IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Comparative Phytochemical, Antimicrobial And Molecular Docking Studies On A Medicinal Tree *Vitex negundo* var. *negundo* And Its Intraspecific Variant "Kali Nirgundi"

Sandhya Alijala[1], Navaneetha Nambigari[2], Venkat Ramana Munigela*[1] & Nethaji Kante[1]

[1] Department of Botany, Plant Systematics, Biodiversity and Conservation Laboratory, University College of Science, Saifabad, Osmania University, Hyderabad -500 004, Telangana State, INDIA.

[2]Department of Chemistry, University College of Science, Saifabad, Osmania University, Hyderabad -500 004, Telangana State, INDIA.

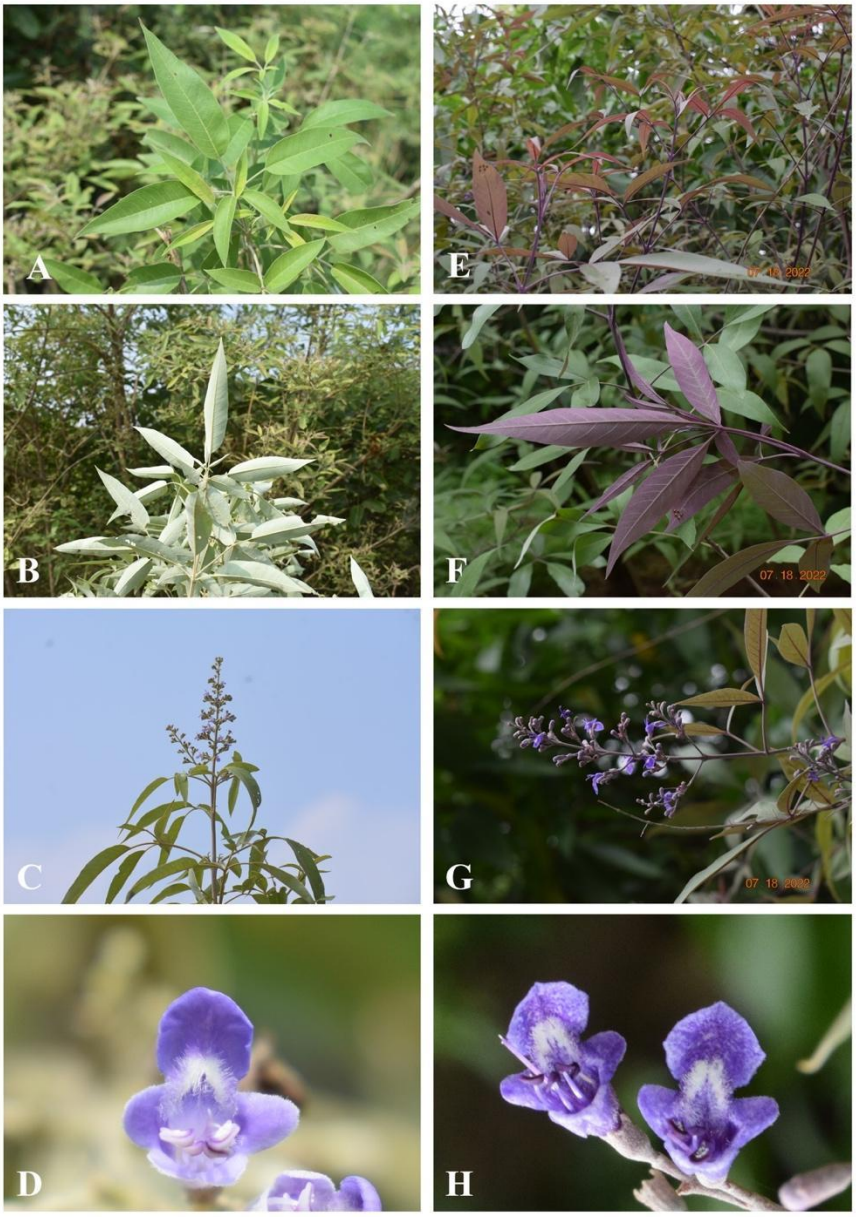
[2]Department of Chemistry, Osmania University, Taranaka, Hyderabad-500007 Telangana State, INDIA.

Abstract: The present studies focused mainly on the comparative phytochemical analysis and antimicrobial activity in the typical form of the *Vitex negundo* var. *negundo* (VN1) and its intraspecific variant Kali Nirgundi or Nalla vavili (VN2). In the traditional systems of medicines as well as in the local/folk medicine the kali Nirgundi is extensively used rather than the typical form of the species for its effective functioning as an antimicrobial and antirheumatic/anti-inflammatory. To find out the variations in the phytochemical components and their activity in the both typical form and variant the studies were carried out to strengthen the local knowledge and by providing scientific evidence. The powdered leaf materials of both were extracted by using Aqueous, ethanol, chloroform and pet-ether extracts the following chemical compounds were reported Alkaloids, Tannins, Saponins, Steroids, Triterpenoids, Glycosides, Flavonoids in VN1 & VN2. The only glycosides and flavonoids showing are absent in the aqueous and chloroform extracts in the VN1 differing with VN2. The antimicrobial activity was carried out by disk-diffusion method by using four different microorganisms with the nutrient agar medium. The high inhibitory activity was observed in the VN2 against all isolates in all extracts. This may be the presence of chemical compounds glycosides and flavonoids in the VN2. The Molecular docking studies were also applied to support the qualitative and quantitative variations in the presence of phytochemicals within the species due to change of geographic regions and environmental changes.

Keywords: Antimicrobial activity, Comparative phytochemical analysis, Intraspecific variant, Molecular docking studies.

Introduction

The genus *Vitex* L. belongs to family Lamiaceae represented with 250 species distributed in tropical and temperate regions. In India the genus is distributed almost throughout the country from dry to semi evergreen and ever green forests. Many of the species of the genus *Vitex* are used therapeutically in ancient Indian systems of medicine and in folk medicines. The most popular and widely used species is *Vitex negundo* L., in the Indian region. Naturally grows in hill slopes, marshy habitats along field bunds, hedges, water streams and also widely introduced in the cultivation and planted as a hedge plant along agricultural fields, near houses and in gardens. The species is represented with two infraspecifics *V. negundo* var. *negundo* (East Africa, Asia & South East Asia, Japan and Pacific Islands) and *V. negundo* var. *thyrsoides* (China) (https://powo.science.kew.org). Sivarajan & Moldenke published a new infraspecific *V. negundo* var. *purpurascens*. The type was collected by V.V. Sivaraja (no. 1849) from the Calicut University Campus, Kerala, India[19]. It differs from the typical form of the species in having its branches, leaves, inflorescences deep purple in colour (slightly fading in age) and there is no other morphological difference in the structure of floral parts and fruit. It is synonymized under the typical form of the species *Vitex negundo* var. *negundo* (https://powo.science.kew.org) [15]. "Kali Nirgundi'' taxonomically similar with its typical form of the species but with its colour variation (dark purple) morphologically it looks distinct even for a common man (local people) and it has been named locally as "Kali Nirgundi" in Kerala (Malayalam) and "Nalla Vavili" in Telugu states Andhra & Telangana (Telugu) and it is commonly called "Black Vitex" by the plant sellers. The local names derived from its unique colour dark purple (young twigs, leaves, inflorescence). The typical form of the species Nirgundi (Sephalika) and it intra specific variant "Kali nirgundi" leaves has been using externally for antiparasitic, antimicrobial and anti-inflammatory diseases (Prashith et al., 2014). There is a strong belief in the locals the intraspecific variants work more effectively than the typical form of the species. The recent comparative studies on Antimicrobial activity of *Vitex negundo* var. *negundo* (Vnvn) and *Vitex negundo* var. *purpurascens* (Vnvp) also supports the presence of high phenolic and flavonoid content in the intraspecific variant Vnvp (Kali nirgundi) than the typical form of the species Vnvn (Nirgundi)[11]. The other unknown local uses of "Kali Nirgundi" (Nalla Vavili) is the young twigs and leaves are widely using for the arthritis (Joint pains & inflammations) and analgesic (Pain relief in Muscle & Joint rub) in Telugu states (Andhra Pradesh and Telangana). Due to its immediate relief and effective control of joint and muscular pains, it has been more popularized and widely introduced in the cultivation in the homesteads and in the gardens rather than its typical form of the species. Even the plant sellers (nursery people) develop by vegetative method from stem cuttings in large scales and selling locally and even online. The present studies are focused on the comparative phytochemical analysis, antimicrobial activity and molecular docking studies in the typical form of the species (Nirgundi / Vavili) and its intraspecific variant (Kali Nirgundi / Nalla Vavili) to determine the variation in the presence chemical compounds and their activity to strengthen the traditional knowledge and the local beliefs of the intraspecific variants are given priority more rather than typical form of the species.



Legend for Figure 1. A – D. *Vitex negundo* var. *negundo* (typical form of the species Nirgundi / Vavili); E – H. *Vitex negundo* var. *negundo* (Intraspecific variant -Kali Nirgundi / Nalla Vavili)**.**

The dynamic replication protein interaction network (PIN) studies the composition of protein complexes formed by the main factors involved in DNA replication in E. coli [1]. Replication proteins, including main DNA replication regulators (initiator protein DNA B, regulatory proteins DiaA, Hda and SeqA), the key component of the mechanisms of regulation of initiation, in E. coli and B. subtilis is certainly the DNA A protein (replication initiator) [2, 3].

2. MATERIALS AND METHODS:

2.1. Plant material:

Young twigs and fresh leaf material of *Vitex negundo* var. *negundo* (typical form of the species) collected from the natural habitats of Ambrabad Tiger Reserve range (Kollapur, Nagarkurnool District) and the intraspecific variant Kali Nirgundi/Nalla Vavili young twigs and fresh leaf material collected from the cultivation (Botanical Garden, University College of Science, Saifabad, Hyderabad). The live images of both the typical form of the species and intraspecific variant provided in the plate 1 for showing the morphological differences and for also to avoid confusion in Taxonomic identity of the species.

2.2. Preparation of extracts:

The fresh collected twigs of the VN and its variant are made free from dust and other undesirable material. Then foliage separated manually with hands by wearing the gloves and kept in separate plastic trays with proper labeling VN1 & VN2. Later kept them in a shade for drying. During drying of the leaf material care was taken to avoid fungal contamination. After three weeks (18 days) of shade dry, the dried leaf material grinded by Blender Machine and coarse powder obtained after grinding. The powdered leaf material (250 gm) was extracted with various solvents such as Ethanol, Chloroform, Petroleum ether and water by cold extraction method. The extract was filtered through a fresh cotton bed and finally with Whatman no. 1 filters papers.

2.3. Preliminary Phytochemical Screening:

Phytochemical screening is performed to identify phytochemicals in the Aqueous, Ethanol, Chloroform and Pet-ether extracts of plant leaves that were used in the present study, the phytochemicals were detected by color tests.

i. Test for alkaloids:

Of each extract 2ml was acidified with a few drops of dilute hydrochloric acid. Then 1ml of Dragendorff's reagent was added. The appearance of orange to red precipitate indicates the presence of alkaloids.

ii. Test for tannins:

To 2ml of each extract a few drops of 10% lead acetate were added. The appearance of white precipitate indicates the presence of tannins.

iii. Test for saponins:

To 1ml of extract taken in a measuring jar,9ml of distilled water was added and shaken vigorously for 15seconds and extract were allowed to stand for 10min.Formation of stable foam(1cm) indicates the presence of saponins.

iv. Test for steroids:

Chloroform 10ml was added to 2ml of all three plant extracts. To these extracts 1ml of acetic anhyride was added and then 2ml of concentrated sulphuric acid was added along the sides of the test tube. Colour formation at the junction is noted. The appearance of blue-green colour indicates the presence of steroids.

v. Test for Triterpenoids:

The test for Triterpenoids is same as that for steroids the appearance of red, pink colour or violet colour at the junction indicates the presence of Triterpenoids.

vi. Test for glycosides:

To 1ml of each extract a few drops of glacial acetic acid and ferric chloride and 3-4 drops of concentration sulphuric acid were added. The appearance of blue-green colour indicates the presence of glycosides.

Vii. Test for flavonoids:

4ml of extract solution was treated with 1.5ml of methanol solution. The solution was warmed and metal magnesium was added to this solution 5-6 drops of Con. HCl acid were added and color was observed for flavonoids and orange color for flavones.

Viii . Test for reducing sugars:

To 0.5ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution was added to the test tube hot and observed for brick red precipitate.

ix. Test for Resins:

10 mL of distilled water was added to extract, to which a few drops of 4% HCl were added. Appearance of turbidity in solution indicates presence of resins.

x. Test for Phenolic compounds:

Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

The phytochemical screening was performed in *Vitex negundo* (VN1) and intraspecific variant (VN2) leaf extracts of polar and non-polar solvents and the results are tabulated in Table-1 & 2 respectively.

Table -1. Showing phytochemical screening in leaf extracts of VN1

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

Table -2. Showing phytochemical screening in leaf extracts of VN2

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

2.4. Antimicrobial Activity:

Disc preparation:

The 6mm (diameter) discs were prepared from what man No.1 filter paper and the discs were sterilized by autoclave at 121OC. After sterilization the moistened discs were kept in a hot air oven at 50OC. Then the disc was impregnated with suitable concentration of extracts from stock of 50mg/ml. Four different micro-organisms: *E. coli* (MTCC: 41), *P. aeruginosa* (MTCC: 424), *B .cereus (*MT CC: 4 30 and *B. subtilis* (MTCC: 441). The solvent without extracts served as negative control. Standard antibiotic streptomycin (10 µg), Ampicillin (10 µg) were employed as positive control.

Disk-Diffusion Method:

Antimicrobial activity was carried out by disk-diffusion method using nutrient Agar medium. 100 micro liters of suspension containing 108 colony forming units mL-1 of bacteria spread over the nutrient agar medium plates by using separate sterile cotton buds. After the microbial lawn preparation extracts of plant discs (aqueous, ethanol and acetone extract) were firmly pressed onto the agar surface of each seeded plate. Petri dishes were incubated at 37^{o}C for 24 h and the average diameter of the inhibition zone surrounding the wells was determined visually.

Antimicrobial activities of the extracts were expressed by – (no zone of inhibition), + zone of

inhibition=8mm in diameter (low zone of inhibition) and ++ zone of inhibition>8mm in diameter (moderate zone of inhibition), +++ zone of inhibition ≥12mm in diameter (high zone of inhibition). All the tests were performed in duplicate and repeated for confirmation of result.

Antimicrobial activity is performed in the leaf extracts of *Vitex negundo* (VN1) and its variant (VN2) against four species of bacteria *viz. Escherichia coli* (MTCC:41), *Pseudomonas aeruginosa* (MTCC:424), *Bacillus cereus (*MTCC: 4 30), *Bacillus subtilis* (MTCC:441).

The results of zone of inhibition (in mm) per 100mg/ml and anti-microbial activity in various polar and non-polar solvents is explained in Table- 3 & 4 respectively. Similarly, zone of inhibition and anti-microbial activity of variant (VN2) are shown in Table-3 & 4. Photos are exhibited in plate No. 1 & 2.

Table-3. Showing zone of inhibition (in mm) of various leaf extracts of plant VN1

Micro organism	**Aqueous (100mg/ml)**	**Ethanol (100mg/ml)**	**Pet-ether (100mg/ml)**	**Chloroform (100mg/ml)**
E.coli (MTCC:41)	**6.3mm**	**16.6mm**	**9.4mm**	**10.6mm**
P. aeruginosa(MTCC:424)	**6.6mm**	**11.6mm**	**10.3mm**	**9.8mm**
*B. cereus (*MTCC: 4 30)	**8.2mm**	**7.8mm**	**16.2mm**	**12.4mm**
B .subtilis(MTCC:441)	**11.4mm**	**41.6mm**	**7.9mm**	**8.8mm**

Table-4. Showing Anti-microbial activity of various leaf extracts of plant VN1

Micro organism	Aqueous	Ethanol	Pet-ether	Chloroform
E.coli (MTCC:41)	**+**	**+++**	**++**	**++**
P.aeruginosa(MTCC:424)	**+**	**++**	**++**	**++**
*B.cereus (*MTCC: 4 30)	**++**	**+**	**+++**	**+++**
B.subtilis(MTCC:441)	**++**	**+++**	**+**	**++**

Antimicrobial activities of the extracts were expressed by – (no zone of inhibition), + zone of inhibition=8mm in diameter (low zone of inhibition) and ++ zone of inhibition>8mm in diameter (moderate zone of inhibition), +++ zone of inhibition ≥12mm in diameter (high zone of inhibition).

Plate-1. Showing zone of inhibition of leaf extract of plant VN 1 in various solvents

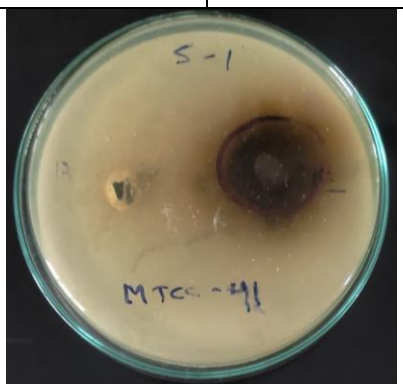
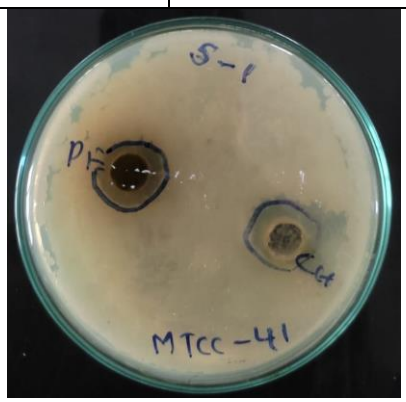
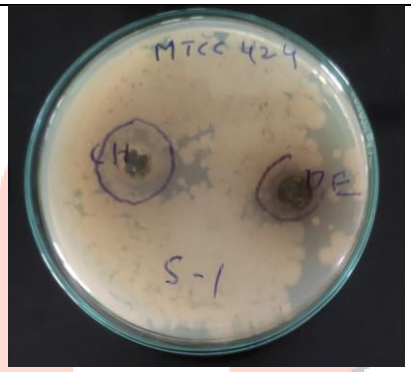
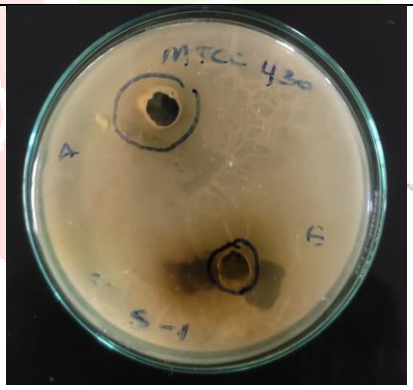
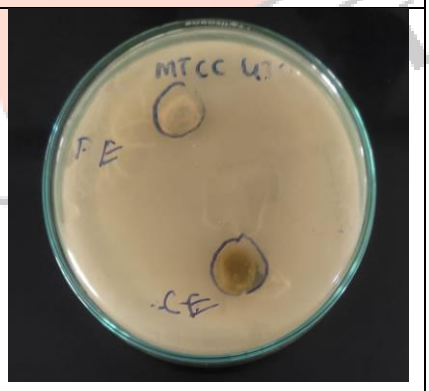
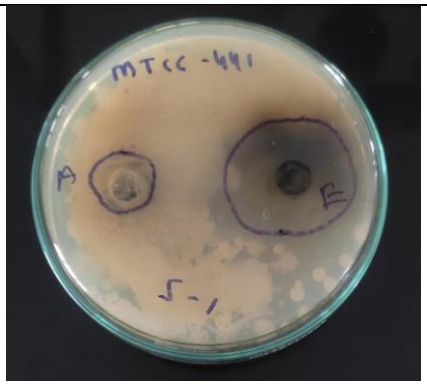
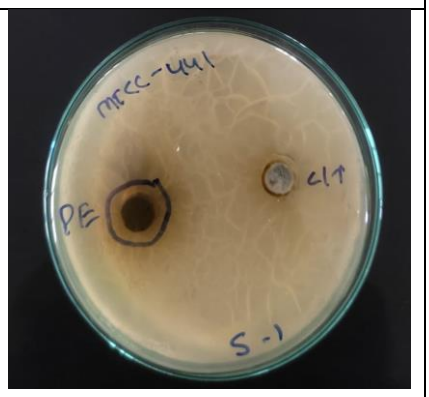
Micro organism	Aqueous	Ethanol	Pet-ether	Chloroform
E.coli **(MTCC:41)**				
P.aeruginosa**(MTCC:424)**				
B.cereus (**MTCC: 4 30)**				
B.subtilis**(MTCC:441)**				

Table-5. Showing zone of inhibition (in mm) of various leaf extracts of plant VN2

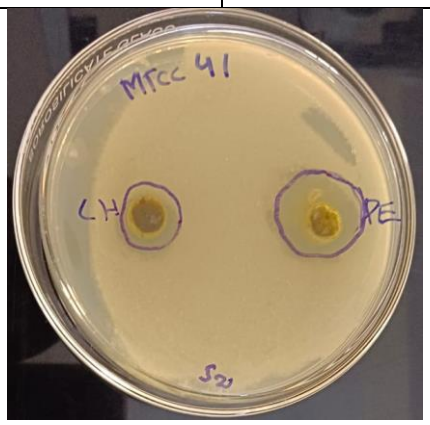
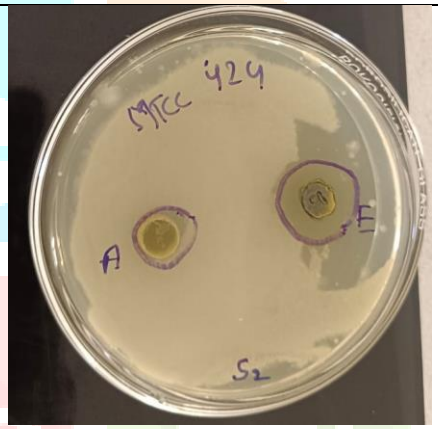
Micro organism	**Aqueous (100mg/ml)**	**Ethanol (100mg/ml)**	**Pet-ether (100mg/ml)**	**Chloroform (100mg/ml)**
E.coli (MTCC:41)	**9.4mm**	**20.6mm**	**10.9mm**	**23.2mm**
P.aeruginosa(MTCC:424)	**10.4mm**	**27.4mm**	**11.7mm**	**24.3mm**
*B.cereus (*MTCC: 4 30)	**11.6mm**	**19.8mm**	**10.9mm**	**17.6mm**
B.subtilis(MTCC:441)	**9.4mm**	**-**	**11.3mm**	**20.4mm**

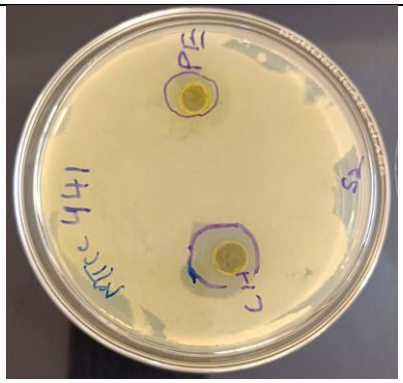
Table-6. Showing Anti-microbial activity of various leaf extracts of plant VN2

Micro organism	Aqueous	Ethanol	Pet-ether	Chloroform
E.coli (MTCC:41)	**++**	**+++**	**++**	**+++**
P.aeruginosa(MTCC:424)	**++**	**+++**	**++**	**+++**
*B.cereus (*MTCC: 4 30)	**++**	**+++**	**++**	**+++**
B.subtilis(MTCC:441)	**+**	**-**	**++**	**+++**

Antimicrobial activities of the extracts were expressed by – (no zone of inhibition), + zone of inhibition=8mm in diameter (low zone of inhibition) and ++ zone of inhibition>8mm in diameter (moderate zone of inhibition), +++ zone of inhibition ≥12mm in diameter (high zone of inhibition).

Plate-2. Showing zone of inhibition of leaf extract of VN2 in various solvents

Micro organism	Aqueous	Ethanol	Chloroform	Pet-ether
E.coli **(MTCC:41)**				
P. aeruginosa**(MTCC:424)**				
B. cereus (**MTCC: 4 30)**				

***B .subtilis*(MTCC:441)**		

2.5. Molecular Docking Studies:

Virtual ligand screening is an insilico method used to dock small molecules (ligand) tomacromolecule (protein) to discover potent compounds that have the necessary biological effect [10, 16]. The molecular docking study of the selected volatiles from *Vitex negundo* var. *negundo* (VN1) and its intraspecific variant "Kali Nirgundi" (VN2) was carried out on the three-dimensional (3D) structures of the DNA B, regulatory replication protein was obtained from the RCSB Protein Data Bank with PDB ID: 1B79[17]. The 3D structures of the selected proteins converted into PDB formats by deleting the water molecules, HETATOMS, and adding polar hydrogens using Biovia Discovery Studio-2021 Client. The compounds from the essential oils for docking studies were selected based on their higher percentage contents and their concerned structures were obtained from the PUBCHEM database (https://pubchem.ncbi.nlm.nih.gov/, accessed on 12 August 2022) in the SDF (structure datafile) format. The selected compounds were 6'-p-hydroxybenzoyl mussaenosidic acid(Pub chem ID: 23955877), 2'-p-hydroxybenzoyl mussaenosidic acid (Pub chem ID: 73298898), 5,3',5'-Trihydroxy-6,7,4'-trimethoxyflavone(Pub chem ID: 21581557), 5,3'-dihydroxy-7,8,4'-Trimethoxyflavanone (Pubchem ID: 13871369), 4-terpineol(Pubchem ID: 11230) , Beta-Sitosterol (Pub chem. ID: 481107734), Casticin (tetramethoxyflavone) (Pub chem. ID: 5315263), protocatechuic acid (Pub chem. ID : 72), Oleanic acid (Pub chem. ID: 10494), and vitamin-C (Pub chem ID:785).The drug discovery research involves virtual screening (VS) as an essential method for evaluating large compound libraries to discover new drugs. The ligand-target approach has become incredibly popular, where the use of the number of latest techniques and software is increasing. [12] The procedure of VS includes the protein preparation, the database preparation of ligands, and the docking. [13, 9] Protein surface atoms and site points are also calculated internally in the docking software. Computational methods predict the best ligand hits that 'dock' ligand library into target protein and 'score' their possible complementarities to binding sites of the target protein.[18]

Structures of the ligands in their SDF format were then imported into PyRx Software using an open babel tool embedded in PyRx software. Energy minimization (optimization) was performed by adding charges and optimizing the universal force field. Further, the ligands were converted into AutoDock Ligand format (PDBQT). To find out the binding affinity and to know the various ligand–receptor interactions responsible for the antioxidant and phytotoxic activity, the molecular docking of the selected major constituents was performed using PyRx with the VinaWizard tool. The protein and multiple ligands to be docked were selected in the PyRx software using the Vina Wizard Control. The "Run Vina'' control was selected to initiate the docking process. The results were observed by selecting the "Analyze Vina'' tool and exported asCSV files.[10] Biovia Discovery Studio-2021 Client was used for the visualization of 2Dand 3D interactions of docking poses.

2.6. In Silico ADMET Study

The structures of the selected compounds from the essential oils were drawn using ChemDraw Ultra 8.0 for the pharmacokinetics (absorption, distribution, metabolism, and excretion (ADME)) studies. The legends were converted into the SMILES format and then the drug-like and pharmacokinetic properties of the selected compounds were predicted using ADME tool by a Swiss ADME online server (http://www.swissadme.ch/,accessed on 12 August 2022), as per the developed protocol.[6] It calculates the prediction based on different parameters such as organ toxicity (hepatotoxicity), oral toxicity, and toxicological endpoints (cytotoxicity, mutagenicity, carcinotoxicity,and immunotoxicity).

2.7. Results and Discussions

The preliminary phytochemical analysis in VN1 & VN2 showed the minor variation in the presence of alkaloids, tannins, saponins, steroids, triterpenoids, glycosides, flavonoids, reducing sugars, phenols and absence of resins in the extracts of aqueous, ethanol, pet-ether and chloroform results shown in the Table 1. In VN 1 tannins shown absent in the chloroform extract, steroids, triterpenoids shown absent in the pet-ether, glycosides and flavonoids shown absent in the extracts of aqueous and chloroform. In VN2 saponins shown absent in the pet ether, steroids shown absent in the chloroform, triterpenoids shown absent in the aqueous extracts results shown in the table 2. Mainly the glycosides and flavonoids compounds shown presence in all the extracts in VN2.

The antibacterial activity was performed in the leaf extracts of VN1 & VN2 against four species of bacteria (*E. coli, B. cereus, B. subtilis, P. aeruginosa*) results shown in the Tables 3 &4 (VN1) 5 & 6 (VN2) and Figures 1 (VN1) & 2 (VN2). The antimicrobial activities were expressed in all extracts against all isolates in leaf extracts of VN1 & VN2 except bacteria *B. subtilis* not shown the inhibitory effect in the ethanol extract in VN2. The high zone of inhibition shown in the leaf extracts of VN 2 in all extracts in all isolates.

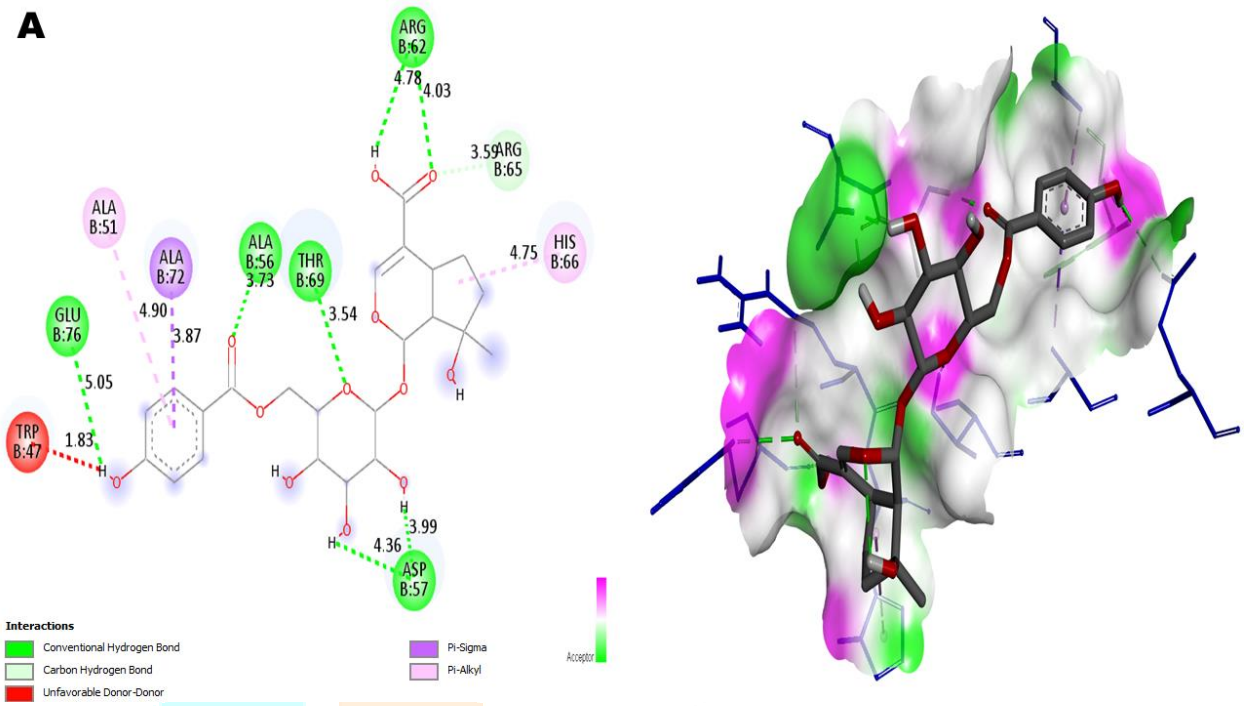
2.8. Chemical Composition

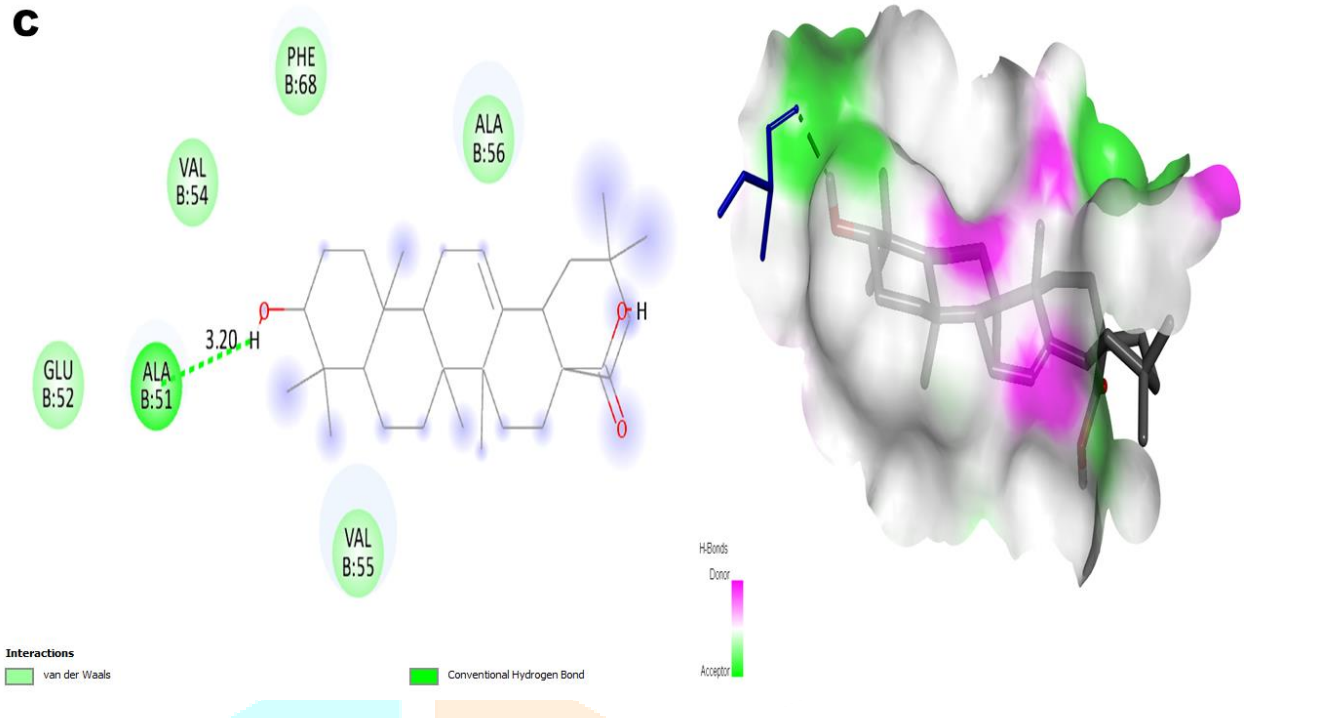
Typical form of the *Vitex negundo* (VN), was mainly dominated by monoterpene hydrocarbons (29.4%), followed by oxygenated sesquiterpenes (24.8%) and oxygenated monoterpenoids (11.3%). Previous researchers have also studied the EOs of *V. negundo* under investigation here in. For instance, the major compounds detected in the *V. negundo*, sabinene (19.4%),viridiflorol (17.8%) and _-caryophyllene (7.5%), were also found to be present in the hydro distilled *V. negundo* leaves EO in variable amounts [11 - 13]. 5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate (5.2%), another majorcompound detected in VNO, was also found to be present in leaves essential oil of *V. negundo* in notable amounts.[8] The chemical composition of EO of Vitex negundo extracted during the spring season from the same location (Pantnagar) revealed the presence of over33 compounds, in which the major compounds detected were viridiflorol (23.8%), sabinene(11.2%), unidentified diterpene M+ = 272 (11.0%), and caryophyllene (6.7%) [12].Both qualitative and quantitative variations in essential oils of *V. negundo* from differentgeographic regions might be due to the different geographical and climatic conditions.

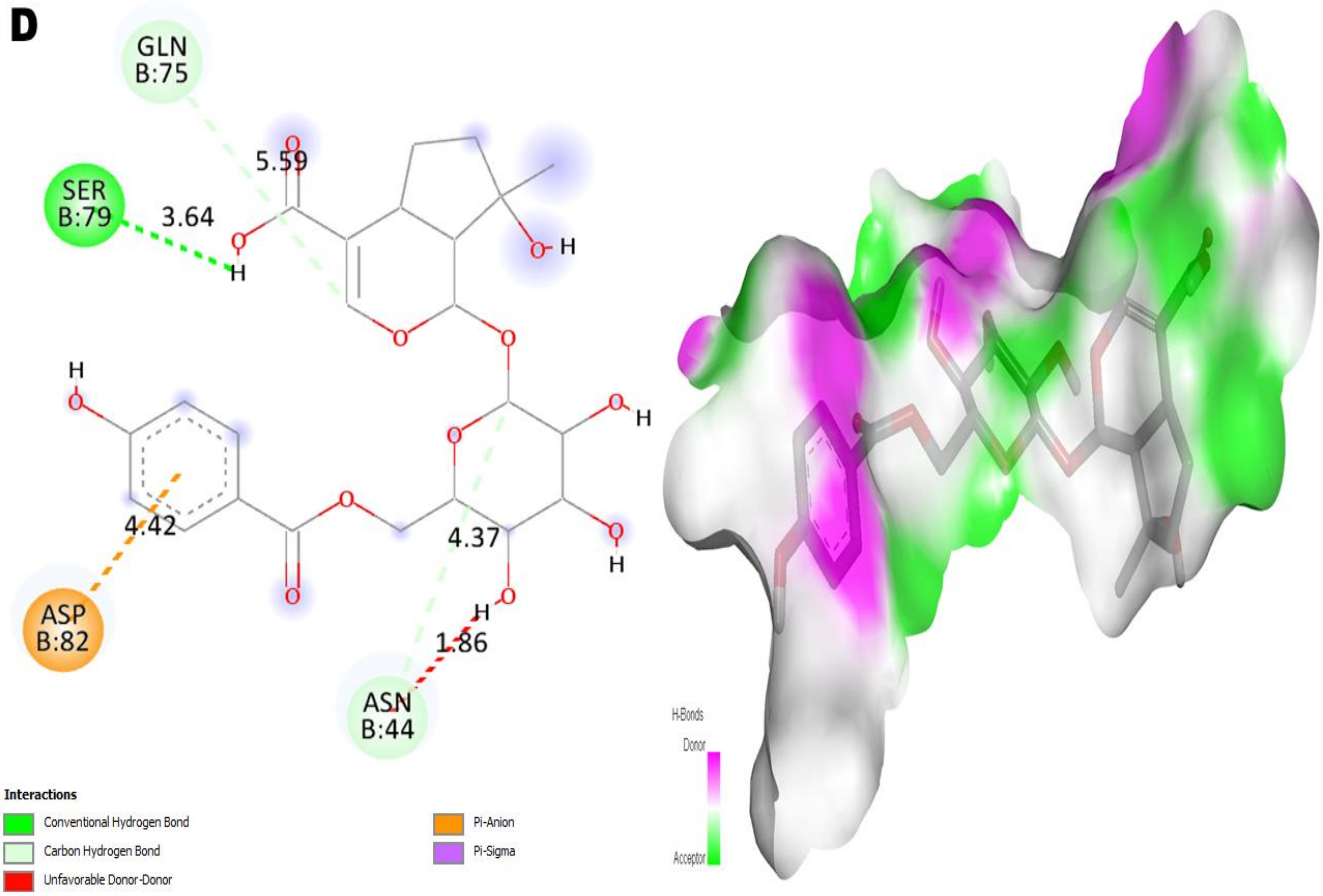
The binding energy for 6'-p-hydroxybenzoyl mussaenosidic acid complexed with 1B79 was −6.6 kcal/mol, which is very close to that of Beta - sisterol (−6.1 kcal/mol). Onthe other hand, binding energy of ascorbic acid a known antioxidant complexed with 1B79 came out to be −6.7kcal/mol, which was higher than most of the compounds. The binding energy for various Flavones ranges between -5.8 to -5.5 kcal/mol, and whereas for Oleanicacid (-6.2kcal/mol) 4-terpineoland protocatechuic acid is least (-4.6kcal/mol) respectively.

The best docked pose of 6'-p-hydroxybenzoyl mussaenosidic acid exhibited 2 pi-alkyl interaction, 1 pi-sigma interaction, and other Van der Waal interactions with 1B79 containing amino acid residues such as B: ALA56, B: ASP57, B:THR 69, B: GLU76, as represented in Figure 1A. Similarly, the best docked pose of Oleanic acid exhibited 1 hydrogen bond with B:ALA51 and vitamin C 1 pi – Anion and other Van der Waal interactions involving B:SER79, B:ASN44, B:GLN75, B:SER79, B:ASN44, B:GLN75, B:ASP82, B:ASP82 amino acids.

The lower values of binding free energy demonstrate more significant interaction between the receptor and the ligand. The ADMET analysis revealed the safety of compounds and also the possibility of designing further with enhanced activity.


A
ARG B:62
4.78
4.03
3.59
ARG B:65
ALA B:51
ALA B:72
ALA B:56
3.73
THR B:69
4.75
HIS B:66
GLU B:76
4.90
3.87
3.54
5.05
TRP B:47
1.83
3.99
4.36
ASP B:57
Interactions
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Unfavorable Donor-Donor
Pi-Sigma
Pi-Alkyl
Acceptor





Docked conformations of molecules in the binding cavity of DNA B Helicase (PDB: 1B79) with least binding energies.

Conclusion

The present studies in *Vitex negundo* var. *negundo* (VN1) and in its intra specific variant "Kali Nirgundi" (VN2) reveals the presence of high amount of glycosides and flavonoids in the intra specific variant (VN2). This may be the reason the leaf material of intraspecific variant Kali Nirgundi widely used and working effectively for anti-inflammatory, anti-arthritis and antibacterial in the traditional and local systems of medicine. The molecular docking study suggested that the compounds (6'-p-hydroxybenzoyl mussaenosidic, Oleanic acid) can be good antimicrobial agents by the analysis of ligand interaction with the proteins.Overall, this study unveiled some interesting biological activities, which justifies the use of the plant species in traditional medicine.

Acknowledgements:

The authors are thankful to the Principal, University College of Science, Saifabad, Osmania University, Hyderabad for the facilities and support.

References

1. POWO. "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 05 January 2024."

2. Sam, M and Monika, G: Interaction networks of Escherichia coli replication proteins under diferent bacterial growth conditions. Joanna Morcinek-Orłowska, BeataWalter, Raphaël Forquet, DominikCysewski, MaximeCarlier, Michał Mozolewski 2023; 10:788. | https://doi.org/10.1038/s41597-023-02710-1.

3. Issa, M, Chandel, S, Singh, HP, Batish, DR, Kohli, RK, Yadav, SS and Kumari, A. Appraisal of phytotoxic, cytotoxic and genotoxic potential of essential oil of a medicinal plant *Vitex negundo*. Ind. Crops Prod 2020; 145: 112083.

4. Katayama, T: Initiation of DNA Replication at the Chromosomal Origin of *E. coli, ori* C. Adv. Exp. Med. Biol., 2017; 1042, 79–98.

5. Raju, S, Dharani, J, and Ravi, S. Essential oil composition of *Vitex negundo*, acetylcholine esterase inhibition activity and molecular docking studies against bacterial proteins. J. Pharm. Sci. Res., 2017; 9, 1677–1681.

6. Daina, A, Michielin, O and Zoete, V. Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 2017; 7: 42717.

7. Jameson, HK and Wilkinson, JA: Control of Initiation of DNA Replication in *Bacillus subtilis* and *Escherichia coli*. Genes 2017; 8: 22. http://doi.org/10.3390/genes8010022

8. Padalia, RC, Verma, RS, Chauhan, A, Chanotiya, CS and Thul, S. Phytochemical diversity in essential oil of Vitex negundo L.populations from India. Rec. Nat. Prod., 2016; 10, 452–464.

9. Huang Y, Zhao J, Song Q et al: Virtual screening and experimental validation of novel histone deacetylase inhibitors. BMC Pharmacol Toxicol. 2016. https://doi.org/10.1186/s40360-016-0075-8

10. Dallakyan, S and Olson, AJ: Small-molecule library screening by docking with PyRx. In Chemical Biology; Humana Press 2015; 243–250.

11. Prashith Kekuda, TR, Vivek, MN, Yashoda, K, Manasa M, and Raghavendra, HL. Comparative Study on Antimicrobial Activity of *Vitex negundo* var. *negundo* and *Vitex negundo* var. *purpurascens*. Sci. Technol. Arts Res. J. 2014, 3(1): 126-131.

12. Lavecchia A and Giovanni C: Virtual screening strategies in drug discovery: a critical review. Curr Med Chem., .2013. https://doi.org/10. 2174/09298673113209990001

13. Lionta E, Spyrou G, Vassilatis D and Cournia Z: Structure-based virtual screening for drug discovery: principles, applications and recent advances. Curr Top Med Chem., 2014. https://doi.org/10.2174/1568026614666140929124445

14. Huang, HC, Chang, TY, Chang, LZ, Wang, HF, Yih, KH, Hsieh, WY and Chang, TM. Inhibition of melanogenesis versus antioxidant properties of essential oil extracted from leaves of Vitex negundo Linn and chemical composition analysis by GC-MS. Molecules 2012; 17: 3902–3916.

15. Govaerts, R. World Checklist of Selected Plant Families Database in ACCESS: 1-216203. The Board of Trustees of the Royal Botanic Gardens, Kew 2003.

16. Jyothi B, Vasavi, M and Navaneetha N: An insilico study of KLK-14 protein and its inhibition with curcumin and its derivatives. Chemical Papers 2022. 76, 4955–496.https://doi.org/10.1007/s11696-022-02209-w

17. Fass D, Bogden, CE and Berger JM: Crystal structure of the N-terminal domain of the DnaB hexameric helicase. Structure 1999; 15:7(6):691-8. doi: 10.1016/s0969-2126(99)80090-2. PMID: 10404598.

18. Kitchen DB, Decornez H, Furr, JR and Bajorath, J: Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov., 2004. https://doi.org/10.1038/nrd1549

19. Sivarajan & Moldenke. 1974. *Vitex negundo* var. purpurascens Sivara. & Moldenke in Moldenke. Phytologia 1974; 28: 404.