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Computer aided screening and evaluation of herbal therapeutics against MRSA infections

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Abstract:
Methicillin resistant *Staphylococcus aureus* (MRSA), a pathogenic bacterium that causes life threatening outbreaks such as community-onset and nosocomial infections has emerged as 'superbug'. The organism developed resistance to all classes of antibiotics including the best known Vancomycin (VRSA). Hence, there is a need to develop new therapeutic agents. This study mainly evaluates the potential use of botanicals against MRSA infections. Computer aided design is an initial platform to screen novel inhibitors and the data finds applications in drug development. The drug-likeness and efficiency of various herbal compounds were screened by ADMET and docking studies. The virulent factor of most of the MRSA associated infections are Penicillin Binding Protein 2A (PBP2A) and Panton-Valentine Leukocidin (PVL). Hence, native structures of these proteins (PDB: 1VQQ and 1T5R) were used as the drug targets. The docking studies revealed that the active component of *Aloe vera*, β-sitosterol (3S, 8S, 9S, 10R, 13R, 14S, 17R) -17- [(2R, 5R)-5-ethyl-6-methylheptan-2-yl] -10, 13-dimethyl 2, 3, 4, 7, 8, 9, 11, 12, 14, 15, 16, 17-dodecahydro-1H-cyclopenta [a] phenanthren-3-ol) showed best binding energies of -7.40 kcal/mol and -6.34 kcal/mol for PBP2A and PVL toxin, respectively. Similarly, Meliantriol (1S-1-[(2R, 3R, 5R)-5-hydroxy-3-[(3S, 5R, 9R, 10R, 13S, 14S, 17S)-3-hydroxy 4, 4, 10, 13, 14-pentamethyl-2, 3, 5, 6, 9, 11, 12, 15, 16, 17-decahydro-1H-cyclopenta[a] phenanthren-17-yl] oxolan-2-yl] -2-methylpropane-1, 2 diol), active compound in *Azadirachta indica* (Neem) showed the binding energies of -6.02 kcal/mol for PBP2A and -8.94 for PVL toxin. Similar studies were conducted with selected herbal compound based on pharmacokinetic properties. All *in silico* data tested *in vitro* concluded that herbal extracts of *Aloe-vera*, Neem, Guava (*Psidium guajava*), Pomegranate (*Punica granatum*) and tea (*Camellia sinensis*) can be used as therapeutics against MRSA infections.

Key words: Superbug, MRSA, PBP2A, PVL toxin, docking, β-sitosterol, Meliantriol

Background:
Methicillin resistant *Staphylococcus aureus* (MRSA) is a notorious pathogenic bacterium causing many infections and the disease control has become a serious issue worldwide **[1]**. The high outbreak of MRSA was observed in closed communities such as schools, prisons and sports teams and the disease has mainly transmitted from fomite to person and from person to person and so on **[2]**. The pathogenecity of the bacteria includes skin and soft tissue infections (SSTI), bone, joint and implant infections, pneumonia, septicemia and various toxicoses such as toxic shock syndrome, bloodstream infections, osteomyelitis, septic arthritis, and device-related infections, necrotizing fasciitis and purpura fulminans and abscesses **[3]**. Recent reports indicated the emergence of multidrug resistant Staphylococci against all classes of β-lactam antibiotics. The antibiotic resistance is mainly due to the expression of PC1 β-lactamase and the acquisition of mecA gene encoding a penicillin-binding protein, PBP2A **[4]**. The bacteria initially penetrate the host's immune system via epidermal and mucosal epithelia and the antimicrobial peptides play necessary role in

the host's innate immune defense against the initial colonization of bacteria **[5]**. It has been studied that many strains of MRSA contain genes that encode the toxin called Panton Valentine Leukocidin (PVL), bi-component leukocidal toxins (synergohymenotropic toxins) consists of F and S components. PVL is encoded by the lukPVoperon encoding the LukF-PV and LukS-PV components (cytotoxins) that lyse leukocyte. Hence, PVL positive Staphylococcal infections exist as life threatening infections of soft tissues and bones **[6]**.

Recent studies revealed that strains of MRSA developed resistance to conventional antibiotics and emerged as multidrug resistant superbugs. Hence, there is a significant demand of finding better therapeutic agents. The active substances present in many medicinal plants could be used as therapeutic alternatives for MRSA infection **[7]**. Computer aided method is a preliminary approach to screening novel therapeutic agents and the discipline is an emerging strategy as it reduces many complexities of drug discovery process. The screening of lead molecule with good pharmacological properties and drug likeness is a tedious task in drug development process. Computer aided method is an easy platform to search such kinds of biologically active compounds with favorable ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) and drug-likeness properties **[8]**. The study of receptor-ligand interaction is a fundamental concept of rational drug design and the prediction of such interactions by computational docking has increasing importance in the field of structure based drug discovery **[9]**.

The study mainly focused to screen potential herbal inhibitors against the drug targets of MRSA by computer based ADMET and docking studies. The data obtained from *in silico* studies were later used for *in vitro* studies. The study also provides a comparative account of the efficiency of herbal compounds to that of known antibiotics.

Methodology:

Identification of potential ligands

Since MRSA has emerged as a multidrug resistant organism the design of alternative therapies are very crucial to treat MRSA outbreaks. There are reports that many herbal based compounds have high medicinal values against many pathogens. Hence, an initial survey of 74 compounds from 13 different medicinal plants was carried out by extensive literature studies. It is possible to find out the druggish properties of herbal compounds by computer aided screening and the data will be useful to screen best lead molecules. The 3D structures of all plant based ligands are available in drug data base and the structures were retrieved from PubChem **[10]**, KEGG **[11]** and Chemspider **[12]**.

ADMET studies and screening of ligands

The ligands with good pharmacological and druggish properties are very crucial for structure based drug discovery. The absorption, distribution, metabolism, excretion and toxicity (ADMET) are the most important part of pharmacological studies of lead molecules and these can be predicted by computational biology tools. Hence, all 74 ligands identified were tested for their drug-likeliness, ADME profile and toxicity analysis by Pre-ADMET. The ADME includes the extent and rate of absorption, distribution, metabolism and excretion. Absorption checks how the substance is entering to the blood circulation, distribution explains how substances are disseminating throughout the fluids and tissues of the body, metabolism is the irreversible transformation of parent compounds into daughter metabolites and excretion explains how the substances are excreted from the body after metabolism. Pre-ADMET uses Caco2-cell (heterogeneous human epithelial colorectal adenocarcinoma cell lines) and MDCK (Madin-Darby Canine Kidney) cell models for oral drug absorption prediction and skin permeability, and human intestinal absorption model for oral and trans-dermal drug absorption prediction. Similarly, the programme uses BBB (blood brain barrier) penetration and plasma protein binding models to predict the distribution. Pre-ADMET predicts toxicity based on the mutagenicity of AMES parameters and rodent carcinogenicity assays of rat and mouse **[13]**.

Selection of target proteins and molecular docking

The receptor and ligand interaction is the key mechanism of computer aided drug design. Previous studies reported that Penicillin Binding Protein 2A (PBP2A) and Panton-Valentine Leukocidin (PVL) are the major virulent factors of MRSA infections **[14, 15]**. Hence, the native structures of the proteins are used as drug targets. The crystal structures of PBP2A (1VQQ) and PVL toxin (1T5R) were retrieved from Protein Data Bank and the coordinate files were used for the study. The selected herbal compounds were docked with target proteins by AutoDock. The program uses a Monte Carlo simulated annealing for configurational exploration using grid based molecular affinity potentials and provides bioactive conformation by energy minimization **[16]**.

***In vitro* studies**

The data generated by computer aided studies should be tested experimentally for further confirmation. Based on the pharmacological properties studied by ADMET & docking studies, five botanicals were identified for *in vitro* testing. The botanicals used in the study are *Azadirachta indica* **[17]**, *Aloe vera* **[18]**, *Camellia sinensis* **[19]**, *Punica granatum* **[20]** and *Psidium guajava* **[21]**. The herbals were collected from Horticulture Centre-Hulimavu, Bangalore. The leaf extracts were prepared by standard solvent extraction method and the antimicrobial activity of each extract was tested in different concentrations. The pure culture of MRSA was swabbed on Muller Hinton agar plates which have many wells. The extracts were added in different concentration to each well (well diffusion method) and the plates were incubated at 37^{o}C for 24 hours. The antimicrobial activity was determined by measuring the zone of inhibition. The efficiency of herbal extracts was also compared with known antibiotics. The antibiotic susceptibility patterns were carried out by disc diffusion method. The sensitivity patterns of each antibiotic were confirmed by measuring the zone of inhibition around the disc and compared with standard antibiotic susceptibility chart.

Discussion:

The solution for MRSA associated outbreaks are critical problem because the organism recently emerged as VRSA. Hence, the discovery of an alternative treatment has profound scope and significance. Drug discovery process is time consuming and multistep process which includes many preclinical and clinical trials. One of the major reasons for drug failures is the poor drug-likeness and pharmacokinetic properties of lead compounds. Computer aided method is a rapid and significant screening approach because it selects the lead molecules with good pharmacological and druggish properties. We have selected 74 active compounds from herbal origin such as leaf extracts of *Azadirachta indica* (Neem), *Psidium*

guajava (Guavas), *Aloe vera*, *Ocimum tenuiflorum* (Tulsi), *Curcuma longa* (Turmeric), *Allium sativum* (Garlic), *Punica granatum* (Pomegranate), *Eucalyptus*, *Camellia sinensis* (Tea) and the oil of *Syzygium aromaticum* (Clove). The 3D structures of the ligands were retrieved from drug databases and most of lead molecules qualified the properties of drugs. However, few ligands were found to be better as per Lipinski's rule of 5, CMC-like rule and MDDR-like rule **(Table 1, see supplementary material).**

Another important concern in drug discovery is the pharmacokinetics properties. The ADME profiles of selected drug candidates are shown in **Table 2 (see supplementary material).** *In silico* toxicity prediction is the final step of pharmacokinetic screening of lead molecules and 30% of drug failures are due to toxicity issues. In this study, we have found that the predicted toxicities of many herbal compounds were suitable for further studies. Meliantriol from *Azadirachta indica*, β-sitosterol from *Alo vera*, ursolic acid and lupeol from *Ocimum tenuiflorum*, brevifolin and ellagic acid from *Punica granatum*, corilagin from *Camellia sinensis*, and many more showed better pharmacokinetic and drug-likeness properties **(Table 3, see supplementary material).** Hence, these lead molecules were identified as the best ligands for docking studies. All these herbal extracts were reported to have high medicinal potential against many pathogenic bacteria and we have tested efficiency of the same against MRSA by computer aided approach and *in vitro* studies.

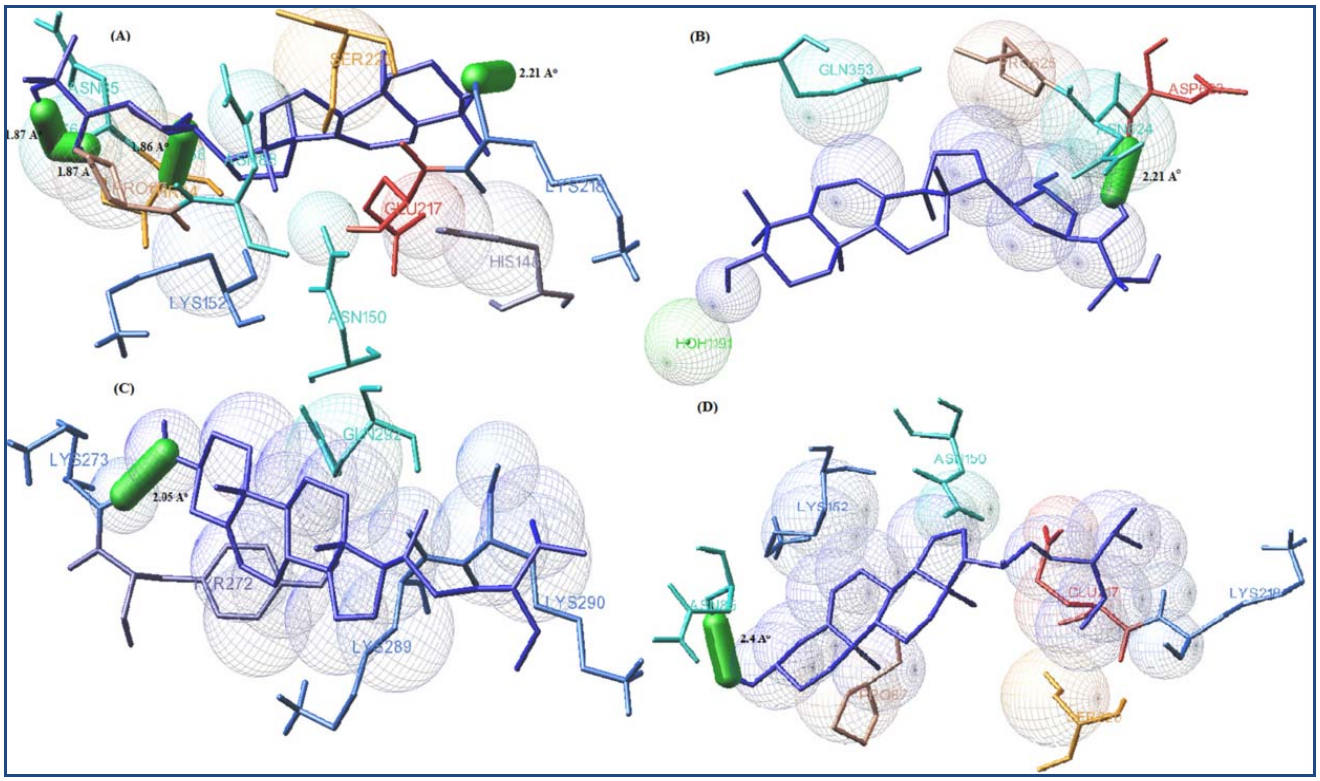

Figure 1: The interaction of Meliantriol and β-Sitosterol against MRSA drug targets- Penicillin Binding Protein 2A (PBP2A) and Panton-Valentine Leukocidin (PVL) generated by computer aided docking. Neem active compound Melantriol interacts with PVL toxin by four hydrogen bonds (represented as green coloured sticks) and the interacting residues are LYS218 of chain A, THR 84, ASN 85 and ASN 86 of chain E. The lengths of the hydrogen bonds are 2.1 Å, 1.86 Å, 1.8 Å and 1.87 Å respectively **(Figure A).** Melantriol interacted with PBP2A by ASP 624 of Chain A and the length of the bond formed was 2.21 Å **(Figure B).** The ligand effectively binds with both PBP2A and PVL toxin with minimum binding energies of -6.02 kcal/mol and -8.94 kcal/mol respectively. **Figure C and D** showed the inhibitory activity of *Aloe vera* active compound β-sitosterol against both the targets. The ligand interacted to PBP2A by LYS273 (Chain A) with hydrogen bond of length 2.05 Å **(Figure C).** Similarly, β-sitosterol interacted with PVL toxin by ASN 85 of Chain A with one hydrogen bond of 2.4 Å **(Figure D).** β-sitosterol is also identified as best lead molecule against MRSA targets with minimum binding energy of -7.40 kcal/mol for PBP2A and -6.34 kcal/mol for PVL toxin. The docking studies revealed that herbal compounds have good pharmacological activities against MRSA targets.

The key proteins involved in MRSA infections are PBP2A and PVL toxins. Hence, these proteins have been selected as probable drug targets. The crystal structure of Penicillin Binding Protein 2A (PDBID: 1VQQ) was retrieved from the PDB. The protein consists of two chains and three penicillin binding protein domains. Chain A is the key domain consists of 646 amino acids and constitutes 33% helices (31 helices; 214 residues) and 26% beta sheet (34 strands; 169 residues) which are important for the functional activity. The crystal structure of Panton-Valentine Leucocidin (PDBID: 1T5R) was also retrieved from PDB and it has 8 chains. All chains have 284 amino acid residues and constitute 3% helices (3 helices; 11 residues) and 59% beta sheet (22 strands; 168 residues). The interactions between selected inhibitors and target proteins were studied by docking simulations. Among antibiotics, Vancomycin, best drug currently available against MRSA, showed the best binding energy value of -8.91 kcal/mol. However, recent reports indicate that the organism developed resistance against Vancomycin and emerged as Vancomycin resistant *Staphlococcus areus* (VRSA). The binding efficiencies of herbal

active compounds also tested against the target proteins by molecular docking. The docking scores and binding energies of all selected ligands with both the targets were shown in **Table 4 (see supplementary material).** As the best, Meliantriol, active inhibitor present in Neem, showed binding energies of -6.02 kcal/mol with PBP2A and -8.94 kcal/mol with PVL toxin. Similarly, β-sitosterol, an antimicrobial agent present in medicinal aloe, showed binding energies of -7.40 kcal/mol and -6.34 kcal/mol for PBP2A and PVL toxin respectively **(Figure 1).** The docking studies reveled that herbal compounds are better inhibitors than chemical drugs; Meliantriol and similar compounds are the potential lead molecules than Vancomycin. As per computer aided studies plant derived inhibitors have high therapeutic value and it should be evaluated through *in vitro* studies.

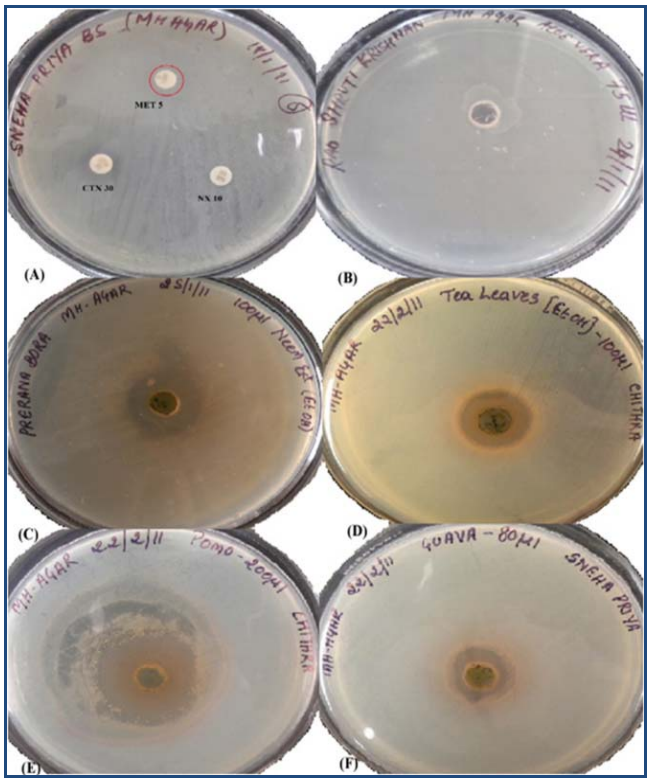

Figure 2: The therapeutic potential of selected herbal based compounds against MRSA confirmed by *in vitro* studies. The antibiotic susceptibility pattern was identified by Kirby-Bauer disc diffusion method. The resistance pattern of *Staphylococcus areus* against Methicillin is encircled and which was characterized by lack of zone of inhibition implies resistance. Similarly the organism shows resistance against Cefatoxime (30 mcg/disc), Norflaxacin (10 mcg/disc) and other antibiotics (**Table 5, see supplementary material**). Hence, the treatment with antibiotics are challenging in future. Figure B to F indicate the efficiency of herbal compounds against MRSA. As confirmed by computer aided screening neem active compound melantriol has significant antimicrobial activity against MRSA, represented by the zone of inhibition (C) around the well (well diffusion method). Figure B shows that the inhibitory activity of β-sitosterol (*Aloe vera* active compound) characterized by zone of clearance around the well, which was selected from docking studies. As screened by the computer aided approach, the active compound present in tea leaf (D), pomogranate (E), and guava (F) showed good inhibitory properties against MRSA. Thus, the study clearly indicates that herbal compounds have good therapeutic value against MRSA than antibiotics.

Since the data generated by computer aided method has just a hypothetical solution it should be tested *in vitro* also. We have studied and compared the efficiency of antibiotics and selected herbal extracts (The active compounds of those extracts found to be effective by docking studies) against pure culture of MRSA. The method employed was disc diffusion and well diffusion techniques. Our study revealed that the organism resistant to wide range of antibiotics such as Moxifloxacin, Cefatoxime, Rifampicin, Oxacillin, Co-Trimoxazole, Chloramphenicol Ciprofloxacin, Ceftazidime, Nalidixic acid, Tetracycline, Streptomycin, Erythromycin, Ampicillin, Norfloxacin and Methicillin. The organism is susceptible to only few antibiotics, Vancomycin is the best one. Moreover, recent studies on Vancomycin resistant *Staphylococcus areus* (VRSA) indicated that antibiotic treatments are challenging and pointing out 'the end of antibiotic era' is very near in future. The susceptibility pattern of various antibiotics against MRSA is shown in **Table 5 (see supplementary material)**. Nonetheless, interestingly, as confirmed by the computer aided approach, the leaf extracts of Neem, Tea, Guava and Pomegranate showed better antimicrobial properties at a concentration of 100 µl, 100 µl, 80 µl and 200 µl respectively, which implies the potential of herbal extract against MRSA **(Figure 2)**. The computer aided method is useful to screen best ligands from wide range of compounds which will reduce the complexities of drug discovery process. Subsequently, the study needs more clinical testing using laboratory animals and further *in vivo* studies should be conducted. The study would provide all initial inputs to design novel therapeutics against MRSA.

Conclusion:

Present studies revealed that the mortality rate of MRSA infection is very high and the organism is resistant to Vancomycin (VRSA), the best chemotherapeutic agent available against MRSA. This study concluded that computer aided screening is an effective alternative for identification of novel remedies when all antibiotics seems to have failed. Several natural herbal compounds are screened and their effectiveness against MRSA is compared with known antibiotics. Computer aided ADME and toxicity prediction favored to screen best ligands with good pharmacological activities. It was found that herbal compounds such as Melantriol, β- Sitosterol and similar compounds have good inhibitory activity against Penicillin Binding Protein 2A and Panton-Valentine Leukocidin toxin of MRSA, key virulent factors of MRSA infections. The *in silico* data have been tested *in vitro* also, as suggested by computer aided method, herbal extracts of neem, medicinal aloe, tea, pomegranate and guava were identified as potential therapeutics against MRSA. The study concluded that, though the organism emerged as a multidrug resistant "superbug" there is high scope and application of herbal remedies towards MRSA infections.

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Supplementary material:

Table 1: Drug-likeliness prediction of selected herbal compounds using Pre-ADMET tool. The main rules used to test the drug likeliness were Lipinski's Rule, Lead like Rule, CMC-like rule, MDDR-like rule and WDI-like rule. Melantriol and β-sitosterol have qualified most of the rules and these compounds have selected for further studies.

Active Component in plant extract	Source	Lipinski's Rule	Lead like Rule	CMC-like rule	MDDR-like rule	WDI-like rule
Nimbin	Neem	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Quercetin	Neem	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Meliantriol	Neem	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Aloin	*Aloe vera*	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Emodin	*Aloe vera*	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Salicyclic acid	*Aloe vera*	Suitable	Violated	Not qualified	Non-druglike	Out of 90% cutoff
Cholestrol	*Aloe vera*	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Campesterol	*Aloe vera*	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Lupeol	*Aloe vera*	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
β -sitosterol	*Aloe vera*	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Oleanolic acid	Tulsi	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Ursolic acid	Tulsi	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Rosmarinic acid	Tulsi	Suitable	Violated	Qualified	Midstructure	In 90% cutoff
Eugenol	Tulsi	Suitable	Suitable	Qualified	Midstructure	Out of 90% cutoff
Carvacrol	Tulsi	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Linalool	Tulsi	Suitable	Suitable	Not qualified	Midstructure	Out of 90% cutoff
Beta-caryophyllene	Tulsi	Suitable	Violated	Qualified	Midstructure	In 90% cutoff
Methyl cinnamate	Tulsi	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Apigenin	Tulsi	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Luteolin	Tulsi	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
curcumin	Turmeric	Suitable	Violated	Not qualified	Midstructure	out of 90% cutoff
Demethoxy curcumin	Turmeric	Suitable	Violated	Qualified	Midstructure	Out of 90% cutoff
Tumerone	Turmeric	Suitable	Violated	Qualified	Midstructure	In 90% cutoff
Diaryl heptanoid	Turmeric	Violated	Violated	Not qualified	Midstructure	Out of 90% cutoff
Allicin	Garlic	Suitable	Suitable	Not qualified	Midstructure	Out of 90% cutoff
Diallyl sulphide	Garlic	Suitable	Suitable	Not qualified	Non-druglike	In 90% cutoff
Diallyl disulphide	Garlic	Suitable	Suitable	Not qualified	Non-druglike	In 90% cutoff
Brevifolin	Pomogranate	Suitable	Suitable	Qualified	Midstructure	Out of 90% cutoff
Ellagic acid	Pomogranate	Suitable	Suitable	Not qualified	Non-druglike	In 90% cutoff
Gallic acid	Pomogranate	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Epigallacatechin gallate	Tea	Violated	Violated	Qualified	Midstructure	Out of 90% cutoff
Corilagin	Tea	Violated	Violated	Not qualified	Midstructure	Out of 90% cutoff
Thymol	Clove	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Alpha-pinene	Eucalyptus	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Benzyl benzoate	Eucalyptus	Suitable	Violated	Qualified	Midstructure	In 90% cutoff

Geranial	Eucalyptus	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Limonene	Eucalyptus	Suitable	Violated	Not qualified	Midstructure	In 90% cutoff
Ethyl cinnamate	Eucalyptus	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Terpinen	Eucalyptus	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Thujene	Eucalyptus	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Cinnamaldehyde	Cinnamon	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Cinnamyl alcohol	Cinnamon	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Cinnamyl acetate	Cinnamon	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Ferulic acid	Guava	Suitable	Violated	Qualified	Midstructure	Out of 90% cutoff
Ascorbic acid	Guava	Suitable	Violated	Not qualified	Midstructure	In 90% cutoff
Gallic acid	Guava	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Casuarinin	Guava	Violated	Violated	Not qualified	Midstructure	Out of 90% cutoff
Cyanidin	Guava	Violated	Violated	Not qualified	Midstructure	Out of 90% cutoff
Geraniol	Lemon Grass	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Citral	Lemon Grass	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Geranic acid	Lemon Grass	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Chlorogenic acid	Lemon Grass	Suitable	Violated	Not qualified	Midstructure	In 90% cutoff
Orientin	Lemon Grass	Violated	Violated	Qualified	Midstructure	Out of 90% cutoff
Myrcene	Lemon Grass	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Beta pinene	Lemon Grass	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Linalool	Lemon Grass	Suitable	Suitable	Not qualified	Midstructure	Out of 90% cutoff
Farnesol	Lemon Grass	Suitable	Violated	Qualified	Midstructure	Out of 90% cutoff
Barneol	Lemon Grass	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Caffeic acid	Lemon Grass	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Alpha Tocopherols	Grape	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Beta carotene	Grape	Violated	Violated	Not qualified	Midstructure	Out of 90% cutoff
Benzethonium	Grape	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Bergamottin	Grape	Suitable	Violated	Qualified	Midstructure	Out of 90% cutoff
Bergapten	Grape	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Citric acid	Grape	Suitable	Violated	Not qualified	Midstructure	Out of 90% cutoff
Azadirachtin	Grape	Violated	Violated	Qualified	Midstructure	Out of 90% cutoff
Limonin	Grape	Violated	Violated	Qualified	Midstructure	Out of 90% cutoff
Methyl paraben	Grape	Suitable	Suitable	Not qualified	Midstructure	In 90% cutoff
Narangenin	Grape	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Narangin	Grape	Violated	Violated	Not qualified	Midstructure	Out of 90% cutoff

Nootkatone	Grape	Suitable	Violated	Qualified	Midstructure	In 90% cutoff
Putrescine	Grape	Suitable	Violated	Not qualified	Non-druglike	In 90% cutoff
Resveratron	Grape	Suitable	Suitable	Qualified	Midstructure	In 90% cutoff
Triclosan	Grape	Suitable	Violated	Qualified	Midstructure	Out of 90% cutoff

Table 2: ADME prediction of herbal compounds in plant extract using Pre-ADMET tool. ADME properties of Melantriol and β sitosterol showed that these compounds are good lead molecules.

Active compound in Plant extract	Source	Human Intestinal Absorption	Caco2 Cell Permeability	MDCK Cell Permeability	Skin Permeability	Blood Brain Barrier Penetration
Nimbin	Neem	Well absorbed	Middle	Low	-2.894	Middle
Quercetin	Neem	Moderate	Low	Low	-4.433	Middle
Meliantriol	Neem	Well absorbed	Middle	Low	-3.095	Middle
Aloin	*Aloe vera*	Poor	Middle	Low	-4.7114	Low
Emodin	*Aloe vera*	Well absorbed	Middle	Middle	-4.29575	Middle
Salicyclic acid	*Aloe vera*	Well absorbed	Middle	Middle	-2.04714	Middle
Cholestrol	*Aloe vera*	Well absorbed	Middle	Middle	-0.64315	High
Campesterol	*Aloe vera*	Well absorbed	Middle	Low	-0.62172	High
Lupeol	*Aloe vera*	Well absorbed	Middle	Low	-1.9778	High
β -sitosterol	*Aloe vera*	Well absorbed	Middle	Low	-0.59344	High
Oleanolic acid	Tulsi	Well absorbed	Middle	Low	-2.354	High
Ursolic acid	Tulsi	Well absorbed	Middle	Low	-2.1509	High
Rosmarinic acid	Tulsi	Moderate	Middle	Low	-3327	Middle
Eugenol	Tulsi	Well absorbed	Middle	Middle	-1.3109	High
Carvacrol	Tulsi	Well absorbed	Middle	Middle	-1.04552	Middle
Linalool	Tulsi	Well absorbed	Middle	Middle	-0.895	High
Beta-caryophyllene	Tulsi	Well absorbed	Middle	Middle	-0.676	High
Methyl cinnamate	Tulsi	Well absorbed	Middle	Middle	-1.423	Middle
Apigenin	Tulsi	Well absorbed	Middle	Middle	-4.1457	Middle
Luteolin	Tulsi	Well absorbed	Middle	Low	-4.28	Middle
curcumin	Turmeric	Moderately absorbed	Middle permeablity	Low permeablity	-3.62686	Middle absorption to CNS
Demethoxy curcumin	Turmeric	Well absorbed	Middle permeablity	Middle permeablity	-2.21821	Middle absorption to CNS
Tumerone	Turmeric	Well absorbed	Middle permeablity	Middle permeablity	-0.92884	High absorption to CNS
Diaryl heptanoid	Turmeric	Well absorbed	Middle permeablity	Low permeablity	-2.25168	Middle absorption to CNS
Allicin	Garlic	Well absorbed	Middle permeablity	Middle permeablity	-1.36911	Middle absorption to CNS
Diallyl sulphide	Garlic	Well absorbed	Middle permeablity	Low permeablity	-1.17034	Middle absorption to CNS
Diallyl disulphide	Garlic	Well absorbed	Middle permeablity	Low permeablity	-1.08723	Middle absorption to CNS
Brevifolin	Pomogranate	Well absorbed	Middle permeablity	Middle permeablity	-2.39945	Middle absorption to CNS
Ellagic acid	Pomogranate	Well absorbed	Middle permeablity	Low permeablity	-1.15024	Middle absorption to CNS
Gallic acid	Pomogranate	Moderately absorbed	Middle permeablity	Low permeablity	-3.62686	Middle absorption to CNS
Epigallacatechin gallate	Tea	Moderately absorbed	Middle permeablity	Low permeablity	-3.9954	Low absorption to CNS
Corilagin	Tea	Poorly absorbed	Middle permeablity	Low permeablity	-4.32617	Low absorption to CNS
Thymol	Clove	Well absorbed	Middle	Middle	-1.06531	High

Alpha-pinene	Eucalyptus	Well absorbed	Middle	Middle	-1.44636	High
Benzyl benzoate	Eucalyptus	Well absorbed	Middle	Low	-2.03706	High
Geranial	Eucalyptus	Well absorbed	Middle	Middle	-0.96121	High
Limonene	Eucalyptus	Well absorbed	Middle	Middle	-0.8341	High
Ethyl cinnamate	Eucalyptus	Well absorbed	Middle	Middle	-1.33427	Middle
Terpinen	Eucalyptus	Well absorbed	Middle	Middle	-1.30527	High
Thujene	Eucalyptus	Well absorbed	Middle	Middle	-1.43448	High
Cinnamaldehyde	Cinnamon	Well absorbed	Middle	Middle	-1.3358	Middle
Cinnamyl alcohol	Cinnamon	Well absorbed	Middle	Middle	-1.38467	Middle
Cinnamyl acetate	Cinnamon	Well absorbed	Middle	Middle	-1.36474	Middle
Ferulic acid	Guava	Well absorbed	Middle	Middle	-1.872	High
Ascorbic acid	Guava	Moderately absorbed	Low	Low	-5.14959	Middle
Gallic acid	Guava	Moderately absorbed	Middle	Low	-3.62686	High
Casuarinin	Guava	Poorly absorbed	Middle	Low	-3.1221	Middle
Cyanidin	Guava	Well absorbed	Low	Middle	-4.13468	High
Geraniol	Lemon Grass	Well absorbed	Middle	Middle	-1.05921	High
Citral	Lemon Grass	Well absorbed	Middle	Middle	-0.9612	High
Geranic acid	Lemon Grass	Well absorbed	Middle	Middle	-1.07	High
Chlorogenic acid	Lemon Grass	Moderately absorbed	Middle	Low	-3.89403	Middle
Orientin	Lemon Grass	Poorly absorbed	Low	Low	-4.684	Middle
Myrcene	Lemon Grass	Well absorbed	Middle	Middle	-0.6329	High
Beta pinene	Lemon Grass	Well absorbed	Middle	Middle	-1.3583	High
Linalool	Lemon Grass	Well absorbed	Middle	Middle	-0.8956	High
Farnesol	Lemon Grass	Well absorbed	Middle	Middle	-0.6274	High
Barneol	Lemon Grass	Well absorbed	Middle	Middle	-1.88173	High
Caffeic acid	Lemon Grass	Well absorbed	Middle	Middle	-2.6699	High
Alpha Tocopherols	Grape	Well absorbed	Middle	Middle	-0.51599	High
Beta carotene	Grape	Well absorbed	Middle	Middle	-0.60917	High
Benzethonium	Grape	Well absorbed	Middle	Low	-0.8867	Middle
Bergamottin	Grape	Well absorbed	Middle	Low	-2.4988	High
Bergapten	Grape	well absorbed	Middle	Middle	-3.7077	High
Citric acid	Grape	Poorly absorbed	Middle	Low	-3.94504	Middle
Azadirachtin	Grape	Moderately absorbed	Middle	Low	-4.67787	Middle
Limonin	Grape	Well absorbed	Middle	Low	-3.733	Middle
Methyl paraben	Grape	Well absorbed	Middle	Middle	-2.009	High
Narangenin	Grape	Well absorbed	Middle	Middle	-4.1802	High
Narangin	Grape	Poorly absorbed	Middle	Low	-4.54	High
Nootkatone	Grape	Well absorbed	Middle	Middle	-0.899	High
Putrescine	Grape	Well absorbed	Middle	Low	-4.091	Middle
Resveratron	Grape	Well absorbed	Middle	Middle	-3.1525	High
Triclosan	Grape	Well absorbed	Middle	Middle	-2.2848	High

Table 3: Toxicity prediction of selected herbal compounds by Pre-ADMET tool. The toxicity testing have given satisfactory results for both Melantriol and β-sitosterol.

Active compound in plant extract	Source	AMES Test							Carcinogenicity	
		TA_{100} +s9	TA_{100} -s9	TA_{1535} +s9	TA_{1535} -s9	TA_{98} +s9	TA_{98} -s9	Result	Mouse	Rat
Quercetin	Neem	-	+	-	-	-	+	Mutagen	-	+
Meliantriol	Neem	-	-	-	-	-	-	Non mutagen	-	-
Aloin	Neem	-	-	-	-	+	-	Mutagen	-	-
Emodin	*Aloe vera*	-	+	-	-	+	+	Mutagen	-	+
Salicyclic acid	*Aloe vera*	+	+	+	-	+	-	Mutagen	-	-
Cholestrol	*Aloe vera*	-	-	-	-	-	-	Non mutagen	+	-
Campesterol	*Aloe vera*	-	-	-	-	-	-	Non mutagen	+	-
Lupeol	*Aloe vera*	-	-	-	-	-	+	Mutagen	-	+
β -sitosterol	*Aloe vera*	-	-	-	-	-	-	Non mutagen	-	-
Oleanolic acid	*Aloe vera*	-	-	-	-	-	-	Non	+	+

								mutagen		
Ursolic acid	Tulsi	-	-	-	-	-	-	Non mutagen	+	+
Rosmarinic acid	Tulsi	-	-	-	-	-	-	Non mutagen	-	+
Eugenol	Tulsi	+	+	+	-	+	+	Mutagen	+	+
Carvacrol	Tulsi	+	-	+	-	+	+	Mutagen	-	-
Linalool	Tulsi	+	-	+	-	-	-	Mutagen	-	-
Beta-caryophyllene	Tulsi	-	-	-	-	-	+	Mutagen	-	+
Methyl cinnamate	Tulsi	+	+	-	-	+	-	Mutagen	-	-
Apigenin	Tulsi	-	+	-	-	-	+	Mutagen	-	+
Luteolin	Tulsi	-	+	-	-	-	=	Mutagen	-	+
curcumin	Tulsi	-	+	+	-	-	-	Mutagen	-	+
Demethoxy curcumin	Turmeric	-	-	-	-	-	-	Non mutagen	-	+
Tumerone	Turmeric	-	-	-	-	+	-	Mutagen	+	+
Diaryl heptanoid	Turmeric	-	-	-	-	-	-	Non mutagen	-	-
Allicin	Turmeric	-	-	+	-	-	-	Non mutagen	+	-
Diallyl sulphide	Garlic	-	-	-	-	-	-	Non mutagen	+	+
Diallyl disulphide	Garlic	-	-	-	-	_	_	Non mutagen	+	+
Brevifolin	Garlic	-	+	-	-	+	-	Mutagen	-	+
Ellagic acid	Pomogranate	-	-	-	-	-	-	Non mutagen	+	+
Gallic acid	Pomogranate	-	+	+	-	-	-	Mutagen	-	+
Punicafolin	Pomogranate									
Epigallacatechin gallate	Tea	-	-	-	-	-	-	Non mutagen	-	+
Corilagin	Tea	-	-	-	-	-	-	Non mutagen	-	-
Thymol	Clove	+	+	+	-	+	+	Mutagen	-	-
Alpha-pinene	Eucalyptus	+	-	-	-	-	-	Mutagen	-	+
Benzyl benzoate	Eucalyptus	+	+	-	-	+	`	Mutagen	-	-
Geranial	Eucalyptus	-	+	+	-	+	-	Mutagen	-	+
Limonene	Eucalyptus	-	-	-	-	+	+	Mutagen	-	+
Ethyl cinnamate	Eucalyptus	+	+	-	-	+	-	Mutagen	-	-
Terpinen	Eucalyptus	-	-	+	-	-	+	Mutagen	+	-
Thujene	Eucalyptus	+	-	-	-	+	-	Mutagen	+	+
Cinnamaldehyde	Cinnamon	+	-	-	-	+	-	Mutagen	-	-
Cinnamyl alcohol	Cinnamon	+	-	-	-	+	-	Mutagen	-	-
Cinnamyl acetate	Cinnamon	+	-	-	-	+	-	Mutagen	-	-
Ferulic acid	Guava	-	+	+	-	+	-	Mutagen	-	+
Ascorbic acid	Guava	-	+	+	-	+	-	Mutagen	-	-
Gallic acid	Guava	-	+	+	-	-	-	Mutagen	-	+
Casuarinin	Guava	-	-	-	-	-	-	Non mutagen	-	+
Cyanidin	Guava	-	+	-	-	out of range	+	Mutagen	-	-
Geraniol	Lemon Grass	-	+	-	-	+	-	Mutagen	+	-
Citral	Lemon Grass	-	+	+	-	+	-	Mutagen	-	+
Geranic acid	Lemon Grass	-	-	+	-	+	-	Mutagen	+	+
Chlorogenic acid	Lemon Grass	-	-	-	-	-	-	Non mutagen	-	-
Orientin	Lemon Grass	-	-	-	-	-	-	Non mutagen	-	-
Myrcene	Lemon Grass	-	-	-	-	+	-	Mutagen	-	+
Beta pinene	Lemon Grass	+	-	-	-	-	+	Mutagen	-	+
Linalool	Lemon Grass	+	-	-	-	+	-	Mutagen	-	-
Farnesol	Lemon Grass	-	-	-	-	-	-	Non mutagen	+	-
Barneol	Lemon Grass	-	-	+	-	-	-	Mutagen	-	+
Caffeic acid	Lemon Grass	-	+	+	-	+	-	Mutagen	-	+
Alpha Tocopherols	Grape	-	-	-	-	-	-	Non	-	-

								mutagen		
Beta carotene	Grape	-	-	-	-	-	-	Non mutagen	+	+
Benzethonium	Grape	-	-	-	-	+	-	Mutagen	-	-
Bergamottin	Grape	-	+	-	-	+	+	Mutagen	+	-
Bergapten	Grape	+	+	+	-	+	+	Mutagen	-	+
Citric acid	Grape	-	-	+	-	-	-	Mutagen	-	+
Azadirachtin	Grape	-	-	-	-	-	-	Non mutagen	+	+
Limonin	Grape	-	-	-	-	-	-	Non mutagen	-	+
Methyl paraben	Grape	+	+	+	-	+	-	Mutagen	-	-
Narangenin	Grape	+	+	-	-	-	+	Mutagen	-	+
Narangin	Grape	-	-	-	-	-	-	Non mutagen	-	-
Nootkatone	Grape	-	-	+	-	-	+	Mutagen	-	+
Putrescine	Grape	-	-	-	-	-	-	Non mutagen	+	+
Resveratron	Grape	-	-	-	-	-	+	Mutagen	-	-
Triclosan	Grape	-	-	-	-	-	+	Mutagen	+	-

Table 4: The binding energies (kcal/mol) of various plant derived compounds and antibiotics with PBP2A and PVL toxin after molecular docking. Melantriol and β -sitosterol have showed better binding energies. The table also indicating that herbal based compounds have better binding affinities towards PBP2A and PVL toxin of MRSA than antibiotics

Compound ID	Antibiotics/ Herbal active compound	Binding Energy Value With PBP2A (kcal/mol)	Binding Energy Value With PVL
DB00512	Vancomycin	-8.91	-5.81
CID:6024	Cephalothin	-5.91	-3.72
CID:6604200	Nitrofurantoin	-5.41	-3.69
CID:3476	Gentamicin	-5.26	-4.36
CID:37768	Amikacin	-5.13	-4.13
CID:298	Chloramphenicol	-4.49	-2.84
CID:149096	Levofloxacin	-4.35	-3.76
C16783	Meliantriol	-6.02	-8.94
CID:108058	Nimbin	-5.84	-3.42
CID:5280343	Quercetin	-5.02	-5.11
CID:222284	β-sitosterol	-7.40	-6.34
CID:5997	Cholestrol	-7.09	-6.70
CID:3220	Emodin	-6.49	-5.15
CID:173183	Campesterol	-6.38	-5.71
C08628	Lupeol	-6.24	-5.65
C10305	Aloin	-4.96	-6.08
CID:338	Salicyclic acid	-4.71	-4.42
CID: 73568	Corilagin	-6.34	-5.85
CID:65064	Epigallacatechin gallate	-5.56	-3.60
CID:445858	Ferulic acid	-4.55	-5.84
CID:128861	Cyanidin	-4.10	-5.09
CID:5785	Ascorbic acid	-4.05	-3.94
CID:138517	Casuarinin	-5.18	-4.70
CID:445858	Ferulic acid	-4.55	-5.84
CID:128861	Cyanidin	-4.10	-5.09
CID:5785	Ascorbic acid	-4.05	-3.94
CID:5281855	Ellagic acid	-5.45	-6.31
CID:9838995	Brevifolin	-5.16	-6.37
CID:370	Gallic acid	-4.10	-2.61
CID:65036	Allcin	-6.52	-3.67
CID:16590	Diallyl sulphide	-2.79	-3.41
CID:11617	Diallyl disulphide	-2.61	-4.65
CID:4445319	Cinnamyl acetate	-4.52	-4.67
CID:21105870	Cinnamyl alcohol	-4.21	-5.20
CID:637511	Cinnamaldehyde	-3.60	-3.89
CID:21105998	Thymol	-4.13	-4.57
CID:573	Beta-carotene	-6.44	-6.46
CID:442428	Narangin	-5.87	-5.76
CID:932	Narangenin	-5.67	-6.43
CID:1268142	Nootkatone	-5.42	-5.46

CID:5564	Triclosan	-5.36	-4.83
CID:1045	Putrescine	-5.27	-4.53
CID:2355	Bergapten	-5.05	-4.36
CID:5471349	Bergamottin	-4.99	-4.91
CID:14985	Alpha-tocopherols	-4.96	-4.36
CID:392875	Resveratron	-4.86	-4.38
CID:5281303	Azadirachtin	-4.43	-4.45
CID:311	Ciric acid	-4.25	-4.67
CID:7456	Methyl paraben	-3.65	-4.89
CID:6507	Limonin	-3.41	-4.53
CID:8478	Benzethonium chloride	-2.98	-4.01
CID:2345	Benzyl benzoate	-4.86	-4.76
CID:22311	Limonene	-4.47	-4.43
CID:6380311	Geranial	-4.37	-3.89
CID:440968	Alpha-pinene	-4.35	-4.86
CID:637758	Ethyl cinnamate	-4.27	-4.35
CID:420384	Thujene	-3.98	-4.02
CID:64945	Ursolic acid	-6.90	-4.48
CID:10494	Oleanolic acid	-6.30	-5.47
CID:5280445	Luteolin	-5.68	-5.47
CID:5280443	Apigenin	-5.54	-5.87
CID:5281515	Beta-caryophyllene	-5.48	-5.52
CID:637520	Methyl cinnamate	-4.03	-5.08
CID:3314	Eugenol	-3.90	-4.86
CID:10364	Carvacrol	-3.86	-3.78
CID:5315615	Rosmarinic acid	-3.79	-4.27
CID:6549	Linalool	-3.49	-4.38
CID:1405788	Chlorogenic acid	-5.27	-4.38
CID:689043	Caffeic acid	-5.21	-4.89
CID:5281675	Orientin	-4.88	-5.47
CID:14896	Beta-pinene	-4.12	-5.23
CID:31253	Myrcene	-4.10	-5.47
CID:5275520	Geranic acid	-4.10	-4.18
CID:6552009	Barneol	-3.93	-4.64
CID:13849989	Geraniol	-3.88	-4.89
CID:638011	Citrial	-3.77	-4.23
CID:3327	Farnesol	-3.66	-4.46

Table 5: Antibiotic susceptibility analysis of MRSA. The study revealed that MRSA has emerged as superbug because it resistant to multiple antibiotics. Vancomycin is the best antibiotics available against MRSA infection. But recent report revealed that the organism developed resistant against Vancomycin (VRSA)

Antibiotic	Symbol	Concentration (mcg)	Diameter of zone of inhibition(mm)	Susceptibility pattern
Moxifloxacin	MO	5	22	Intermediate
Cefatoxime	CTX	30	14	Resistant
Rifampicin	RIF	5	Nil	Resistant
Oxacillin	OX	1	Nil	Resistant
Co-Trimoxazole	COT	25	Nil	Resistant
Ciprofloxcin	CIP	5	16	Intermediate
Ceftazidime	CAZ	30	10	Resistant
Nalidixic acid	NA	30	9	Resistant
Gentamicin	GEN	10	15	Sensitive
Tetracycline	TE	30	12	Resistant
Levofloxacin	LE	5	20	Sensitive
Nitrofurantoin	NIT	300	19	Sensitive
Amikacin	AK	30	18	Sensitive
Streptomycin	S	10	8	Resistant
Chloramphenicol	C	30	26	Sensitive
Erythromycin	E	15	Nil	Resistant
Ampicillin	AMP	10	11	Resistant
Vancomycin	VA	30	15	Sensitive
Cephalothin	CEP	30	23	Sensitive
Norfloxacin	NX	10	8	Resistant
Methicillin	MET	5	Nil	Resistant