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Phytochemical and In Silico Prediction on a Medicinal tree *Vitex negundo* for Antimicrobial Potential

Hajera Sana[1], Dharmasoth Rama Devi[2], Shetkar Suchita Lahuraj[3], Khanapure Priti Parmeshwar[4], Kapil Chouhan[5], Pravin S. Uttekar[6], Rasika Manoj Rewatkar[7], Anwesha Das[8] and Manasa Ravindra Walmiki[9*]

[1]Veeranari Chakali Ilamma Women's University, Koti, Hyderabad - 500095, Telangana, India

[2]Vignan Institute of Pharmaceutical Technology, Duvvada, Visakhapatnam - 530046, Andhra Pradesh, India

[3,4]Dayanand College of Pharmacy Latur, Swami Ramanand Teerth Marathwada University Nanded, Latur - 413512, Maharashtra, India

[5]Samrat Prithviraj Chauhan College of Pharmacy, Kashipur - 244713, Uttarakhand, India

[6]Dnyan Kala Krida & Krushi Prathishthan's, Late Laxmibai Phadtare College of Pharmacy

A/P-Kalamb, Walchandnagar, Tal: Indapur Dist: Pune - 413114, Maharashtra, India

[7]Department of CSE-ET, Ramdeobaba University, Nagpur - 440013, Maharashtra, India

[8]Department of Pharmacy, Sanaka Educational Trust's Group of Institutions (SETGOI), Malandighi, Durgapur - 713212, West Bengal, India

*[9]Department of Microbiology, JSS Academy of Higher Education and Research, Mysore- 570006, Karnataka, India

***Corresponding Author:**

Dr. Manasa Ravindra Walmiki, Department of Microbiology, JSS Academy of Higher Education and Research, Mysore-570006, Karnataka, India

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ABSTRACT

The main aim of this study was to look at the plant chemicals and antibacterial effects of *Vitex negundo* (VN1) in its usual form and its different version. In traditional medicinal systems and local/folk medicine, Kali Nirgundi is commonly used in place of the species' typical form due to its strong antibacterial and anti-rheumatic/anti-inflammatory qualities. Tests were carried out to ascertain the variations in the phytochemical components and their activity in both the standard form and variant to bolster local knowledge and offer scientific proof. We extracted the powdered leaf components from both varieties using aqueous, ethanol, chloroform, and pet-ether extracts. The following list of chemical compounds is documented. Alkaloids, steroids, tannins, saponins, glycosides, triterpenoids, and flavonoids are all present in VN1 and VN2. The only apparent glycosides and flavonoids in VN1 are not present in the aqueous and chloroform extracts, in contrast to VN2. We tested the antibacterial activity using the disk-diffusion method with nutritional agar medium and four different microorganisms. VN2 exhibited strong inhibitory activity against each isolate in the extract. This might be as a result of the presence of chemicals known as flavonoids and glycosides in the VN2. Molecular docking studies showed more proof of the differences in the types and amounts of phytochemicals in the species because of different geographic areas and environmental changes.

Keywords: *Vitex negundo, Antimicrobial activity, Phytochemicals, In silico, Molecular docking*

1. INTRODUCTION

The escalating threat of antimicrobial resistance has intensified the global search for novel therapeutic agents derived from natural sources [1]. Medicinal plants, long revered in traditional systems of medicine, offer a rich repository of bioactive compounds with potential antimicrobial properties [2]. Of these, *Vitex negundo* also referred to as the Chinese chaste tree or five-leaved chaste tree has drawn a lot of interest due to its diverse ethnopharmacological applications [3].

Native to South and Southeast Asia, this perennial shrub is traditionally utilized to treat a variety of ailments including inflammation, respiratory disorders, wounds, and infections [4].

Phytochemical investigations have revealed that *Vitex negundo* contains diverse classes of secondary metabolites such as flavonoids, alkaloids, terpenoids, glycosides, and essential oils, many of which are known for their antimicrobial activity. While traditional use provides a strong foundation for its therapeutic potential, modern scientific validation is essential to identify, characterize, and predict the mechanisms of these bioactive constituents [5, 6].

Drug development benefits greatly from the use of in silico methods like molecular docking and ADMET prediction, which allow for the economical and timely assessment of pharmacokinetic profiles and compound-target interactions. By integrating phytochemical screening with computational modeling, researchers can gain deeper insights into the antimicrobial potential of natural compounds and prioritize the most promising candidates for further biological testing [7, 8].

This study employs in silico methods to analyze the phytochemical composition of *Vitex negundo* and assess the antibacterial efficacy of its bioactive constituents. The findings may contribute to the development of novel antimicrobial agents from plant-based sources and support the conservation and scientific utilization of this valuable medicinal species [9, 10].

Figure 1: Plant of *Vitex negundo*

2. MATERIAL AND METHODS

Plant Material:

We gathered fresh leaves and young twigs of *Vitex negundo* from the intraspecific variety and its natural habitats. We gathered fresh leaf material and young branches from the crops for Kali Nirgundi/Nalla Vavili. Live photos of the species' usual form and intraspecific variant are included in the plate to highlight morphological distinctions and prevent misunderstandings about the species' taxonomic identity [11, 12].

Preparation of extracts of *Vitex negundo*:

Dust and other unwanted materials are removed from the newly acquired VN and its variation twigs. After that, the foliage was manually separated using gloves and stored in distinct plastic trays labeled VN1 and VN2. Later, they were left to dry in a shaded area. Care was taken to prevent fungus contamination as the leaf material dried. The dried leaf material was ground using a blender machine, and a coarse powder was produced following three weeks (18 days) of shade drying. The 250 g of powdered leaf material was extracted using a cold extraction process using various solvents, including water, petroleum ether, ethanol, and chloroform. After passing through a clean cotton bed, the extract was filtered using Whatman No. 1 filter papers [13, 14].

Phytochemical Screening:

In order to find phytochemicals in the plant leaf extracts employed in this study—aqueous, ethanol, chloroform, and pet-ether phytochemical screening is carried out. The phytochemicals were found using color tests [15].

Test for alkaloids: In order to acidify each extract, a few drops of hydrochloric acid that had been diluted were added to a total volume of 2 milliliters. Next, one milliliter of Dragendorff's reagent was added to the mixture. On the other hand, the production of a precipitate that ranges from orange to scarlet is an indication that alkaloids are present.

Test for tannins: It was decided to add a few drops of lead acetate at a concentration of 10% to two milliliters of each extract. It is possible to determine the presence of tannins by observing the production of a white precipitate.

Test for saponins: After vigorously agitating the mixture for fifteen seconds, nine milliliters of distilled water was added to one milliliter of extract that had been extracted from a measuring jar. The mixture was then allowed to stand for ten minutes. The creation of a stable foam that is one centimeter in height is evidence that saponins are present.

Test for steroids: A total of 10 milliliters of chloroform was combined with two milliliters of each of the three plant extracts. Immediately following the addition of one milliliter of acetic anhydride to these extracts, two milliliters of strong sulfuric acid were poured down the sides of the test sample container. It is possible to detect the creation of color at the intersection. The appearance of a blue-green tint indicates the presence of steroids.

Test for Triterpenoids: Triterpenoids are detected by the same test used for steroids: the presence of triterpenoids is indicated by the junction appearing red, pink, or violet.

Test for glycosides: After adding a few drops of glacial acetic acid and ferric chloride, three to four drops of concentrated sulfuric acid were added to one milliliter of each extract. Additionally, a few drops of sulfuric acid were added. The appearance of a blue-green tint on the surface of the substance is an indication that glycosides are present.

Test for flavonoids: After adding 4 ml of extract solution, 1.5 ml of methanol solution was added to the mixture. The magnesium metal was added to the solution after it had been heated initially. There are five to six drips of Con. It was observed that flavonoids and flavones exhibited distinct coloration subsequent to the addition of HCl acid.

Test for reducing sugars: The addition of 0.5 milliliters of extract solution, 1 milliliter of water, and 5–8 drops of Fehling's solution to a test tube that had been heated was followed by the observation of a brick-red precipitate.

Test for Resins: Following the addition of ten milliliters of distilled water, a few drops of 4% hydrochloric acid were added to the extract. The appearance of turbidity in the solution is a sign that resins are present in the solution.

Test for Phenolic compounds: The extract should be treated with a ferric chloride solution; if there are hydrolyzable tannins present, the extract will change blue; if there are condensed tannins present, the extract will turn green [16-18].

Antimicrobial Activity:

Disc Preparation:

We created the 6mm (diameter) discs from Whatman No. 1 filter paper and autoclave sterilized them at 121°C. Following sterilization, the moistened discs were maintained at 50°C in a hot air oven. The disc was then impregnated with extracts from the stock at a sufficient concentration of 50 mg/ml. *E. coli, P. aeruginosa, B. cereus, and B. subtilis* are the four distinct microorganisms. We used the extract-free solvent as a negative control. Ampicillin (10 µg) and streptomycin (10 µg), two common antibiotics, were used as positive controls [19].

Disk-Diffusion Method:

Using nutritional agar medium and the disk-diffusion method, antimicrobial activity was measured. Using individual sterile cotton buds, 100 microliters of a solution containing 108 colony-forming units mL^{-1} of bacteria were spread out across the nutritional agar medium plates. Following the microbial lawn preparation, plant disc extracts (aqueous, ethanol, and acetone extracts) were firmly applied to each seeded plate's agar surface. The average diameter of the inhibition zone surrounding the wells was visually assessed after the petri dishes were incubated for 24 hours at 37 °C. For the purpose of confirming the results, each test was run twice [20-24].

Molecular Docking Studies:

We can utilize a computer technique known as virtual ligand screening to find powerful chemicals that have the required biological effect. smaller molecules, known as ligands, to larger molecules, known as proteins. The molecular docking analysis of the chosen compounds from *Vitex negundo* (VN1) and its variation "Kali Nirgundi" (VN2) was done using the 3D shapes of the DNA B replication control protein. For the docking investigations, we chose molecules from the essential oils based on the higher percentage concentrations of those compounds. We retrieved the pertinent structures from the PUBCHEM database using the SDF format. The chosen compounds were 6'-p-hydroxybenzoylmussaenosidic acid (PubChem ID: 23955877), 2'-p-hydroxybenzoylmussaenosidic acid (PubChem ID: 73298898), 5,3',5'-Trihydroxy-6,7,4'-trimethoxyflavone (PubChem ID: 21581557), 5,3′-dihydroxy-7,8,4′-Trimethoxyflavanone (PubChem ID: 11230), beta-sitosterol (PubChem ID: 481107734), Casticin (tetramethoxyflavone) (PubChem ID: 5315263), protocatechuic acid (PubChem ID: 72), oleanic acid (PubChem ID: 10494), and vitamin C (PubChem ID: 785). Drug discovery research employs virtual screening (VS) as a crucial method. It is utilized to evaluate large chemical libraries to locate new drugs. The ligand-target strategy has seen a meteoric rise in popularity, and as a result, sophisticated techniques and software are being utilized in an increasing number of applications. The VS process consists of several phases, including docking, development of the ligand database, and preparation of the protein. Additionally, the docking program is responsible for the computation of site positions and atoms on the surface of proteins[25-30]. Computational methods are used to "dock ligand libraries into the target protein" and "score" their potential complementarities to the binding sites, providing predictions about the best ligand hits. After that, an open Babel tool that was made within the PyRx software was utilized to import the ligand structures in

the SDF standard format. We achieved energy minimization, also known as optimization, by introducing charges and refining the universal force field. Furthermore, we transformed the ligands into the AutoDock Ligand format, referred to as PDBQT. Using PyRx and the Vina Wizard tool, we performed molecular docking of the chosen main components to find out how well they bind and the different interactions between the ligands and receptors that contribute to their antioxidant and phytotoxic effects. The Vina Wizard Control was utilized by the PyRx program to pick the protein as well as a large number of ligands for docking. It was necessary to activate the "Run Vina" control in order to initiate the docking procedure. When we chose the "Analyze Vina" function, we were able to view the findings and export them as CSV files simultaneously. The Biovia Discovery Studio-2021 Client was utilized in order to see the two-dimensional and three-dimensional interactions of docking positions [31-34].

***In silico* Study:**

For the purpose of the pharmacokinetics research, ChemSketch Ultra 8.0 was utilized to create blueprints of the structures of the selected compounds derived from the essential oils. Using the set method, a Swiss ADME web server converted the descriptions into SMILES format and then performed the ADME program in order to estimate the drug-like and pharmacokinetic features of the compounds that were chosen. In order to make the forecast, it takes into account a number of different criteria, such as toxicological endpoints, oral toxicity, and organ toxicity [35 - 39].

3. RESULTS AND DISCUSSIONS

In the extracts of aqueous, ethanol, pet-ether, and chloroform results displayed in Table 1, the preliminary phytochemical analysis in VN1 and VN2 revealed a slight difference in the presence of alkaloids, saponins, tannins, steroids, glycosides, reducing sugars, triterpenoids, phenols, flavonoids, and the absence of resins. In *Vitex negundo* (VN1) and intraspecific variant (VN2) leaf extracts of polar and non-polar solvents, phytochemical screening was carried out; the findings are shown in Tables 1 and 2, respectively.

Table 1: Phytochemical screening in leaf extracts of *Vitex negundo* VN1

Name of the Phytochemical	Aqueous	Ethanol	Pet-ether	Chloroform
Glycosides	_	+	+	_
Alkaloids	+	+	+	+
Tannins	+	+	+	_
Flavonoids	_	+	+	_
Saponins	+	+	+	+
Steroids	+	+	_	+
Triterpenoids	+	+	_	+
Phenols	+	+	-	+
Resins	-	-	-	-
Reducing sugar	+	_	+	+

Table 2: Phytochemical screening in leaf extracts of VN2

Name of the Phytochemical	Aqueous	Ethanol	Pet-ether	Chloroform
Glycosides	+	+	+	+
Alkaloids	+	+	+	+
Tannins	+	+	+	+

Flavonoids	+	+	+	+
Saponins	+	+	-	+
Steroids	+	+	+	_
Triterpenoids	-	+	+	_
Phenols	+	+	-	-
Resins	-	-	-	-
Reducing sugar	+	+	+	+

In VN 1, the chloroform extract had no tannins, the pet-ether had no steroids or triterpenoids, and the aqueous and chloroform extracts had no glycosides or flavonoids. Table 2 shows the results of the aqueous extracts in VN2, which did not contain triterpenoids, steroids, or saponins in the pet ether and chloroform extracts. The chemicals that are mostly present in all of the VN2 extracts include glycosides and flavonoids. Tables 3 and 4 (VN1), 5 and 6 (VN2), and Figures 1 (VN1) and 2 (VN2) show the results of the antibacterial tests done on the leaf extracts of VN1 and VN2 against four types of bacteria. All extracts showed they could fight off bacteria in VN1 and VN2, except for the B. subtilis bacterium, which did not show any effect in the VN2 ethanol extract. All extracts exhibited antimicrobial activity against all isolates in VN1 and VN2 leaf extracts, with the exception of bacterium B. subtilis, which did not exhibit an inhibitory effect in the VN2 ethanol extract. All isolates and extracts exhibit a high zone of inhibition in VN 2 leaf extracts. The leaf extracts of *Vitex negundo* (VN1) and its variation (VN2) exhibit antimicrobial action against four bacterial species: Pseudomonas aeruginosa (MTCC: 424), Bacillus cereus (MTCC: 4-30), Bacillus subtilis (MTCC: 441), and Escherichia coli (MTCC: 41). Tables 3 and 4 show how much the leaf extracts can stop bacterial growth (measured in millimeters) for every 100 mg/ml and their antibacterial effects in various types of solvents. Likewise, Tables 3 and 4 demonstrate the variant's zone of inhibition and antimicrobial activity (VN2). Plates No. 1 and 2 display the photos.

Table 3: Zone of inhibition of various leaf extracts of plant VN1

Micro organism	Aqueous (100mg/ml)	Ethanol (100mg/ml)	Pet-ether (100mg/ml)	Chloroform (100mg/ml)
E. coli	6.3mm	16.6mm	9.4mm	10.6mm
P. aeruginosa	6.6mm	11.6mm	10.3mm	9.8mm
B. cereus	8.2mm	7.8mm	16.2mm	12.4mm
B. subtilis	11.4mm	41.6mm	7.9mm	8.8mm

Table 4: Antimicrobial activity of various leaf extracts of plant VN1

Micro organism	Aqueous	Ethanol	Pet-ether	Chloroform
E. coli	+	+++	++	++
P. aeruginosa	+	++	++	++
B. cereus	++	+	+++	+++
B. subtilis	++	+++	+	++

Table 5: Zone of inhibition of various leaf extracts of plant VN2

Micro organism	Aqueous (100mg/ml)	Ethanol (100mg/ml)	Pet-ether (100mg/ml)	Chloroform (100mg/ml)
E. coli	9.4mm	20.6mm	10.9mm	23.2mm
P. aeruginosa	10.4mm	27.4mm	11.7mm	24.3mm
B. cereus	11.6mm	19.8mm	10.9mm	17.6mm
B. subtilis	9.4mm	-	11.3mm	20.4mm

Table 6: Antimicrobial activity of various leaf extracts of plant VN2

Microorganism	Aqueous	Ethanol	Pet-ether	Chloroform
E. coli	++	+++	++	+++
P. aeruginosa	++	+++	++	+++
B. cereus	++	+++	++	+++
B. subtilis	+	-	++	+++

The extracts' antimicrobial properties were demonstrated by the following: -(no zone of inhibition), +(low zone of inhibition) = 8 mm in diameter, ++(moderate zone of inhibition) > 8 mm in diameter, and +++(high zone of inhibition) ≥12 mm in diameter.

Chemical Composition

In the typical form of *Vitex negundo* (VN), 29.4% was made up of monoterpene hydrocarbons, while oxygenated sesquiterpenes made up 24.8% and oxygenated monoterpenoids made up 11.3%. Monoterpene hydrocarbons were the most abundant component. These essential oils of V. negundo, which are currently being investigated, have also been investigated by researchers who came before us. An example of this would be the discovery that the essential oil extracted from the leaves of V. negundo, which was hydro-distilled, contains varied concentrations of the primary compounds that are found in the plant. These chemicals include sabinene (19.4%), viridiflorol (17.8%), and β-caryophyllene (7.5%). An additional substantial component that was detected in VNO, 5-(1-isopropenyl-4,5-dimethylbicyclo [4.3.0] nonan-5-yl)-3-methyl-2-pentenol acetate, was also found to be present in considerable levels in the essential oil of V. negundo leaves. This component was determined to be 5.2%. In the chemical composition of the essential oil (EO) of *Vitex negundo* that was extracted in the spring from the same area (Pantnagar), the most prominent chemicals that were discovered were viridiflorol (23.8%), sabinene (11.2%), an unidentified diterpene M+=272 (11.0%), and caryophyllene (6.7%). It is possible that the qualitative and quantitative variances in essential oils derived from V. negundo that are found in different locations of the world are due to the variations in climate and geography that exist in those regions. The binding energy of the complex formed by 6'-p-hydroxy benzoyl mussaenosidic acid and 1B79 was -6.6 kcal/mol, which is remarkably comparable to the binding energy of beta-sisterol, which was -6.1 kcal/mol. However, ascorbic acid, which is a well-known antioxidant, complexed with 1B79 and had a binding energy of -6.7 kcal/mol, which was higher than the binding energy of the rest of the compounds. The binding energies of various flavones range from -5.8 to -5.5 kcal/mol, with oleanic acid having the lowest binding energy (-6.2 kcal/mol), followed by 4-terpineol and protocatechuic acid (-4.6 kcal/mol). Different flavones have different binding energies. Figure 2 shows that in the best-docked position of 6'-p-hydroxybenzoyl mussaenosidic acid, there are two pi-alkyl contacts, one pi-sigma connection, and additional Vander Waals interactions with 1B79. These interactions involved amino acid residues such as B: ALA56, B: ASP57, B: THR 69, and B: GLU76. Likewise, the best-docked position of oleanic acid revealed a single hydrogen bond with B: ALA51 and vitamin C 1 pi-anion, along with additional Vander Waals interactions involving the amino acids B: SER79, B: ASN44, B: GLN75, B: ASP82, and B: ASP82. The presence of lower binding free energy values is indicative of a more robust link between the ligand and the receptor.

The compounds' safety and the potential for future design with increased activity were both demonstrated by the ADMET study.

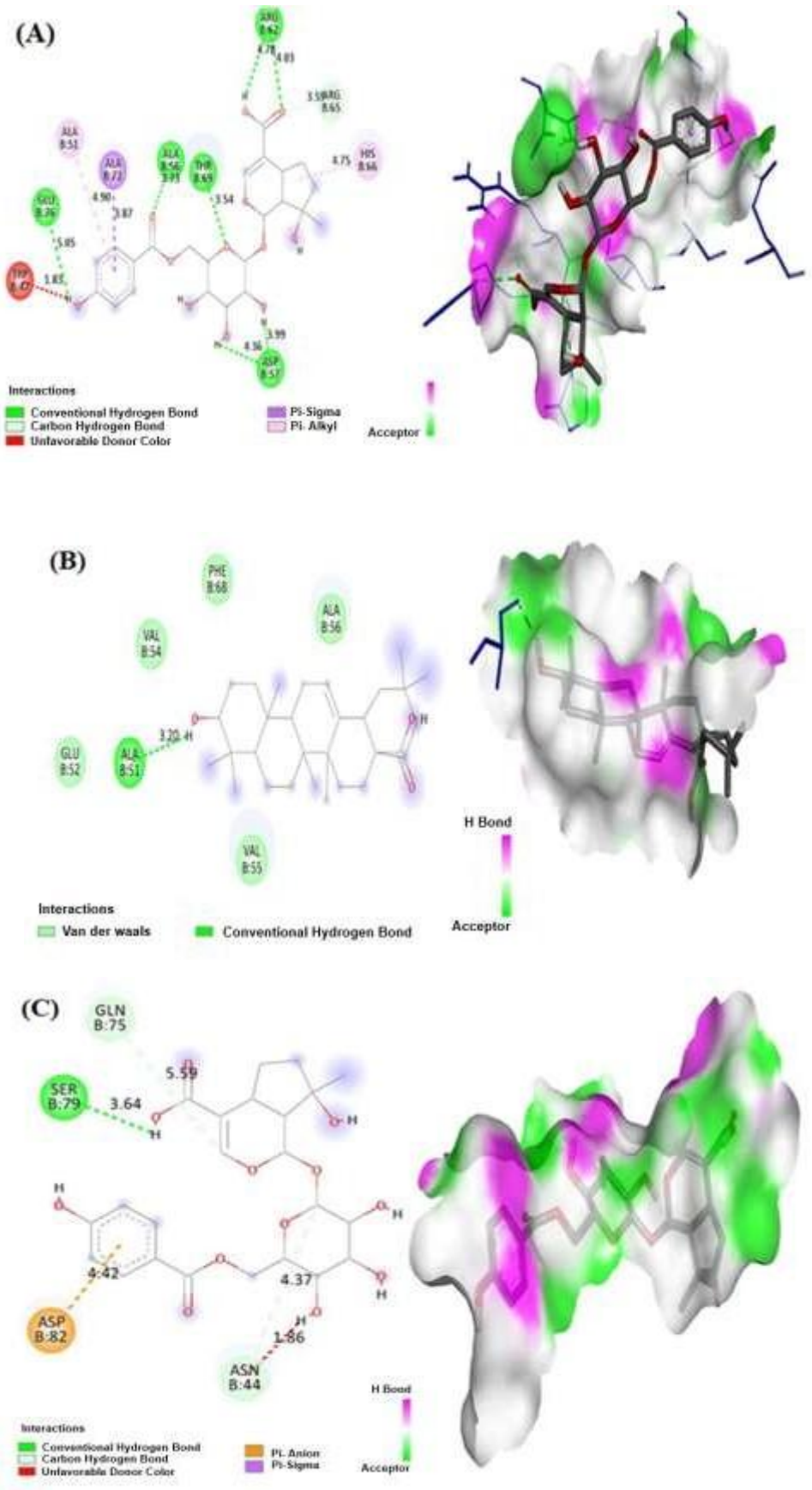

Figure 2: Molecular Docking interaction of molecules in the binding cavity of DNA B Helicase (PDB: 1B79) with amino acid sequence.

4. CONCLUSION

Recent studies on *Vitex negundo* (VN1) and its variant "Kali Nirgundi" (VN2) indicate that the variant (VN2) has a lot of glycosides and flavonoids. This phenomenon could be the cause of the intraspecific Kali Nirgundi leaf material's widespread use and efficacious anti-inflammatory, anti-arthritic, and antibacterial properties in both traditional and regional medical systems. By studying how the compounds 6'-p-hydroxybenzoyl mussaenosidic and oleanic acid interact with proteins, the molecular docking studies showed that they might be good at fighting bacteria. Overall, this study's intriguing biological activities justify the use of these plant species in traditional medicine.

5. DECLARATIONS

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

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