An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

MOLECULAR DOCKING STUDIES AND ANTIBACTERIAL ACTIVITY OF LEAVES OF *TABERNAMONTANA DIVARICATA*

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ARTICLE INFO

Key Words
Antibacterial, Ciprofloxacin, Docking analysis, Conofoline, Histidine Kinase.

Access this article online
Website: https://www.jgtps.com/
Quick Response Code:

ABSTRACT

Tabernamontana Divaricata is a medicinal shrub belonging to family Apocynaceae. In the present research an attempt has been made to extract the phytoconstituents from leaves of *Tabernamontana Divaricata* using different solvents and to evaluate its antibacterial activity. The selected phytoconstituents Conofoline, Pachysiphine and Voacanine from plant are also evaluated for its enzyme inhibition activity against Histidine kinase using molecular docking analysis. The extraction was carried out using water, ethanol, methanol, chloroform and ethyl acetate employing maceration and soxhlet methods. Phytochemical investigation for each extract was performed using standard chemical test for alkaloids, glycosides, flavonoids, tannins, terpenoids, phytosterols and saponins. Antibacterial activity against *Escherichia coli* of each extract was carried out by disk diffusion method using Ciprofloxacin as standard. The results of the study showed that each extracts yields different phytoconstituents with varying antibacterial potentials. Methanolic extract showed good antibacterial activity with maximum zone of inhibition 22.8 mm against *Escherichia coli*. Binding energy of Conofoline, Pachysiphine and Voacanine obtained from docking analysis was found to be -6.8, -2.4 and -2.4 respectively.

INTRODUCTION

Plants are the great source of primary and secondary metabolites and have ability to protect body against many degenerative diseases [1]. They are reported for their antioxidant, antimicrobial, anticancer and antimutagenic effect and also few phytoconstituents are used to synthesize many drugs [2, 3]. Various steps involved in the extraction of bioactive compounds are Extraction, isolation, Fractionation, Characterization and biological evaluation. Extraction is a very crucial step in which the selection of solvent systems play an important role [4]. During extraction, the solvents solubilize the compounds of similar polarity by diffusing into the solid plant tissue. A good solvent should have low toxicity, do not destruct the active metabolites, easy to evaporate, preservative action, inability to cause complex or dissociation of extract, should not interfere with the bioassay [5]. Phytochemicals from plants have a different mechanism of action compared to that of conventional antibiotics and this could be of great help in the treatment of resistant microbes

[6]. *Tabernamontana Divaricata* is a medicinal shrub belonging to family Apocynaceae. Leaves and flowers of this plant are medicinally important. The leaves of plant found to possess various chemical constituents such as alkaloids, tannins, resins, proteins, saponins, glycosides, flavonoids, phenols, steroids, terpenoids, fixed oils and fats. The presence of wide range of phytoconstituents responsible to show astringents, anti-diarrhoeal, antioxidants, anti-infection, anti-tumor, analgesic, enhancement of cholinergic activities. The extracts of plant are also used in the treatment of neurodegradative disease, anti-inflammatory, anti-diabetic, anti-bacterial. Nature and types of solvent used play an important role in extraction of phytoconstituents from crude drug. Conofoline, Pachysiphine and Voacanine are important constituents present in *Tabernamontana Divaricata* [7]. The increase in bacterial resistance to most of the antibiotics is a threat to public health across the world [8]. Two component systems are widely used by the bacteria to respond to external stimuli for their survival. Two compartment systems typically comprises of Histidine kinase for receiving external input signals and response regulator for a proper change in bacterial cell physiology [9]. Histidine kinase enzyme of bacterial two component systems reported for promising target for discovery of new class of antibiotics or antibacterial drugs [10]. In the present research work an attempt has been made to extract the phytoconstituents from leaves using different solvents and evaluate it for antibacterial effect against *Escherichia coli* also to study molecular docking interaction between Histidine kinase and selected phytoconstituents from *Tabernamontana Divaricata.*

MATERIALS AND METHODS

Collection and Identification of Plant Material: The plant *Tabernamontana Divaricata* was collected from the local area of Belagavi district Karnataka, India. Identified and authentification was done by the taxonomist Dr. Harsha Hegde ICMR, Belagavi and the voucher specimen (RMRC-1428) was deposited in the Department of Medicinal plants of Western Ghats in ICMR Belagavi.

Chemicals and reagents: All solvents methanol, ethanol, chloroform and ethyl acetate used for extraction purpose were purchased from Merk. The chemicals and reagents used for phytochemistry research were obtained from store house of KLE College of Pharmacy, Belagavi. Standard drug Ciprofloxacin was procured from Micro Labs, Bengaluru.

Preparation of leaf extracts: The leaves of plant were collected, washed and dried under shade. The dried and moisture free leaves were ground into powder and stored in air tight containers for extraction. The powdered plant sample was subjected to extraction process. Two different methods namely maceration and soxhlet method were used. Extraction of powdered leaves were carried out by using water, ethanol and methanol as solvents using maceration whereas extraction by using soxhlet method was carried out by using different sets of solvents namely ethyl acetate and chloroform. The obtained extracts were dried to remove the solvents and weighed and % yield was calculated and then stored in well closed container for the further investigation. The solvent-free extracts were subjected to phytochemical investigation and biological evaluation.

Phytochemical Screening of the Leaf extracts: The qualitative phytochemical tests were performed for all the extracts as per the standard procedure. Chemical qualitative tests for alkaloids, glycosides, flavonoids, tannins, terpenoids, phytosterols and saponins were performed [11-12].

Antibacterial Activity: Antibacterial activity of different leaves extract of plant was evaluated by using disc diffusion method [13]. Petri plates were prepared by using dextrose agar media and organisms *Escherichia coli* were swabbed onto the medium. All the extracts were dissolved in Dimethyl Sulfoxide (DMSO) and three different concentrations (500 µg, 1000 µg and 1500 µg) were prepared for each extract. 25 µL of the extracts were loaded onto the sterile discs and placed in the incubator for 24 h at 37 ℃. The antibacterial zones formed were measured in millimeters and the results were tabulated. DMSO was taken as a negative control and Ciprofloxacin was used as standard antibacterial agent [14, 15].

Table 1: Nature and Percentage Yield of Leaf Extracts of Tabernamontana Divaricata

Type of extract	Aqueous Extract	Ethanol Extract	Methanol Extract	Chloroform extract	Ethyl Acetate extract
State	Solid	Solid	Solid	Semi-solid	Semi-solid
Colour	Brownish	Brownish	Brownish	Brownish	Brownish
Yield	5%	5.93%	5.86%	3.37%	2.30%

Table 2: Phytochemical analysis of Leaf extracts of Tabernamontana Divaricata

Phytochemicals	AQ	E	M	C	EA
Alkaloids	+ve	+ve	+ve	-ve	-ve
Glycosides	+ve	+ve	+ve	-ve	-ve
Flavonoids	+ve	+ve	+ve	-ve	-ve
Tannins	+ve	+ve	+ve	-ve	-ve
Steroids	-ve	+ve	+ve	+ve	-ve
Carbohydrates	+ve	+ve	+ve	-ve	-ve
Proteins	-ve	+ve	+ve	-ve	-ve
Saponins	+ve	-ve	-ve	-ve	-ve
Terpenoids	-ve	+ve	+ve	-ve	+ve

Note: AQ- Aqueous; C-Chloroform; EA-Ethyl acetate; E- Ethanol; M-Methanol.

Table 3: Anti-bacterial activity of leaf extracts of T. Divaricata against Escherichia coli

Concentration	AQ	E	M	C	EA
500 µg/mL	12.3 mm	13 mm	14.6 mm	2.4 mm	1.3 mm
1000 µg/mL	15.9 mm	16.8 mm	18.5 mm	6.8 mm	1.6 mm
1500 µg/mL	18.0 mm	20.3 mm	22.8 mm	8.1 mm	2.1 mm
CIP 10 µg/mL	26 mm	28 mm	27 mm	26 mm	28 mm

Note: AQ- Aqueous; C-Chloroform; EA-Ethyl acetate; E- Ethanol; M-Methanol.

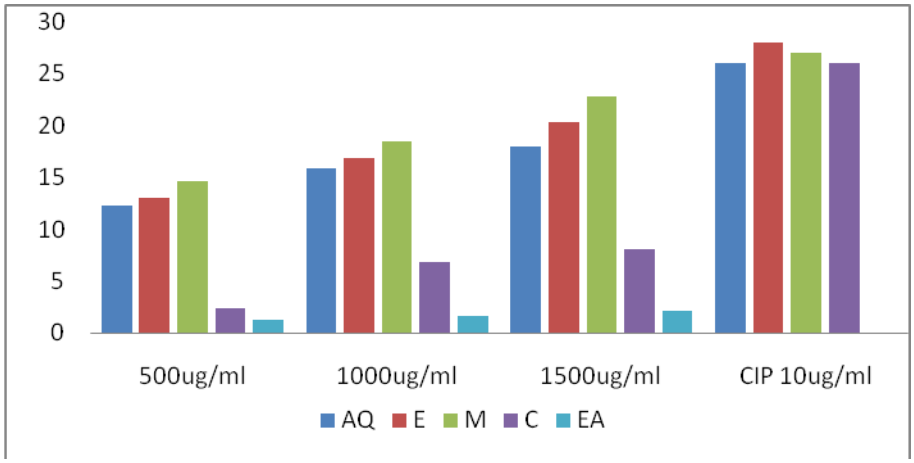


Figure 1: Relative percentage of inhibition of the extract against Escherichia coli

Table 4: Drug Likeness Properties of Selected Ligands from T. Divaricata

Ligands	Molecular formula	Molecular weight	Number of HBA	Number of HBD	Mol. logP	Drug Likeness Score
Conofoline	$C_{43}H_{52}N_4O_7$	736.38	9	2	4.24	1.12
Pachysiphine	$C_{21}H_{24}N_2O_3$	352.18	4	1	2.53	1.30
Voacanine	$C_{43}H_{52}N_4O_5$	704.39	7	2	5.90	1.20

Table 5: Molecular structure and 2D interaction with different amino acids on target

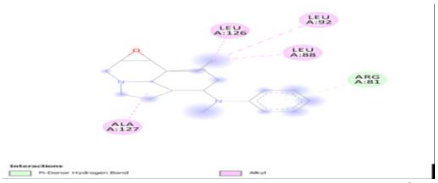
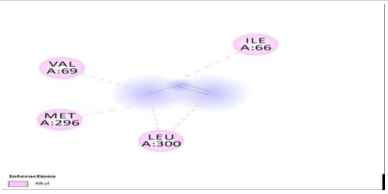
Sr. No	Ligands Name	Molecular structure and Interaction with Protein Kinase	Amino acids involved in the interaction
1	Conofoline		LEU 126, LEU 88, LEU A92, ALA 127, ARG A81
2	Pachysiphine		VAL-69, ILE-66, MET-296, LEU-300
3	Voacanine		VAL-69, ILE-66, MET-296, LEU-300

Table 6: Binding energies of Conofoline, Pachysiphine and Voacanine

Compounds and Rank	Binding energies of the compounds based on their rank (kcal/mol)								
	1	2	3	4	5	6	7	8	9
Conofoline	-6.8	-6.6	-6.5	-6.4	-6.3	-6.3	-6.2	-6.1	-6.0
Pachysiphine	-2.4	-2.4	-2.3	-2.3	-2.0	-2.0	2.0	-2.0	-2.0
Voacanine	-2.4	-2.4	-2.3	-2.3	-2.2	-2.2	-2.1	-2.0	-2.0

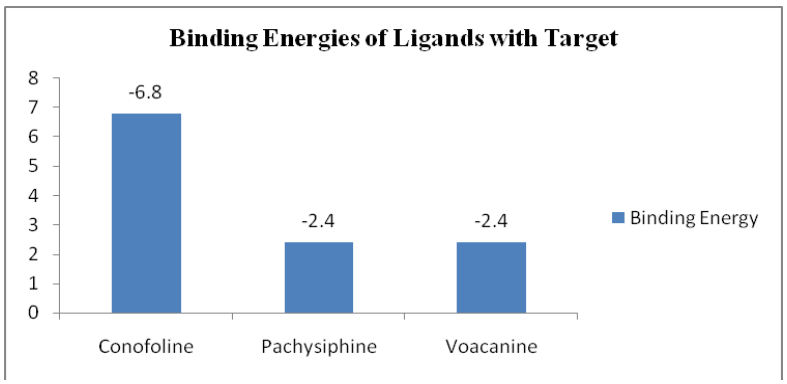


Figure 2: Binding Energies of Conofoline, Pachysiphine and Voacanine with target

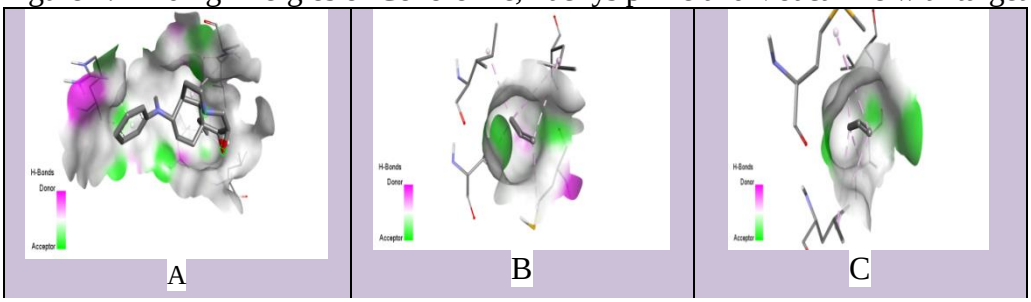


Figure 3: 3D interaction between target and ligands (A), Conofoline (B), Pachysiphine (C) Voacanine

Determination of relative percentage inhibition: The relative percentage inhibition was calculated for all the extracts with respect to the standard. The formula used is as follows Relative Percentage Inhibition = 100 (X−Y)/(Z−Y) Where X is the total area of inhibition of the test extract, Y is the total area of inhibition of the solvent and Z is the total area of inhibition of the standard drug.

Molecular Docking Analysis of Selected Phytoconstituents:

Phytoconstituents and their targets: The lists of phytoconstituents present in *Tabernaemontana divaricata* were identified from published literature. The compounds predicting for anti microbial activity and targeting Histidine kinase were identified using PASSONLINE.

Determination of Drug like properties: The drug likeness index of selected phytoconstituents was determined using Molsoft online. The drugs like properties are explained by Lipinskies Rule of 5. It gives information about molecular weight, LogP, number of hydrogen bond donor and number of hydrogen bond acceptor present in molecules.

Preparation of Ligand: All the 3D structures of top 3 hit molecules for positive drug likeness were scored i.e Conofoline, Pachysiphine and Voacanine were retained from PubChem database in SDF format. These compounds were converted into PDB format using Discovery studio 2019. These ligand molecules were minimized using MMFF94 force field and their PDBQT format was used for docking.

Preparation of Target molecule: FASTA Sequence of Histidine kinase was retrained from NCBI protein database (Accession number ALB28461). Homology of the protein was camed using SWISS-MODEL and the homology model was visualized in Ranmachandran plot to access the phi and psi distribution of amino acid.

Molecular Docking: Molecular interaction of protein *Histidine kinase* with selected phytochemical was carried using Auotodock under PyRx 0.8 platform. The pose scoring lowest binding energy was chosen for visualized ligand protein interaction.

RESULT AND DISCUSSION

Percentage yield of leaf extracts: The percentage yield of all the extracts was calculated. Aqueous extract was in solid state and have brownish colour with yield of about 5%. The ethanolic and methanolic extracts were in solid state with brownish colour and yields about 5.93 % and 5.86%. The chloroform and ethyl acetate extracts were in semi-solid form with brownish colour. The yield was found to be 3.37% for chloroform, 2.30% for ethyl acetate extract. The state, colour and % yield of extract were presented in Table 1.

Phytochemical Investigation of Leaf extracts

All the five extracts were subjected to phytochemical analysis. Aqueous extracts showed the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins and saponins. Ethanolic extract showed the presence of alkaloids, glycosides, flavonoids, tannins, steroids, carbohydrates, proteins, terpenoids. Methanolic extract found to show the presence of alkaloids, glycosides, flavonoids, tannins, steroids, carbohydrates, proteins and terpenoids. Chloroform extract showed the presence of Steroids. Ethyl acetate showed the presence of terpenoids. The results of phytochemical investigation were presented in Table 2.

Antibacterial activity: All the five extracts were tested for their antibacterial activity against *Escherichia coli* and the results were tabulated Table 3. Aqueous leaves extract showed moderate antibacterial activity, chloroform, and ethyl acetate extract shows poor antibacterial activity. Ethanol and methanol extract showed good antibacterial activity with highest zone of inhibition 20.3 mm and 22.8 mm respectively against *Escherichia coli*. We have prepared three different concentration of each extract. Out of which extracts having 1500 μg/mL concentration found to show maximum inhibitory activity (mm).

Relative percentage inhibition: The relative percentage inhibition was calculated for all the extracts with respect to the Ciprofloxacin as positive control and the results were represented as bar graph in Figure 1.

Determination of Drug Likeness Properties of Selected Phytoconstituents: The drug likeness properties of Conofoline, Pachysiphine and Voacanine were determined as per Lipinskies R5. Results showed that molecular weight of Conofoline is 736.38, Hydrogen Bond Acceptors (HBA) is 9 and Hydrogen Bond Donors (HBD) is 2. The molecular weight of Pachysiphine is 352.18, HBA are 4 and HBD is 1. Voacanine, molecular weight is 704.39, HBA are 7 and HBD are 2. Molecular logP values of Conofoline, Pachysiphine and Voacanine was observed to be 4.24, 2.53 and 5.90 respectively. Drug likeness of Conofoline, Pachysiphine and Voacanine was found to be 1.12, 1.30 and 1.20 respectively. All the three phytoconstituents obeys the Lipinskies rule of 5. The data were presented in Table 4. Amino acids present on protein plays vital role for interaction with ligand molecules and forms different types of bonds such as hydrogen bonds, electrostatic pi bonds and hydrophobic bonds etc. Structure of ligand molecules and their 2D interaction with different amino acids on Histidine kinase target were presented in Table 5. Conofoline found to show interaction with LEU 126, LEU 88, LEU A92, ALA 127, ARG A81 amino acids of target. Pachysiphine found to show interaction with VAL-69, ILE-66, MET-296, LEU-300 amino acids of target. The VAL-69, ILE-66, MET-296, LEU-300 amino acids of target are interacted with Voacanine. The results showed that Pachysiphine and Voacanine are found to show interaction with similar amino acids VAL-69, ILE-66, MET-296, LEU-300 of target. The drug candidates were docked against Histidine Kinase target using. Conofoline, Pachysiphine and Voacanine found to show binding energies -6.8, -2.4 and -2.4 respectively. Pachysiphine and Voacanine found to show same binding energy were as Conofoline was found to show maximum binding energy as compared to other to compounds with Histidine Kinase. Binding Energies of the Compounds Based on their Rank were presented in Table 6. Binding energies data also compared using bar diagram and presented in Figure 2. As showed in table 4, Pachysiphine and Voacanine are found to interact with same amino acids of target namely VAL-69, ILE-66, MET-296, LEU-300. Because of this the both phytochemicals found to show same binding energies (-2.4) with target. Docking analysis visualization of target and bioactive compounds were performed and their 3D interaction was presented in Figure 3.

CONCLUSION

From the present investigation it can be concluded that different solvents extract different phytoconstituents from leaves of *Tabernaemontana divaricata* due to the different polarity of solvents and active phytoconstituents responsible for maximum antibacterial activity are extracted in methanolic extract. Also an attempt was made by the researchers to find out the standard methodology for extraction of phytochemicals for potential antibacterial activity against *Escherichia coli*. Docking of the three selected compounds has promising inhibitor of *Histidine kinase* protein. In future there is need to carry out the systematic fractionation, isolation and characterization of target compound from active fractions of leave extracts of *Tabernaemontana Divaricata.*

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