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In silico and *in vitro* Analysis Identifies the Effective Role of Isovaleric Acid Extracted from *Musa acuminata* against *Staphylococcal aureus* Infection Causing Brain Abscess

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Abstract

Staphylococcus aureus can cause severe infections, including meningitis and brain abscesses, which can be fatal if untreated. This study evaluates the antibacterial potential of *M. acuminata* extracts against *S. aureus* using *in vitro* and *in silico* analyses. Transcriptome samples from GSE217832 of the GEO database were analysed on GEO2R for the identification of differentially expressed genes. Further, the GeneCard database was also explored to identify genes governing brain abscess. Protein-protein interactions between these genes were analysed using the STRING plugin of Cytoscape at a confidence score of 0.90. The network was further analysed on CytoHubba for identifying the top 20 genes involved in the network based on MCC, DMNC, MNC, Degree, EPC, BottleNeck, EcCentricity, Closeness, radiality, Betweenness, Stress, and ClusteringCoefficient. AKT1 protein was identified as the key protein for brain abscess. The structure of the AKT1 protein was modelled using SWISS-MODEL. Active ingredients of *M. acuminata* were identified through the IMPPAT database and evaluated for their drug-like properties. Molecular docking studies revealed the antibacterial potential of isovaleric acid (-4.9 kcal/mol) as the most effective treatment against brain abscesses. Further, *M. acuminata* leaves and peels were dried, crushed, and mixed with organic solvents (acetone, methanol, chloroform, ethanol). Extracts were incubated with bacteria on Luria-Bertani media, and inhibition zones were measured to evaluate antibacterial activity to estimate the largest zone of inhibition.

Major Findings: This study elucidates the antibacterial mechanisms of *M. acuminata*, demonstrating its promising activity against *S. aureus*, particularly in combination with acetone and chloroform, respectively. The study identifies *M. acuminata* as a promising candidate for alternative treatments targeting brain abscesses caused by *S. aureus*.

Keywords: Brain abscess, *Musa acuminata*, Network pharmacology, *Staphylococcal aureus*

1. Introduction

Brain abscesses, serious infections with high morbidity and mortality, pose a significant global health and financial burden[1-3]. The increasing prevalence of Multi-Drug-Resistant (MDR) bacteria, particularly *S. aureus* and its Methicillin-Resistant Strain (*MRSA*), has made treatment more challenging[4-6]. The misuse of antibiotics has contributed to this resistance, highlighting the urgent need for alternative antimicrobial therapies[7]. Traditional

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medicine, relied upon by 80% of people in developing nations, has long used plant-based remedies[8-10], including those derived from the banana plant (Musa sp.), which originated in the Southwestern Pacific and later spread to India and other tropical regions. *M. acuminata*, a species of banana, contains bioactive compounds such as flavonoids, alkaloids, tannins, and phenolics, which possess antimicrobial, anti-inflammatory, and neuroprotective properties. These compounds can help in brain abscess management by inhibiting bacterial

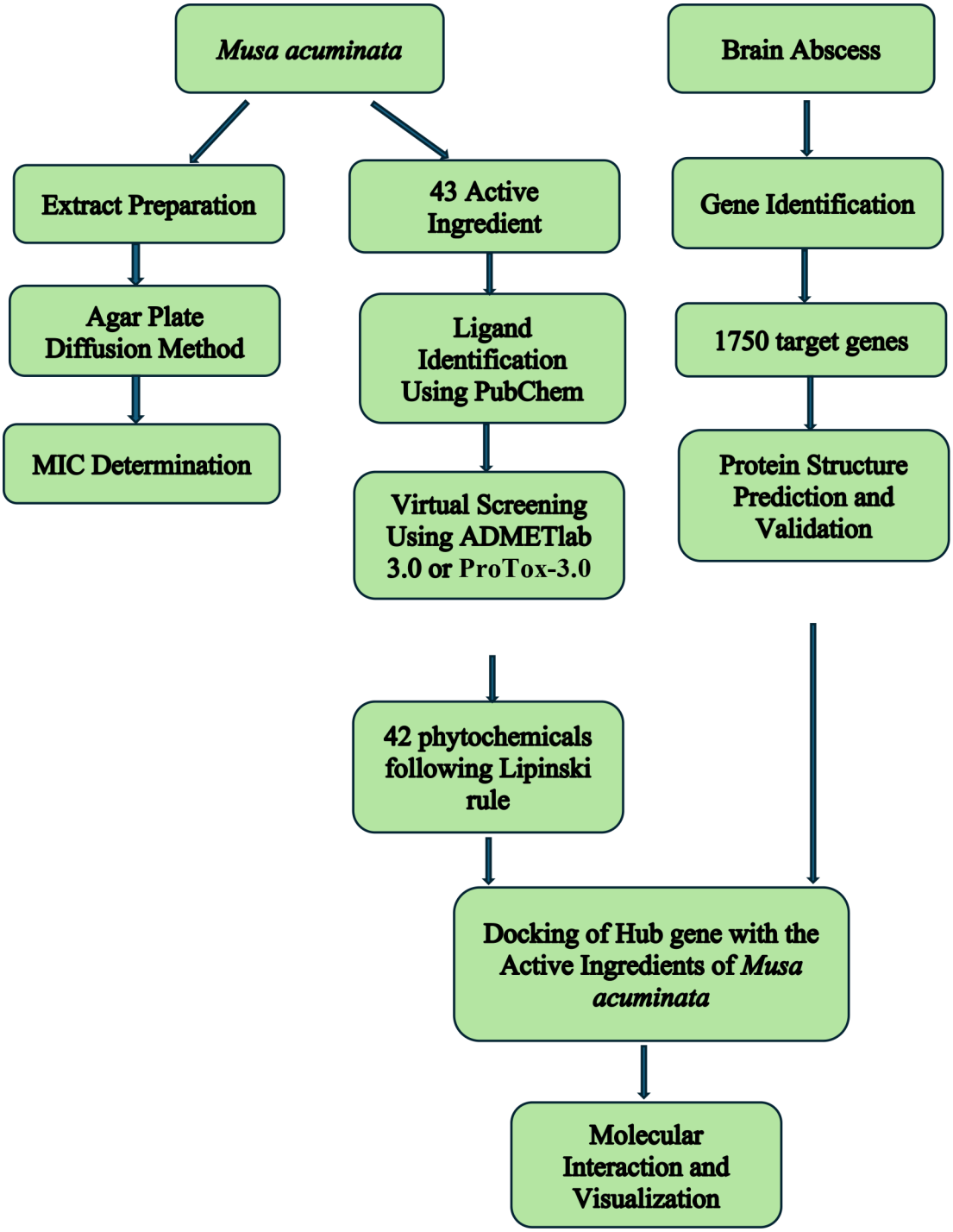


Figure 1. Schematic representation of the complete workflow used in the study, including data retrieval, DEG analysis, PPI construction, molecular modelling, and antibacterial validation.

growth, regulating cytokine activity and reducing oxidative stress, which may improve clinical outcomes and reducing neuroinflammation. Studies show that banana stem extracts exhibit antibacterial activity against *S. aureus, Proteus mirabilis, Bacillus subtilis, Aspergillus niger, Candida albicans, Micrococcus sp., and Salmonella sp.* due to the presence of glycosides, tannins, saponins, steroids, phenolics, alkaloids, anthraquinones, flavonoids, and carbohydrates[11]. Similarly, the ethanolic extract of *Musa paradisiaca* has demonstrated strong antibacterial effects against *B. subtilis, E. coli, Pseudomonas aeruginosa,* and *S. aureus*, suggesting that in silico approaches like molecular docking could be used to explore its antibacterial potential further[12]. Since brain abscesses often require surgical drainage[13], wound healing is another critical aspect of recovery[14]. Wounds, defined as physical injuries that disrupt normal skin structure and function, heal in four phases: haemostasis, inflammation, proliferation, and resolution[15]. Scientific research has identified the banana plant as an effective aid in wound healing, as its antioxidant-rich fruit peels offer protection against free radicals[16-18], while its oligosaccharides act as prebiotics, promoting gut health and boosting immune function[19-21]. Historically, various parts of the banana plant have been used to treat ailments such as dysentery, colitis, diabetes, burns, eczema, fevers, epilepsy, leprosy, migraines, and high blood pressure[22-24]. Its fruit peels, rich in antioxidants, phytochemicals, and potassium, have also been studied for their potential anti-cancer and heart-protective properties. Considering its high nutritional value, it ranks among the top five most nutritious foods globally[25,26]. Further research into its wound healing and therapeutic bioactivity is warranted. The integration of in silico and in vitro approaches can reveal Musa's neuroprotective properties, aiding bacterial inhibition, reducing neuroinflammation antibiotic-resistant infections. Its bioactive compounds exhibit antimicrobial, anti-inflammatory, and neuroprotective properties, aiding bacterial inhibition, reducing neuroinflammation, and promoting faster recovery, making it a promising addition to therapeutic strategies.

2. Materials and Methods

The complete methodology opted for in the current research work has been described in Figure 1.

2.1 Retrieval of Gene Information

Gene targets related to brain abscess were retrieved from the GeneCard database. These databases provide detailed information about human genes. GeneCard is a comprehensive database that provides extensive information on human genes, covering various aspects such as genomics, transcriptomics, proteomics, and genetic data. It gathers data from over 150 sources, including gene expression, protein interactions, pathways, diseases, and genetic variants, making it a valuable tool for researchers studying gene-disease associations and molecular functions.

2.2 Transcriptomic Data Analysis

The differentially expressed genes in patients with brain abscess were also identified through transcriptome data analysis. RNA-Seq data from patients with brain abscesses were retrieved from the Gene Expression Omnibus (GEO) database hosted by the National Centre for Biotechnology Information (NCBI). The transcriptomic data were analysed using the GEO2R tool to identify Differentially Expressed Genes (DEGs). The selection criteria for DEGs included an absolute log2 fold change ($|\log2FC| \geq 1$ and an adjusted pvalue<0.05. These thresholds were applied to ensure the biological significance and statistical reliability of the identified genes.

2.3 Protein-Protein Interaction Study

In this study, network biology methods were used to identify key targets. We used the STRING and CytoHubba plugins in Cytoscape to find important genes in *S. aureus*. A network of all identified genes encoding for *S. aureus* was built using the STRING plugin in Cytoscape, with a 0.95 confidence level to ensure reliable interactions. Further analysis of the hub network aimed to pinpoint key target genes that could be targeted for various *S. aureus* infections. The cytohubba plugin of Cytoscape was used to evaluate the STRING network on the basis of various parameters like MCC, DMNC, MNC, Degree, EPC, BottleNeck, EcCentricity, Closeness, Radiality, and Maximum Neighbourhood Component to identify the genes with maximum interactions in the network.

2.4 Molecular Docking Studies

We used the protein sequences from Cytoscape to create 3D protein structures using the Swiss Model tool. These

structures were downloaded in PDB format for further analysis. The active compounds of *M. acuminata* were identified through the IMPPAT database. To identify the drug-like potential of the identified active compounds, we gathered their names, sources, and canonical SMILES from PubChem, creating a list of 43 active phytochemicals. To create a library of potential lead compounds, phytochemicals were screened using ADMETlab following Lipinski's Rule of Five, that is, molecular weight less than 500 Daltons, a lipophilicity (Log) of 5 or less, fewer than 5 hydrogen bond donors, and fewer than 10 hydrogen bond acceptors. To complement this, toxicity prediction was performed using the ProTox-3.0 webserver. Virtual screening was then performed to identify phytochemicals with favourable pharmacokinetic and pharmacodynamic properties. To estimate the binding affinity of the phytochemicals, we performed molecular docking using SeamDock, targeting the hub protein identified through network biology approaches. For accurate ligand-protein interaction predictions in SeamDock using AutoDock software, after getting the chemical structure information (canonical smiles) of the ligand from PubChem, the PDB file of the receptor was uploaded. Docking parameters were configured as follows: grid spacing set to 0.375 Å, number of docking modes = 10, energy range = 5, and exhaustiveness = 8.

2.5 *In vitro* Analysis

To validate *in silico* analysis results, *in vitro* experiments were performed by taking a plant sample. On the basis of available literature and results of *in silico* following samples were taken: fruit peel and leaf. Luria Bertani media was used for the activation of the culture. Fruit peel and leaf were used for the extraction of compounds by using organic solvents, *viz.*, acetone, methanol, ethanol, and chloroform, by following the protocol[27]. Antimicrobial potential was studied by the agar plate diffusion method[28,29].

2.5.1 Preparation of Luria–Bertani (LB) Broth and Agar

To prepare the LB broth, 6 g of NaCl, 10 g of tryptone, and 3 g of yeast extract were dissolved in 1 L of distilled water. The solution was then divided into two parts: 100 mL was reserved for broth culture, and the remaining 600 mL was used to prepare LB agar by adding 9 g of agar powder (1.5% w/v concentration). The agar solution was autoclaved, cooled, and poured into sterile Petri dishes, with agar concentration adjusted if required by the experimental setup.

2.5.2 Preparation of Plant Extracts

Exactly 100 mg of each plant sample (*e.g.*, fruit peel and leaf) was weighed and transferred into four separate test tubes containing 50 mL each of acetone, methanol, ethanol, and chloroform. The mixtures were vortexed briefly and allowed to stand at room temperature for 24-48 h to enable optimal extraction of bioactive compounds. The extracts were later filtered, stored in airtight tubes, and refrigerated until further use.

2.5.3 Bacterial Strain Activation

A 10 µL aliquot of the *S. aureus* bacterial stock was transferred into a sterile Eppendorf tube containing 1 mL of LB broth and incubated at 37°C overnight to promote bacterial growth. Post-incubation, the culture was stored at 4°C. For experimental use, 50 µL of the activated bacterial culture was further diluted in 1 mL of LB broth for inoculation.

2.5.4 Extraction from Dried Fruit Samples

Fresh banana peel and leaf samples were air-dried in Petri dishes for two days. Dried samples were then treated with Dimethyl Sulfoxide (DMSO), mixed thoroughly, and allowed to stand. The resulting extracts were collected and stored in sterile Eppendorf tubes for use in the diffusion assay.

2.5.5 Agar Well Diffusion Assay

Sterile Petri dishes were prepared with LB agar and allowed to solidify. The activated bacterial suspension was evenly spread on the agar surface using a sterile glass spreader. Wells were bored into the agar using a sterile cork borer. Each well was filled with 100 µL of plant extracts prepared in acetone, methanol, ethanol, or chloroform. Control wells contained the respective solvents alone. Plates were sealed with parafilm and incubated at 37°C for 18-24 h. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition around each well using a ruler. The diameter of the zone of inhibition was measured for each Petri dish using a ruler, excluding the control. Each treatment—fruit extract in acetone, methanol, ethanol, and chloroform—was tested in triplicate against *S.*

aureus. The average zone of inhibition (in mm) and standard deviation were calculated for each group. Statistical analysis was conducted using Student's t-test to determine the significance of differences among the extract treatments. Measurements were recorded in millimetres for all treatment and control groups.

The experiment assessed the efficacy of *M. acuminata* extracts against *S. aureus*. The diameter of the zone of inhibition was measured for each Petri dish using a ruler, excluding the control. Each treatment—fruit extract in acetone, methanol, ethanol, and chloroform—was tested in triplicate against *S. aureus*. The average

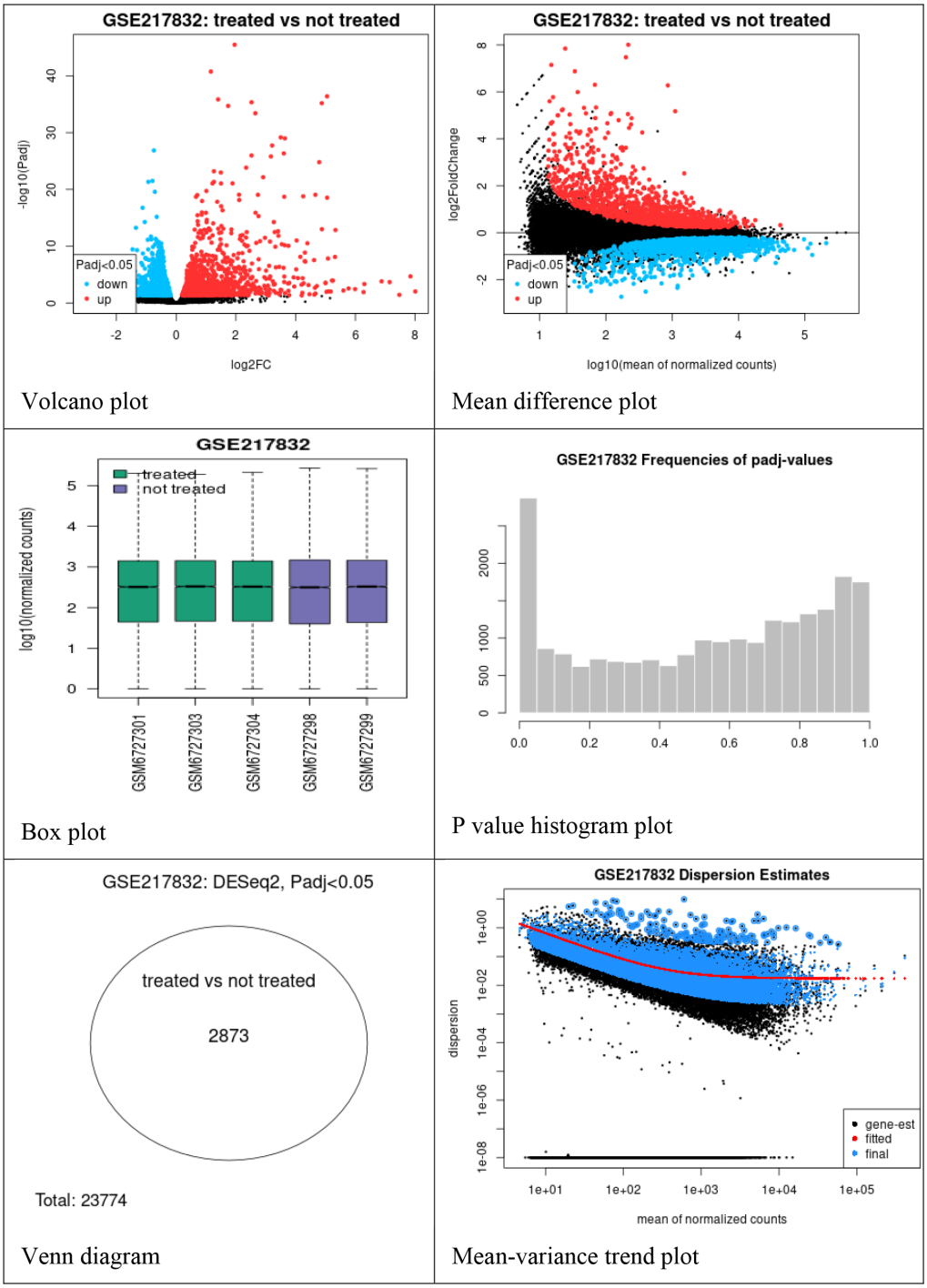


Figure 2. Visual representation of Differentially Expressed Genes (DEGs) identified from the GEO2R transcriptome analysis.

zone of inhibition (in mm) and standard deviation were calculated for each group. Statistical analysis was conducted using Student's t-test to determine the significance of differences among the extract treatments.

3. Results

3.1 Transcriptomic Data Analysis

The transcriptomic dataset was analysed using the GEO2R tool, which identified differentially expressed genes. The samples from the GEO database, GSE217832, were used for the identification of differentially expressed genes in the current study. This study examined seven samples in total, comprising three control and four treated samples. The control group, which did not undergo treatment, was composed of cerebral organoids derived from induced pluripotent stem cells (iPSCs). The corresponding accession numbers for these control samples are GSM6727298 (Control organoid replicate 2), GSM6727299 (Control organoid replicate 3), and GSM6727300 (Control organoid replicate 4). In contrast, the treated group, which was inoculated with CMV Merlin, also included cerebral organoids generated from iPSCs. The patient samples are represented by accession numbers GSM6727301 (CMV-infected organoid replicate 1), GSM6727303 (CMV-infected organoid replicate 3), and GSM6727304 (CMV-infected organoid replicate 4). Both the control and treated organoids were produced under the same experimental conditions, enabling a direct comparison between the two groups. Out of 250 expressed genes, 168 were found to be upregulated, while 82 were downregulated. The representation in Figure 2 allows researchers to identify key genes and pathways that are affected by the experimental conditions, providing a focused list of candidates for further biological investigation or validation studies.

3.2 Protein-Protein Interaction Study and Identification of Hub Gene

The *S. aureus* protein-protein interaction network consisted of 634 nodes and 15182 edges, shown at a 0.90 confidence level. CytoHubba used criteria like Degree, Betweenness, Closeness, EcCentricity and others to analyse the network shown in (Figure 3). To identify important hub genes, the degree parameter, which measures the number of direct connections a node has, was particularly useful. The gene "AKT1" had the highest degree, indicating maximum interactions with other genes in the network. This suggests that *AKT1* plays a crucial role in *S. aureus* pathogenesis and may be a target for antibacterial treatment.

3.3 Virtual Screening and Molecular Docking Studies

Literature Review and IMPPAT created a list of phytochemicals, resulting in a library of compounds. Canonical SMILES, names, and sources were obtained from PubChem. We confirmed interactions between active drugs and important targets identified through network pharmacology screening. Lower binding energies suggested higher affinity and greater stability in the interaction. The results of the molecular docking study revealed Isovaleric acid as the top candidate showing a with a binding energy of -4.9 kcal/mol with the AKT1 protein. This moderately strong binding affinity indicates a potential interaction between the ligand and target, though it may require further optimization for enhanced efficacy and specificity in clinical application and the ProTox-3.0 prediction for the Isovaleric acid revealed a predicted LD50 of 91mg/kg, classifying it under toxicity Class 3. Followed by Butyric acid (-4.81kcal/mol) and Hexanoic acid (-4.64kcal/mol) shown in Table 1 and Figure 4 shows the 3D visualisation of ligand, receptor, and interactively on the SeamDock.

3.4 *In vitro* Experiments

To validate *in silico* analysis results, *in vitro* experiments were performed by taking a plant sample. On the basis of available literature and results of *in silico* following samples were taken: fruit peel and leaf.

The findings of this study reveal that the fruit peel extract of *M. acuminata* in chloroform gave the maximum zone of inhibition against *S. aureus.* Chloroform extracts showed a mean inhibition zone value, depicting their efficiency in preventing the growth of bacteria as opposed to ethanol and acetone extracts. These results showed that Chloroform can be used as a solvent for extracting bioactive compounds with possible antibacterial activity from *M. acuminata* fruit peels. However, leaf extract in Acetone showed maximum zone of inhibition against *S aureus* (Figure 5).

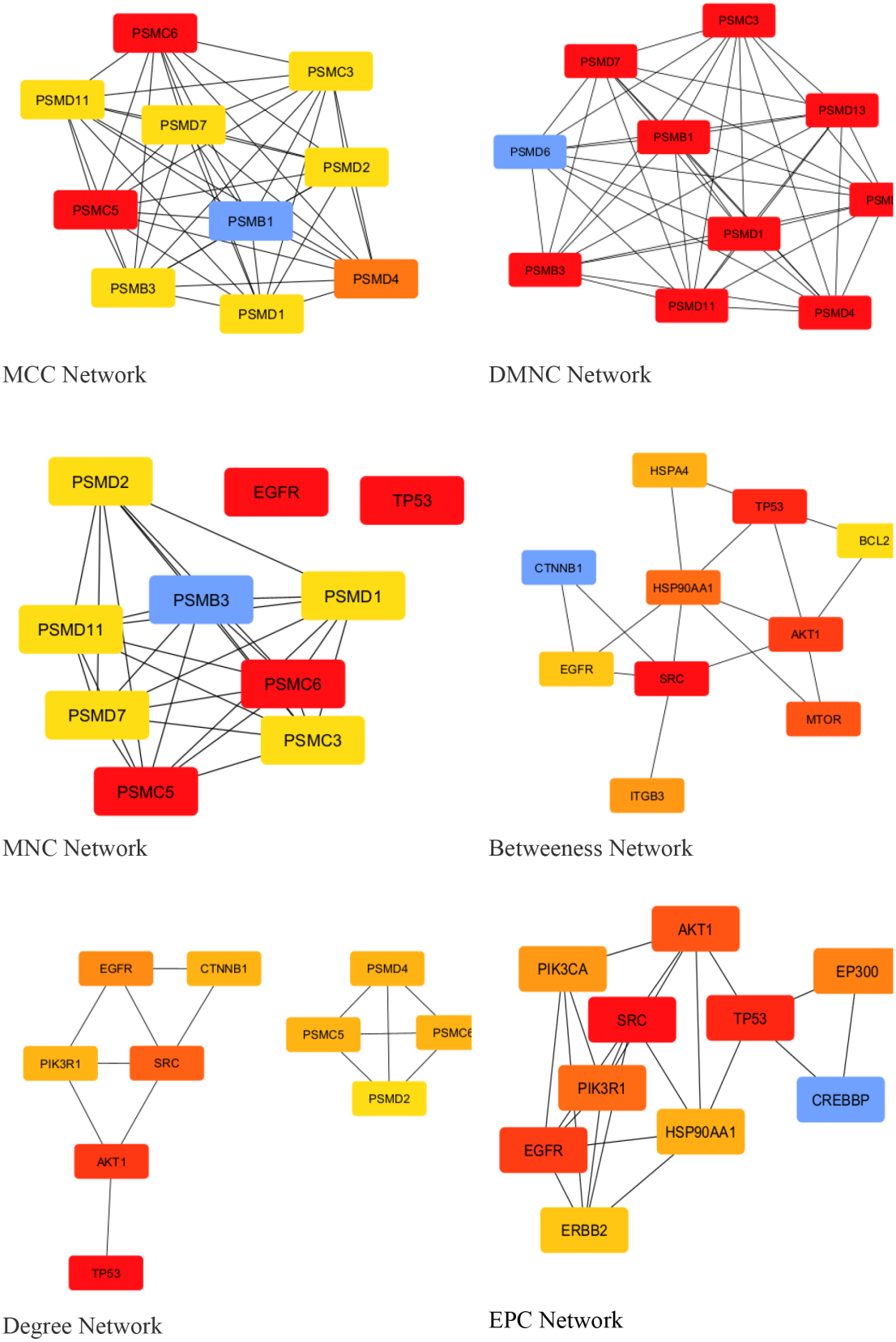
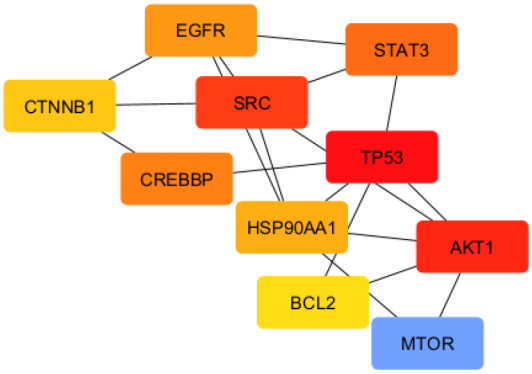
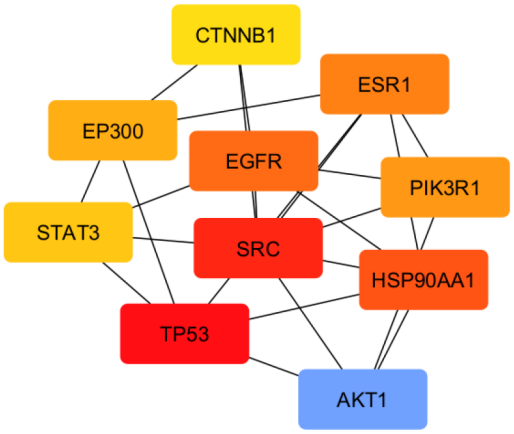


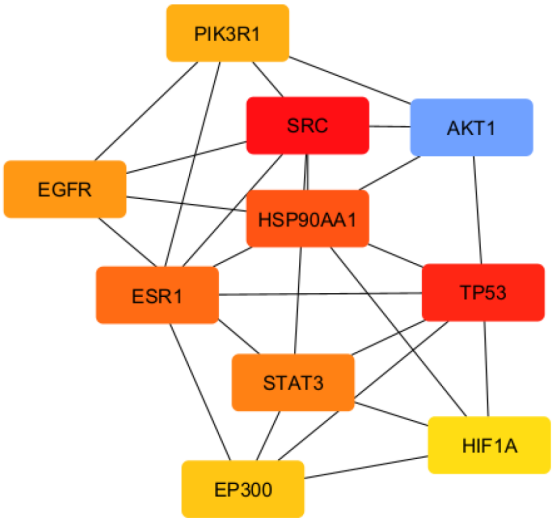
Figure 3. Protein-Protein Interaction (PPI) network of DEGs constructed using STRING and visualised in Cytoscape, highlighting key hub genes.



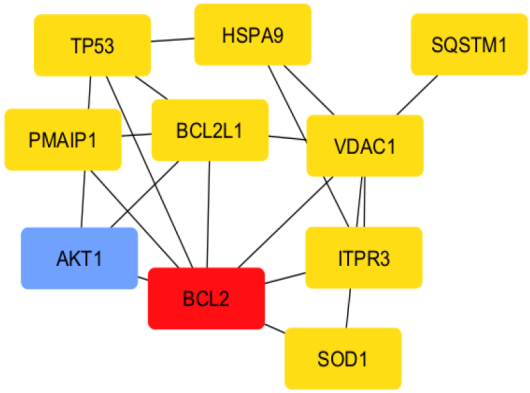
Bottleneck Network



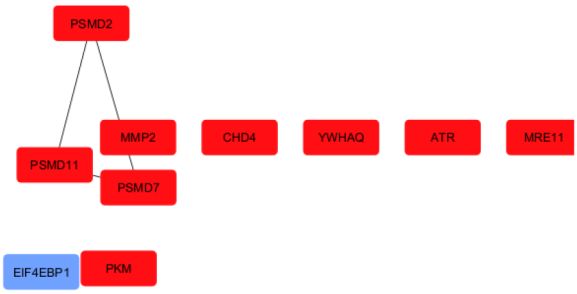
Closeness Network



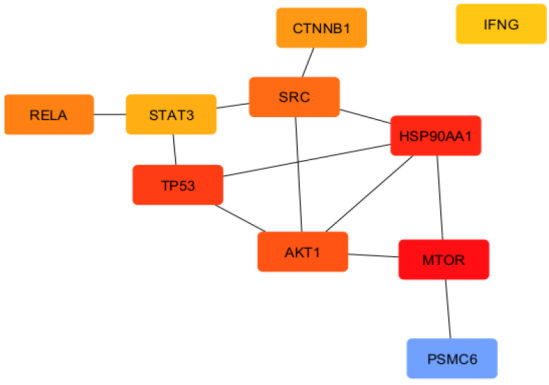
Radiality Network



EcCentricity Network



Clustering Coefficient Network



Stress Network

Figure 3. Continued...

Table 1. Results of virtual screening and molecular docking studies of selected bioactive compounds from *M. acuminata* against the AKT1 protein. The table includes canonical SMILES, binding affinity (kcal/mol), and Lipinski's rule of five status for drug-likeness.

Phytochemical name	Canonical SMILES	Binding energy	Lipinski rule
2-Heptanol	CCCCCC(C)O	-3.24	accepted
Isovaleric acid	**CC(C)CC(=O)O**	**-4.9**	**accepted**
Elemicin	COC1=CC(=CC(=C1OC)OC)CC=C	-3.78	accepted
1-Penten-3-OL	CCC(C=C)O	-3.47	accepted
2-Hexanol	CCCCC(C)O	-3.37	accepted
1-Butanol	CCCCO	-2.95	accepted
3-Phenylpropyl acetate	CC(=O)OCCCC1=CC=CC=C1	-4.39	accepted
Ethyl hexanoate	CCCCCC(=O)OCC	-2.93	accepted
Isoamyl alcohol	CC(C)CCO	-3.9	accepted
Eugenol	COC1=C(C=CC(=C1)CC=C)O	-4.39	accepted
2-Heptanone	CCCCCC(=O)C	-3.36	accepted
Ethyl 3-phenylpropionate	CCOC(=O)CCC1=CC=CC=C1	-4.36	accepted
2-Pentanol	CCCC(C)O	-3.07	accepted
Ethyl butyrate	CCCC(=O)OCC	-3.28	accepted
2-Phenylethanol	C1=CC=C(C=C1)CCO	-3.61	accepted
Hexanal	CCCCCC=O	-3.02	accepted
2-Pentanone	CCCC(=O)C	-3.14	accepted
Acetophenone	CC(=O)C1=CC=CC=C1	-4.4	accepted
Furfural	C1=COC(=C1)C=O	-3.66	accepted
1-Hexanol	CCCCCCO	-3.11	accepted
Hexanoic acid	CCCCCC(=O)O	-4.64	accepted
Ethyl acetate	CCOC(=O)C	-3.18	accepted
1-Octanol	CCCCCCCCO	-3.1	accepted
1-Pentanol	CCCCCO	-2.91	accepted
Butyl acetate	CCCCOC(=O)C	-3.26	accepted
Ethyl palmitate	CCCCCCCCCCCCCCCC(=O)OCC	-3.42	accepted
Methyl 2-methylbutyrate	CCC(C)C(=O)OC	-3.28	accepted
Hexyl isovalerate	CCCCCCOC(=O)CC(C)C	-3.82	accepted
Benzaldehyde	C1=CC=C(C=C1)C=O	-3.99	accepted
Ethyl isovalerate	CCOC(=O)CC(C)C	-3.6	accepted
Cyclohexanol	C1CCC(CC1)O	-3.89	accepted
Butyric acid	CCCC(=O)O	-4.81	accepted
cis-3-Hexen-1-ol	CCC=CCCO	-3.34	accepted
Methyl cinnamate	COC(=O)C=CC1=CC=CC=C1	-4.76	accepted
Ethyl cinnamate	CCOC(=O)C=CC1=CC=CC=C1	-4.36	accepted
Isoamyl butyrate	CCCC(=O)OCCC(C)C	-3.53	accepted
Methyl butyrate	CCOC(=O)CCC1=CC=CC=C1	-4.36	accepted
3-Nonen-2-ol	CCCCCC=CC(C)O	-3.99	accepted
Diethyl succinate	CCOC(=O)CCC(=O)OCC	-3.31	accepted
(E)-Hept-4-en-2-ol	CCC=CCC(C)O	-3.82	accepted
Methyl hexanoate	CCCCCC(=O)OC	-3.25	accepted
Dimethyl eugenol	CC1=C(C(=C(C=C1CC=C)OC)O)C	-4.26	accepted
4-Hexenyl butyrate	CCCC(=O)OCCCC=CC	-3.43	accepted
Tannic acid	C1=C(C=C(C(=C1O)O)O)C(=O)OC2=CC(=CC(=C2O)O)C(=O)OCC3C(C(C(C(O3)OC(=O)C4=CC(=C(C(=C4)OC(=O)C5=CC(=C(C(=C5)O)O)O)O)O)O)OC(=O)C6=CC(=C(C(=C6)OC(=O)C7=CC(=C(C(=C7)O)O)O)O)O)OC(=O)C8=CC(=C(C(=C8)OC(=O)C9=CC(=C(C(=C9)O)O)O)O)O)OC(=O)C1=CC(=C(C(=C1)OC(=O)C1=CC(=C(C(=C1)O)O)O)O)O		**rejected**

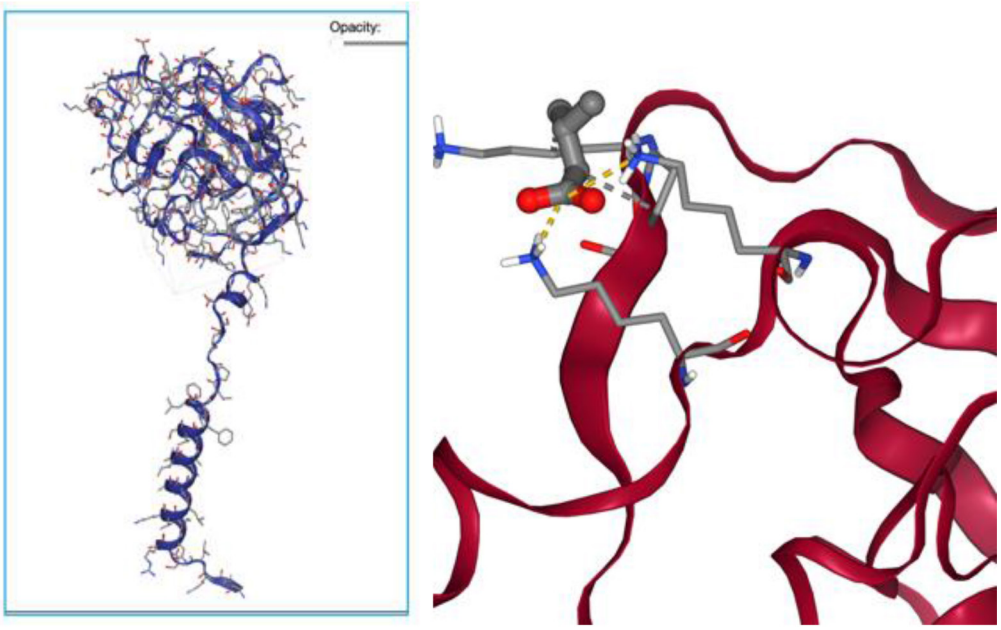


Figure 4. **(a)** Predicted 3D structure of the AKT1 protein generated using SWISS-MODEL. **(b)** Molecular docking interaction between Isovaleric acid and AKT1 protein, visualised with binding site residues.

Table 2. Mean diameter (mm)of inhibition zones for *M. acuminata* extracts against *S. aureus*. Values represent mean ± SD. ***p***values indicate statistical significance between different extracts (n = 3)

Solvents	Acetone	Chloroform	Methanol	Ethanol	Solvents	Acetone	Chloroform	Methanol	Ethanol
leaf	4	2	2	1	peel	2	3	2	1
leaf	5	3	1	2	peel	1	4	3	1
leaf	3	2	1	1	peel	0	3	1	1
Leaf	4	2	0	2	peel	0	2	2	1
Leaf	3	2	0	1	peel	2	2	4	1
mean	3.8 mm	2.2 mm	0.8 mm	2.4 mm	mean	1 mm	2.8 mm	2.4 mm	1 mm

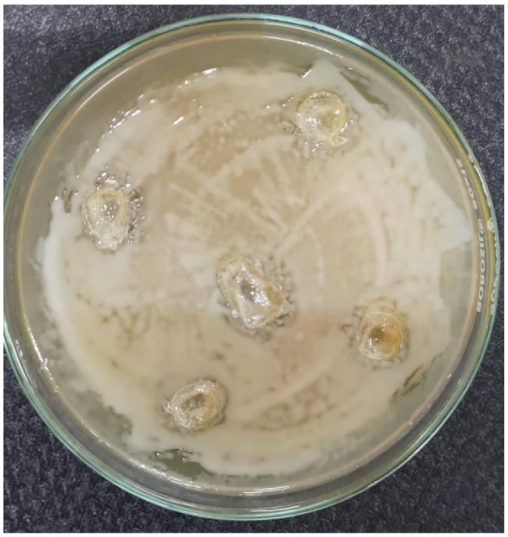
These results show that acetone can be used as a solvent for extracting bioactive compounds with possible antibacterial activity from *M. acuminata* leaves.

4. Discussion and Conclusion

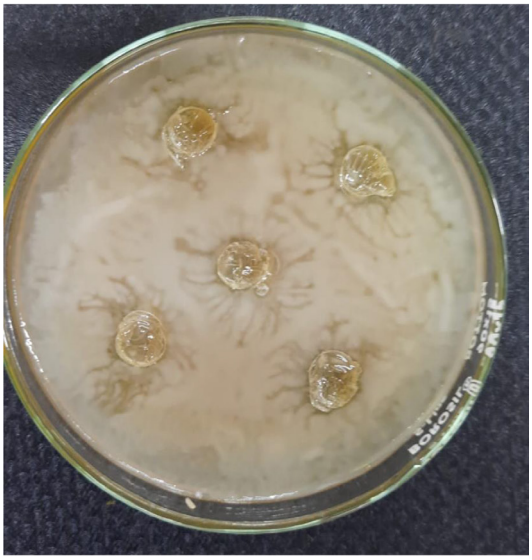
The study employed transcriptome analysis to explore silica- induced disease mechanisms in *Homo sapiens*, identifying 1,500 differentially expressed genes with 33 target genes prioritized through network biology. Among them, AKT1 emerged as a key hub gene linked to silicosis, making it a promising molecular target. Molecular docking revealed Isovaleric acid, a compound from *M. acuminata*, as the top candidate due to its strong binding affinity with AKT1. Despite its classification under toxicity class 3 (LD50 value of 91 mg/kg), the compound's biological activity and structural similarity to known therapeutic agents indicate drug development potential, contingent upon further structural optimisation and dosage evaluation.

Complementing the computational findings, *in vitro* antibacterial assays demonstrated the efficacy of chloroform-extracted *M. acuminata* peel against *S. aureus*, highlighting its potential as a natural antimicrobial agent.

In summary, this integrated *in silico* and *in vitro* study suggests that *M. acuminata* derived phytochemicals may offer therapeutic value in treating *in vivo* studies, pharmacokinetic profiling, and safety assessments



(a)



(b)

Figure 5. Zones of inhibition observed for *S. aureus* using. **(a)** Acetone-extracted *M. acuminata* leaf, **(b)** Chloroform-extracted *M. acuminata* peel.

essential to validate their clinical applicability and ensure translational success.

5. References

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