

Evaluation of Bioorganic Compound from *Vitex negundo* Linn and its Inhibitory Effect on Biofilm Forming Urinary Tract Infectious agent *Enterococcus faecium* HAUT119

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Abstract

Enterococci are the main pathogen to cause human urinary tract infection is rapidly enhancing the antimicrobial resistant leads to develop biofilm associated infections throughout the globe. The present work aimed to evaluate the inhibition activity carried out by traditional plant of *Vitex negundo* Linn. A clinical sign of 25 urine samples was collected and processed to identify the biofilm bacteria and their antibiofilm activity against *Vitex negundo*. The drug sensitivity pattern was determined among the four selected enterococci strains by disc diffusion method. Among the four enterococci isolates, the biofilm forming strain was identified as *Enterococcus faecium* HAUT119 by 16S rRNA gene sequence with the accession number MG002173. The biofilm isolates used for further studies against medicinal plant extract of *Vitex negundo* Linn by disc diffusion method, well diffusion method and minimal inhibitory concentration for biofilm inhibition. The application of ethyl acetate leaves extract of *Vitex negundo* showed antibacterial and biofilm inhibition activity compared to amikacin as standard antibiotics. The structural compound was analyzed by FT-IR and NMR detected as 1-(3-anilino-5-(2H-1,3-benzodioxol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl

) ethan-1-one. This finding concludes that the confirming the critical need to achieve novel antimicrobial metabolite derived from well-known traditional plant in India *Vitex negundo* Linn is a promising source and its implementation in medical field used to eradicate all common types infection raised from multidrug resistant and biofilm producing bacterial pathogens.

Keywords: Biofilm, *Enterococci*, Drug resistant, *Vitex negundo*, Ethyl acetate, Bioactive compound.

Introduction

Enterococci are the most prevailing bacterial strains which cause nearly 95% of enterococcal infection in humans and also described the most regularly isolated multi drug resistant bacterial pathogen from clinical infection (1). They are Gram positive bacteria and able to cause bacteremia, intra-abdominal infection, endocarditis and urinary tract infection in the various body sites (2). The control of bacterial infection was done by the development of new antibiotics. However, the antibiotics overuse leads to the development of multidrug resistance among organisms. Enterococci emerged as potential pathogen and are resistant to many routinely used broad spectrum antibiotics (3).

Inhibitory effect of *Vitex negundo* Linn. on biofilm forming *Enterococcus faecium* HAUT119

The multidrug resistant enterococci from UTI site that can form biofilms which are most important global public health issues present in almost all dangerous environments (4). In several ways, Biofilm can contribute various virulence factors and additionally contributes to the bacterial pathogenesis and bacterial resistant to antibiotics which lead to the prolonged infection and their eradication process is highly difficult (5). A huge collection of environmental and medical sources contains Enterococcal species isolated from urinary tract infection which are more virulent than others (6,7). Biofilm formation is accountable for antibiotic resistant mediated by plasmids transfer and a medium for intercellular communication. Currently, enterococci act as reservoir for diverse mobile genetic elements and proficient at acquiring transferable antimicrobial resistance (8). The biofilm forming ability could be a best approach to increase the survival percentage under unfavorable situations such as host invasion or antibiotic treatment (9). There is need to assess the antimicrobial resistance among enterococcal pathogen to control urinary tract infection is challenging due to the extensive ability of antibiotic resistant as it may extend the enterococcal pathogenesis in infection (10). Biofilm formation is the principle cause of numerous lives threatening diseases particularly urinary tract infection which is controlled by developing novel anti-biofilm drugs. Indeed, the application of new therapeutic compounds synthesized by traditionally involved medicinal plant extract which is an alternative to antibiotics resistant and formation of biofilm threats (11). A quality of bioactive compounds from traditional plants are widely exploited in the pharmaceutical, agricultural and food industries and showed as good anti-biofilm agents against many bacteria (12, 13). In this present investigation, *Vitex negundo* Linn, Indian traditional and medicinal plant belongs to the Verbenaceae family. They are growing small tree with 2- 5 meters in height and have their ability to produce metabolically active compounds with different biological properties especially strong antibiotics activities against drug resistant and

biofilm producing bacteria (14). Biofilm formation shows significant challenges in processing of food, medical, water treatment and various aspects directly impose the healthcare system (15). *Vitex negundo* as a medicinal plant herb are representing as hotspots discovery of new drugs which highlight their huge biosynthetic potential to produce therapeutics agent to inhibit the biofilm associated infection (16).

This study aimed to investigate the *in-vitro* antibacterial properties of *Vitex negundo* Linn and its effectiveness against multidrug resistant of enterococci that can lead to cause biofilm production.

Materials and Methods

Collection and Identification of Samples

The clinical urine samples of total 25 were collected from patient having diabetes, pregnancy, pyuria, painful urine and inflammation. All the collected specimens were processed for microbiological examination within 24 hrs. Microbiological identification test such as Gram staining, coagulase test, catalase test, salt concentration test, blood agar and esculin bile salt agar were utilized to identify the bacteria from clinical urinary tract sources.

Sensitivity test and MAR Index

The antibiotics sensitivity pattern of urine isolates was carried out on Muller- Hinton agar medium by using different antibiotics disc with various concentration. According to guidelines recommended by the Clinical and Laboratory Standard Institute (17), the drug resistance pattern of urobacteria was identified due to presence of less than 12mm in diameter zone followed by MAR Index calculation.

$$\text{MAR Index} = \frac{\text{Number of antibiotics to which the isolate was resistant}}{\text{Total number of antibiotics tested}}$$

Isolation of bacterial plasmid

Based on the resistant pattern among all the four enterococcal strains were subcultured into LB broth at 37°C and incubated for 12 -16 hrs. The alkaline lysis method was used

to isolate plasmid DNA (18). Following purification, the DNA was precipitated using ethanol for long-term storage at -20°C. To analyze the size of resistant plasmids, the purified samples were loaded alongside a DNA marker onto a 0.8% agarose gel and electrophoresed. Following electrophoresis, the gel containing the separated DNA stained with ethidium bromide was visualized using a UV transilluminator and documented with a gel documentation system.

Qualitative biofilm assay

The qualitative method of biofilm production assay was employed using Congo Red Agar media (19). This medium is formulated with BHI agar (Brain Heart Infusion agar) supplemented with 5% sucrose and 0.08% Congo red dye. The selected strains were cultivated and inoculated on CRA media. After the incubation for 24 hours at 37°C, colony color formation was assessed to evaluate biofilm formation.

Biofilm formation assay by microtiter plates

The enterococci biofilm production assay was determined by quantitatively by microtiter plates (19). All the four enterococci strains were grown at 37 °C for 24 hrs by using trypticase-soy broth (TSB) which were diluted 1:100 in TSB supplemented with 2% glucose. The diluted solution was added to the wells and incubated for 24 hrs at 37 °C. Negative controls consisted of wells containing only sterile trypticase soy broth with 2% glucose. Following incubation, wells were washed three times with 300 µL of phosphate-buffered saline (PBS) to remove unattached cells. Biofilms were then fixed with 95% ethanol, stained with 100 µL of 1% crystal violet, and washed again three times with 300 µL of sterile distilled water and then air dried. An ELISA reader was used to measure the stained bacteria in the microtitre plate at 570 nm.

Molecular phylogenetic analysis of 16S rRNA gene sequence

DNA extraction from multiple drug resistant and biofilm forming bacteria was performed using Sodium dodecyl sulfate (SDS) - based method (20). The forward primer (16S rRNA F)

and the reverse primer (16S rRNA R) were employed for 16S rRNA gene sequencing and was documented with Photonyx (Model: Slite140) Gel documentation system (Nyx Technic, Inc. USA). The phylogenetic tree was constructed using the NJ (neighbor-joining) method by the software MEGA 5.05 (21).

Sample collection and extraction

The *Vitex negundo* plant leaves were collected at Tenkasi district, Tamil Nadu, India and authenticated. The collected plant leaves were washed and rinsed with distilled water. They dried at room temperature for nine days and powdered using a blender. The powder was stored in airtight containers within sealed polyethylene bags. The powdered leaves of 50 gm were extracted using soxhlet apparatus with 150 mL of ethanol at 50°C. Filtered extract was placed until the evaporation of solvent phase at room temperature. The dried solvent was taken and redissolved in ethyl acetate and methanol at 5mg per ml concentration for further analyses.

Primary screening by disc diffusion test

Primary screening of *Vitex negundo* plant leaves extract by disc diffusion method on MHA medium (22). The 24 hrs nutrient broth culture of *Enterococcus faecium* HAUT119 was inoculated on the agar medium. After 15 min, sterile disc was loaded with the known concentration of 50, 100, 150 and 200 µg *Vitex negundo* leaf extract on the agar medium. The diameter of inhibitory zone was recorded after 24 hrs at 37°C.

Secondary screening by agar well diffusion method

The chloroform-ethyl acetate extracts (2:1 v/v) were used for the antibacterial and antibiofilm activity after performing the TLC bio-assay. The secondary screening was carried out by well diffusion method (23). The 24 hrs culture of *Enterococcus faecium* HAUT119 was bio assayed against standard antibiotics. 50, 100, 150 and 200 µg leaf extract of *Vitex negundo* Linn, were loaded and incubated at 37°C.

After 24 hrs, the diameter of inhibitory zone was observed around the well. Amikacin (30µg) antibiotic and solvent were placed as positive and negative control.

Antibacterial and antibiofilm assay by MIC

The minimum inhibitory concentration was carried out by the broth dilution method using alamar blue assay. The various concentration of *Vitex negundo* leaves extract such as 100, 50, 25, 12.5, 6.5, 3.25, and 1.5 µg/mL were prepared by diluting two fold concentrations (200 µg) of plant extract in one mL broth was serially diluted. 25µL *Enterococcus faecium* HAUT119 was inoculated and incubated. After incubation, inhibitory effect was observed by the adding the resazurin indicator (24).

FT-IR spectrum analysis

The compound was analyzed using FT-IR spectroscopy to identify the functional groups. The spectrum covered a range of 4000 – 400 cm⁻¹ with a resolution of 0.9 cm⁻¹. The observed absorption peaks were compared with known spectra to confirm the presence of specific functional groups using the FT-IR spectrometer V-70 (Bruker, Billerica, MA, USA).

NMR spectrum

The structure of the compound exhibiting antibacterial activity was analyzed using nuclear magnetic resonance (C¹³ and H¹NMR) spectroscopy. The analysis was performed using on a 400 MHz JOEL NMR spectrophotometer.

Molecular docking

The structural compound analyzed through FT-IR and NMR is identified as 1-(3-anilino-5-(2H-1,3-benzodioxol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one. This compound matches the standard compound 1-(2H-1,3-benzodioxol-5-yl) ethan-1-ol. Molecular docking studies were conducted using Discovery Studio to evaluate the interaction of 1-(2H-1,3-benzodioxol-5-yl) ethan-1-ol, a compound derived from the traditional medicinal plant *Vitex negundo* Linn., with alanine racemase (AlaR). The AlaR is a bacterial enzyme

belonging to the fold-type III group of pyridoxal 5'-phosphate (PLP)-dependent enzymes. It catalyzes the interconversion of L- and D-alanine, which is crucial for peptidoglycan biosynthesis. The 3D structure of AlaR was obtained from the RCSB Protein Data Bank (PDB: 3E5P). Using PyMOL, water molecules were removed, polar hydrogen atoms and charges (Gasteiger and Kollman) were added, and the structures were optimized for bond angles, orders, and topology. The prepared structures were saved in PDBQT format. The 3D structure of the ligand, 1-(2H-1,3-benzodioxol-5-yl) ethan-1-ol, was downloaded from PubChem in SDF format and converted to PDB using PyMOL. Ligand preparation included removing water molecules, adding hydrogen bonds, and applying Kollman and Gasteiger charges. The final ligand structure was saved in PDBQT format for docking. A three-dimensional grid box was created using AutoDock to calculate docking scores. The grid box was sized to encompass the entire protein receptor, allowing ligands to dock anywhere on the receptor surface. The dimensions of the box were adjusted for X, Y, and Z coordinates with 0.5 Å spacing, centering the ligand in the active site. The grid parameters were exported as grid.txt for docking calculations. The analysis of hydrophobic and polar interactions between the protein and its best-binding ligand was conducted using LigPlus. The complex structure was generated and visualized in Discovery Studio, with PDB files exported and saved in PNG format.

Results and Discussion

Isolation of *Enterococcus* sp

The investigation of the present work performed the screening of multidrug resistant enterococci isolated from urine specimens. Totally, 25 urine samples were collected from infectious patients signed with clinical syndrome such as diabetes, painful urination, pregnancy, inflammation during urination and pyuria. Among 25 samples, 9 samples were noticed in positive cases and 16 urine cases were obtained negatively. The highest cases of infected

patient executed with painful urination (Table 1). Positively obtained urine samples were further tested by microbiological analysis. Among the 9 positive cases, *Enterococcus* sp were observed in four samples which was experimentally tested by morphological and biochemical tests. As per guidelines of Bergey's manual of systematic bacteriology, *Enterococcus* sp grew at a NaCl concentration of 6.5%. The black colour colonies were developed on esculin bile salt agar medium (25). Similarly, hemolytic activity of *Enterococcus* sp absent in blood agar medium. The catalase, oxidase and coagulase test for used as negative reaction for enterococci. Studies indicate that *Enterococcus* sp is a most emerging bacterial pathogen able to cause urinary tract infection in worldwide (26).

Table.1: Collection and Distribution of urine samples.

Characterization of Patient	Urine samples collection	Positive cases
Diabetes	7	1
Painful urination	5	4
Pregnancy	5	2
Inflammation during urination	6	2
Pyuria	2	0
Total	25	09

Resistant pattern of enterococcus sp

The *Enterococcus* sp drug resistant pattern was performed on Muller hinton agar medium by using 20 antibiotic discs at different concentration. Four strains of *Enterococcus* sp

coccus faecium using Disc diffusion test.

were 100% resistant to ampicillin, ceftazidime, methicillin and penicillin followed by 75% resistant to cefepime, cefixime, cefpirome, cefuroxime, linezolid, teicoplanin and vancomycin. The least resistant measured in ceftizoxime antibiotic. The cephalothin and tetracycline antibiotics were noted as intermediate resistant to all four strains of *Enterococcus* sp. The antibiotic methicillin, penicillin and cephalosporin group resistant strains to create dangerous situation in clinical setting and are currently recognized throughout the world (27). The 100% sensitive strains were found in antibiotics such as azithromycin, doxycycline, and ofloxacin (Table 2). The least sensitivity was explored in the gentamycin. In this work, 75% of *Enterococcus* strains were resistant to linezolid, teicoplanin and vancomycin. Studies showed that vancomycin resistant enterococci are significantly higher among the global concern (28, 29). The antibiotics such as linezolid, teicoplanin and vancomycin resistant enterococci strains are making persistent problems in clinical infection (30,31). The multiple antibiotics resistance index of *Enterococcus* sp was calculated as 0.65 where several antibiotics are used as resistance to more commonly used antibiotics including ampicillin, penicillin and cephalosporin groups (32). These agents are act as opportunistic pathogen which was found in the environment to cause clinical disease. Indeed, the multiple antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium* are emerging as the rise in hospital-acquired infections particularly in UTI site infection become international concern (33,34).

Table.2: Susceptibility pattern among *Enterococcus*

Antimicrobial agent	Resistant Cases	Percentage of Resistant	Intermediate cases	Percentage of Intermediate	Sensitive cases	Percentage of Sensitivity
ampicillin (10 µg)	4	100%	-	-	-	-
azithromycin(15µg)	-	-	-	-	4	100%
cefepime (30µg)	3	75%	1	25%	-	-
cefixime (5µg)	3	75%	1	25%	-	-
cefpirome (30µg)	3	75%	1	25%	-	-
cefpodoxime(10µg)	2	50	2	50%	-	-

ceftazidime (30µg)	4	100%	-	-	-	-
ceftizoxime (30µg)	2	25%	3	75%	-	-
cefuroxime (30µg)	3	75%	1	25%	-	-
cephalothin (30µg)	-	-	4	100%	-	-
cefotaxime (10µg)	2	50%	2	50%	-	-
doxycycline (30µg)	-	-	-	-	4	100%
gentamycin (10µg)	-	-	2	50%	2	50%
linezolid (30µg)	3	75%	-	-	1	25%
methicillin (5µg)	4	100%	-	-	-	-
ofloxacin (5µg)	4	-	-	-	4	100%
penicillin (10µg)	4	100%	-	-	-	-
teicoplanin (30µg)	3	75%	-	-	1	25%
tetracycline (30µg)	-	-	4	100%	-	-
vancomycin (30µg)	3	75%	1	25	-	-

Plasmid profile of *Enterococcus* sp

The multidrug-resistant bacteria were subjected to alkaline lysis followed by electrophoresis to determine the plasmid content. Among the four lanes, plasmid analysis of *Enterococcus* sp was observed from lane 1-3. The fifth lane served as a marker DNA. The lane four showed the absence of plasmid. The plasmid bands were found from lane 1 to 3 which showed the multi-drug resistant strains were plasmid mediated (Fig. 1). As previously reported, the loss of plasmid content in enterococci in the environments has less selective pressure with lower antimicrobial level (35,36) Studies indicating that presence of multiple drugs resistant represents a high abundance of plasmid among clinical environmental origin of enterococci which facilitate the transfer of resistant plasmid from one strain to another strain (37).

Biofilm formation by *Enterococcus* sp

The biofilm formation was performed with all multidrug resistant strains of enterococci. Among the four, a single strain produced black color colonies with dark consistency on congo red agar medium was noted as strong biofilm producers. The remaining strains, with pink or red colonies, were classified as non-producers of biofilm. The experiments were performed in triplicate to quantitatively analyze biofilm pro-

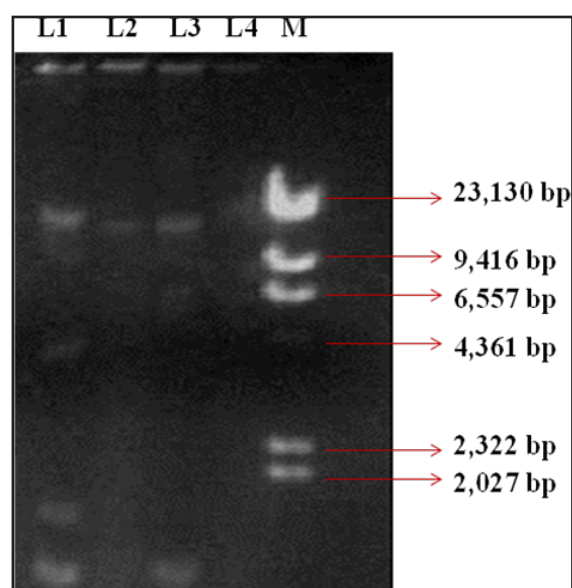


Fig.1. Plasmid Profile of *Enterococcus* sp

duction by enterococci using the microtiter plate method. Among four strains, the OD values of the congo red agar medium containing strain was produced as > 0.244 in the 570 nm which indicates as strong biofilm producer of enterococci. The other three strains were determined the OD values as less than 0.120 which denoted the negative biofilms producers of enterococci. The studies of congo red agar method and microtiter plate methods were evaluated

as excellent, sensitive and positive approaches and values (38,39). The effectiveness of bio-film formation in microorganisms depends on the factors including environmental conditions, nutrient availability, specimen type, geographic origin, surface adhesion properties, and the organism's genetic makeup (40). The multiple antibiotics resistant enterococci sp has potential to develop biofilm formations are clinically important because their treatments with many commonly used antibiotics are very complicated (41).

Genomic DNA amplification and phylogenetic analysis

The multiple drug-resistant biofilm forming enterococci was isolated and their genomic DNA was then extracted and 16S rRNA gene was amplified by the specific primers. *Enterococcus faecium* HAUT119 was submitted in GenBank and received the accession number MG002173. The phylogenetic analysis included the *Enterococcus faecium* HAUT119 16S rRNA was compared with retrieved sequences from the NCBI database. A phylogenetic tree was constructed using MEGA 5.05 software (Fig. 2). The Neighbor-Joining method (42) was employed to analyze the evolutionary history based on 7 nucleotide sequences, with a final dataset of 52 positions (43). The phylogenetic sequence analysis starts with the appropriate alignment of the data as it's the poorly understood molecular data analysis. The phylogenetic analysis helps in search for evidence of molecular evolution (44).

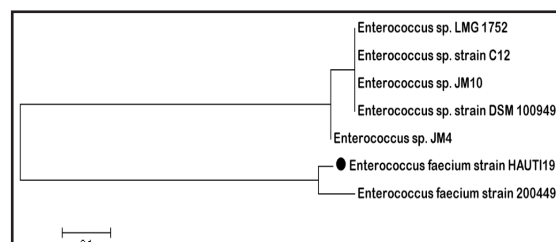


Fig. 2. Molecular Phylogenetic analysis of *Enterococcus faecium* HAUT119

Antibacterial and antibiofilm analysis of *Vitex negundo* Linn

This study investigated to analyze the antibacterial and antibiofilm activity of *Vitex negundo* Linn, plant leaf extract against the test pathogen *Enterococcus faecium* HAUT119. The primary screening method was performed by disk diffusion test. The maximum zone of inhibition was recorded as 25mm in 200 µg concentration. No zone of inhibition was noted in the 50µg concentration (Table 3). Based on the separation of a bioactive component in the ethyl acetate extract identified by TLC bioassay, a secondary screening was carried out using the agar well diffusion method. The antibacterial activity of Ethyl acetate extract was showed as 22mm maximum zone of inhibition in 200µg concentration whereas chloroform extract was recorded the least zone of inhibition was evaluated as 8mm in 200µg concentration (Table 4). Amikacin (30µg) antibiotics was used as positive control and the results of tested leaves extracts were very promising source in comparison to standard antibiotics as positive control.

Table.3: Primary Screening of Leaves extract of *Vitex negundo* Linn

Sample	Leaves extract of <i>Vitex negundo</i>				Positive control amikacin(30µg)
	50 µg	100 µg	150 µg	200 µg	
<i>Enterococcus faecium</i> HAUT119	-	9mm	16mm	25	21

Table.4: Secondary Screening of Leaves Extract of *Vitex negundo* Linn

Sample	chloroform extract of <i>Vitex negundo</i>				Ethyl acetate extract of <i>Vitex negundo</i>				Positive control amikacin (30µg)
	50 µg	100 µg	150 µg	200 µg	50 µg	100 µg	150 µg	200 µg	
<i>Enterococcus faecium</i> HAUT119.	-	-	-	8mm	-	-	13mm	22	18

Inhibitory effect of *Vitex negundo* Linn. on biofilm forming *Enterococcus faecium* HAUT119

The determination of antibacterial and antibiofilm activity was performed by Minimal inhibitory concentration by alamar blue assay against the *Enterococcus faecium* HAUT119. The ethyl acetate extract of *Vitex negundo* Linn, leaves were prepared as 100, 50, 25, 12.5, 6.5, 3.2, and 1.5 µg/ml. The minimum inhibitory concentration was found in 1.5 µg/ml against the *Enterococcus faecium* HAUT119. Plants extract of *Vitex negundo* Linn, are the potential source for antibacterial activity against many clinical types of infections (45). In previous studies documented that the solvent extracts from *Vitex negundo* plant exhibited potent antibacterial activity against MRSA (Methicillin-Resistant *Staphylococcus aureus*) especially in ethyl acetate and chloroform extract (46). In another study, plant leaves extract is used to detect zone of inhibition against *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (47). The studies indicating that the broad-spectrum activity of *Vitex negundo* leaves extract with Minimum inhibitory concentration values of 1.5 µg/ml exhibited potent antibacterial activity (48). The *Vitex negundo* most frequently focused medicinal plants in India and the natural compound of *Vitex negundo* can donate the novel drugs to combat the infectious diseases.

FT-IR and NMR Analysis of Bioactive Compound of *Vitex negundo* Linn

The FT-IR spectroscopy was analysed by the functional groups present in the *Vitex negundo* with the spectral range of 4000 - 400 cm⁻¹. The spectral data revealed the peak around 3301 cm⁻¹ indicate the N-H group, peak at 3124 cm⁻¹ correspond to aliphatic (C-H) group. The absorption peak at 1675 cm⁻¹ denote the C=O stretching, peak at 1450 and 1488 cm⁻¹ determines the aromatic C - C stretching and the peak at 1258 cm⁻¹ exhibits the C-N stretching. The absorption properties indicate the presence of functional groups of *Vitex negundo* Linn plant leaves extract (Fig. 3).

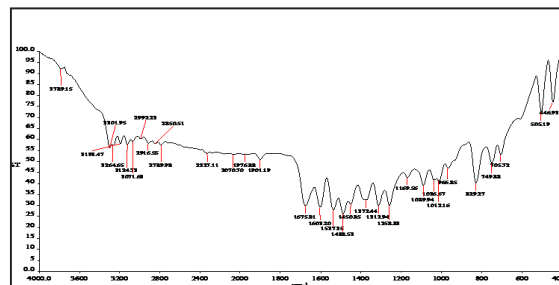


Fig.3. FTIR analysis of active compound derived from *Vitex negundo* Linn

The active compound isolated from *Vitex negundo* Linn leaves was analyzed using NMR to determine its structure. The chemical shift values indicated the presence of specific functional groups N-H proton (δ 3.3 ppm), aromatic protons (multiplet at δ 7.61 – 7.12), pyrazoline proton (doublet at δ 2.04), acetyl proton (δ 2.5 ppm), phenyl group of benzodioxol proton (singlet at δ 6.17) (Fig. 4). Based on this analysis, the structure of the compound was found to be 1-(3-anilino-5-(2H-1,3-benzodioxol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one (Fig. 5).

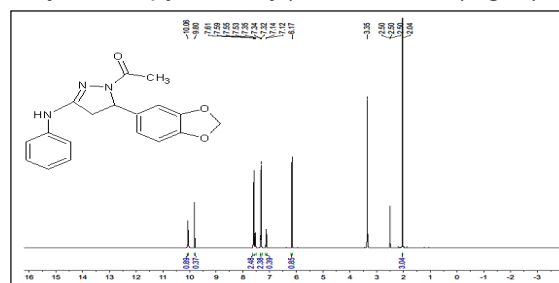


Fig. 4. NMR studies on active compound derived from *Vitex negundo* Linn

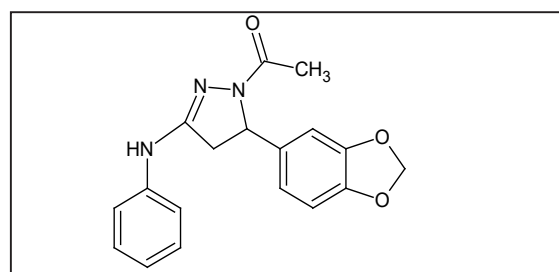


Fig. 5. Active compound derived from *Vitex negundo* Linn as 1-(3-anilino-5-(2H-1,3-benzodioxol-5-yl)-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one

The molecular weight of the compound was 323.34 with its formula $C_{18}H_{17}N_3O_3$. The FT-IR and NMR spectroscopy are valuable tools for identifying functional groups in plant extracts for the identification of pharmaceutical compounds (49). In this study, the *Vitex negundo*, an aromatic plant exhibits the presence of functional groups commonly found in aromatic plants like phenols, amines, alcohol, flavonoids, alkenes, proteins and lipids found because of the presence C-H vibration. These groups along with many organic acids and derivatives are associated with numerous medicinal properties. The result of FT-IR and NMR studies detect the structure of secondary metabolites from leave extract of *Vitex negundo* Linn. The similar observation was discussed by many researchers (50,51). The identification of novel plant drugs for clinical use match with other reports (52). These results suggested that the *Vitex negundo* Linn, has the unique distinction that have broad source of diverse secondary metabolites leads to the applications of the antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory and anticancer activity.

Molecular docking of Alanine racemase with 1-(2H-1,3-benzodioxol-5-yl) ethan-1-ol

Docking of alanine racemase from *Enterococcus faecium* exhibited binding affinities of -5 kcal/mol with the compound 1-(2H-1,3-benzodioxol-5-yl) ethan-1-ol shown in the (Fig. 6). AlaR catalyzes the conversion between L- and D-alanine, which is essential for peptidoglycan biosynthesis. This enzyme is found in prokaryotes but not in eukaryotes, making it a promising antibacterial drug target for treating tuberculosis and urinary tract infections (53). The study demonstrates that the crystal struc-

ture of AlaR from *Enterococcus faecalis* forms hydrogen bonds with key active site residues (TYR:44, HIS:169 and LYS:132) represented in the Table 5. The ForcefieldBase is calculated using CHARMM whose energy is -21. In molecular docking, more negative values indicate a higher binding affinity between the receptor and ligand, suggesting greater efficacy of the bioactive compound. The docking result indicates the stabilization of the cell wall structure, which influences bacterial virulence and modulates the host immune response via alanine racemase.

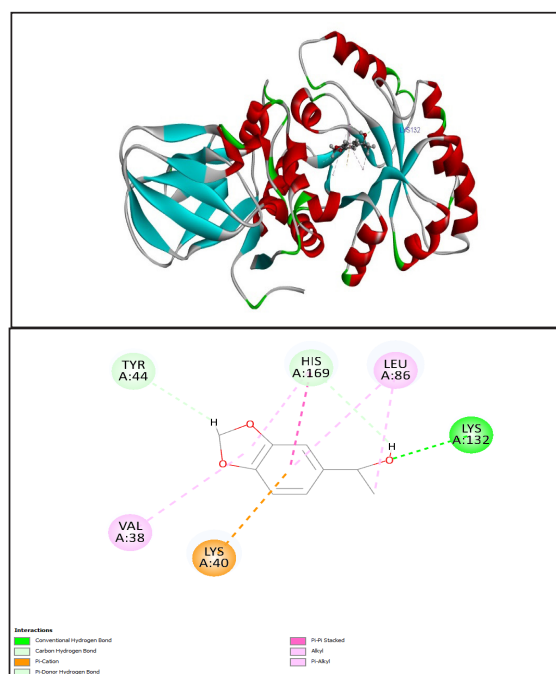


Fig. 6 Figure 3D molecular docking interaction between 3E5P and the compound 1 - (2H - 1,3 - Benzodioxol - 5 - yl) ethan - 1- ol of docking score -5 kcal/mol

Table.5: Binding score of compounds against antibacterial and biofilm inhibition activity of *Enterococcus faecium*

S. No	Compound name/MW	Binding energy (kcal/mol) 3E5P/ MW	Protein and compound interaction
1	1 - (2H - 1,3 - Benzodioxol - 5 - yl) ethan - 1- ol / 166.17	-5	LYS:40, Val:38, TYR:44, HIS: 169, LEU:86, LYS:132

Conclusions

The obtained results conclude that the multidrug resistant *Enterococcus faecium* HAU-TI19 leads to cause biofilm associated infection is continuing to be a major threat in worldwide concern and play an unconditional role in human population. This agent is isolated from urinary tract infection which exhibited multidrug resistance to several commonly tested antimicrobial agents. The resistant biofilm forming bacteria to antibiotics can be donated to the persistent issues in human clinical environments. Increased knowledge regarding the development of novel bioactive compounds as appropriate control measures to inhibit the resistant strains. The application of antibacterial metabolite derived from *Vitex negundo* Linn provides a promising source that can destroy the biofilm bacteria. In the present investigation, the plant of *Vitex negundo* Linn which are the back bone of traditional medicine. They play an abundant role in the development and application of various bioactive compounds which can ruin the biofilm bacteria due to its high reactivity in order to acquire resistant bacteria to all antibiotics and also control the human and health care associated infections.

Credit authorship contribution statement

M. Mohamed Mahroop Raja: Writing Original draft preparation, Methodology, Visualization and Data curation. **A. Asrar Ahamed:** Supervision, Formal analysis and Conceptualization. **N. Reehana:** Reviewing and Editing. **K. Vijayalakshmi:** Investigation, Formal analysis and Validation. **Anis Ahamed Nazeer:** Resources and Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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