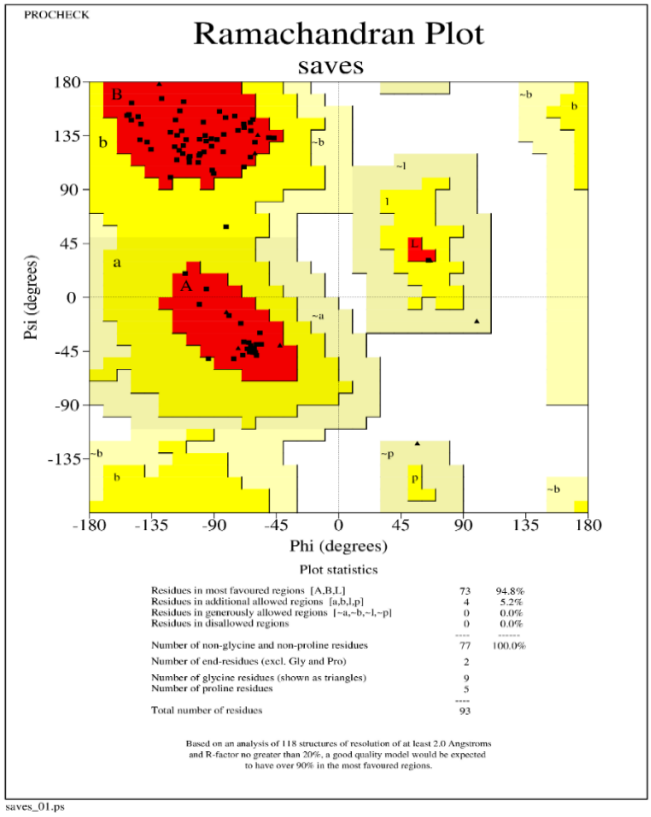

Fig. 7: **Ramachandran plot for ribosomal protein L23**

Fig. 8 shows the phylogenetic tree of protein named ribosomal protein L23. Table 3 illustrated the percentage of identity of proteins present in different organisms with the protein in this tree, ranging between 29 and 100%. This has comprehensively and scientifically been proven to have substantial pharmacological efficacy.

Integration of experimental and bioinformatics

While the experimental results provided insights into the pharmacological properties of *J. mimosifolia* seed extracts, the bioinformatics analysis of ribosomal protein L23 remains somewhat disconnected from these findings. The potential link between the protein's predicted antioxidant and anti-inflammatory roles and the observed biological activities of plant extracts need to be further elucidated. Also, the functional studies, including protein-ligand docking and protein-protein interaction analysis, could offer valuable insights into the molecular mechanisms underlying the plant's pharmacological effects. The findings demonstrate that *J. mimosifolia* seeds contain a variety of bioactive compounds with significant antioxidant, antimicrobial, and anti-inflammatory properties. These results, in combination with the bioinformatics analysis of ribosomal protein L23, provide a comprehensive understanding of plant's potential as a

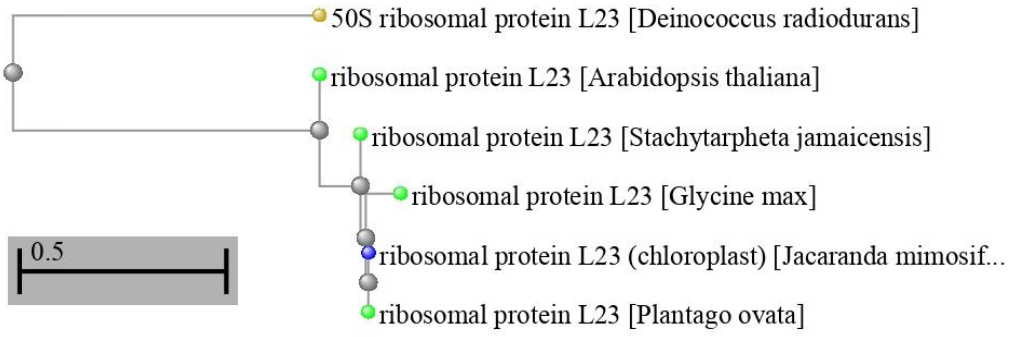

Fig. 8: Phylogenetic tree of ribosomal protein L23

Table 3: Phylogeny of ribosomal protein L23

Accession No.	Description	Identity
NP_051100.1	ribosomal protein L23 [*Arabidopsis thaliana*]	94.62%
YP_538806.1	ribosomal protein L23 [*Glycine max*]	91.40%
WP_010886958.1	50S ribosomal protein L23 [*Deinococcus radiodurans*]	29.89%
YP_010721646.1	ribosomal protein L23 [*Stachytarpheta jamaicensis*]	98.92%
YP_009573006.1	ribosomal protein L23 [*Plantago ovata*]	100.00%

natural source of therapeutic agents. However, further studies are needed to establish a more direct connection between the bioinformatics data and the observed pharmacological activities. Molecular docking studies and functional analysis of the ribosomal protein L23 could provide valuable insights into its role in the plant's therapeutic potential.

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