RESEARCH ARTICLE

Optimized Leads for Antimicrobial Activity of Volatile Components from Leaves of *Vitex negundo* using Molecular Docking Studies

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ABSTRACT
The greatest challenge facing humanity and researchers is bacterial resistance. There is an urgent need to address this issue to stop additional risk from microbial diseases. Herbal medicine has been used to heal illnesses since ancient times, particularly in African nations with rich natural resources. This study focuses on finding optimized leads for the antibacterial activity of *Vitex negundo* using molecular docking techniques. *Vitex negundo* from the Lamiaceae family is abundant in tropical Eastern and Southern Africa and Asia. Different leaf powder extracts were tested against resistant microorganisms (*Acinetobacter baumannii* and Methicillin-resistant Staphylococcus aureus). The docking outcomes showed that 2 phytochemicals named beta-Eudesmol and viridiflorol out of 20 essential oil compounds exhibited good binding affinity with target molecules (3PJD and 3LBS) as compared to standard drugs. Amoxycillin, Penicillin G, and Cephalosporin were used as the standard antibacterial drugs. The antibacterial properties and in silico molecular docking study exhibited by the constituents validate the traditional application of the plant against bacterial pathogens. Moreover, the present work significantly underscores the necessity for additional investigations focused on the separation of pure chemicals and their application against resistant bacteria.

Keywords: Antimicrobial activity, Volatile components, *Vitex negundo*, Molecular docking, Bacterial resistance
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INTRODUCTION

Vitex negundo is a big, fragrant shrub with quadrangular, thickly white, tomentose branchlets also known as the Chinese chaste tree, five-leaved chaste tree, horseshoe vitex, or nisinda[1]. Folk medicine makes extensive use of it, especially in South and Southeast Asia. *Vitex negundo*, also known as nirgundi in Hindi and Sanskrit, is a deciduous shrub that has spread around the world. According to some, it started in the Philippines and India. The plant is used for a variety of purposes in India, including basketry, dyeing, fuel, food, stored-grain protection, field pesticide, growth stimulant, manure, and human, animal, and poultry medicine[2]. Every medical system, including Ayurveda, Unani, Siddha, homoeopathy, and allopathy, uses it. In India, Bangladesh, the Philippines. China, Japan and Sri Lanka, it is frequently utilised in traditional medicine[3]. In keeping with its Sanskrit meaning, which is "that which keeps the body free from all diseases," it is used to treat a wide range of conditions, including headaches, migraines, wounds, skin infections, swelling, asthma, and issues related to male and female sex and reproduction[4]. Nirgundi, also known as sindhuvara in Ayurveda, has been used as medicine for ages. It is interpreted both internally and externally in a number of ways. Certain ailments are treated with the entire plant, including the roots, fruits, seeds, leaves, and leaf oil[5-8]. But according to Ayurveda, the most crucial components are the leaves, roots, and bark. To determine this plant's antibacterial potential, molecular docking methods were used rather than bioactivity-guided fractionation. In order to investigate the antimicrobial potential of *Vitex negundu's* volatile components, the authors used AutoDock-Vina17 to perform molecular docking against targets 3PJD and 3LBS and three common drugs, namely amoxycillin, cephalosporin, and penicillin G.

MATERIAL AND METHODS

Plant Material

Leaves of *Vitex negundo* were collected from Srinagar region (Pauri Garhwal District) of Uttarkhand and were identified by Department of Botany, H.N.B.G.U Srinagar Uttarakhand. The fresh leaves were hydro distilled for 8hrs to get the yellow-colored essential oil. Dried leaves were Successive extracted for extracts in different solvent (methanol, petroleum ether, water and chloroform). The essential oil was stored in an air tight bottle for further analysis[9].

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Table 1: Essential Oil Composition of *Vitex negundo*

S.N.	Compound	Molecular Formula	Molecular Weight	RI
1.	2,6,6-Trimethylbicyclo[3,1,1]hept-2-ene	$C_{10}H_{16}$	136	1022
2.	1,8-Cineole	$C_{10}H_{18}O$	154	1031
3.	Sabinene	$C_{10}H_{16}$	136	1020
4.	Pheno, bis(1,1-dimethyethyl)	$C_{14}H_{24}O$	206	1581
5.	Veridifloro	$C_{15}H_{24}$	204	1589
6.	Pyrrolo(3,2,1-jk) carbazole	$C_{14}H_9$	191	1258
7.	Limonene	$C_{10}H_{16}$	136	1027
8.	β-Eudesmol	$C_{15}H_{26}O$	222	1650
9	α-terpineol	$C_{10}H_{18}O$	154	1187
10.	β-Mycrene	$C_{10}H_{16}$	136	988
11.	Levomenol	$C_{15}H_{26}O$	222	987
12.	Camphene	$C_{10}H_{16}$	136	954
13.	β-pinene	$C_{10}H_{16}$	136	978
14.	γ-teripnene	$C_{10}H_{16}$	136	1165
15.	β-bisabolene	$C_{15}H_{24}$	204	1506
16.	β-sesquiphellandrene	$C_{15}H_{24}$	204	1523
17.	α-terpinene	$C_{10}H_{16}$	136	1018
18.	2 β-Pinene	$C_{10}H_{16}$	136	978
19.	δ-terpineol	$C_{10}H_{18}O$	154	1191
20.	β-Salinene	$C_{15}H_{24}$	204	1485

***In-silico* Activity**

The structures of X-ray crystallography of various protein targets 3PJD and 3LBS were downloaded with RCSB-PDB (Research Collaboratory for Structural Bioinformatics-Protein Data Bank). The protein targets 3PJD and 3LBS, which have shown the best X-ray crystallographic structures at resolution 2.50Å and 2.15A respectively, were used as the receptor in the molecular docking experiments. After retrieving the structure from the RCSB-PDB, all the water molecules as well as hetro atoms were discarded and polar hydrogen atoms were added to the protein by using Biovia discovery studio visualizer (BDSV). The various active sites were determined using the site sphere method, and energy minimization was done using the MMFF94 force field. After that, the protein targets were ready for docking. The PROCHECK SAVESv6.0 server was widely used to check the integrity of generated 3D crystallographic and NMR structure model of various proteins 3PJD and 3LBS by uploading the (.pdb) file of the prepared proteins. The 3D structure of *Vitex negundu* phytochemicals were downloaded from PubChem and ChemDraw-3D in .sdf and .mol2 format which was further converted into .pdb format by using software Open Bable GUI23. The ligands were then activated for docking by energy minimization through MMFF94s force field. Molecular docking is considered as the most admired virtual screening techniques, particularly when the target protein's 3D structure is available as it identifies the protein-ligand complex structure and the binding affinity between them, which is crucial knowledge for lead optimization. Once the protein targets and ligands were prepared, molecular docking was done with the aid of software autodock-vina embedded in PyRx24. After completion of docking, the best optimal conformations were further analysed for binding energy (Kcal/mol) with the help of docking score[10-13].

Table 2: Antioxidant activity of different extracts of *Vitex negundo*

Extracts and Standard	DPPH free radical scavenging activity (IC50)
PEE	0.358±0.003
CE	0.494±0.045
ME	0.663±0.006
AQE	1.711±0.331
Standard (Ascorbic acid)	0.02±0.034

Table 3: Antimicrobial Activity of Solvent Extracts of Leaves of *Vitex negundo*

Bacterial Pathogen	Diameter of zone of inhibition (mm)			
	ME (100 µg/ml)	AQE (100 µg/ml)	CE (100 µg/ml)	PE (100 µg/ml)
B. subtilis (MTCC 441)	-	-	-	-
S. aureus (MTCC 441)	15	7	11	8
Pseudomonas aeriginosa (MTCC 441)	17	13	12	8
Proteus vulgaris (MTCC 441)	-	-	-	-
E. coli (MTCC 441)	14	15	18	11
Klebsiella pneumnia (MTCC 441)	11	10	6	6

Determination of Antioxidant Activity

The following *in vitro* techniques were used to assess each test sample's antioxidant activity[14-20]:

Reducing Power Method

Gallic acid was utilised as a standard reference to determine the extract's reducing power using the Oyaizu method. Three duplicates of the test were run. Alkaline dimethyl sulfoxide technique for scavenging superoxide radicals. Gallic acid served as a standard reference while the superoxide scavenging activity was measured. The inhibitory concentration 50% (IC50) value was determined after the test was run in triplicate.

Scavenging Activity

$$Scavenging\ activity\ (\%) = \frac{(Test\ absorbance\ -\ Control\ absorbance)}{Test\ absorbance} \times 100$$

The IC50 is a measure of antioxidant activity. The concentration of a medication in µg/ml that inhibits 50% of free radicals is known as the IC50 value.

Antimicrobial Activity

The Disc Diffusion Method was utilised to ascertain the antibacterial activity. The antibacterial activity of the herbal concoction was assessed using the impregnated filter paper discs. To create a grass culture for antibacterial purposes, 0.1 ml of a bacterial solution containing 10 5CFU ml-1 was swabbed onto a Nutrient Agar plate. The corresponding solvents were used to prepare the petroleum ether, chloroform, methanol, and aqueous extracts.

Table 4: Lipinski's properties of the essential oil composition of *Vitex negundo*

Compound name	Molecular weight (< 500kD)	Log P (< 5)	H-bond donor (< 5)	H-bond acceptor (< 10)
Pyrrolo[3,2,1-jk] carbazole	305.4 g/mol	2.3	0	2
Delta-Terpineol	154.25 g/mol	1.9	1	1
beta-Pinene	136.23 g/mol	3.1	0	0
alpha-Terpinene	136.23 g/mol	2.8	0	0
Beta-Sesquiphellandrene	204.35 g/mol	5.4	0	0
Beta-Bisabolene	204.35 g/mol	5.2	0	0
gamma-Terpinene	136.23 g/mol	2.8	0	0
Camphene	136.23 g/mol	3.3	0	0
levomenol	222.37 g/mol	3.8	1	1
beta-Myrcene	136.23 g/mol	4.3	0	0
Alpha-Terpineol	154.25 g/mol	1.8	1	1
beta-Eudesmol	222.37 g/mol	3.7	1	1
Limonene	136.23 g/mol	3.4	0	0
Viridiflorol	222.37 g/mol	3.7	1	1
SABINENE	136.23 g/mol	3.1	0	0
1,8-Cineole	154.25 g/mol	2.5	0	1
Phenol, 2,4-bis-(1,1-dimethylethyl), TMS	278.5 g/mol	Not given	0	1
beta-Selinene	204.35 g/mol	5.4	0	0
2,6,6-trimethylbicyclo [3.1.1] hept-2-ene	136.23 g/mol	2.8	0	0
2 beta-pinene	136.23 g/mol	3.1	0	0

After being individually impregnated with varying quantities of extract, the filter paper discs (6 mm in diameter) were put on the agar plates that had already been infected with the test microorganisms. As a negative control, discs were soaked in different organic solvents, dried, and then put on lawns.

The zone of growth inhibition was measured in millimetres during a 24-hour incubation period at 37°C. The various extracts' percentage inhibitory concentrations were determined and contrasted with the conventional antibiotics.

RESULTS AND DISCUSSION

GC-MS of Essential Oil

The GC-MS analysis of yellow colored leaf oil of *Vitex negundo* resulted in the identification of large numbers of sesquiterpenes, aliphatic components, monoterpenes and diterpenes. Twenty components were identified, both the major as well as minor constituents were identified by their retention time. The main compounds having the highest peak were cinole, mycerene, sabenine. Salinene emerged as the minor component of the *Vitex nigundo* essential oil under tropical conditions. Results are shown in Figure 1 and Table 1.

Anti-oxidant Activity

The results of the antioxidant activity suggest that, polar extracts of *Vitex negundo* had significant antioxidant potential in comparison to non-polar extracts. The result of antioxidant activity follows the order as

Methanolic extract>chloroformic extract> petroleum ether extract> water (aqueous) extract[16]. The results are shown in Table 2.

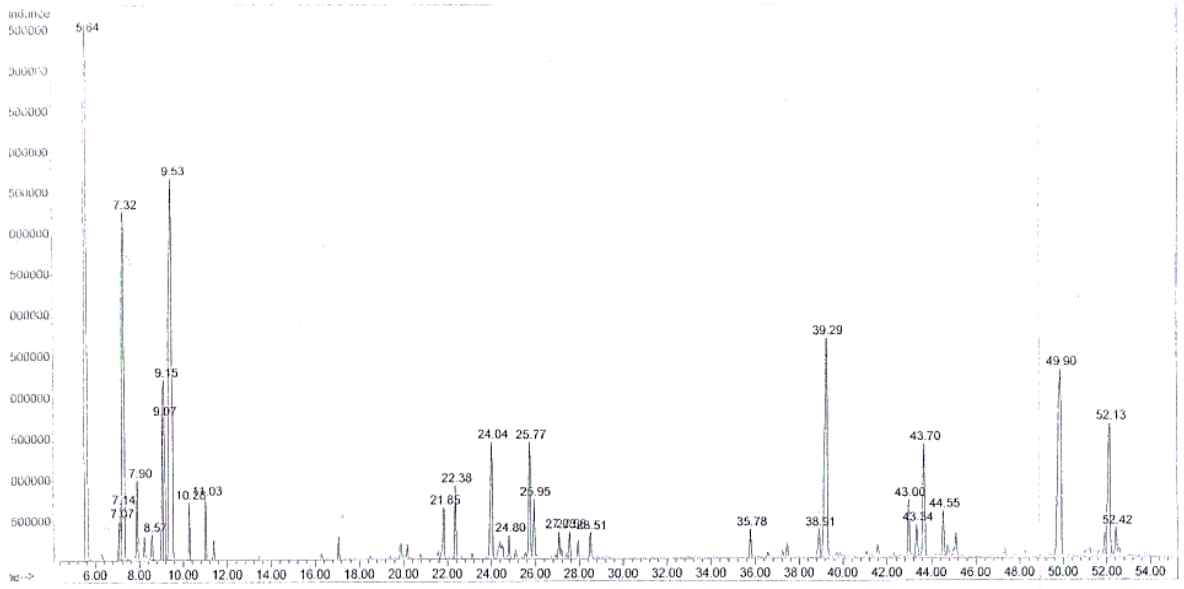


Figure 1: GC-MS Spectra of composition of Essential oil

Table 5: The binding energy of 20 essential oil compounds from leaves of *Vitex negundo* against therapeutic targets for antimicrobial activity

S. No.	PHYTO-CHEMICALS AND CIDs	SMILES	Binding energy of 3PJD (Kcal/mol)	Binding energy of 3LBS (Kcal/mol)
1.	Pyrrolo[3,2,1-jk] carbazole CID: 12545891	C1=CC=C2C(=C1) C3=CC=CC4=C3N2C=C4	-8.5	-7.1
2.	Delta-Terpineol CID :81722	CC(C)(C1CCC(=C)CC1)O	-6.6	-6.6
3.	beta-Pinene CID:14896	CC1(C2CCC(=C)C1C2)C	-6.4	-6.4
4.	alpha-Terpinene CID:7462	CC1=CC=C(CC1) C(C)C	-6.6	-6.7
5.	Beta-Sesquiphellandrene CID:12315492	C[C@@H] (CCC=C(C)C)[C@H]1CCC(=C)C=C1	-7.1	-8.3
6.	Beta-Bisabolene CID:10104370	CC1=CC[C@H](CC1)C(=C)CCC=C(C)C	-7.4	-8
7.	gamma-Terpinene CID: 7461	CC1=CCC(=CC1)C(C)C	-6.7	-6.8
8.	Camphene CID: 6616	CC1(C2CCC(C2)C1=C)C	-6.5	-6.1
9.	levomenol CID:442343	CC1=CC[C@H](CC1)[C@](C)(CCC=C(C) C)O	-7.4	-7.3
10.	beta-Myrcene CID:31253	CC(=CCCC(=C)C=C)C	-5.3	-5.6
11.	Alpha-Terpineol CID:17100	CC1=CCC(CC1)C(C)(C)O	-6.8	-6.9
12.	beta-Eudesmol CID:91457	C[C@]12CCCC(=C)[C@@H]1C[C@@H](CC2)C(C)(C)O	-8.1	-8.7
13.	limonene CID:22311	CC1=CCC(CC1)C(=C)C	-6.6	-6.5
14.	Viridiflorol CID:11996452	C[C@@H]1CC[C@H]2[C@@H]1[C@H]3 [C@H](C3(C)C)CC[C@]2(C)O	-8.1	-8.4
15.	Sabinene CID:18818	CC(C)C12CCC(=C)C1C2	-6.3	-6.6
16.	1,8-Cineole CID:2758	CC1(C2CCC(O1)(CC2)C)C	-6.1	-7.2
17.	Phenol, 2,4-bis-(1,1-dimethylethyl), TMS CID:528937	CC(C)(C)C1=CC(=CC(=C1)O[Si](C)(C)C) C(C)(C)C	-7.1	-6.2
18.	beta-Selinene CID:442393	CC(=C)[C@@H]1CC[C@]2(CCCC(=C)[C @@H]2C1)C	-6.4	-6.1
19.	2,6,6-trimethylbicyclo [3.1.1] hept-2-ene CID:72201054	CC1=CCC2CC1C2(C)C.CC1=CCC2CC1C 2(C)C	-5.1	-6.1
20.	2-beta-Pinene CID:14896	CC1(C2CCC(=C)C1C2)C	-6.4	-6.4
21.	Standard drug 1(Amoxycillin) CID:33613	CC1(C(N2C(S1)C(C2=O)NC(=O)C(C3=C C=C(C=C3)O)N)C(=O)O)C	-9	-8.3
22.	Standard drug 2 (cephalosporin) CID: 25058126	C=C1CSC(N=C1C(=O)O)C(C(=O)O)NC(= O)CCCCC(C(=O)O)N	-8.1	-7.3
23.	Standard drug 2 (penicillin G) CID: 5904	CC1(C(N2C(S1)C(C2=O)NC(=O)CC3=CC =CC=C3)C(=O)O)C	-9.1	-8.1

Antimicrobial Activity

The antimicrobial activities of polar and non-polar solvent extracts of the leaves of *Vitex nigundo*were determined against *E. coli*, *B. subtilis* and *S. aureus* via well diffusion method. The significant highest zone of inhibition was recorded of polar extracts against all the bacterial strains studied in comparison to non-polar extracts. The results of antimicrobial activity follow the order as- Methanolic extract>chloroformic extract>> petroleum ether extract>water (aqueous) extract[16]. The results are shown in Table 3 and Figure 2.

Molecular Docking

Additionally, 20 bioactive substances from *Vitex nigundo* that were discovered by GC-MS analysis were chosen and

put through molecular docking with Enoyl-[acyl-carrier-protein] reductase [NADH] FabI (Escherichia coli K-12)(3PJD) and Maltose/maltodextrin-binding periplasmic protein (Escherichia coli K-12)(3LBS).

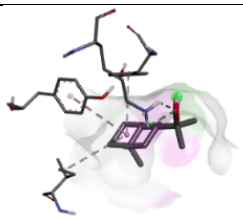
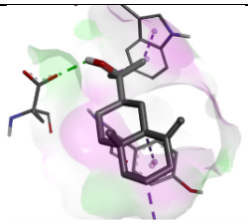
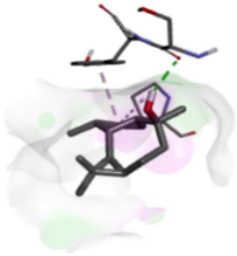
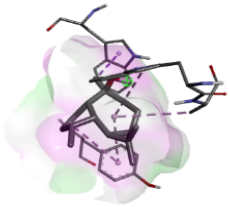
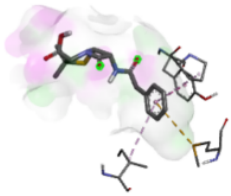
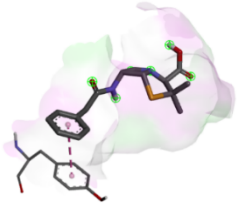
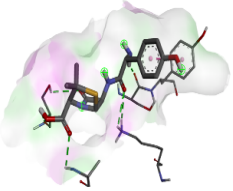
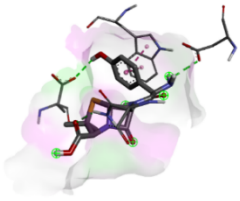
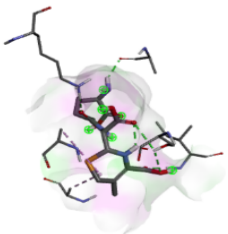
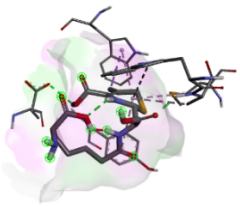
As standard controls, amoxycillin, penicillin G, and cephalosporin (an antibiotic) were employed. Initially, the PubChem database was used to obtain the three-dimensional structures of bioactive chemicals. We choose these compounds based on Lipinski's rule of five, which incorporates molecular weight, log P, the number of hydrogen bond donors, and the number of hydrogen bond acceptors. (Table 4).

Molecular docking generates several potentials adduct structures which are ranked and categorized by using the scoring function in the software programme. The docking outcomes showed that 2 phytochemicals named **beta-Eudesmol and viridiflorol** out of 20 essential oil compounds exhibited good binding affinity with target molecules (3PJD and 3LBS) as compared to standard drug i.e. Amoxycillin, Penicillin G and Cephalosporin. The binding affinity of various active phytochemicals along with reference drugs has been given in Table 5.

Molecular docking is the most effective approach used to assess the correct binding position along with the binding affinity between the drug and receptor. Also the physicochemical interactions among ligand and receptor are deduced from the 3D structures. So, in present study docking interaction has been performed using Biovia Discovery Studio (BDS).

The flexible docking studies of all the isolated compounds were done using Auto-dock Vina version 1.1.2 embedded in PyRx version 0.8. The macromolecules 3PJD and 3LBS showed the favorable interaction with 2 **(beta-Eudesmol and viridiflorol)** out of 20 compounds and best 3D interaction poses as compared to standard drug (Cephalosporin) as shown in Table 6.

Table 6: The 3D interactions of 3PJD and 3LBS proteins with different ligands

S.No.	Components	3PJD	Bonds	3LBS	Bonds
1.	beta-Eudesmol CID: 91457		CONVENTIONAL HYDROGEN BONDS: LYS A:163 Pi-Alkyl bond:TYR A:146 Alkyl bond:TYR A:156,ILE A:200		CONVENTIONAL HYDROGEN BOND: GLU A:153,0Pi-Alkyl bond:TYR A:155 Pi-sigma: TRP A :230, TYR A:155, TRP A:340
2.	Viridiflorol CID:11996452		CONVENTIONAL HYDROGEN BONDS: SER A :145 Pi-Alkyl bond:TYR A:146 Alkyl bond: PRO A :191		Pi-Alkyl bond:TRP A:62, ALA A:63, Alkyl bond:TYR A:155 Pi-Sigma:TRP A:340
3.	Standard drug 1 (penicillin G) CID: 5904		Pi-PI STACKED bond:TYR A:146 Alkyl bond:ILE A:200,PRO A:191		Pi-Pi stacked: TYR A :210
4.	Standard drug 2 (Amoxycillin) CID:33613		CONVENTIONAL HYDROGEN BONDS: SER B:145, LYS B:163,SER B:91,ALA B :93 Pi-Pi stacked bond:TYR B:146		CONVENTIONAL HYDROGEN BONDS: ASN A:12, GLU A:153, ASP A:65, Pi-Pi stacked bond:TRP A :340
5.	Standard drug 3 (cephalosporin) CID: 25058126		CONVENTIONAL HYDROGEN BOND: ALA A:189,LYS A:163,SER A:91,ILE A:20 Alkyl bond:ALA A:93,ALA A:196		CONVENTIONAL HYDROGEN BONDS: ALA A:63, GLU A: 153, Pi-Alkyl bond:TYR A: 155 Alkyl bond:MET A:310, TRP A:62 Pi-Sigma bond:TRP A :340

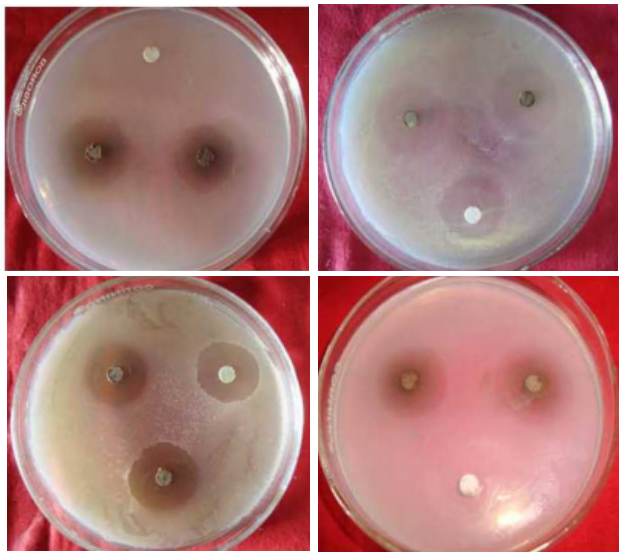


Figure 2: Antimicrobial Activity of Different Extracts of *Vitex negundo*

CONCLUSION

The essential oil content of *Vitex nigundo* is reported in this study and showed twenty essential oil constituents which were identified based on GC-MS. The various extracts displayed excellent antioxidant activity and antimicrobial activities. Because of the existence of large number of compounds in *Vitex negundo,* it has many important applications in the treatment of various ailments. The current study's findings demonstrated a broad range of antibacterial activity against every bacterial infection mentioned above. In-silico study, it has been observed that beta-Eudesmol and Viridiflorol may be act as potential antimicrobial agents. Therefore, these can be further explored for antimicrobial activity for the development of new therapeutic agents.

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