Pharmacognostic Study and Antimicrobial Screening of *Justicia adhatoda* L. Using *in Silico* and *in Vitro* Strategies

Murad Khan[*], Imtiaz Ahmad[†], Haseebullah[‡], Fayaz Asad[§], Adil Khan[**]

Abstract

This study was carried out to investigate the antibacterial, antifungal activity, physicochemical (extractive values, total ash, fluorescence) and organoleptic characteristics of Justicia adhatoda L. *Highest extractive value for leaves was found in methanol (7.96%) followed by chloroform (0.21%) and n-hexane (0.13%) respectively. Total ash content value for powdered leaves was recorded as 7.82%. The plant shows different colors under visible light as pale green, under short wavelength UV (254) nm(brownish) and under long wavelength (366) nm (dark brown) were observed during powder study. Based on the results obtained the extracts showed significant to low activities against Citrobacter koseri, Proteus vulgaris and Providencia stuartii while show no activity against Streptococcus pneumoniae, Klebsiella aerogenes, Acinetobacter baumannii and Staphylococcus aureus. The antifungal activity of methanolic extracts was assessed against six pathogenic strains i.e. Alternaria solani, Aspergillus niger, Candida albicans, Rhizopus arrhizus, Fusarium oxysporum and Colletotrichum trifolii. Extracts show no activity against any of the tested fungal strain. Additionally, 1EA1 protein was docked with Betaine, Anisotine, Vasicinone and Vasicine. With a docked score of -12.89 kcal/mol, Anisotine demonstrated the highest binding affinity against the target protein, followed by Vasicine (-9.68 kcal/mol), Vasicinone (9.00 kcal/mol) and Betaine (-8.54 kcal/mol). The results will be useful to establish the identification, authentication of plant drug. And the results of in silico studies can be used as base for developing novel bioactive substances with therapeutic potentials.*

***Keywords*:** Antibacterial; Antifungal; Ash; Pharmacognosy; *Justicia adhatoda*

Introduction

The word pharmacognosy came from two Greek words, i.e. Pharmakon, meaning drug, and gnosis, means knowledge. Early in the 19th century, an Austrian scientist named Johann Adam Schmidt (1759–1809), followed by Wilhelm Joseph Schmitt (1760–1827), introduced the

[*]Department of Botany, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, murad.botmphil4th@bkuc.edu.pk

[†] Corresponding author: Department of Botany, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, drimtiazahmad@bkuc.edu.pk

[‡]Department of Botany, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, Hasebs38@gmail.com

[§]Department of Botany, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, drfayazasad@bkuc.edu.pk

[**]Department of Botany, Bacha Khan University Charsadda, Charsadda 24420, Pakistan, dradikhan@bkuc.edu.pk

term of "Pharmacognosy" C. A. Seydler used this word in 1811 (Sharma et al., 2015). In the broader context the pharmacognosy referred to the study of morphological, physical, chemical, biological, and biochemical properties of drugs of natural origin. Along with the research of novel medications derived from natural resources, it also covers the history, distribution, sources, collecting technique, isolation, formulations, and standardization (Balakrishnan et al., 2013). Pharmacognosy supplies enough information regarding the morphology, physical properties, purity, quality, identity, selection, and processing of natural products designated for restorative purposes (Fofie et al., 2017). Worldwide, the incidence of illnesses brought on by bacteria has increased. These are the main causes of morbidity and death in underdeveloped countries among patients who are unable to mount a defense against microbial diseases (Ahmed et al., 2014; Begum et al., 2018; Asad et al.,2018; Begum et al., 2021). Plant-based medications and growth performance are the main and abundant source of many kinds of active antibacterial agents (Begum et al., 2018; Basit et al., 2020; Asad et al., 2021; Shahid et al., 2023).

Justicia Adhatoda, a shrub of the family Acanthaceae, grows quickly and can reach a height of 5 m (16 ft) in ideal conditions. However, it is usually 2 to 3 m (7 to 10 ft) tall, with several branches supporting a crown that spreads widely and is frequently as wide as the plant is tall. The leaves have whole basal attenuation, elliptic-lanceolate lamina, glabrous above, pubescent on nerves beneath, and petioles that are 1.5–3.5 cm long. Blooms are white, up to 10 cm long, almost sessile, and borne in terminal and axillary spikes. lacking in fruits. The dry winter months are when flowers bloom (Pachaiappan et al., 2021). *Justicia adhatoda* is a widely spread plant that thrives on waste lands up to 1300 meters in Pakistan. Due to the antispasmodic qualities of the roots and leaves, they are frequently employed in the Ayurvedic and Unani medical systems to treat bronchitis, asthma, fever, and jaundice (Ningthoujam et al., 2023).

In silico methods can be used to investigate, simulate, depict, or predict chemicals. In silico research encompasses all methods for assessing chemical and biological features that are generally based on a chemical structure representing a real or hypothetical (i.e., virtual) substance. Today, in silico processes are routinely utilized in conjunction with other tests; but, depending on the decision context, the approaches are beginning to be used to generate toxicity assessment information without the need for any in vitro or in vivo research. To predict a chemical's potential toxicity and, in some situations, to quantify the dangerous dose or potency, in silico toxicology utilizes models that can be integrated into software tools. This study intended to assess *J. adhatoda* physicochemical characteristics antifungal and antibacterial activity.

Methodology

Plant Collection and Identification

Healthy leaves of *Justicia adhatoda* were collected from plants growing in District Malakand (Lat. 34.56°, and long. 71.90°) in September to November 2023. Plant was identified through Standard Literature and further confirmed through Herbarium, Department of Botany, Bacha Khan University Charsadda. The identified specimen was kept at the Herbarium, Bacha Khan University Charsadda, for the record (Reference No. HBKU-903).

Following the botanical authentication, the plant leaves were rinsed under tap water to get rid of any sticking dust. The leaves were shade-dried for further use in research. After complete evaporation of moisture content, the plant material is grinded to make fine powder, using electric grinder (Ahmad et al., 2023).

Extract Preparation and Extractive Value Determination

To prepare methanolic extract the dried leaves were soaked in methanol at the rate of 100 g powder per 250 ml methanol in an airtight clean container for 72 hours. The mixture was stirred/shacked vigorously at regular intervals. Thereafter, the mixture was filtered using Whatman filter paper (Grade I). The process was repeated three times. The filtrate was then concentrated using rotary evaporator. The concentrated filtrate was then placed on water bath for complete removal of solvent (Ahmad et al., 2023).

Extractive values were calculated using the following (Sharma & Kumar, 2016): $\left(\frac{W}{W}\right) = \frac{Extract\ weight}{Sample\ weight} \times 100$

Organoleptic and Morphological Characters

Using normal procedures, the powdered medication of *Justicia adhatoda* (leaves) was thoroughly examined for its physical attributes, including color, odor, and touch.

Ash Content: The leaves of the plants were ground into a powder, balanced, and burned to produce ash, a solid material with a greyish color. The method provided by Kumar was used to measure the amount of ash (Jamwal et al., 2023). After being weighed, the leaf sample was heated for five hours at 600°C in a muffle furnace. After quickly cooling, the resulting ash was weighed. This process was then repeated until the ashes' weight stabilized. Ash (%) is calculated as 100 times the ash weight per sample.

Fluorescence Study: The samples were seen in day and UV light of both short and long wave lengths to do the fluorescence analysis of the

powdered plant leaves (1 g each) (Brain and Turner, 1975; Chase Jr. and Pratt, 1949; Nikam et al., 2009; Trease, 2002).

Antibacterial Activity

Nutrient agar (28g) was mixed with 1.0 liters of distilled water and heated to 100^{o} C to dissolve the methanol extract (10 mg) of the leaves. After that, it was autoclaved for 15 minutes at 121 degrees Celsius to sterilize it. *J. adhatoda's* antibacterial activity was assessed in the following cultures: Gramme-negative bacteria are *Citrobacter, Klebsiella, P. volgarous, E. aerogenes,* and *ovidencia*; gram-positive bacteria are *Acinetobacter and S. aureus*. The antibacterial activity of *J. Adhatoda* leaf methanolic extract was assessed against pathogenic microorganisms. To achieve uniform distribution of microorganisms on Petri plates, microbial culture dilution was prepared by taking a loop full of microbial culture and mixing it with distilled water. The bacterial cultures were maintained in nutrient broth and stored at 4°C. Cultures were reactivated by streaking on a nutrient agar plate before every test. The methanolic extract of *J. Adhatoda* leaves did not show good antibacterial activity against bacterial strains. Before the test, a loop full of microbe-containing broth was taken from this broth tube, transferred to a nutrient agar plate, and incubated for 16–18 hours at 37°C. Next, 100 µl of the appropriate plant extract was applied to the well. As a negative control. The zone of inhibition was estimated after 72 hours in millimeters (Balkrishna et al., 2022).

Antifungal Activity

Fully dissolved Potato dextrose agar (PDA) (39g), 1 litter of distilled water was heated to 100^{o} C. After that, it was thoroughly combined and autoclaved for 15 minutes at 121 degrees Celsius to sterilise it before pouring. Fungal strains (*Alternaria solani, Aspergillus niger, Candida albicans, Rhizopus arrhizus, Fusarium oxysporum*, and *Curvularia trifolii*) were autoclaved at 121°C for 30 minutes while being present at a density of 15 lbs/sq. inch to create nutrient agar. Petri dishes were filled with the medium, which was then left to harden. To ensure that the microorganisms were distributed uniformly throughout the Petri plate, distilled water was added to a loop filled with microbial culture. The bacterial cultures were kept alive at 4°C in nutrient broth. Before each test, cultures were revived by streaking them on a nutrient agar plate. Before the test, a loop full of microbe-containing broth was taken from this broth tube, transferred to a nutrient agar plate, and incubated for 16–18 hours at 37°C. The negative control was performed using the blank solvent. After being cautiously closed, the petri plates were left in the incubator for a

whole day. Following the duration, the millimetre-scale zones of inhibition beneath every petri dish were measured (Giordani et al., 2020).

In Silico Screening

Target Protein Receptor: The potential bioactivity of the identified compounds, including potential anti-bacterial and anti-fungal effects, was calculated and evaluated for a subset of target proteins. To this end, the crystal structures of 3VSL and 1EA1, which are derived from easily available data and biological mechanisms, are illustrated. These three-dimensional structures were retrieved from the RCSB Protein Data Bank at https://www.rcsb.org in PDB format. While the 3VSL protein was used as an Anti-bacterial, the 1EA1 protein has Anti-Fungal properties.

Protein Preparation: X-Ray crystallographic structure of all the target proteins i.e. 3VSL and 1EA1 structures were downloaded from Protein Data Bank (PDB). All of the proteins were prepared using MOE 2015.10's prior to molecular docking. This required changing the protein's link ordering, adding hydrogen atoms, and removing water molecules that weren't needed. The significant water molecules that were discovered to be interacting with the enzyme's active site residues were kept. In addition to adding side chains and missing atoms, partial charges were assigned (Shivani et al., 2017). After reducing their energy using the Molecular Operating Environment (MOE) 2015.10 program, the proteins were purified and stored in PDB format for future analysis.

Active Site Prediction: The site discovery software in the Molecular Operating Environment was used to anticipate the active site. Additional computations were performed for ligand binding sites and restriction sets for partial molecular rending, per good ford (1985) (Afshan *et al*., 2022). The active sites of 3VSL and 1EA1 were also predicted by the CASTp web server (http://cast.engr.uic.edu). The protein structure's pockets and cavities are all found by CASTp, which also offers a thorough explanation of the atoms that made each one. Additionally, it computes each pocket's area and volume analytically or by fusing the Richards surface - a solvent-accessible surface model with the Connolly surface, a molecular surface model (Binkowski et al., 2003).

Ligand Preparation: The PubChem website (http://pubchem.ncbi.nlm.nhi.gov/) was utilized by the researchers to identify different compounds from the previously published *J. adhatoda* data. The molecular docking structures were modified, and 3D protonation and power reduction were carried out using the automatic MOE settings.

Molecular Docking Analysis: Molecular docking was used to determine the binding affinity and optimal orientation of the ligand to the corresponding receptor. Different conformations were produced by using

flexible ligand architectures (Friesner et al., 2006). Rescoring 1 and 2 were set up in both cases to record the ligand-protein contact, after the receptors' molecular docking process selected the ligand atom. With BIOVIA Discovery studio visualize b21.1.0.20298, the results were analyzed. On the DSV, you can also see the binding site, which is where the ligand binds to the protein.

Pharmacokinetics Parameters: The Swiss ADMET online database was used to calculate the physiochemical properties of the drugs, including their molecular weight (MW), predicted octanol / water partition coefficient (QPlogPo/w), number of hydrogen bond donors (donor HB), acceptors (accept HB), and percentage of human oral absorption. Examined were instances of breaking Lipinski's rule of five (Alhazmi et al., 2015).

Molecular Dynamic Simulation: Since MD simulation offers a more complete insight of the physical bases of the complexes under study, it was used to investigate the molecular activity and stability of protein-ligand complexes (López-Blanco et al., 2014). The proposed protein and its inter-ligand complexes' 3D structures will be examined in the current investigation using the IMODs web server.

Statistical Analysis

The results were analysed using one way ANOVA, followed by Tukey (Post ANOVA test) at $p < 0.05$.

Results and Discussion

Determination of Extractive Values

The highest extractive value for leaves was observed in methanol (7.96%), followed by n-hexane (0.13%) and chloroform (0.21%), respectively. The maximum extractive value was obtained for methanolic extract. This shows that determining extractive values is a useful method for assessing medications and identifying various deliberate and inadvertent drug adulterations. According to a quantitative investigation, *J. adhatoda* may contain a wealth of bioactive components that are useful for medicinal preparations in the future (Table 1).

Organoleptic and Morphological Characters

Organoleptic evaluation can be performed using the sense organs, which contain the sensory parameters and define certain specific features of the substance. This can be seen as a preliminary step towards determining the identification and purity level of the material. The organoleptic analyses (taste, color, shape, and odor) were carried out.

Organoleptic characteristics, including color, taste, texture, odor, and size, are displayed in Table 2.

Table 1: Extractive values in different organic solvents for J. Adhatoda leaf powder.

S. No.	Plant parts	Solvents	(%) Extracts
1	Leaves	Chloroform	0.21
2	Leaves	N-Hexane	0.13
3	Leaves	Methanol	7.96

Table 2: Organoleptic and morphological characters leaf powder of J. Adhatoda.

S. No.	Parts/Characters	Leaves/Observations
1	Size	1-2.5m
2	Shape	Oblong
3	Fracture	Rough
4	Color	Pale green
5	Odor	Pungent
6	Height	2.5m
7	Taste	Bitter
8	Width	4-6cm
9	Petiole	Leaf stalk winged
10	Leaves	Simple
11	Base	Symmetrical
12	Nature	Shrub/Evergreen
13	Apex	Acuminate

Ash Content: An essential component for identifying adulteration or the presence of inorganic particles in drug tests for *J. adhatoda* leaves is the total ash analysis of the powdered medication (Jarald & Jarald, 2007). Three iterations of *J. Adatoda's* ash action was conducted (Table 3).

Table 3: Total ash content of J. adhatoda leaves powder

S. No.	Total Ash		Average Ash
	Powder	ash	
1	21g	7.77g	
2	21g	8.79g	7.82%
3	21g	6.91g	

Fluorescence Analysis: The powdered leaves of *J. adhatoda* were examined for fluorescence characteristics in three different light wavelength ranges: ultraviolet (white), short (254 nm), and long (366 nm). Regarding the characterization, authenticity, and identification of the plant material, the fluorescence investigations provided insightful information.

It's a natural medication's efficacy. Biswal et al., (2011) and Chand et al., (2012) found that crude pharmaceuticals have the ability to change color when UV light passes through them. It is a phase in the process of verifying and removing contaminants from crude pharmaceuticals. According to Gersbach, 2002, the powder displayed various color tints when exposed to visible and ultraviolet light with short (254 nm) and long (366 nm) wavelengths as mentioned in the Table 4.

Table 4. Florescence properties of leaves powder of J. adhatoda under UV light

S. No.	White	Short Wavelength/254nm	Long Wavelength/366nm
1.	Pale green	Brownish	Dark Brownish

Antibacterial Activity: In the present investigation of antibacterial activity, three concentrations (100 µg/ml, 200 µg/ml, and 300 µg/ml) of the methanolic extracts of *Justicia adhatoda* leaf were tested against seven pathogenic bacterial strains: two gram-positive bacteria (*Klebsiella pneumoniae and Staphylococcus aureus*) and five gram-negative bacterial strains (*Citrobacter koseri, Enterobacter aerogenes, Proteus vulgaris, Acinetobacter baumannii,* and *Providencia staurtii*). At varying doses, the plant extract's antibacterial efficacy against a variety of bacterial species was evaluated (Table 5). For *Citrobacter koseri*, the zone of inhibition (ZI) rose from 7.333 mm at 100 µg/ml to 9.667 mm at 300 µg/ml. With 3.333 mm at 100 µg/ml, 2.333 mm at 200 µg/ml, and 2.667 mm at 300 µg/ml, *Proteus vulgaris* showed declining ZI values (Tabassum et al., 2018). ZI values for *Providencia staurtii* were 1.667 mm at 100 µg/ml and 2 mm at 200 µg/ml and 300 µg/ml. On the other hand, ZI values of 0 mm were recorded by *Klebsiella pneumoniae*, *Enterobacter aerogenes, Acinetobacter baumannii*, and *Staphylococcus aureus*, which did not exhibit any inhibition at any concentration (Figure 1 & 2).

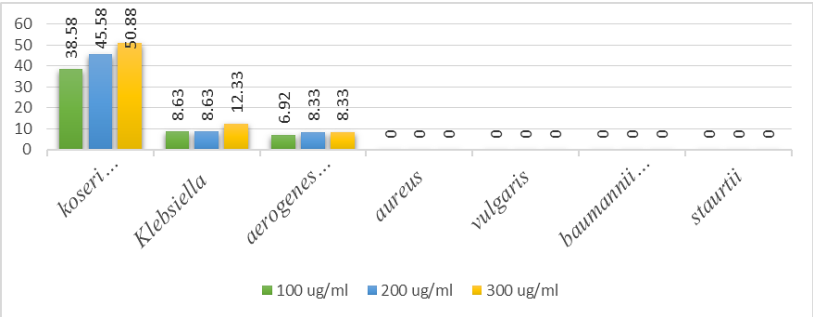

Figure 1: Antibacterial potential of methanolic extracts of J. adhatoda against selected pathogenic bacteria.

Table 5. Antibacterial potential of crude methanolic extracts of J. adhatoda

Treatments	Conc. (µg/ml)	Zone of Inhibition in mm (Mean ± Standard deviation)						
		Citrobacter koseri	Proteus vulgaris	Providencia staurtii	Klebsiella pneumoniae	Enterobacter aerogenes	Acinetobacter baumannii	Staphylococcus aureus
CME	100	7.333± 0.577*	3.333± 0.933NS	1.667± 0.57NS	0±0	0±0	0±0	0±0
	200	8.667± 1.528*	2.333± 0.528NS	2.00± 0.01 NS	0±0	0±0	0±0	0±0
	300	9.667± 2.082*	2.667± 1.528NS	2.00± 0.1 NS	0±0	0±0	0±0	0±0
S. D		19.00± 0.01	27.00± 0.02	24.00± 0.01	23.00± 0.12	21.00± 0.01	26.00± 0.01	39.00± 0.13

CME = Crude methanolic extract, S.D = standard antibiotic,
* = Significantly different from negative control at $p < 0.05$.

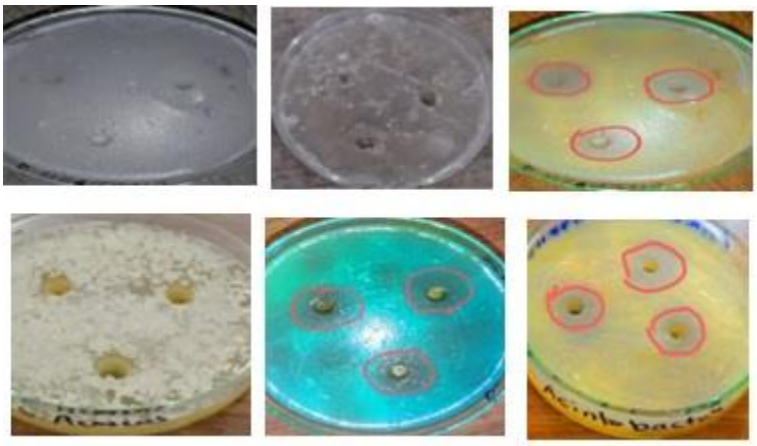

Figure 2: Antibacterial Activity of J. adhatoda.

Antifungal Activity: In the current study crude methanolic extracts of *J. adhatoda* were tested against selected strains of pathogenic fungi, namely *Alternaria solani, Aspergillus niger, Candida albicans, Rhizopus arrhizus, Fusarium oxysporum* and *Colletotrichum trifolii* (Figure 3). Methanolic extracts exhibited no significant activity at any concentration against any fungal strains. These results highlight the need for additional research or changes to identify the effective phytochemicals using other extracts/fractions.

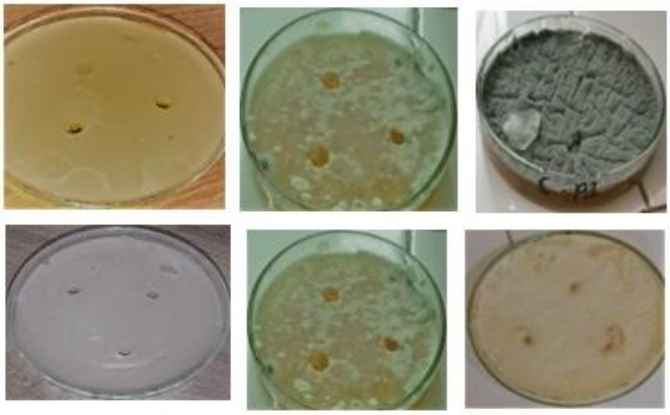

Figure 3. Antifungal Activity of Methanolic Extract of J. adhatoda Against Selected Fungal Strains.

In silico screening

In silico ADMET and Target prediction: The ADMET of Betaine, Anisotine, Vasicinone and Vasicine was predicted using the Swiss ADMET Online Database (Table 6, Figure 4).

Table 6. ADMET prediction of ligands.

Compounds	Blood brain barrier	Intestinal absorption	Mo. Weight (g/mol)	TPSA	Water solubility	Skin permeability (cm/s)	Lipinsik rule	CYP3A4 inhibitor
Betaine	No	Yes	117.15	40.13	-0.35	-7.11	Yes	No
Anisotine	Yes	Yes	349.38	73.22	-4.10	-6.29	Yes	Yes
Vasicinone	No	Yes	202.21	55.22	-1.91	-7.16	Yes	No
Vasicine	No	Yes	188.23	35.83	-1.60	-7.16	Yes	No

Active Site Prediction: The CASTp web server (http://cast.engr.uic.edu) was also used to forecast the 3vsl active site (Figs. 4. A and 1. B). CASTp is used to identify the pockets and cavities of the protein structure and to offer a detailed breakdown of the atoms that make them up. VAL67, PRO68, ARG69, ASN83, TRP228, ASP229, ARG230, VAL245, ARG270, VAL271, GLU281, ARG285, GLY286, LYS287, and LYS288 are the amino acids that make up 3vsl. LYS52, GLU65, SER66, VAL67, PRO68, ARG69, ASN83, TRP228, ASP229, ARG230, VAL245, ARG270, VAL271, GLU281, ARG285, GLY286,

LYS287, and LYS288 are the amino acids that make up 1EA1. The molecular docking process made use of these residues. Aminoacids of 3VSL are LYS52, GLU65, SER66, VAL67, PRO68, ARG69, ASN83, TRP228, ASP229, ARG230, VAL245, ARG270, VAL271, GLU281, ARG285, GLY286, LYS287 and LYS288. While amino acids of 1EA1 are LYS52, GLU65, SER66, VAL67, PRO68, ARG69, ASN83, TRP228, ASP229, ARG230, VAL245, ARG270, VAL271, GLU281, ARG285, GLY286, LYS287 and LYS288. These residues were used for molecular docking.

Figure 4: Active site of 3VSL (A) and 1EA1 (B) protein found through CASTp server.

Molecular Docking Study: A molecular modeling study was conducted using the docking tool Molecular Operating Environment (MOE version 2015.10) to examine potential binding modes of target compounds (Betaine, Anisotine, Vasicinone and Vasicine) to 3VSL and 1EA1 enzymes. Three powerful molecules were docked into the active sites of 3VSL and 1EAfor this purpose. To shed light on the interactions between target chemicals and enzymes, the best docked poses (3VSL & 1EA1) were further examined. The binding affinities and amino acid interactions of the chemicals in the docking results of Betaine, Anisotine, Vasicinone and Vasicine (Table 7). Anisotine had the highest docking score of -13.79 kcal/mol against 3VSL, followed by Vasicine (-11.18kcal/mol), Vasicinone (-10.87 kcal/mol) and Betaine (-10.09 kcal/mol). The docked structure Anisotine was photographed to show how hydrogen bonds and Vander Waal forces interact with important amino acids such as SER66, VAL67, ASN83, TRP228, ARG270, ARG285 and GLY286. Important amino acids including SER66, VAL67, ARG270 and GLY286 of 3VSL interact with Vasicine. To examine the intramolecular correlation circumstances, the optimal position for every molecule was considered. Important amino acids including LYS52, VAL67, ARG69, ARG270 and GLY286 of 3VSL with Vasicinone. Betaine, the ligand, docks with 3VSL indicating binding interactions with important and functionally crucial amino acids such as LYS52, ARG69 and GLY286 (Figure 5 & 6).

Additionally, 1EA1 protein was docked with Betaine, Anisotine, Vasicinone and Vasicine; the binding affinity values are displayed in the Table 7. With a docked score of -12.89 kcal/mol, Anisotine demonstrated the highest binding affinity against the target protein, followed by Vasicine (-9.68 kcal/mol), Vasicinone (9.00 kcal/mol) and Betaine (-8.54 kcal/mol).

The docking of Anisotine with 1EA1 showed hydrogen bond interaction with amino acids like MET99, PHE387 and HIS392. Vasicine interacts with target protein 1EA1 had shown carbon-hydrogen bond interactions with amino acids like GLN72 and ALA73. The docking of Vasicinone with target protein through various amino acids as GLN72, MET79, MET99, HIS392 and CYS394. The docking of Betaine with target protein through various amino acids asGLN72, TYR76, ARG96, ARG326, PHE387 and HIS392. The results are summarized in Table 7 and Figure 5 to Figure 8.

Table 7. Docking score and interaction of amino acid residues.

Plant Source	Compound	PubChem ID	Docking Score		Amino Acid Residue	
			3VSL	1EA1	3VSL	1EA1
Justicia adhatoda	Betaine	247	-10.09	-8.54	ARG69, VAL67, ARG27, GLY286	GLN72, ARG96, TYR76, ARG32, PHE387, HIS392
	Anisotine	442884	-13.79	-12.89	SER66, VAL67, ASN83, TRP228,ARG27, ARG22, GLY286	MET99, PHE387, HIS392
	Vasicinone	442935	-10.87	-9.00	LYS52, VAL67, ARG69, ARG27, GLY286	GLN72, MET79, MET99, HIS392, CYS394
	Vasicine	667496	-11.18	-9.68	SER66, VAL67, ARG27, GLY286	GLN72, ALA73

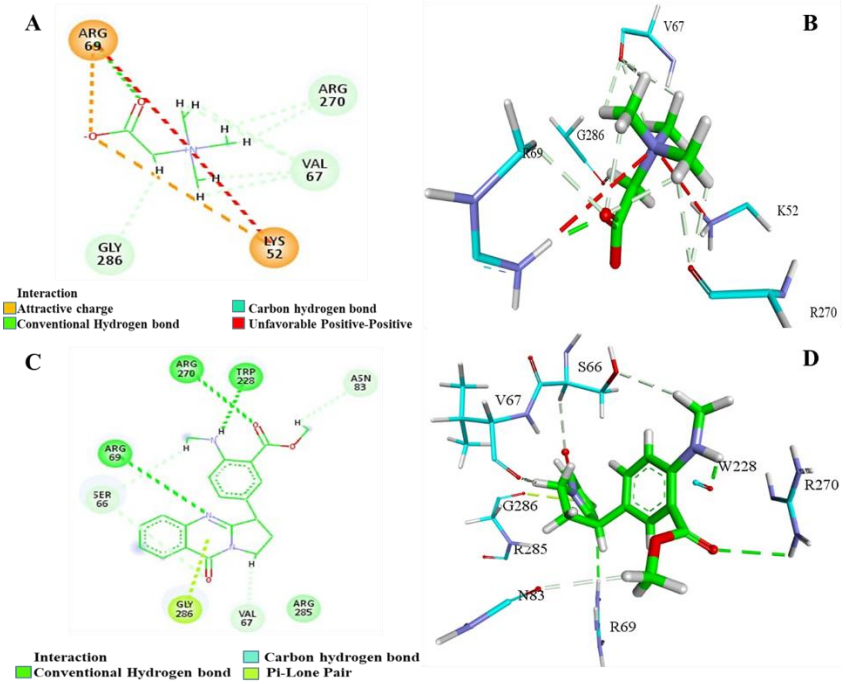

Figure 5: 2D&3D interaction of 3VSL protein with Betaine (A & B) and Anisotine (C & D).

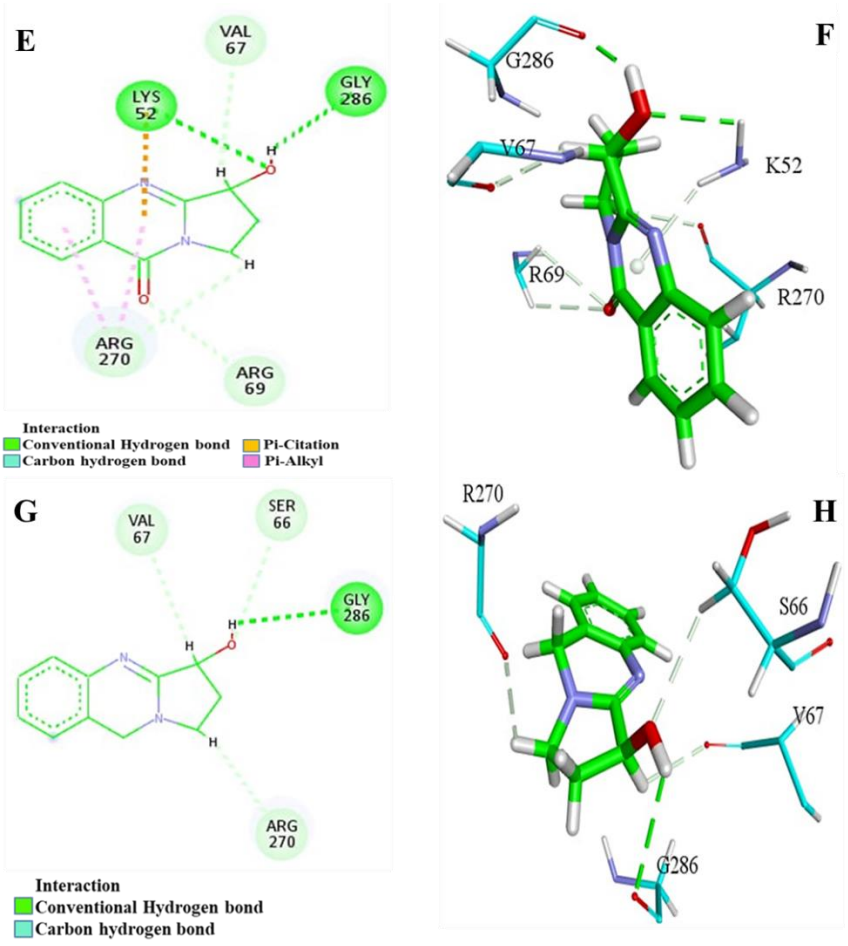

Figure 6: 2D & 3D interaction of 3VSL with Vasicinone (E & F) and Vasicine (G & H).

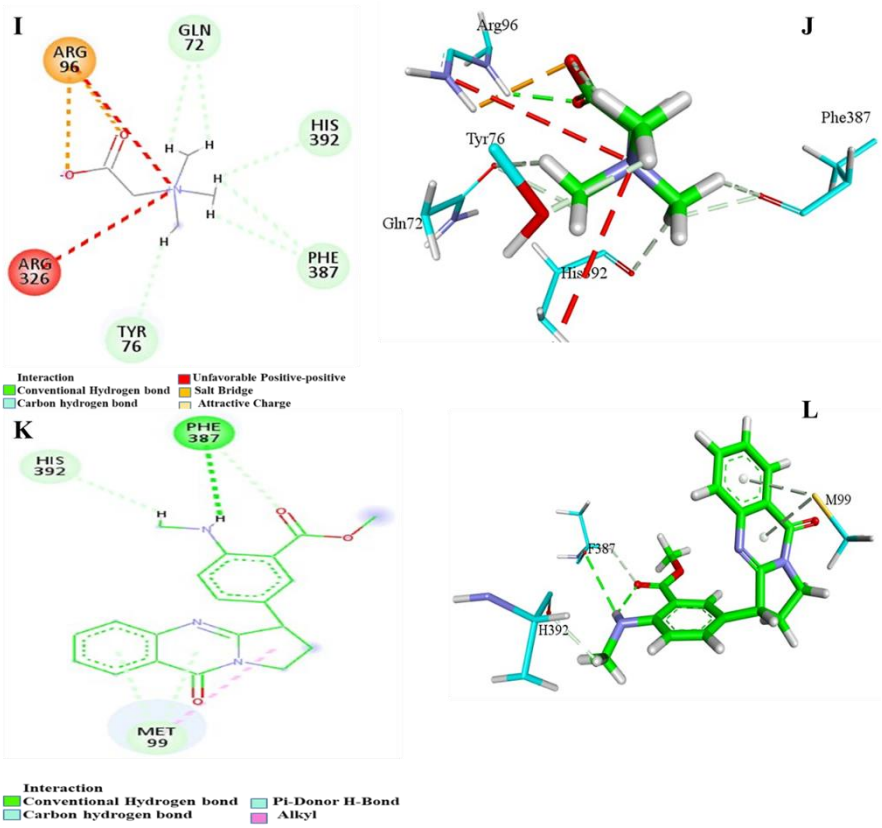

Figure 7: 2D & 3D interaction of 1EA1 protein with Betaine (I &J) and Anisotine (K & L).

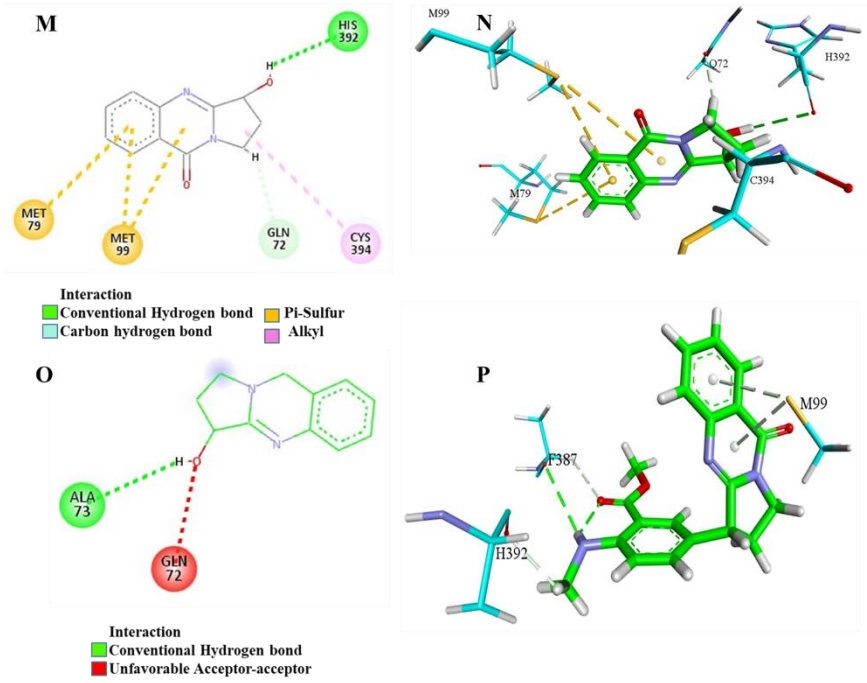

Figure 8: 2D & 3D interaction of 1EA1protein with Vasicinone (M & N) and Vasicine (O & P).

Conclusion

Medicinal plants are essential for preventing a wide range of illnesses. When developing novel medications to address a range of illnesses, pharmaceutical businesses and academic institutions both see commercial value in the phytochemical analysis of medicinal plants. The plant *J. adhatoda* has long been used as a remedy for skin conditions, bronchitis, asthma, and bio-insecticides, among other ailments. This plant has a lot of promise for use in the pharmaceutical industry. A methanol extract of the leaves of *J. adhatoda*. demonstrated a notable ability to inhibit the growth of *Citrobacter koseri*, *Acinetobacter baumannii*, and *Providensia staurtii*. The findings of this investigation suggested that *J. adhatoda*, might be a novel and effective source of antibiotics for use as medicinal agents. Computational studies confirmed the effectiveness of Anisotine, Vasicine and Vasicinone against 3VSL, which can be used for developing novel medications in future.

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