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-methylheptyan-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 pathogenicity 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 (synergohymenotrophie toxins) consists of F and S components. PVL is encoded by the lukPV operon 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-likeness, 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 herbs 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°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
guava (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 Aloe 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 Leukocidin (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 Staphylococcus aureus (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 revealed 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.

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, Oxacinilin, Co-Trimoxazole, Chloramphenicol Ciprofloxacin, Cefazidime, 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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References:

[1] Kuehnert MJ et al. Emerg Infect Dis. 2005 11: 868 [PMID: 15963281]

[2] David MZ et al. PLoS One. 2011 6: e18217 [PMID: 21533238]

[3] Monecke S et al. PLoS One. 2011 6: e17936 [PMID: 21494333]

[4] Llarrull LI et al. Antimicrob Agents Chemother. 2009 53: 4051 [PMID: 19470504]

[5] Ouhara K et al. J Antimicrob Chemother. 2008 61: 1266 [PMID: 18367458]

[6] Diep BA & Otto M. Trends Microbiol. 2008 16: 361 [PMID: 18585915]

[7] Chomnawang MT et al. Fitoterapia 2009 80:102 [PMID: 19022354]

[8] Khanna V & Ranganathan S. BMC Bioinformatics. 2009 10: S10 [PMID: 19958509]

[9] Lyskov S & Gray JJ. Nucleic Acids Res. 2008 36:W233 [PMID: 18442991]

[10] Wang Y et al. Nucleic Acids Res. 2010 38: D255 [PMID: 19933261]

[11] Kanehisa M et al. Nucleic Acids Res. 2010 38: D355 [PMID: 19880382]

[12] Anthony JW et al. J Cheminform. 2010 2: O16 [PMCID: PMC2867124]

[13] Seal A et al. Bioinformation 2011 5: 430 [PMID: 21423889]

[14] Lim D & Strynadka NC. Nat Struct Biol. 2002 9: 870 [PMID: 12389036]

[15] Guillet V et al. J Biol Chem. 2004 279: 41028 [PMID: 15262988]

[16] Morris GM et al. J Comput Aided Mol Des. 1996 10: 293 [PMID: 8877701]

[17] Kareru PG et al. Afr J Tradit Complement Altern Med. 2010 7: 214 [PMCID: PMC3025622]

[18] Habeeb F et al. Methods. 2007 42: 315 [PMID: 17560318]

[19] Cho YS et al. Carr Microbiol. 2008 57: 542 [PMID: 18781360]

[20] Aqil F et al. J Basic Microbiol. 2005 45: 106 [PMID: 15812867]

[21] Metwally AM et al. Pharmacogn Mag. 2010 6: 212 [PMID: 20931082]

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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        | qualified      | Midstructure   | In 90% cutoff   |
| Melantriol                        | Aloe vera | Suitable | qualified      | Midstructure   | In 90% cutoff   |
| Aloin                             | Aloe vera | Suitable | Violated       | Midstructure   | In 90% cutoff   |
| Emodin                            | Aloe vera | 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 | 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        | Violated       | Qualified      | Midstructure    | In 90% cutoff   |
| Carvacrol                         | Tulsi   | Suitable        | Violated       | Not qualified  | Midstructure    | Out of 90% cutoff |
| Linalool                          | Tulsi   | Suitable        | Not qualified  | Midstructure   | Out of 90% cutoff |
| Beta-caryophyllene                | Tulsi   | Suitable        | Violated       | Qualified      | Midstructure    | In 90% cutoff   |
| Methyl cinnamate                  | Tulsi   | Suitable        | Violated       | Qualified      | Midstructure    | In 90% cutoff   |
| Apigenin                          | Tulsi   | Suitable        | Violated       | Qualified      | Midstructure    | In 90% cutoff   |
| Luteolin                          | Tulsi   | Suitable        | Violated       | Qualified      | Midstructure    | In 90% cutoff   |
| curcumin                          | Turmeric | Suitable | Violated       | Not qualified  | Midstructure    | Out of 90% cutoff |
| Demethoxy curcumin                | Turmeric | Suitable | Violated       | Not qualified  | Midstructure    | Out of 90% cutoff |
| Tumerone                          | Turmeric | Suitable | Violated       | Qualified      | Midstructure    | In 90% cutoff   |
| Diaryl heptanoid                  | Turmeric | Suitable | Violated       | Not qualified  | Midstructure    | Out of 90% cutoff |
| Allicin                           | Garlic  | Suitable        | Not qualified  | Midstructure   | Out of 90% cutoff |
| Diallyl sulphhide                 | Garlic  | Suitable        | Not qualified  | Non-druglike   | In 90% cutoff   |
| Diallyl disulphide                | Garlic  | Suitable        | Not qualified  | Non-druglike   | In 90% cutoff   |
| Brevifolin                        | Pomogranate | Suitable | Not qualified  | Midstructure   | Out of 90% cutoff |
| Ellagic acid                      | Pomogranate | Suitable | Not qualified  | Non-druglike   | In 90% cutoff   |
| Gallic acid                       | Pomogranate | Suitable | Not qualified  | Midstructure   | Out of 90% cutoff |
| Epigallacatechin gallate          | Tea     | Violated        | Not qualified  | Midstructure   | Out of 90% cutoff |
| Corilagin                         | Tea     | Violated        | Not qualified  | Midstructure   | Out of 90% cutoff |
| Thymol                            | Clove   | Suitable        | Not qualified  | Midstructure   | Out of 90% cutoff |
| Alpha-pinene                      | Eucalyptus | Suitable | Not qualified  | Midstructure   | In 90% cutoff   |
| Benzyl benzoate                   | Eucalyptus | Suitable | Violated       | Qualified      | Midstructure    | In 90% cutoff   |
| Compound          | Source          | Suitable Status | Violated Status | Qualification Status | Midstructure Status | Cutoff Value       |
|-------------------|-----------------|-----------------|-----------------|----------------------|---------------------|--------------------|
| 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      |
| Thuene            | Eucalyptus      | Suitable        | Not qualified   | Midstructure         | In 90% cutoff       |
| Cinnamaldehyde    | Cinnamon        | Suitable        | Not qualified   | Midstructure         | In 90% cutoff       |
| Cinnamyl alcohol  | Cinnamon        | 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  |
| Benzenzethonium   | 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        | Violated        | Qualified            | Midstructure        | In 90% cutoff      |
| Narangin          | Grape           | Suitable        | Violated        | Not qualified        | Midstructure        | Out of 90% cutoff  |
| 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                      |
| Aloe                             | Aloe vera| Poor                       | Middle                  | Middle                 | -4.7114            | Low                         |
| Emodin                           | Aloe vera| Well absorbed              | Middle                  | Middle                 | -4.29575           | Middle                      |
| Salicylic 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                        |
| Oleamolic acid                   | Tulsi    | Well absorbed              | Middle                  | Low                    | -2.354             | High                        |
| Ursolic acid                     | Tulsi    | Well absorbed              | Middle                  | Low                    | -2.1509            | High                        |
| Rosmarinic acid                  | Tulsi    | Moderate                   | Low                     | Middle                 | -3.327             | 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                 | -4.157             | High                        |
| Luteolin                         | Tulsi    | Well absorbed              | Middle                  | Low                    | -4.28              | Middle                      |
| curcumin                         | Turmeric | Moderately absorbed        | permeability            | permeability           | -3.62686           | Middle absorption to CNS   |
| Demethoxy curcumin               | Turmeric | Well absorbed              | Middle                  | permeability           | -2.21821           | Middle absorption to CNS   |
| Tumerone                         | Turmeric | Well absorbed              | Middle                  | permeability           | -0.92884           | High absorption to CNS     |
| Diaryl heptanoid                 | Turmeric | Well absorbed              | Middle                  | permeability           | -2.25168           | Middle absorption to CNS   |
| Allicin                          | Garlic   | Well absorbed              | Middle                  | permeability           | -1.36911           | Middle absorption to CNS   |
| Diallyl sulphide                 | Garlic   | Well absorbed              | Middle                  | permeability           | -1.17034           | Middle absorption to CNS   |
| Diallyl disulphide               | Garlic   | Well absorbed              | Middle                  | permeability           | -1.08723           | Middle absorption to CNS   |
| Brevifolin                       | Pomogranate| Well absorbed            | Middle                  | permeability           | -2.39945           | Middle absorption to CNS   |
| Ellagic acid                     | Pomogranate| Well absorbed            | Middle                  | permeability           | -1.15024           | Middle absorption to CNS   |
| Gallic acid                      | Pomogranate| Moderately absorbed      | Middle                  | permeability           | -3.62686           | Middle absorption to CNS   |
| Epigallactechin gallate          | Tea      | Moderately absorbed        | Middle                  | permeability           | -3.9954            | Low absorption to CNS      |
| Corilagin                        | Tea      | Poorly absorbed            | Middle                  | permeability           | -4.32617           | Low absorption to CNS      |
| Thymol                           | Clove    | Well absorbed              | Middle                  | permeability           | -1.06531           | 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 |
|----------------------------------|----------------------|--------------------|-----------------|
|                                  |                      | TA100 TA1535 TA98  | Mouse Rat       |
|                                  |                      | +s9 ±s9 +s9 ±s9 +s9 | ±s9             |
| Quercetin                        | Neem                 | - + - - +          | Mutagen -       |
| Meltantriol                      | Neem                 | - - - - -          | Non -           |
| Aloin                            | Neem                 | - - - - +          | Mutagen -       |
| Emodin                           | Aloe vera            | - - - + +          | Mutagen -       |
| Salycyclic acid                  | Aloe vera            | + + + - -          | Mutagen -       |
| Cholestrol                       | Aloe vera            | - - - - -          | Non +           |
| Campesterol                      | Aloe vera            | - - - - -          | Non +           |
| Lupeol                           | Aloe vera            | - - - - +          | Mutagen -       |
| β-sitosterol                     | Aloe vera            | - - - - -          | Non +           |
| Oleanolic acid                   | Aloe vera            | - - - - -          | Non +           |
| Compound          | Species  | Mutagenicity | Description                  |
|-------------------|----------|--------------|------------------------------|
| Ursolic acid      | Tulsi    | Non mutagen  | Ursolic acid Tulsi - - - - - |
| Rosmarinic acid   | Tulsi    | Non mutagen  | Rosmarinic acid Tulsi - - - - |
| Eugenol           | Tulsi    | Mutagen      | Eugenol Tulsi + + + + + +   |
| Carvacrol         | Tulsi    | Mutagen      | Carvacrol Tulsi + + + + + +  |
| Linalool          | Tulsi    | Mutagen      | Linalool Tulsi + + + + + +   |
| Beta-caryophyllene| Tulsi    | Mutagen      | Beta-caryophyllene Tulsi + + |
| Methyl cinnamate  | Tulsi    | Mutagen      | Methyl cinnamate Tulsi + + + |
| Apigenin          | Tulsi    | Mutagen      | Apigenin Tulsi - + - - - +   |
| Luteolin          | Tulsi    | Mutagen      | Luteolin Tulsi + + - - - +   |
| curcumin          | Tulsi    | Mutagen      | curcumin Tulsi + + - - - -   |
| Demethoxy curcumin| Turmeric | Mutagen      | Demethoxy curcumin Turmeric - |
| Tumerone          | Turmeric | Mutagen      | Tumerone Turmeric - - - - +  |
| Diaryl heptanoid  | Turmeric | Mutagen      | Diaryl heptanoid Turmeric - - |
| Allicin           | Turmeric | Mutagen      | Allicin Turmeric - - + - -   |
| Diallyl sulphide  | Garlic   | Mutagen      | Diallyl sulphide Garlic - -  |
| Diallyl disulphide| Garlic   | Mutagen      | Diallyl disulphide Garlic - -|
| Brevifolin        | Garlic   | Mutagen      | Brevifolin Garlic + + - - -  |
| Ellagic acid      | Pomogranate| Mutagen   | Ellagic acid Pomogranate - + |
| Gallic acid       | Pomogranate| Mutagen    | Gallic acid Pomogranate - + |
| Punicaflavin      | Pomogranate| Mutagen    | Punicaflavin Pomogranate - +|
| Epigallacatechin gallate | Tea | Mutagen | Epigallacatechin gallate Tea - - - - |
| Corilagin         | Tea      | Mutagen      | Corilagin Tea - - + - - +   |
| Thymol            | Clove    | Mutagen      | Thymol Clove + + + + + +     |
| Alpha-pinene      | Eucalyptus| Mutagen     | Alpha-pinene Eucalyptus + + |
| Benzyl benzoate   | Eucalyptus| Mutagen     | Benzyl benzoate Eucalyptus + |
| Geranial          | Eucalyptus| Mutagen     | Geranial Eucalyptus - + - - |
| Limonene          | Eucalyptus| Mutagen     | Limonene Eucalyptus - + - - |
| Ethyl cinnamate   | Eucalyptus| Mutagen     | Ethyl cinnamate Eucalyptus + |
| Terpinen          | Eucalyptus| Mutagen     | Terpinen Eucalyptus - + - - |
| Thujene           | Eucalyptus| Mutagen     | Thujene Eucalyptus + - - - |
| Cinnamaldehyde    | Cinnamon | Mutagen      | Cinnamaldehyde Cinnamon - - |
| Cinnamyl alcohol  | Cinnamon | Mutagen      | Cinnamyl alcohol Cinnamon - |
| Cinnamyl acetate  | Cinnamon | Mutagen      | Cinnamyl acetate Cinnamon - |
| Ferulic acid      | Guava    | Mutagen      | Ferulic acid Guava - + - - |
| Ascorbic acid     | Guava    | Mutagen      | Ascorbic acid Guava - + - - |
| Gallic acid       | Guava    | Mutagen      | Gallic acid Guava - + - -   |
| Casuarinin        | Guava    | Mutagen      | Casuarinin Guava - - - -   |
| Cyanidin          | Guava    | Mutagen      | Cyanidin Guava - + - - out of range + |
| Geraniol          | Lemon Grass| Mutagen     | Geraniol Lemon Grass - + - |
| Citral            | Lemon Grass| Mutagen     | Citral Lemon Grass - + - |
| Geranic acid      | Lemon Grass| Mutagen     | Geranic acid Lemon Grass - + |
| Chlorogenic acid  | Lemon Grass| Mutagen     | Chlorogenic acid Lemon Grass - |
| Orientin          | Lemon Grass| Mutagen     | Orientin Lemon Grass - - - |
| Myrcene           | Lemon Grass| Mutagen     | Myrcene Lemon Grass - - - |
| Beta pinene       | Lemon Grass| Mutagen     | Beta pinene Lemon Grass - + |
| Linalool          | Lemon Grass| Mutagen     | Linalool Lemon Grass - + - |
| Farnesol          | Lemon Grass| Mutagen     | Farnesol Lemon Grass - - - |
| Barneol           | Lemon Grass| Mutagen     | Barneol Lemon Grass - + - |
| Caffeic acid      | Lemon Grass| Mutagen     | Caffeic acid Lemon Grass - + |
| Alpha Tocopherols | Grape    | Mutagen      | Alpha Tocopherols Grape - |

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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                         |
| CID:1678    | Melantriol                           | -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                         |
| CID:8628    | Lupel                                | -6.24                                    | -5.65                         |
| CID:10305   | Aloin                                | -4.96                                    | -6.08                         |
| CID:338     | Salicylic 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:12885   | 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:5785    | Ellagic acid                         | -5.45                                    | -6.31                         |
| CID:9838995 | Brevifolin                           | -5.16                                    | -6.37                         |
| CID:370     | Gallic acid                          | -4.10                                    | -2.61                         |
| CID:65036   | Allein                               | -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:21105598| 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                         |
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              |
| Ciprofloxacin  | 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              |