Ind. J. Pharm. Edu. Res., 2026; 60(1):283-293.
https://www.ijper.org

Original Article

In vitro Antioxidant, Antimicrobial, *in silico* Molecular Docking Analysis, and Phylogenetic Analysis of *Anoectochilus elatus*

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ABSTRACT

Background: Medicinal plants have long been valued for their therapeutic properties, offering a rich reservoir of bioactive compounds. Across millennia, diverse cultures worldwide have developed intricate systems of traditional medicine, utilizing plant-based remedies to treat a wide range of ailments. **Objectives:** This study investigates the *in vitro* antioxidant and antimicrobial activities, along with *in silico* analyses, of the roots of *Anoectochilus elatus*. **Materials and Methods:** Crude saponins were isolated from the root extracts, and their free radical scavenging and antimicrobial activities were evaluated using standard methods. *In silico* analyses and phylogenetic investigations were performed to identify key protein constituents associated with the medicinal properties of *A. elatus* roots, focusing on Ribulose-1,5-bisphosphate carboxylase and Maturase K. **Results:** Isolated saponins from *A. elatus* root extracts exhibited significant antioxidant and antimicrobial activities against various pathogens. *In silico* and phylogenetic analyses highlighted the potential therapeutic roles of Ribulose-1,5-bisphosphate carboxylase and Maturase K proteins in *A. elatus*. **Conclusion:** This study demonstrates the antioxidant potential and antimicrobial efficacy of saponins derived from *A. elatus* roots. Furthermore, *in silico* findings suggest a crucial role for Ribulose-1,5-bisphosphate carboxylase and Maturase K in mediating the plant's therapeutic effects.

Keywords: Pathogens, Antioxidant, *Anoectochilus elatus*, *In silico* analysis, Maturase K.

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Received: 07-04-2025;
Revised: 24-06-2025;
Accepted: 18-08-2025.

INTRODUCTION

The use of medicinal plants in the management of diseases has a rich and extensive history, dating back to ancient civilizations. Throughout the millennia, diverse cultures around the world have developed intricate systems of traditional medicine, utilizing a vast array of plant-based remedies to treat several ailments.[1] Recorded history provides ample evidence of the long-standing utilization of herbal plants. The Indian medical system, rooted in ancient Ayurvedic traditions, has long recognized the remarkable therapeutic efficacy of herbal plants. Ayurveda has documented the use of extensive plants and formulations to treat numerous diseases. The rich diversity of India's flora, with an estimated 250,000 plant species, has served as the foundation for a vast array of plant-based remedies.[2]

Traditional healing systems, such as Ayurveda, Siddha, and Unani, have been extensively studied for their applications in treating a wide range of diseases. As per the World Health Organization, approximately 70% of the world's population depends on plants for their primary health care. In the global market, more than 50 major drugs have been derived from tropical plants, underscoring the immense potential of plant-based medicine.[3,4] As human civilization progressed, the use of herbal plants continued to evolve and expand. The knowledge of the healing properties of these plants was passed down from generation to generation, serving as a primitive form of health insurance. Over time, ancient records meticulously documented the various applications of herbal plants to treat diseases, creating a rich verbal materiamedica.[5] With the advancement of modern science and technology, the focus shifted from the use of whole plant extracts to the isolation and synthesis of active components. Nevertheless, botanicals continue to serve as the foundation for many modern medical therapeutics, and the study of traditional plant-based medicines has become an area of growing interest and research.[6]

Saponins, a diverse class of naturally occurring compounds found in various medicinal plants, have garnered significant attention in

DOI: 10.5530/ijper.20262104

the scientific community due to their remarkable pharmacological properties and potential therapeutic applications.[7] One of the most intriguing aspects of saponins is their cytotoxic potential against cancer cells. Numerous studies have demonstrated that saponins can induce apoptosis in several cancer cells, making them attractive as potential anti-cancer agents. This is particularly significant, as eliminating tumor cells through apoptosis can help reduce the side effects associated with traditional cancer therapies.[8] In addition to their anti-cancer efficacy, saponins have also been shown to exhibit anti-inflammatory, antioxidant, and antimicrobial properties, further expanding their potential therapeutic applications. For instance, saponin-rich plant extracts have been utilized in conventional medicine for centuries to manage numerous diseases, including infections, metabolic disorders, and even cognitive impairment.[9]

Despite the growing interest in the therapeutic effects of saponins, further study is required to fully understand their roles, optimize their bioavailability, and develop effective delivery systems. As our understanding of these remarkable plant-derived compounds continues to expand, the future holds great interest in developing saponin-based therapeutics that can revolutionize the way we approach the treatment of human diseases.[10] *A. elatus* is a medicinal plant originating in India and China, which is well known for its therapeutic effects. The phytochemical screening of *A. elatus* root extracts revealed a variety of bioactive compounds, including saponins, flavonoids, phenols, alkaloids, tannins, and terpenoids. Saponins are significant due to their pharmacological relevance, while flavonoids and phenolic compounds contribute to the plant's antioxidant potential. Alkaloids and tannins may enhance the root extracts' therapeutic efficacy.[11] The therapeutic effects of *A. elatus* are harnessed from various parts, including leaves, stems, and roots.[12,13] Historically, it has been utilized to manage several ailments, including hepatic diseases, respiratory issues, and gastrointestinal illnesses. The herb has additionally been utilized to promote cardiovascular wellness and exhibit anticancer qualities.[14]

Anoectochilus elatus, a member of the Orchidaceae family, is traditionally valued in certain indigenous medicinal systems for its purported health benefits. However, unlike its more extensively studied relatives within the genus, *A. elatus* remains largely neglected in modern pharmacological literature. Its phytochemical composition, biological properties, and therapeutic mechanisms are poorly understood. This study represents a novel and integrated investigation into the species, combining *in vitro* assays and *in silico* analyses to uncover its antioxidant and antimicrobial potential. By focusing on a scientifically underexplored yet ethnobotanically significant plant, this research contributes new insights into natural drug discovery and highlights the pharmacological promise of an overlooked orchid species. Despite its several beneficial properties, this plant has not undergone scientific examination, necessitating thorough research of its therapeutic properties. The existing evidence regarding this plant is quite limited and insufficient. This work investigates the *in vitro* antioxidant, antimicrobial, and *in silico* analyses of *A. elatus* roots.

MATERIALS AND METHODS

Collection and identification of the plant

The root parts of the plant *A. elatus* were collected from the top hill of Yercaud at Salem District, and authenticated by Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, and India. The authentication certificate number is No.PARC/2020/4319. Soon after the collection, the root was shade-dried for 15 days to remove moisture content. Then coarsely powdered by a mechanical blender and passed through sieve No.40. Powered plant material was used for the extraction.

Extraction procedure

The shade-dried coarsely powdered root of *A. elatus* (100 g) was extracted separately with 500 mL of 50% water, methanol, chloroform, petroleum ether, benzene, and ethyl acetate. Initially, it was macerated at 37°C for 72 hr. Then the plant material was extracted with specific solvents using the Soxhlet apparatus. After extraction, the extract was filtered and stored at 4°C for further investigations.

Extraction of crude saponins

Initially, the plant powder was defatted using petroleum ether and n-hexane. The defatted substance was subsequently extracted using methanol. The methanolic extract was concentrated via rotational evaporation under vacuum conditions. Then it was suspended in distilled water and agitated with n-butanol, leading to the precipitation of a crude saponin mixture with the addition of diethyl ether. A 100 g of the sample was placed into a conical flask, and 500 mL of 20% aqueous methanol was added. The solution is heated for 4 hr with constant stirring at approximately 55°C. The mixture is filtered, and the residue is further extracted with 200 mL of 20% ethanol. The amalgamated extracts are concentrated to approximately 40 mL using a water bath at around 90°C. After evaporation, the sample is subjected to drying in an oven until a constant weight is attained, and the saponin content is determined as a percentage.

In vitro antioxidant analysis of crude saponins extracted from *A. elatus* roots

The effect of saponins from the *A. elatus* roots on the DPPH scavenging effect was assessed using an earlier technique.[15] The effect of saponins from the *A. elatus* roots on the Hydrogen Peroxide (H_2O_2) radical scavenging was studied by previous techniques.[16] The ABTS scavenging property of the saponins from *A. elatus* roots was scrutinized using the previously established technique.[17] The influence of saponins from *A. elatus* roots on

the scavenging of NO radicals was studied using the earlier technique.[18]

Analysis of antimicrobial activity

The well diffusion method was utilized to study the antibacterial and antifungal effectiveness of the saponins extracted from *A. elatus* roots against *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Micrococcus luteus* (ATCC 4698), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 6539), *Proteus vulgaris* (ATCC 6380), *Klebsiella pneumoniae* (ATCC 13883), *Vibrio parahaemolyticus* (ATCC 17802), *Aspergillus niger* (ATCC 16404), *and Candida albicans* (ATCC 10231). The present experiment utilized the appropriate agar growth media. Following the inoculation of the plates with the respective pathogens, saponins extracted from *A. elatus* roots at 25, 50, 75, and 100 mg/mL dosages were introduced into wells created with a cork borer on the agar plate surface and incubated at 37°C for 24 hr. Following an incubation time, the diameter of the inhibitory zones was recorded. Amoxicillin served as a positive control in the trials.

In silico analysis to identify the protein components responsible for the therapeutic activities of *A. elatus*

Sequence retrieval and structure predictions

The FASTA sequence of proteins was acquired from the GenBank database managed by the NCBI at http://www.ncbi.nlm.nih.gov. We determined the theoretical Isoelectric Point (pI), molecular weight, total counts of positive and negative residues, extinction coefficient, instability index, aliphatic index, and Grand Average of Hydropathy (GRAVY) for physicochemical characterization utilizing the ExpasyProtparam server at http://us.expasy.org/tools/protparam.html. The Self Optimized Prediction Method with Alignment (SOPMA) was employed to identify the secondary structure. The SWISS MODEL was employed to forecast the tertiary structures.

Functional characterization

The protein was analyzed utilizing the SOSUI and TMHMM v.2.0 databases to evaluate its solubility or transmembrane characteristics. The InterPro database comprises a comprehensive compilation of protein families, domains, and functional sites. InterPro integrates the principal protein signature databases into a cohesive resource. These include: PROSITE, which employs regular expressions and profiles; PRINTS, which utilizes Position Specific Scoring Matrix-based (PSSM) fingerprints; ProDom, which implements automatic sequence clustering; and Pfam, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D, and PANTHER, all of which utilize Hidden Markov Models (HMMs). The superfamily and molecular function were detected utilizing the InterPro protein sequencing and categorization platform accessible at http://www.ebi.ac.uk/interpro/.

Sequence alignment

Pairwise sequence alignment was done utilizing the NCBI-BLAST program accessible at http://blast.ncbi.nlm.nih.gov/Blast.cgi. Multiple sequence alignment was executed utilizing the EBI-CLUSTAL OMEGA tool accessible at http://www.ebi.ac.uk/Tools/msa/clustalo/. Clustal Omega offers strong functionalities for integrating sequences into and analyzing data within established alignments, utilizing the comprehensive updated information accessible in public databases like Pfam. The main aim of this study is to pinpoint regions of sequence similarity, hence facilitating the derivation of functional and evolutionary relationships among the proteins examined in this paper.

Phylogenetic analysis

A phylogenetic study was performed to detect the quantity of proteins exhibiting common structural and functional characteristics. All sequences in FASTA format were submitted to Clustal Omega using the default settings. A comprehensive analysis was performed on the output of fully aligned sequences, including scores, alignment, conserved residues, substitutions, and patterns of semi-conserved substitutions. A phylogenetic tree was constructed with the bootstrap Neighbour Joining (NJ) technique.

RESULTS AND DISCUSSION

Effect of saponins from the *A. elatus* roots on the *in vitro* free radical scavenging effects

The analysis of free radicals scavenging effect of plant extracts has become an increasingly important field of study due to the growing recognition of the role of oxidative stress in various disease states. Free radicals, such as ROS and RNS, are generated during normal cellular metabolism and can cause damage to cellular components, leading to a range of health issues, including atherosclerosis, aging, cancer, and cardiovascular diseases.[19] The major group of antioxidant phytochemicals present in plants have been found to play a pivotal role in adsorbing and neutralizing free radicals. The capacity of plants to scavenge free radicals can be assessed using *in vitro* assays, which provide valuable insights into the antioxidant potential of these extracts.[20]

A series of free radical scavenging tests were done to assess the *in vitro* antioxidant effects of saponins from the *A. elatus* roots, with the findings illustrated in Table 1. The treatment with saponins from the *A. elatus* roots at varying dosages (20-100 µg/mL) significantly reduced the free radical levels. The saponins from the *A. elatus* roots at 20 to 100 µg/mL dosages effectively inhibited the levels of several free radicals, including DPPH, H2O2, ABTS, and NO, in dose-dependently. The ability of saponins from the *A. elatus* roots to neutralize several free radicals *in vitro* was demonstrated by a reduction in DPPH, H_2O_2, ABTS, LPO, and NO radicals after exposure to increased quantities of the saponins from the *A. elatus* roots (Table 1). These data further demonstrate

that saponin from the *A. elatus* roots exhibits exceptional antioxidant properties.

The DPPH assay is an extensively employed technique for evaluating the free radical scavenging effect of plant extracts. This method involves the reduction of the stable DPPH radical by antioxidants in the extract. Similarly, the H_2O_2 scavenging assay measures the capacity of the plant extract to neutralize H_2O_2, which is a reactive oxygen species. The ABTS assay, on the other hand, measures the capacity of the extract to neutralize the ABTS radical cation, while the NO scavenging assay evaluates the extract's capacity to scavenge NO.[21] It has been already demonstrated the free radical scavenging effect of various plant extracts.[22] The present results also proved that *A. elatus* root extracts have an exceptional capacity to scavenge the various free radicals, including DPPH, H_2O_2, ABTS, and NO radicals.

Antimicrobial activity of the saponins from the *A. elatus* roots

The analysis of the antimicrobial effect of plants is a crucial field of study with significant implications for various applications. Plant extracts have long been known for their therapeutic properties, including antimicrobial effects. The increasing prevalence of drug-resistant microbial strains has further highlighted the importance of exploring alternative antimicrobial sources, such as those found in medicinal plants.[23] The well diffusion method (Figure 1) was employed to study the antibacterial efficacy of the saponins from the *A. elatus* roots against several pathogens, including *S. aureus, S. pyogenes, B. subtilis, E. faecalis, M. luteus, E. coli, S. typhi, P. vulgaris, K. pneumoniae, V. parahaemolyticus*, and a fungi *A. niger* and *C. albicans*. The treatment of different doses of saponins from the *A. elatus* roots has demonstrated a significant impact in inhibiting the growth of these pathogens. The treatment of saponins from the *A. elatus* roots markedly inhibited the growth of various pathogens, especially *S. aureus, S. pyogenes, E. choli*, and *P. vulgaris* (Figure 1). The existence of increased inhibition zones around the wells treated with saponins from the *A. elatus* roots signifies the suppression of pathogen growth.

One of the primary applications of analyzing the antimicrobial effect of plant extracts is in the development of novel antimicrobial drugs. The vast diversity of plant-derived compounds, including secondary metabolites, can provide a rich source of novel

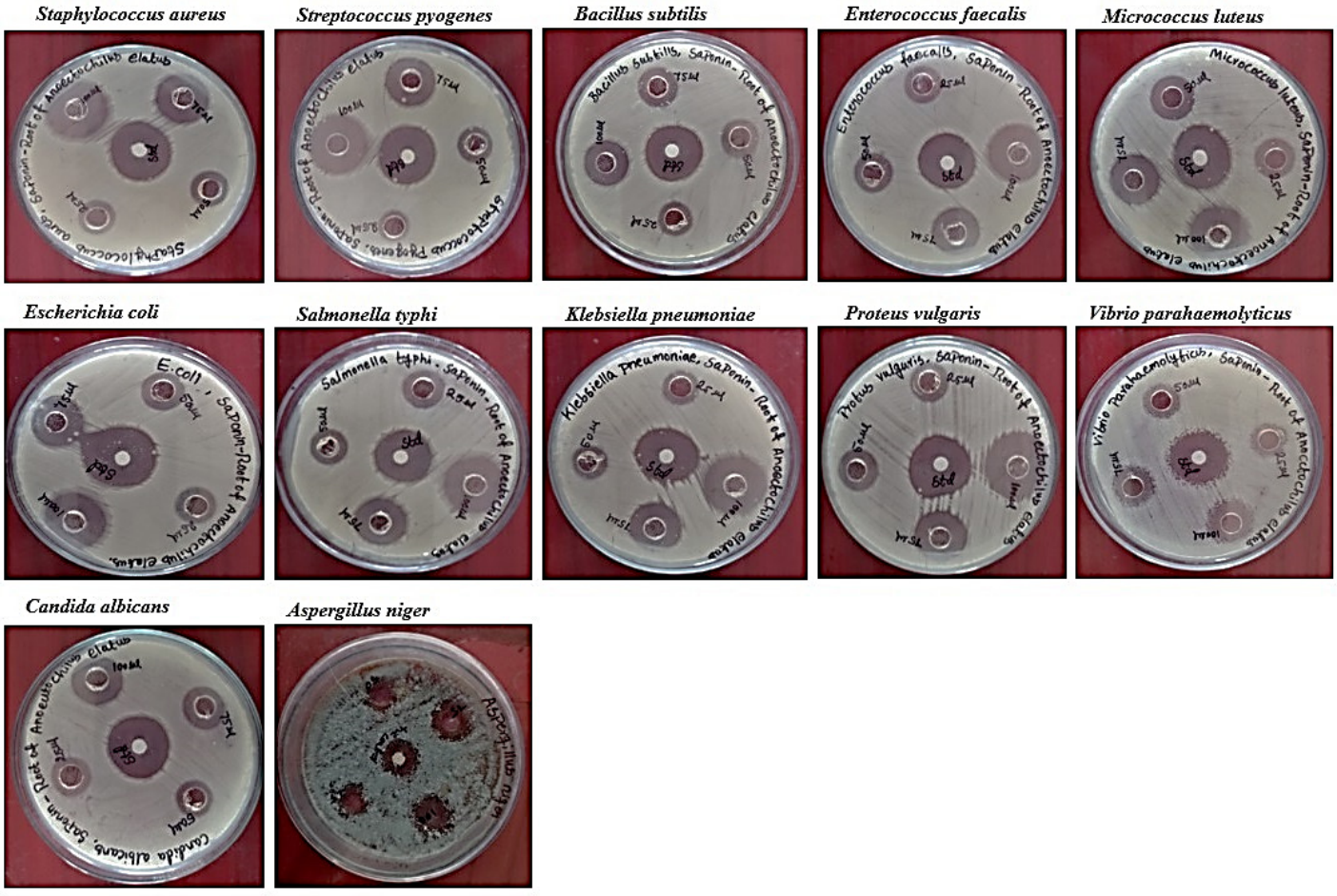

Figure 1: Antimicrobial activity of the saponins from the *A. elatus* roots.

antimicrobial compounds. These compounds can be utilized to develop new antimicrobial formulations, potentially overcoming the limitations of conventional antibiotics and addressing the growing problem of antimicrobial resistance.[24] Additionally, plant extracts with demonstrated antimicrobial activity can be employed for several applications. The use of plant-based antimicrobial agents can help reduce the dependence on synthetic medicines, which can have undesirable effects on human health. The analysis of the antimicrobial activity of medicinal plant extracts is also crucial for traditional medicine systems, as it can provide scientific validation for the use of these plants to treat infectious diseases.[25] The results of this work proved that the *A. elatus* root extracts effectively inhibited the growth of various pathogens, which proves its antimicrobial properties.

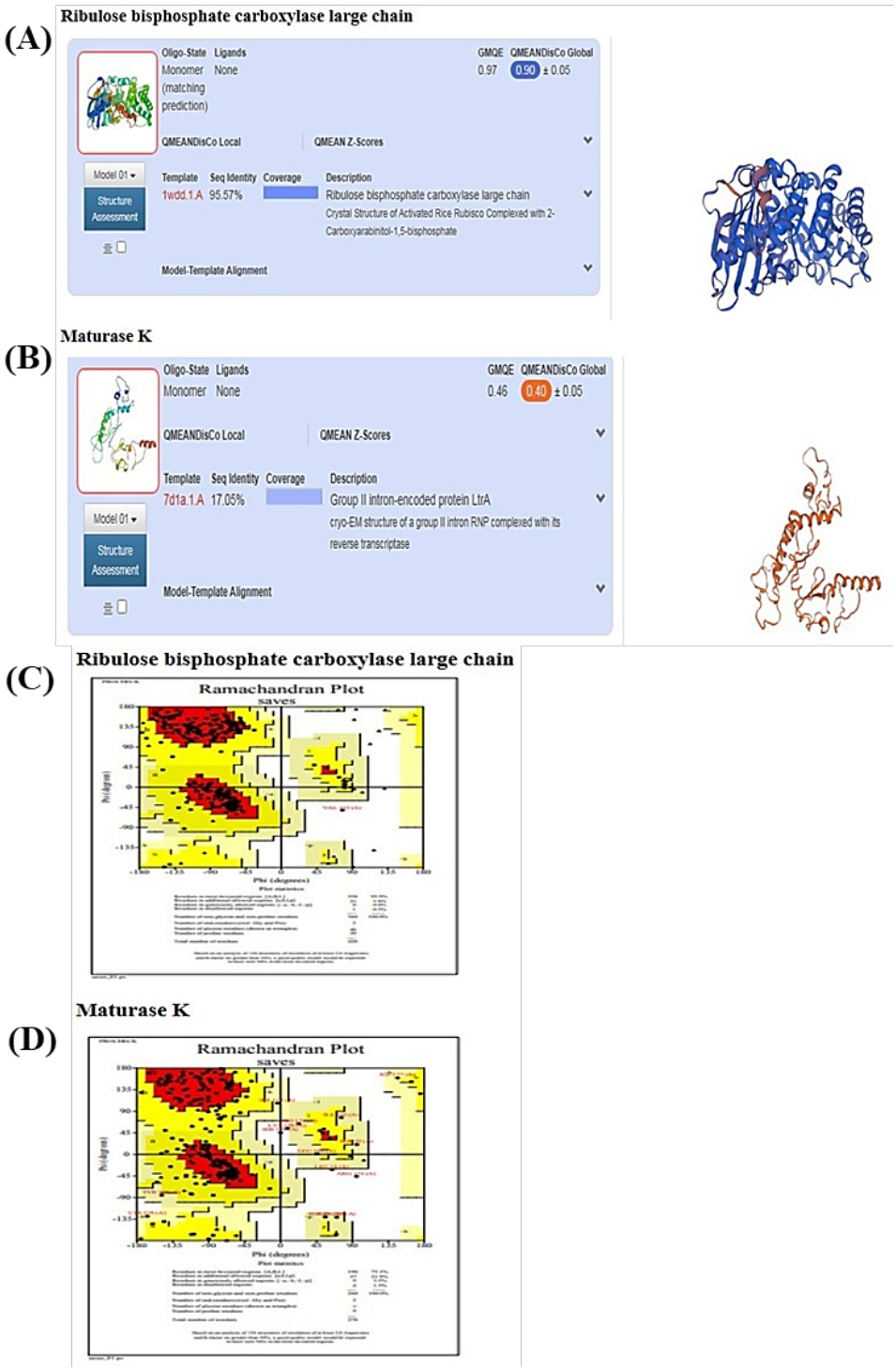

Figure 2: (A and B): Tertiary structures of Ribulose bisphosphate carboxylase large chain and Maturase K proteins; (C and D): Ramachandran plot analysis of Ribulose bisphosphate carboxylase large chain and Maturase K proteins.

Results of *in silico* molecular docking analysis

Molecular docking is a powerful computational method that has gained significant attention in the field of drug development. This method involves predicting the preferred orientation of a molecule (known as a ligand) when it binds to a target protein and evaluating the strength of this interaction. In the context of plant proteins, *in silico* molecular docking analysis has become an increasingly valuable tool, offering numerous applications and insights.[26] The importance of *in silico* molecular docking analysis of plant proteins lies in its ability to provide a cost-effective and time-efficient approach to understanding protein-ligand interactions. This dry lab approach can help researchers identify potential lead compounds, screen large chemical libraries, and optimize the binding affinity and selectivity of drug candidates, ultimately accelerating the drug discovery process.[27]

The primary structural prediction was conducted utilizing the ProtParam program. The molecular weights of several proteins were determined by calculating the markers using Expasy's ProtParam program. The molecular weight of Maturase K and Ribulose-1,5-bisphosphate carboxylase was found to be 47460.93 and 33758.81, respectively (Table 2). The Ribulose-1,5-bisphosphate carboxylase protein exhibited a pI value of 6.57, signifying acidity, whereas Maturase K demonstrated a pI value over 9.87, suggesting basicity. The proteins are noted to be

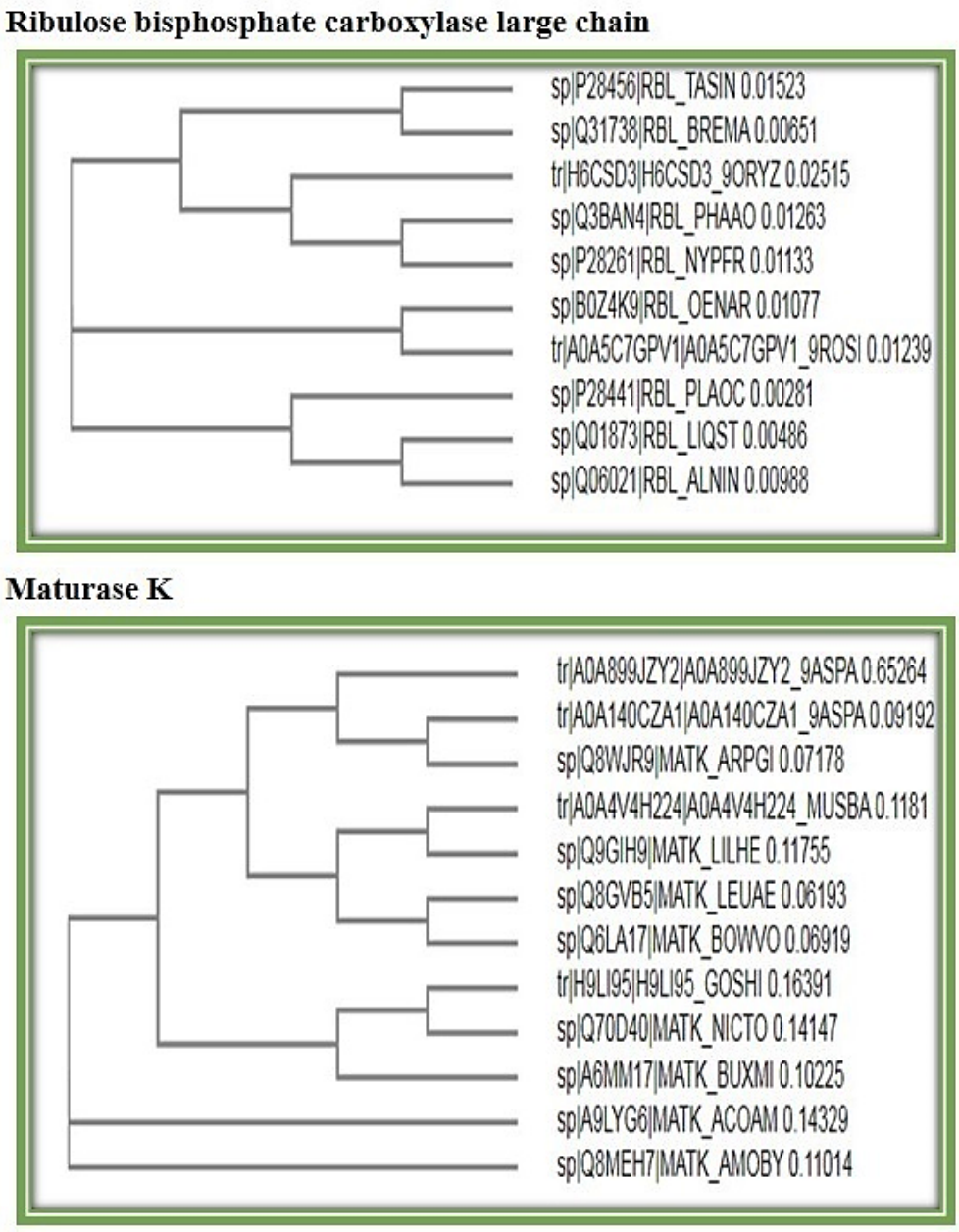

Figure 3: Phylogenetic analysis of plant species with Ribulose bisphosphate carboxylase large chain and Maturase K proteins.

Table 1: *In vitro* antioxidant activities of saponin from *A. elatus* roots.

	Samples	Concentration (µg/mL)	Inhibition (%)
DPPH radical scavenging activity of saponin from *A. elatus* roots	Saponin from *A. elatus* roots	100	95.2±3.31
		80	82.5±2.8
		60	68.0±1.76
		40	46.9±2.72
		20	24.7±3.36
	Rutin	100	98.8±0.32
		80	87.5±3.53
		60	73.1±2.82
		40	57.0±5.02
		20	35.6±2.12
H_2O_2 radical scavenging activity of saponin from *A. elatus* roots	Saponin from *A. elatus* roots	100	76.3±1.53
		80	55.8±2.84
		60	36.1±1.83
		40	22.0±1.49
		20	10.9±1.90
	Rutin	100	86.5±2.77
		80	71.7±2.38
		60	57.4±3.15
		40	45.2±1.80
		20	30.0±4.39
NO radical scavenging activity of saponin from *A. elatus* roots	Saponin from *A. elatus* roots	100	69.1±3.51
		80	46.3.815±
		60	34.7±3.92
		40	20.2±1.93
		20	14.6±1.21
	Rutin	100	80.7±4.85
		80	66.4±3.10
		60	57.8±4.61
		40	43.0±2.93
		20	27.5±1.90
ABTS radical scavenging activity of saponin from *A. elatus* roots	Saponin from *A. elatus* roots	100	91.0±2.35
		80	78.7±3.38
		60	70.5±1.80
		40	53.8±2.82
		20	37.5±1.38
	Rutin	100	95.7±1.34
		80	83.9±3.04
		60	65.6±2.78
		40	51.1±4.01
		20	45.4±1.80
LPO radical scavenging activity of saponin from *A. elatus* roots	Saponin from *A. elatus* roots	100	
		80	83.3±2.88
		60	70.5±2.61
		40	55.1±2.71
		20	33.0±4.70
	Rutin	100	17.9±3.06
		80	94.0±2.7
		60	81.9±4.32
		40	60.8±3.24
		20	48.3±2.30

densely packed and stable at their isoelectric points. The Aliphatic Index (AI) values for both proteins ranged from 78.44 to 100.50. The range of GRAVY of *A. elatus* proteins was found to be -0.281 and 0.065. The secondary structure prediction of Maturase K and Ribulose-1,5-bisphosphate carboxylase was studied by SOPMA which exhibited that alpha helix, extended strand, beta-turn, and random coil, were more predominant. The alpha helix percentage for Maturase K and Ribulose-1,5-bisphosphate carboxylase was found to be 43.42% and 38.93%, respectively. The random coil percentage of both proteins was found to be 36.83% and 33.45%, respectively. The calculated extinction coefficients facilitate the quantitative assessment of protein-protein and protein-ligand interactions (Figures 2A and B).

One of the primary applications of *in silico* molecular docking in the plant sciences is the identification of bioactive compounds from natural sources. Plant-derived compounds have long been recognized as a rich source of therapeutic candidates, and molecular docking can be used to screen these compounds against target proteins of interest. This approach can help researchers prioritize the most talented agents for additional studies, reducing the need for expensive and time-consuming studies.[28] Additionally, molecular docking can be used to study the structural determinants necessary for efficient ligand-receptor binding, providing valuable insights into the mechanisms underlying the biological activities of plant-derived compounds. Another application of *in silico* molecular docking in the plant sciences is the rational design of enzyme inhibitors. Many plant proteins, such as those involved in secondary metabolism, are of great interest for their potential therapeutic applications. Molecular docking can be used to identify and optimize small-molecule inhibitors that can modulate the activity of these enzymes, paving the development of novel plant-based drugs and agrochemicals.[29]

Results of Ramachandran plot analysis

The study of medicinal plant extracts has gained more interest in recent times, as researchers strive to uncover the pharmacological

Table 2: **Primary structures of Ribulose-1,5-bisphosphate carboxylase and Maturase K computed using Expasy's.**

Sl. No.	Accession number	Protein	Length	Mol. Wt	PI	- R	+ R	EC	II	AI	GRAVY
1	A0A899JZY2	Ribulose-1,5-bisphosphate carboxylase	429	47460.93	6.57	50	47	61685	37.87	78.44	-0.281
2	A0A140CZA1	Maturase K	281	33758.81	9.87	15	38	53860	38.28	100.50	0.065

Table 3: **Lists of plant species showing similarity of 95% and above with the Ribulose bisphosphate carboxylase large chain and Maturase K.**

Ribulose bisphosphate carboxylase large chain				
Sl. No.	**Plant species**	**Family name**	**Accession number**	**Identity (%)**
1.	Phalaenopsis aphrodite subsp. formosana (Moth orchid)	Orchidaceae	Q3BAN4	97.2
2.	Brexiamadagascariensis	Celastraceae	Q31738	96.2
3.	*Platanus occidentalis* (Sycamore) (American plane tree)	Platanaceae	P28441	96.5
4.	*Nypa fruticans* (Nypa palm)	Arecaceae	P28261	96.7
5.	*Liquidambar styraciflua* (Sweetgum tree) (*Liquidambar macrophylla*)	Altingiaceae	Q01873	96.0
6.	*Alnus incana* (White alder)	Betulaceae	Q06021	96.3
7.	*Tasmannia insipida* (Pepperbush) (*Drimys insipida*)	Winteraceae	P28456	96.8
8.	*Oryza meridionalis*	Oryzeae	H6CSD3	97.5
9.	*Oenothera argillicola* (Appalachian evening primrose)	Onagraceae	B0Z4K9	95.8
10.	*Acer yangbiense*	Sapindaceae	A0A5C7GPV1	95.6
Maturase K				
Sl. No.	**Plant species**	**Family name**	**Accession number**	**Identity (%)**
1.	*Arpophyllum giganteum* (Hyacinth orchid)	Orchidaceae	Q8WJR9	83.4
2.	*Musa balbisiana* (Banana)	Musaceae	A0A4V4H224	70.5
3.	*Leucojum aestivum* (Summer snowflake)	Amaryllidaceae	Q8GVB5	71.1
4.	*Lilium henryi* (Henry's lily)	Liliaceae	Q9GIH9	68.1
5.	*Bowiea volubilis* (Climbing onion) (*Ophiobostry volubilis*)	Hyacinthaceae	Q6LA17	69.1
6.	*Acorus americanus* (Sweetflag) (*Acoruscalamus* var. americanus)	Acoraceae	A9LYG6	65.7
7.	*Buxus microphylla* (Littleleaf boxwood) (Japanese boxwood)	Buxaceae	A6MM17	66.2
8.	*Amorphophallus abyssinicus* (Black arum) (*Arum abyssinicum*)	Araceae	Q8MEH7	66.3
9.	*Nicotiana tomentosiformis* (Tobacco)	Solanaceae	Q70D40	64.5
10.	*Gossypium hirsutum* (Upland cotton) (*Gossypium mexicanum*)	Malvaceae	H9LI95	66.9

capacity of these natural resources. One crucial tool in this endeavor is the Ramachandran plot, a powerful analytical technique that provides invaluable insights into the structural and functional properties of these extracts. The Ramachandran plot is a graphical representation of the backbone dihedral angles, φ, and ψ, in protein structures. While primarily used in the area of protein structural analysis, this technique has also found applications in the analysis of medicinal plant extracts. By examining the Ramachandran plot of the phytochemicals present in these extracts, researchers can gain a deeper understanding of their structural characteristics, which in turn, can shed light on their potential bioactivities and pharmacological properties.[30]

The secondary structures of polypeptides in proteins are detected by hydrogen bonds between the negatively charged carbonyl oxygen atoms and the positively charged amide hydrogen atoms in the molecular backbone. The angle ψ can be modified within the range of-180° to 180°, which translates to 360° of rotation for each angle. Nevertheless, numerous combinations of these angles are hardly encountered, while others are exceedingly common in proteins. The values of ψ concerning the values of φ for a globular protein are depicted in Figure 2 (C&D). The objective of this work is to obtain a dataset with the spatial coordinates of each atom. This data can be acquired from many protein structural data sites. We will utilize XRD data, as it provides the utmost precision. Despite its lack of perfect accuracy, crystal packing pressures often induce tiny aberrations in proteins. Molecular Magnetic Resonance (NMR) data for proteins in solution lack precision but are thought to be more accurate due to the protein being in its natural environment. This data may be analyzed to determine the amino acid residues, as the XRD ".PDB" data format categorizes all atoms and assigns them to specific residues virtually automatically. Subsequently, we employ a computer to compute the dihedral angles that determine the values of ψ and φ. A software application may examine a ".PDB" file and deliver the recorded values of ψ and φ angles for each specific residue. Initially, we shall plot these values for the yeast protein hexokinase. The plot of ψ against φ is referred to as a Ramachandran plot (Figures 2C and D).

The growing importance of Ramachandran plot analysis in the field of medicinal plant research is evident from the recent advancements in analytical techniques and methods. The challenges posed by the complexity of medicinal plant matrices and the variability of their phytochemical compositions have been largely overcome through the application of modern qualitative and quantitative techniques, such as liquid chromatography, countercurrent chromatography, and ultrasound-assisted extraction.[31] These analytical tools have enabled researchers to identify, characterize, and quantify the bioactive compounds present in medicinal plant extracts, paving the way for a more comprehensive understanding of their structural and functional properties. The structural and functional analysis of plant extracts is crucial for correlating the type and amount of phytochemicals present with their bioactivities, which in turn, can guide the selection of specific applications for these natural products. The Ramachandran plot analysis has emerged as a valuable tool in this regard, providing insights into the conformational preferences and spatial arrangements of the phytochemicals, which can be directly linked to their biological activities.[32]

Phylogenetic analysis of *A. elatus*

Phylogenetic analysis, the study of the evolutionary relationships between organisms, has become an essential tool in the area of medicinal plant research. The economic and pharmaceutical importance of herbal plants has led to their increased exploitation, often resulting in the depletion of their natural habitats. To address this issue, phylogenetic analysis has emerged as a valuable technique for identifying closely related species, understanding their evolutionary history, and developing effective conservation strategies.[33] The current findings indicate that the plant species exhibiting a similarity of 90% or greater with the Ribulose-1,5-bisphosphate carboxylase include *Phalaenopsis aphrodite* (Orchidaceae) at 97.2%, *Brexia madagascariensis* (Celastraceae) at 96.2%, *Platanus occidentalis* (Platanaceae) at 96.5%, *Nypa fruticans* (Arecaceae) at 96.7%, *Liquidambar styraciflua* (Altingiaceae) at 96.0%, *Alnus incana* (Betulaceae) at 96.3%, *Tasmannia insipida* (Winteraceae) at 96.8%, *Oryza meridionalis* (Oryzeae) at 97.5%, *Oenothera argillicola* (Onagraceae) at 95.8%, and *Acer yangbiense* (Sapindaceae) at 95.6% (Table 3 and Figure 3). The phylogenetic analysis identified a list of plant species with a similarity of 90% or greater to the Maturase K including, *Arpophyllum giganteum* (Orchidaceae) at 83.4%, *Musa balbisiana* (Musaceae) at 70.5%, *Leucojum aestivum* (Amaryllidaceae) at 71.1%, *Lilium henryi* (Liliaceae) at 68.1%, *Bowiea volubilis* (Hyacinthaceae) at 69.1%, *Acorus americanus* (Acoraceae) at 65.7%, *Buxus microphylla* (Buxaceae) at 66.2%, *Amorphophallus abyssinicus* (Araceae) at 66.3%, *Nicotiana tomentosiformis* (Solanaceae) at 64.5%, *Gossypium hirsutum* (Malvaceae) at 66.9% (Table 3 and Figure 3).

Phylogenetic analysis has played an essential role in the identification and characterization of medicinal plants, particularly those with known or potential therapeutic properties. By mapping the evolutionary relationships between different plant species, researchers can identify closely related taxa that may share similar chemical profiles and pharmacological activities. This knowledge can guide the discovery of new drug candidates, as well as the development of alternative sources for existing medicinal compounds. Furthermore; phylogenetic analysis has been instrumental in the identification and preservation of endangered medicinal plant species. By understanding the evolutionary relationships and genetic diversity within a plant group, conservation efforts can be tailored to target specific taxa or populations that are at risk of extinction.[34]

CONCLUSION

The present study has highlighted the antioxidant capacity, and antimicrobial activity of saponins isolated from *A. elatus*. *In silico* analyses further revealed the potential interaction of these compounds with key therapeutic targets, such as Maturase K and Ribulose-1,5-bisphosphate carboxylase, suggesting a possible molecular basis for the observed bioactivities. These findings underscore the promising clinical potential of *A. elatus* root-derived saponins as candidates for the development of novel antimicrobial and antioxidant therapies. However, to fully validate their therapeutic applicability, comprehensive *in vivo* studies and clinical trials are warranted. Future research should focus on elucidating the precise molecular mechanisms and pharmacokinetics of these bioactive compounds, paving the way for their translation into effective clinical interventions.

ACKNOWLEDGEMENT

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2025R98), King Saud University, Riyadh, Saudi Arabia for financial support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The authors extend their appreciation to the Researchers Supporting Project number (RSP-2025R98), King Saud University, Riyadh, Saudi Arabia for Financial support.

ABBREVIATIONS

H_2O_2: Hydrogen Peroxide; **pI:** Isoelectric Point; **GRAVY:** Grand Average of Hydropathy; **SOPMA:** Self Optimized Prediction Method with Alignment; **PSSM:** Position Specific Scoring Matrix; **HMMs:** Hidden Markov Models; **NJ:** Neighbour Joining.

SUMMARY

Saponins, a diverse class of naturally occurring compounds found in various medicinal plants, have garnered significant attention in the scientific community due to their remarkable pharmacological properties and potential therapeutic applications. The present study has highlighted the antioxidant capacity, and antimicrobial activity of saponins isolated from *A. elatus*. These findings underscore the promising clinical potential of *A. elatus* root-derived saponins as candidates for the development of novel antimicrobial and antioxidant therapies.

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Cite this article: Muthusamy S, Sellappan S, Arulselvan P, Alarfaj AA. *In vitro* Antioxidant, Antimicrobial, *in silico* Molecular Docking Analysis, and Phylogenetic Analysis of *Anoectochilus elatus*. Indian J of Pharmaceutical Education and Research. 2026;60(1):283-93.