SYNTHESIS OF 4-HYDROXY-3-METHOXYBENZALDEHYDE (VANILLIN) DERIVATIVES AND PHARMACOPHORE MODELLING FOR ANTIMICROBIAL AGENTS Staphylococcus aureus (S.aureus)

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Bachelor of Science with Honours
(Resource Chemistry)
2015
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A final project submitted to fulfill the requirement for the degree of Bachelor of Science with Honours (Resource Chemistry)

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DECLARATION

I hereby declare that this Final Year Project 2015 dissertation is based on my original work except for quotations and citations, which have been duly declared that it has not been or concurrently submitted for any degree at UNIMAS or other institutions of higher education.

______________________________

Nur Sabrina Binti Mohd Raime

Resource Chemistry Programme

Faculty of Resource Science and Technology

Universiti Malaysia (UNIMAS)
**TABLE OF CONTENTS**

ACKNOWLEDGEMENT .................................................................................. i

DECLARATION ......................................................................................... ii

TABLE OF CONTENTS .............................................................................. iii

LIST OF ABBREVIATIONS ........................................................................ vi

LIST OF FIGURES AND TABLES ............................................................. vii

ABSTRACT/ABSTRAK .............................................................................. 1

CHAPTER 1 .......................................................................................... 2

1. INTRODUCTION .............................................................................. 2

CHAPTER 2 .......................................................................................... 4

2. LITERATURE REVIEWS .................................................................... 4

  2.1 Staphylococcus aureus Infections (Staph infection) ....................... 4

  2.2 Vanillin ...................................................................................... 7

  2.3 Pharmacophore modeling .............................................................. 8

  2.4 Ligand Scout 3.12 ......................................................................... 10

  2.5 Biological Evaluation ................................................................... 11

    2.5.1 Cytotoxicity Test .................................................................. 11

    2.5.2 Antimicrobial Test ................................................................. 12
CHAPTER 3………………………………………………………………………….. 13

3. MATERIAL AND METHODOLOGY………………………………………… 13

3.1 Pharmacophore Modeling……………………………………………………… 14

3.1.1 Hypothesis Generation……………………………………………………… 15

3.1.2 Target Compound…………………………………………………………… 17

3.1.3 The review on S.aureus FabI model from PDB (code ID 4BNN)…………… 19

3.2 Chemical Synthesis…………………………………………………………….. 19

3.2.1 General Procedure…………………………………………………………… 20

3.2.2 Chemicals…………………………………………………………………… 21

3.2.3 Syntheses of Vanillin Derivatives………………………………………… 22

3.2.4 Process of Synthesis compound………………………………………… 26

3.3 Compound Characterisation…………………………………………………… 26

3.3.1 Thin-Layer Chromatography (TLC)……………………………………… 26

3.3.2 Fourier Transform Infra-Red (FT-IR) Spectroscopy……………………… 27

3.3.3 Nuclear Magnetic Resonance (NMR)……………………………………. 27

3.4 Biological Evaluation……………………………………………………………. 27

3.4.1 Brine Shrimp Cytotoxicity Test……………………………………………. 28

3.4.1.1 Sample preparation…………………………………………………… 28

3.4.1.2 LC_{50} Determination………………………………………………… 29

3.4.2 Kirby Bauer Test……………………………………………………………. 30
CHAPTER 4. RESULTS AND DISCUSSIONS

4.1 Pharmacophore modeling

4.1.1. Structure-based Pharmacophore

4.1.2 Structure-based interaction in D-JUS1257 with the synthesized compound

4.2 TLC Test

4.3 Synthesized compounds

4.4 Cytotoxicity test

4.5 Antimicrobial Test

CHAPTER 5. CONCLUSION

CHAPTER 6. REFERENCES

APPENDICES

Appendix A: TLC test

Appendix B: Raw data of cytotoxicity test

Appendix C: Cytotoxicity Test
LIST OF ABBREVIATIONS

TSS  Toxic Shock Syndrome
MRSA  Methicillin Resistance Staphylococcus Aureus
\textsuperscript{1}H and \textsuperscript{13}C NMR  Proton and Carbon Nuclear Magnetic Resonance
FTIR  Fourier Transform Infra-Red
NMR  Nuclear Magnetic Resonance
TLC  Thin Layer Chromatography
KBr  Potassium Bromide
SBDD  Structure Based Drug Design
HBA  Hydrogen Bond Acceptor
HBD  Hydrogen Bond Donor
HY  Hydrophobic Interaction
R  Ring Aromatic Interaction
Cat  Cation
Ani  Anion
Aro  Aromatic center
BSLT  Brine Shrimp Lethality Test
LC\textsubscript{50}  Lethal concentration for 50\% of brine shrimp population
PDB  Protein Data Bank
saFabI  \textit{Staphylococcus aureus} enoyl-ACP reductase
EtOH  Ethanol
Rf  Retention Factor
DMSO  Dimethyl sulfoxide
## LIST OF FIGURES AND TABLES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Caption</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1</td>
<td>Image of <em>Staphylococcus aureus</em></td>
<td>2</td>
</tr>
<tr>
<td>Figure 2</td>
<td>9 Years old who developed <em>Staphylococcus aureus</em></td>
<td>4</td>
</tr>
<tr>
<td>Figure 3</td>
<td><em>Penicillium</em> sp. Mould</td>
<td>6</td>
</tr>
<tr>
<td>Figure 4</td>
<td>4-hydroxy-3-methoxybenzaldehyde (Vanillin)</td>
<td>7</td>
</tr>
<tr>
<td>Figure 5</td>
<td>Flow chart of pharmacophore modeling. (Larger &amp; Wolber, 2004)</td>
<td>9</td>
</tr>
<tr>
<td>Figure 6</td>
<td>Sample of pharmacophore modeling using Ligand Scout 3.12 software</td>
<td>10</td>
</tr>
<tr>
<td>Figure 7</td>
<td>Brine shrimp larvae <em>Artemia salina</em></td>
<td>11</td>
</tr>
<tr>
<td>Figure 8</td>
<td>Kirby- Bauer test</td>
<td>12</td>
</tr>
<tr>
<td>Figure 9</td>
<td>Ligand Scout software 3.12</td>
<td>14</td>
</tr>
<tr>
<td>Figure 10</td>
<td>The flowchart on operating structure-based pharmacophore modelling</td>
<td>16</td>
</tr>
<tr>
<td>Figure 11</td>
<td>A 3D structure of <em>S.aureus</em> generated by LigandScout 3.12</td>
<td>17</td>
</tr>
<tr>
<td>Figure 12</td>
<td>Schiff base reaction of vanillin</td>
<td>21</td>
</tr>
<tr>
<td>Figure 13</td>
<td>The interaction of D-JUS1257 in the active site</td>
<td>30</td>
</tr>
<tr>
<td>Figure 13(a-i)</td>
<td>Structure-based Pharmacophore for compound (1-9)</td>
<td>31</td>
</tr>
<tr>
<td>Figure 14(a)</td>
<td>FT-IR spectra compound (1)</td>
<td>42</td>
</tr>
<tr>
<td>Figure 14(b)</td>
<td>$^1$H NMR (500 MHz, DMSO) compound (1)</td>
<td>43</td>
</tr>
<tr>
<td>Figure 14(c)</td>
<td>$^{13}$C NMR (500 MHz, DMSO) compound (1)</td>
<td>44</td>
</tr>
<tr>
<td>Figure 15(a)</td>
<td>FT-IR Spectra compound (2)</td>
<td>47</td>
</tr>
<tr>
<td>Figure 15(b)</td>
<td>$^1$H NMR (500 MHz, DMSO) compound (2)</td>
<td>48</td>
</tr>
<tr>
<td>Figure 15(c)</td>
<td>$^{13}$C NMR (500 MHz, DMSO) compound (2)</td>
<td>49</td>
</tr>
<tr>
<td>Figure 16(a)</td>
<td>FT-IR Spectra compound (3)</td>
<td>52</td>
</tr>
<tr>
<td>Figure 16(b)</td>
<td>$^1$H NMR (500 MHz, DMSO) compound (3)</td>
<td>53</td>
</tr>
<tr>
<td>Figure 16(c)</td>
<td>$^{13}$C NMR (500 MHz, DMSO) compound (3)</td>
<td>54</td>
</tr>
<tr>
<td>Figure 17(a)</td>
<td>FT-IR Spectra compound (4)</td>
<td>57</td>
</tr>
<tr>
<td>Figure 17(b)</td>
<td>$^1$H NMR (500 MHz, DMSO) compound (4)</td>
<td>58</td>
</tr>
<tr>
<td>Figure 17(c)</td>
<td>$^{13}$C NMR (500 MHz, DMSO) compound (4)</td>
<td>59</td>
</tr>
<tr>
<td>Figure 18(a)</td>
<td>FT-IR Spectra compound (5)</td>
<td>62</td>
</tr>
<tr>
<td>Figure 18(b)</td>
<td>$^1$H NMR (500 MHz, DMSO) compound (5)</td>
<td>63</td>
</tr>
<tr>
<td>Figure 18(c)</td>
<td>$^{13}$C NMR (500 MHz, DMSO) compound (5)</td>
<td>64</td>
</tr>
<tr>
<td>Figure 19(a)</td>
<td>FT-IR Spectra compound (6)</td>
<td>67</td>
</tr>
<tr>
<td>Figure 19(b)</td>
<td>$^1$H NMR (500 MHz, DMSO) compound (6)</td>
<td>68</td>
</tr>
<tr>
<td>Figure 19(c)</td>
<td>$^{13}$C NMR (500 MHz, DMSO) compound (6)</td>
<td>69</td>
</tr>
<tr>
<td>Figure 20(a)</td>
<td>FT-IR Spectra compound (7)</td>
<td>72</td>
</tr>
<tr>
<td>Figure 20(b)</td>
<td>$^1$H NMR (500 MHz, DMSO) compound (7)</td>
<td>73</td>
</tr>
</tbody>
</table>
Figure 20(c) \(^{13}\)CNMR (500 MHz, DMSO) compound (7) 74
Figure 21(a) FT-IR Spectra compound (8) 77
Figure 21(b) \(^{1}\)HNMR (500 MHz, DMSO) compound (8) 78
Figure 22(a) FT-IR Spectra compound (9) 81
Figure 22(b) \(^{1}\)HNMR (500 MHz, DMSO) compound (9) 82
Figure 22(c) \(^{13}\)CNMR (500 MHz, DMSO) compound (9) 83
Figure 23 Cytotoxicity graph for 9 compounds 85

<table>
<thead>
<tr>
<th>Table</th>
<th>Caption</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1</td>
<td>\textit{S.aureus} interactions</td>
<td>18</td>
</tr>
<tr>
<td>Table 2</td>
<td>Synthesized of proposed vanillin derivatives</td>
<td>23</td>
</tr>
<tr>
<td>Table 3</td>
<td>Summarized of the interactions and fit value between synthesized compound and active site</td>
<td>38</td>
</tr>
<tr>
<td>Table 4</td>
<td>Retention factor for TLC test</td>
<td>40</td>
</tr>
<tr>
<td>Table 5</td>
<td>LC\textsubscript{50} value of synthesized compound</td>
<td>77</td>
</tr>
<tr>
<td>Table 6</td>
<td>The zone of inhibitions of synthesized compounds</td>
<td>78</td>
</tr>
</tbody>
</table>
Synthesis of 4-hydroxy-3-methoxybenzaldehyde (Vanillin) Derivatives and Pharmacophore Modelling for Antimicrobial Agents Staphylococcus aureus (S.aureus)

by

Nur Sabrina Bt. MohdRaime

ABSTRACT

Staph infections and its complications have increased rapidly in this recent year due to the resistance of S.aureus strains to the current antibiotics. Currently there is no potent vaccine available to against S.aureus since the bacteria are so widespread and will cause so many different diseases. In this study, vanillin was used as the starting material to synthesize a new drug since vanillin show their properties for antimicrobial agent. Pharmacophore modelling was a new approach to search for potential antimicrobial agents with 9 vanillin derivatives were proposed as a test set (synthesized compound). Enoyl-ACP reductase (saFabI) was selected from Protein Data Bank website as target compound and their 3D structure was generated in LigandScout 3.1 for structure-based pharmacophore. The target compound and test set were merged to determine their interactions between both compounds. The vanillin derivatives were synthesized via Schiff base reaction. The characterization of the synthesized compounds was determined by using Fourier Transform Infra-Red and Nuclear Magnetic Resonance (1H and 13C NMR). Biological evaluation was conducted using Brine Shrimp lethality test for cytotoxicity. The antimicrobial test, Kirby Bauer test was also conducted. The pharmacophore modelling showed that 2-methoxy-4-{(Z)-(phenylimino)methyl}phenol (6) from the reaction of vanillin and aniline was the highest number of interaction to D-JUS1257 active site. Biological evaluation showed that the compound was cytotoxic and the antimicrobial assay also showed that compound (6) has antimicrobial property which can inhibit the growth of S.aureus. Therefore, compound (6) was selected as lead compound to against the staph infection.

Keywords: Staph infection, vanillin, pharmacophore modelling, cytotoxicity, Kirby Bauer test

ABSTRAK


Kata kunci :Jangkitan Staph, Vanila, Pemodelanfarmakofor, tahaptoksik, Ujian Kirby Bauer
CHAPTER 1

INTRODUCTION

*Staphylococcus aureus* (*S.aureus*) is a group of Gram-positive cocci bacteria that is a member of the Firmicutes, and is normally found in the human respiratory tract and on the human skin. *Staphylococcus sp.* was first identified by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint in 1880 in Aberdeen, United Kingdom. “Staphylo” comes from Greek’s word, which means the grapes (Schaechter et al., 1993), because of their colonies appearance. *S.aureus* has large, round, golden-yellow colonies and appears as grape-like clusters when viewed under a light microscope, often with hemolysis, when grown on blood agar plates. *Staphylococci sp.* is classified as non-motile, non-spore forming facultative anaerobes that can grow by the aerobic respiration or by the fermentation process as well (Harris et al., 2002).

![Image of Staphylococcus aureus](image)

**Figure 1:** Image of *Staphylococcus aureus*

Figure 1 shows the morphology of *S.aureus*. *S.aureus* is the most common species of *staphylococcus sp.* that cause staph infections and is said to be the most pathogenic because of the combination of nasal carriage and bacterial immuno-evasive strategies. *S.aureus* can cause a several severe of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic
shock syndrome (TSS), bacteremia, and sepsis. Every year, there are 500,000 patients in United States' hospitals got infected by this staphylococcal infection.

*S.aureus* is the most pathogenic and major pathogen because of the rise in antibiotics resistance. It is grouped as the hardiest of the non-spore forming bacteria and can survive for a long periods plus it is hard to eliminate once it is introduced in the human body (Schaechter et al., 1993). In the early 1940s the penicillin has been introduced since the infection of *S.aureus* has become serious and worst. However, some bacteria show their ability to produce the resistance, penicillase in order to break down and purify the effect of penicillin. Therefore, scientist has created semi-synthetic derivatives of penicillin to replace penicillin due to the ineffectiveness of penicillin itself. The synthetic drug which is Methicillin that derived from penicillin in 1960 but however it does not last long because of the Methicillin resistance *S.aureus* (MRSA). In order to synthesis a new kind of antimicrobial agent to against *S.aureus* infection, a pharmacophore modelling will be used to create and develop a new one.

The pharmacophore modelling approaches have become one of the major tools in drug discovery after the past century’s development. It also will assist to reduce expensive cost that involve with the discovery and development of new drug. The design was created using computer software which is ligandscout 3.1 that produce a 3D model of structural of organic molecule. The main objective of this project is to improve pharmacophore model for searching the potential lead compound to cure the infection. The synthesis of Vanillin derivatives will react with several proposed primary amine compound via Schiff base reaction and followed by characterization by FTIR spectrometry,\(^1\)H and \(^{13}\)C NMR spectrometry, melting point test, cytotoxicity test and biological test.
CHAPTER 2

LITERATURE REVIEW

2.1. *Staphylococcus aureus* Infections (Staph infection)

*Staphylococcus aureus* is said to be the most pathogenic bacteria and *S.aureus* bacterium that were usually related with the higher morbidity and mortality compared to the bacterium that caused by other pathogen (Naber, 2007). *Staphylococcus sp.* is group of bacteria that can cause a multitude of diseases. Staph infections may cause many diseases due to direct infection or production of toxins by the bacteria. The diseases that may be caused by this *Staphylococcus sp.* are boils, impetigo, food poisoning, cellulitis, and toxic shock syndrome. Staph infection usually involves a collection of pus, such as a boil, furuncle, or abscess. The area is normally will be painful and may be reddened and swollen.

![Image](image.png)

**Figure 2:** 9 year old who developed *Staphylococcus aureus* infection

Figure 2 shows the staph infection that infects the skin. Methicillin-resistant *Staphylococcus aureus*, known as MRSA, is a type of *Staphylococcus aureus* which is resistant to the antibiotic methicillin and other drugs. Methicillin-resistant *Staphylococcus aureus* (MRSA)
were reported as a major cause of hospital-acquired infections that becomes more challenging to combat because of the emerging resistance to all current drug classes (Enright et al., 2002). *S.aureus* infections and its complications show increased rapidly in this recent year due to the resistance of *S.aureus* strains to the current antibiotics (Naber, 2007). Over 30 different types of *Staphylococci sp.* may infect humans, but mostly the infections are caused by *Staphylococcus aureus*.

*Staphylococci sp.* can be found normally in human’s nose and on the skin (and less commonly in other locations) of around 25%-30% of healthy adults and in 25% of hospital workers. In the majority of most cases, the bacteria will not cause disease but however it will cause damage to the skin or other injury may allow the bacteria to overcome the natural protective mechanisms of the human’s body, leading to the infection. Skin infections are the most common type of disease that produced by *Staphylococcus sp.* Staph infections of the skin can progress to impetigo (a crusting of the skin) or cellulitis (inflammation of the deeper layers of skin and connective tissue under the skin, leading to swell and redness of the area). Staph also can result in mastitis (inflammation of the breast) or in abscess of the breast in breastfeeding women. Staphylococcal breast abscesses may release bacteria into the mother's milk and is dangerous to the kid.

Basically, when the bacteria enter into the bloodstream and spread to other organs in human’s body, a number of serious infections may occur and this spread of the organisms to the bloodstream is known as bacteremia or sepsis. In addition, Staphylococcal food poisoning is an illness of the bowels that causes nausea, vomiting, diarrhea, and dehydration which caused by eating contaminated food with toxins produced by *Staphylococcus aureus* rather than a true infection with the bacteria. The Symptoms normally develop within one to six
hours after a person eating the contaminated food and the illness usually lasts for one to three days and will resolve on its own. Patients with this illness are will not contagious since the toxins are not transmitted from one person to another.

Due to the increasing number of the infections cases, the first antibacterial was introduced which were extracted from mould of *Penicillin* *sp.* In 1943, Alexander Fleming was invented a drug called Penicillin that come from *Penicillium* *sp.* showed in figure 3 to treat the *S.aureus* infections and it showed an effectiveness in decreasing the morbidity and mortality. Penicillin is a β-lactam antibiotics that function by inhibit the bacterium cell wall synthesis and inactivated the penicillin proteins (PBP). In the late 1940’s, Penicillin is no longer show effectiveness to treat the infections due to the emerging resistance, Penicillase and therefore in 1960, Methicillin was introduced to overcome this problem. After the production of Methicillin, the Methicillin-resistant *S.aureus* (MRSA) has been identified and spreading to worldwide as nosocomial pathogens (Harris et al., 2002). In 2000, The Central Public Health Laboratory, UK found that 61% of the nosocomial *S.aureus* infections in the 96 hospitals have been infected with this methicillin resistant.

![Figure 3: Penicillium sp mould](image)

*Figure 3: Penicillium sp mould*
2.2 Vanillin

Vanilla is a flavor derived from orchids of the genus which is Vanilla, primarily from the Mexican species. Based on figure 4, Vanilin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla, which is widely used and important in flavoring especially in sweet food. Vanilin may be easily synthesized via Schiff base reaction and normally used for pharmaceutical approach for the drug development.

Vanilin show their properties likes antifoaming agents and antimicrobial agent (Hocking, 1997) and antioxidant as well which is used as food preservatives (Davidson and Naidu, 2000). According to Asaruddin et al.(2012), vanilin also has been used as a starting material to synthesize drugs such as papaverine, I-dopa, I-methyldopa and trimethoprim as antimicrobial agent. Vanillin derivatives are considerable interest in this work was because of its potential biological activities, such as antiviral, antibacterial, antimalaria and antitumor activities.

![Figure 4: 4-hydroxy-3-methoxybenzaldehyde (Vanillin)](attachment:image.png)
2.3 Pharmacophore modeling

Pharmacophore modeling is defined as 3D arrangement of chemical groups common to active molecules and essential for their biological activities. Pharmacophore become one of the important tools in drug discovery which can be used either in Ligand-based or Structure based pharmacophore modeling. Pharmacophore modeling will be carried out by using LigandScout 3.1 software. Ligand-based pharmacophore modeling is a computational way to discover new drug in the absent of target structure, where as a structure-based pharmacophore modeling needs the target structure and 3D structure to work directly by seeing the new compound fits the active site of the target structure. Structure based drug design (SBDD) method is developing rapidly with the development of modern technology for searching cure for disease (Kalyaanamoorthy& Chen, 2011). Pharmacophore modeling features could be hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), cations (Cat), anions (Ani), aromatic center (Aro), and hydrophobic areas (Hyd) (Sun,2008).

Pharmacophore modelling is widely used due to the applications of 3D pharmacophore based has shown successful result in medicinal chemistry, thus demonstrate its utility in modern drug chemistry (Dror et al., 2004 ;Lyne et al., 2004). Nowadays, many researchers used pharmacophore approaches in the virtual screening, de novo and other application to lead optimization and multitarget drug design (Sheng, 2010). In this study, pharmacophore model will be used to synthesize new drug using vanillin derivatives. In this method, the active site of the bacteria S.aureus will be aligned with the synthesized compound to see their interactions and thus the pharmacophore hypothesis will be determined to choose the best fit value. The molecular docking is an effective method to recognize or increase the efficiency in the new lead drug (Arulalapperumal et al., 2012). The fit value may indicate how well the
features in the pharmacophore overlap with the chemical features in the molecule (Suresh et al., 2010).

According to Mustapha et al.(2011), the top rank of the pharmacophore hypothesis will be selected which it will be used for database search later on. The best pharmacophore model which has the highest interaction and fit value will be chosen as the best pharmacophore. Figure 5 shows the flowchart for pharmacophore modeling.

Figure 5: Flow chart for pharmacophore modeling. (Larger & Wolber, 2004)
2.4 Ligand Scout 3.12

Figure 6: Sample of pharmacophore modeling using Ligand Scout 3.12 software

Figure 6 shows the LigandScout software is one of the several computational approaches for building pharmacophore models either based on the protein structure or ligands. LigandScout creates a pharmacophore from structure based complex data and allows sophisticated pharmacophore analysis and fine tuning to create selective pharmacophoric screening filters for a specific target. The position of the ligand within the macromolecule is visualized using an animated protein-ligand handling that allows the user to zoom back into the protein without modifying the macromolecule at any time (Larger & Wolber, 2005).
2.5 Biological Evaluation

2.5.1 Cytotoxicity Test

A new microplate assay for cytotoxicity test using *Artemiasalina* has been developed and gives comparable result to a previously published test tube method (Solis, et al., 1992). A brine shrimp larvae (nauplii) have been widely used as a bioassay for a variety of toxic substance and this method has also been apply to plant extract in order to assist the isolation of biologically active compound. In order to determine pharmacological action and the toxicity of medicinal plant, the brine shrimp cytotoxicity assay was considered as a convenient probe for preliminary assessment of cytotoxicity (Tawaha, et al., 2005). Brine shrimp lethality test (BSLT) is an important method for preliminary assessment of cytotoxicity of drugs, by determine the LC$_{50}$ values. Figure 7 shows the brine shrimp larvae *Artemiasalina sp.*

![Brine shrimp larvae](image)

**Figure 7:** Brine shrimp larvae *Artemia salina sp.*
2.5.2 Antimicrobial Test

The Kirby-Bauer test for antibiotic susceptibility, called the disc diffusion test, is a standard that has been used for years. First developed in the 1950s, it was refined and by W. Kirby and A. Bauer, then standardized by the World Health Organization in 1961. Kirby-Bauer Test is a valuable standard tool for measuring the effectiveness of antimicrobics against pathogenic microorganisms. Kirby-Bauer Test is a test which uses antibiotic-impregnated wafers to test the effectiveness of antibiotics on the desired bacteria. This test is used to determine the resistance or sensitivity of aerobes or facultative anaerobes to specific chemicals, which can then be used by the clinician for treatment of patients to against bacterial infections. The presence or absence of an inhibitory area around the disc identifies the bacterial sensitivity to the drug. Figure 8 shows a disc containing antibiotics disk placed on agar where bacteria are growing.

Figure 8: Kirby-Bauer test
CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Pharmacophore Modeling

Pharmacophore modeling is a computational method to identify a new potential drug starting with the finding of training set to form hypothesis generation model. Pharmacophore models represent chemical functions that are essential for binding to the target and due to their simplicity, they are suitable for large scale virtual screening. In this project, LigandScout 3.12 OMEGA software was selected to perform virtual screening of synthesized compounds. LigandScout 3.12 OMEGA is a fully integrated platform for precise virtual screening based on 3D chemical feature of selected target compound and antimicrobial drugs. The most critical aspect in generation of pharmacophore hypothesis is the training set. According to Asaruddin et al., (2012), the selection of a suitable training set is essential for the quality of automatically generated pharmacophore model. This LigandScout 3.12 OMEGA can be performed either in ligand and structure based pharmacophore modeling which can be applied to identify lead compound for optimization processes in drug discovery and development.
3.1.1 Hypothesis Generation

Figure 9 shows the target structure that was downloaded into the LigandScout software 3.12. The structure based pharmacophore modeling was carried out to determine the interactions between the synthesized compounds with the target compound which is called as shared features. The selection featured of pharmacophore generation was carried out according to the chemical nature of the compound where the hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic interaction (HY), ring aromatic interaction (R) were selected for calculation with different featured combinations, minimum, maximum, and total numbers for each selected feature (Ravikumar, et al., 2008). In structure based pharmacophore modeling approach, the synthesized compounds were aligned with the selected target compound that was obtained from the protein data bank to determine their shared features. After that both