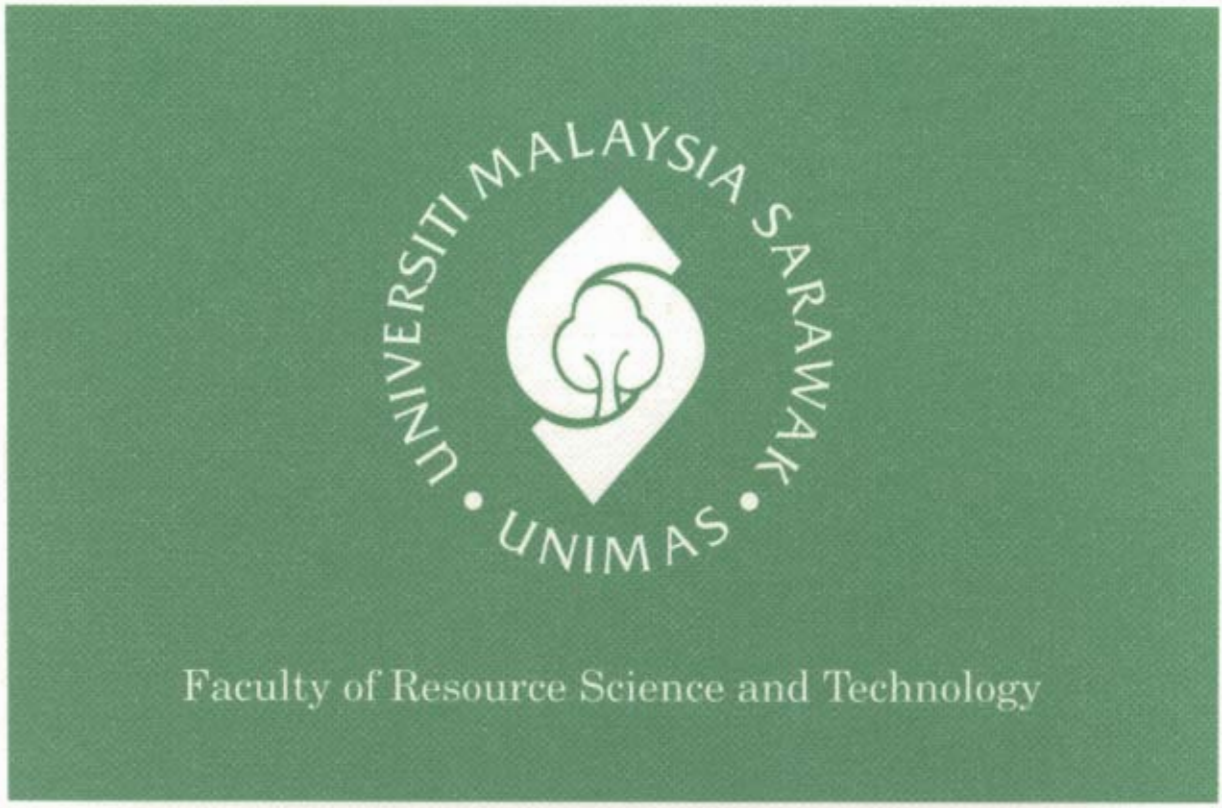

SYNTHESIS AND CHARACTERIZATION OF CHALCONE AND ITS BIOLOGICAL PROPERTIES

Luna Ak. Lium

Bachelor of Science with Honours
(Resource Chemistry)
2008

Faculty of Resource Science and Technology

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APPROVAL SHEET

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Title of Dissertation : Synthesis and characterize of chalcone and its biological properties.

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DECLARATION

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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Synthesis and characterization of chalcone and its biological properties.

Student's name: Luna Ak. Lium

Final report submitted as partial requirement of the Course Final Year Project 1 (STF 3012)

Supervisor's name: Dr. Zainab Bt. Ngaini

Programme name: Resource chemistry

Deaprtment name: Department of chemistry.

Faculty of Resource And Science Technology

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Date: 27/05/08

Abstracts:

Flavonoids and their corresponding chalcone are groups of natural products that are produced from secondary metabolite in plants for defenses against microorganisms. Chalcone is a compound that has high potential in medicinal chemistry and has been widely used in natural product. Chalcone have served as importance starting material to synthesis more complex medicinal compound and used widely in pharmaceutical. For recent years, chalcone showed interested among scientific research due to its diverse properties against bacteria and its biological activity. By considering limited source of plants that contain chalcone it is more effective to synthesis chalcone in laboratory using cross aldol condensation or claisen-schmidt condensation. Chalcone has been prepared by condensation of 4-alkoxyacetophenone and substituted benzaldehyde in the presence of base. Hydroxylated chalcone was assayed against E.coli by dilution method. Increase of bacteriostatic action due to present of hydroxyl group and alkyl group.

Keywords: Claisen- Schmidt condensation, biological activity, cross aldol condensation.

Abstrak:

Flavonoid dan chalcone adalah dalam kumpulan sebatian semulajadi yang dihasilkan dalam metabolit sekunder pada tumbuhan sebagai perlindungan daripada serangan mikrob. Kalkon merupakan sebatian yang mempunyai potensi yang tinggi dalam kimia ubatan dan banyak digunakan dalam sebatian biologi yang berasaskan sumber semulajadi. Kalkon sangat penting dalam bahan pemula sintesis untuk menghasilkan ubatan yang lebih komplek dan digunakan dalam bidang farmasi. Kalkon menjadi tumpuan para saintis kerana sifat kalkon yang antibakteria. Sumber kalkon dari tumbuhan adalah terhad dan adalah lebih efektif untuk kalkon yang disintesis dalam makmal melalui tindak balas claisen-schmidt atau tindak balas "cross aldol". Kalkon boleh disediakan melalui tindal balas claisen-schmidt diantara 4-alkoxyacetophenone dan benzaldehyde dengan kehadiran alkali. Kalkon yang mempunyai kumpulan hydroksi diuji dengan bacteria *E.coli* dengan kaedah pemelarutan. Kadar pertumbuhan bacteria akan direncat dengan kehadiran kalkon yang mempunyai kumpulan hydroksi dan kumpulan alkil.

Kata kunci: Tindak balas Claisen- Schmidt , aktiviti biologi, tindak balas "cross aldol".

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CHAPTER 1

1.0 INTRODUCTION:

This research was mainly about synthesis and characterization of chalcone and application for their biological activity. Chalcone derivative is a very potential molecule which can be extracted from plants. Synthetic chalcone have shown good biological properties and have potential for use as lead compounds to discovery of new potent drug (Yar et al., 2006). The different of this chalcone from the previous research is the presence of alkyl group at para position of acetophenone side. The attachment of alkyl group to OH group at para position of acetophenone known as etherfication process. This process occurs in the presence of strong base and catalyst.

In this research, chalcone has been synthesis in laboratory using cross aldol condensation to give hydroxylated chalcone, OH group in ortho and para position (scheme 6 and 7). This reaction refers to as Claisen- Schmidt condensation that involved dehydration of reactant to yield a chemical compound which has double bond that conjugated to both aromatic rings. Claisen-Schmidt condensation is general method that used to synthesis chalcone from substituted benzaldehyde and 4-alkoxyacetophenone in the present of KOH as a base. The reaction must run in high temperature instead room temperature to get chalcone compound. Synthesis of chalcone at room temperature not suitable for chalcone that have long alkyl group due difficulty to created a bond between benzaldehyde and acetophenone.

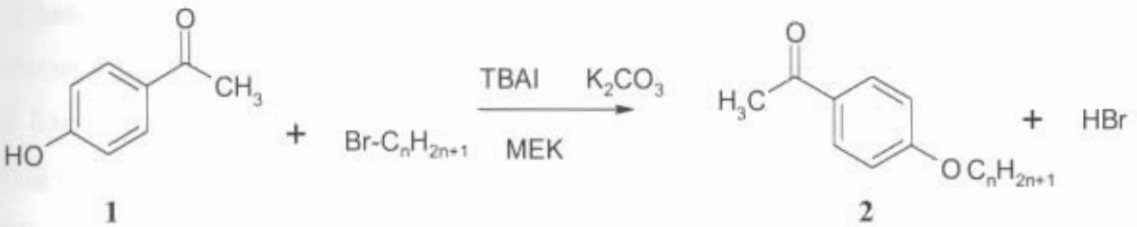
Introducing electron donating group (OH) at ortho and para position in the aromatic ring causes the chalcone compound increase bioactivity (Devia et al., 1998). The compound that have two aromatic ring joined by an α,β-unsaturated carbonyl system such as hydroxylated chalcone shown to bind to receptors and can induces activities of certain enzymes (Makita et al., 1996). Modification of molecules by alkylation will make the compound have high tendency of compound to penetrate into bacteria cell (Silverman, 1992). Introducing of alkyl group to chalcone molecules inhibit the growth of *E.coli.*

In addition to chalcone structure, the alkyl chain length influences the reactivity of chalcone structure against bacteria. However the effect in not too cleared due to lake of information regarding the effect of alkyl length in the research. The bacteriostatic activity of chalcone increase due to the presence of hydroxyl group. This supported by (Devia et al., 1998) that OH groups at ortho and para position increase the reactivity of chalcone structure against bacteria. Biological activity of chalcone also supported by (Yar et al 2006), they indicated that chalcone have the properties such as antimalarial, antioxidant, anti-inflammatory and antibacterial activity.

The objective of this paper is to investigate the effect of alkyl group and hydroxyl group on the chalcone structure. The research also includes characterization of new chalcone structure using analytical technique such as IR, H^1NMR and C^{13} NMR spectroscopy. Determination of functional group that presence in chalcone structure to proved the presence of chalcone compound.

Other objectives of this project are,

1. Alkylation of 4-hydroxyacetophenone using different alkyl length (C_3, C_4 and C_5).



1 **2**

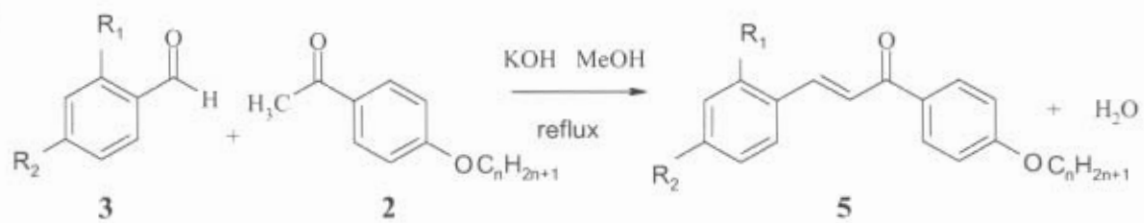
Scheme 1

n = 3: **2a**

n = 4: **2b**

n = 5: **2c**

2. Synthesis chalcone derivative with different hydroxyl position. The hydroxyl group at ortho and para position at B ring.



3 **2** **5**

Scheme 2

R_1= OH, n = 3: **5a**

R_1= OH, n = 5: **5b**

R_2= OH, n = 3: **5c**

R_2= OH, n = 4: **5d**

R_2= OH, n = 5: **5e**

R_1 = OH: **3a**

R_2= OH: **3b**

3. Test antibacterial properties of chalcone derivative using Turbidimetric-kinetic method.

CHAPTER 2

2.0 Literature review:

2.1 Chalcone

Chalcone **6** (figure 1) can be found naturally in plants and has unique molecular structure. The basic structure of chalcone contained two aromatic rings that connected with conjugated double bond with the presence of carbonyl group. Chalcone **7** that found in plants by the previous researcher showed the different substituent attach to chalcone structure and makes its more potent compound.

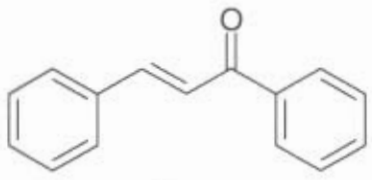


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Figure 1: General structure of chalcone.

Naturally occurring chalcone **8** in plants and synthetic chalcone **6** have shown potent biological activity and used as lead compound for the discovery of new drugs (Buzzi et al., 2007). Recently, Hijova published a review of bioavailability of chalcone showed in figure 2, which constitute of unique structure and associated with diverse biological activities.

Chalcone **10** in figure 2 are the major yellow flower pigment in plants produced from intermediate in the biosynthesis of all flavonoids. The most common chalcone founds in foods are phloretin, chalconaringenin, and arbutin (Hijova, 2006). Flavonoid play importance role in plant- microbe interaction and act as protection against microbe.

Flavonoids compounds are derived from chalcone precursors that derived from the condensation of p-coumaryl CoA and three malonyl CoA's by the enzyme chalcone synthase (CHS). CHS are members of plants polyketide synthases which form variety of natural products (Gonzalez and Rosazza, 2004).

Chalcone contain aromatic ketone that forms variety of importance biological compound. Chalcones is important compound that have right applications in medicine (Palleros, 2004). Most medicine contains chalcone derivative as active molecules against bacteria and fungal. Chalcone can exist in plants and extracted to test its biological activity. Chalcone is compound that can be found in plants or synthesis in the laboratory.

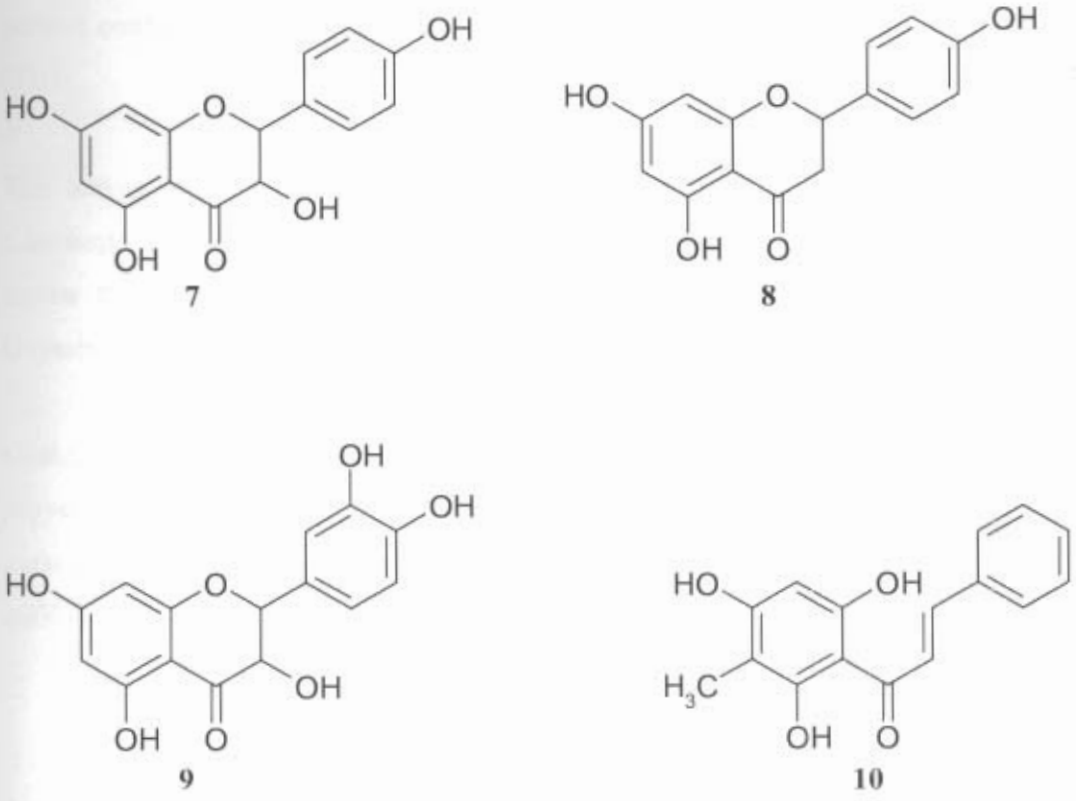

Figure 2: Subclasses of flavonoid with high variation in chemical structure.

Chalcone is one of flavonoid subclasses that can be found in plant with different substituted but still have the chalcone **6** general structure. The biological properties of chalcone varies depend on the different type molecules that attach to its general structure. Figure 2 showed subclasses of flavonoid such as naringenin **7**, kaempferol **8**, quercetin **9** and chalcone **10**.

There are several different structure can be found in plant, most of its has structure similarities with chalcone **6** structure. Figure 2 showed variations of potent flavonoid compound that has different position of OH group in there chemical structure.

In plant, chalcone can be extracted from *Artocarpus nobilis* in the family Moraceae (Jayasinghe et al., 2004). This plant have been investigated in Sri Lanka and have been proved contain bioactive properties in the previous research work (Jayasinghe et al., 2004).

The leaves of *A. nobilis* showed positive response in antifungal bioassay against *Clasdosporium cladosporioides* by TLC bio-authography method. The previous research shows that chalcone also act as antibacterial (Devia et al., 1998) and antifungal (Jayasinghe et al., 2004).

Chalcone belongs to flavonoids group that can be found in plants and animals. It is proven that flavonoid have biological and pharmacological activities such as antiviral, antioxidant, anti-inflammatory, antimutagenic and antiallergic (Alam and Mostahar, 2005).

Both the flavones and their corresponding chalcone were screened in vitro for their antibacterial and antifungal activity against four human pathogenic bacteria such as *Bacilus subtillis, Sarcina lutea, Shigella dysenteriae* , *Pseudomonas aeruginosa* and five plant as well as molds fungi such as *Colletorichum gloeosporioides, Candida albicans Aspergillus nigar, Aspergillus flavus* and *Penicillium* sp (Alam and Mostahar, 2005).

Claisen- Schmidt condensation (scheme 1) also known as aldol condensation frequently used in organic chemistry to formed carbon-carbon bonds. Aldol condensation referred to producing of α, β-unsaturated benzaldehyde or acetophenone in the present of organic solvent driving the reaction to completion. The approach in utilizing substituted benzaldehyde **3** and various symmetric acetophenone **1** to prepare chalcone derivative have been reported (Vyvyan et al, 2002).

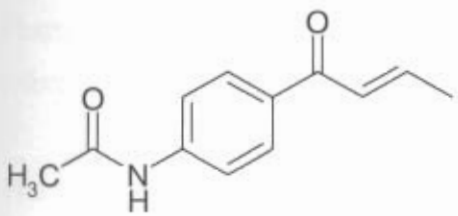

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Figure 3: Example of chalcone structure that have been synthesis in laboratory by other researcher.

Chalcone can be synthesized by condensing 4-hydroxy-3-methylacetophenone with appropriate aromatic aldehydes in dilute methanolic potassium hydroxide solution at room temperature (Yar, Siddiqui and Ali, 2006).

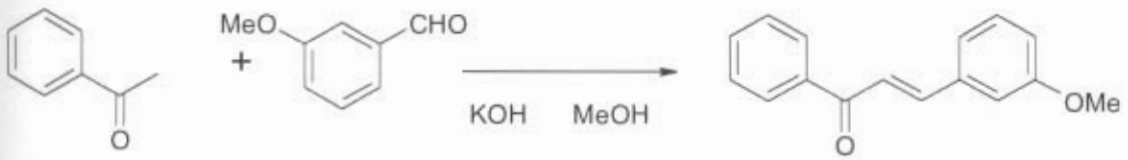
The in vitro activities of the synthesized compounds are for tuberculosis inhibition against the *Mycobacterium tuberculosis* (Yar, Siddiqui and Ali, 2006). The research conclude that chalcone derivatives interested lead molecules for further biological evaluation and the new class of this compounds can pursuit to discover novel class of antimycobial agents (Yar, Siddiqui and Ali, 2006).

Buzzi et al, 2007 demonstrated the significant of acetamidochalcone **11** as importance class of novel and potent analgesic agents. The compound synthesizes using cross aldol condensation in the present of NaOH and MeOH as solvent to give chalcone compound. Pharmacological study showed that the chalcone synthesized have more active than the reference drugs, acetylsalicylic acid and acetaminophen (Buzzi et al, 2007).

The basic structure of chalcone contains the flavan nucleus, which consist of 15 carbon atoms derived from a C_6-C_3-C_6 skeleton. Different type of chalcone can be distinguished by the presence of oxygen heterocyclic rings and the different position of hydroxyl, methyl, and methoxy group in the rings (Das and Rosazza, 2005).

2.2 Synthesis of Chalcone

Extracting chalcone from plants normally gives low yield of product and scientist has been created other ways by synthesizing chalcone in laboratory. By considering the limit in plants resources and environmental issues, chalcone can be synthesis in the laboratory. In laboratory, the synthesis of chalcone derivative can be done by cross aldol condensation. The reaction involves Claisen- Schmidt condensation. The reaction occurs in the presence dilute base.



12 **13** **14**

Scheme 3: Synthesis of chalcone using claisen- schmidt condensation.

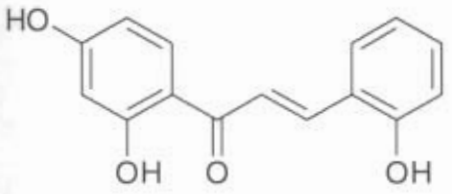
By adding equivalent amount of acetophenone and aldehyde, with the present of potassium hydroxide, ethanol as solvent (50ml), and HCL (10%) solution can yield chalcone compound in room temperature during 24 hour (Cabrera et al, 2006). It is expected the reaction between benzaldehyde **12** and acetophenone **13** can yield chalcone **14** at room temperature.

2.3 Physical properties of chalcone

The other name of chalcone is 2-benzalideneacetophenon, 2- benzalacetophenone and 2-phenylvinyl ketone. Chalcone is compound that have yellow color and have unique physical properties, boiling point at 345°C (653°F), melting point at 54°C (129.2 F). Chalcone soluble in cold water and was a stable compound.

2.4 Hydroxyl chalcone (OH).

Chalcone have high potential in medicine chemistry where the previous research showed that the modification of chalcone molecules by introducing to OH group in previous work showed chalcone **15** have reactive properties against bacteria and fungal (Devia et al, 1998). The relationships between hydroxyl groups with chalcone **15** have been determined and gave positive result as antimicrobial.



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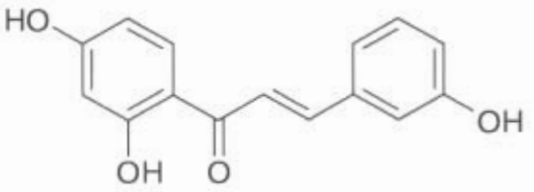

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The research was conducted by Devia et al., 1998, showed 2', 4', 2-trihydroxychalcone **15**, 2', 4', 3-trihydroxychalcone **16** and 2', 4', 4-trihydroxychalcone gave inhibition effect to *Staphylococcus aureus*. Bacteriostatic action becomes higher in the presence of hydroxyl group. The position of OH group in chalcone molecule can gives effect to chalcone antimicrobial properties. The closes the active region (C=O) to the hydroxyl group, the strongest the inhibition effect (Devia et al., 1998).

From the previous work Devia et al, 1998 suggest that chalcone synthesis should be directed to select compound with greater bioactivity with introduction of hydroxyl groups in the B-ring of the molecule. According to Maria et al, 2004, the presence of OH group at four position of chalcone can increase bacteriostatic activity of the base compound. The research showed that, increase efficacy on the part 2, 4, 3,-trihydroxychalcone against bacteria attributed to the presence of OH group at four position (Alvarez et al., 2004).

Hijova, 2006 proposed a potent activity of hydroxychalcone is due to stabilization of its radical tautomerization. The research find that 2-Hydroxychalcone have good antioxidant compared to unsubstituted chalcone.

Trihydroxylated chalcone **17** has been demonstrated to give high bacteriostatic action against *Staphylococcus aureus* (Olivella et al, 2001). The study related to the number and position of hydroxyl groups in aromatic ring of chalcone. The compound prepared by Claisen- Schmidt condensation in KOH solution with equimolar amount of 4-hydroxybenzaldehyde and 2, 4- dihydroxyacetophenone solution in ethyl acetate: water (1:1). The reaction kept at 25 ^{0}C for five days (Olivella et al, 2001). The chalcone structure obtained from this research is favorable for bacteriostatic activity due to the presence of hydroxyl groups at the chalcone rings.

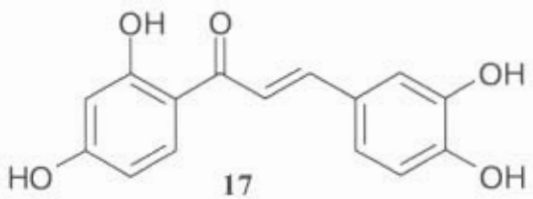

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Figure 4: Example structure of chalcone that have biological properties.

2.5 Effect of alkylation

According to Silverman 1992, by lengthening of saturated carbon side chain from one (methyl) to five to nine atom will increase pharmacological effect. The effect of alkylation has been proved in 4-n-alkylresorcinols and mandelate ester (Silverman, 1992). The structure obtained give biological effect to bacteria with the present of alkyl group in 4-n-alkylresorcinols structure. The Alkylation to molecules that have ability against microbial produced more reactive compound. But further lengthening result in decrease pharmacological effect (Silverman, 1992). Previous study show that, alkylation can increase lipophilicity of molecules, which permit penetration into cell membrane of bacteria and fungal (Silverman, 1992). Maximum potential of alkylation of 4-n-alkylresorcinols and mandelate ester against bacteria are at C3, C4 and C5 (Silverman, 1992).

Research has been done and found that alkylation of 7-hydroxycoumarin can inhibit growth of yeast (Jurd et al., 1970). Alkylation gives more effect on the growth of bacteria and can control their growth. This alkylation have been applied to the structure chalcone and showed supportive result as predicted.

CHAPTER 3

3.0 Material and method:

3.1 Material and chemical

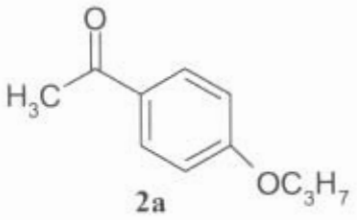
The entire chemical that used in this research are used as received in the organic laboratory. The ^{1}H NMR and ^{13}C NMR spectra were recorded in acetone D6 and $CDCl_3$ on a JEOL spectrometer operating at 500 MHz. The IR spectra were recorded on a FTIR 5300 spectrophotometer. The bacteria inhibition read by using Optima sp- 300, spectrophotometer.

3.2 General procedure to synthesis 4-alkoxyacetophenone.

The strategy for synthesis of 4-alkoxyacetophenone **2** is shown in scheme 1. The synthesis of 4-alkoxyacetophenone were accomplished starting from 4-hydroxyacetophenone **1** and bromoalkane as shown in scheme 1 in introduction. All the starting material mixed in the three neck round bottom flask. MEK as solvent, TBAI as catalyst, and K_2CO_3 give basic medium for reaction. The reaction reflux for 5 hours. The product was filtered and washed with dichoromethane (DCM). The filtrate was extracted with distilled water (2x 30ml). The organic layer was dried, filtered and concentrated under reduced pressure with rotovap. The product was obtained in yellow oils. The reaction was completed and confirmed with TLC give one spot. This compound was obtained as light yellow liquid. The structure of the compound confirmed using IR spectroscopy to determine its functional group.

3.4 Synthesis of alkoxyacetophenone.

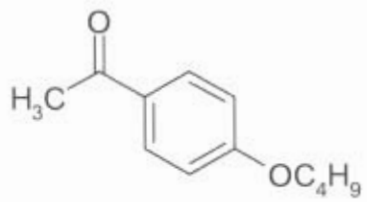
3.4.1 Synthesis of propoxyacetophenone 2a.



2a

Placed 4-hydroxyacetophenone **1** (0.03mol, 4.10g) into bromoalkane (0.036mol, 4.43g) in three neck round bottom flask with K_2CO_3 (4.98g), TBAl (1.108g) and MEK (60ml) showed in scheme 3. The mixture was refluxed for 4-6 hours. The reaction produced propoxyacetophenone **2a**. The workup procedure was according to general procedure. The compound yield gives 57% as an yellow oil. IR^{KBr} 3073, 2967, 2939, 2879, 1670, 1603, 1575, 1509, 1472, 1421, 1393, 1359, 1175, 1116, 1065, 1045, 1015, 975, 957cm^{-1}.

3.4.2 Synthesis of butoxyacetophenone 2b.



2b

Placed 4-hydroxyacetophenone **1** (0.05 mole, 6.83 g) into bromobutane (0.06mole, 6.46ml) and in MEK (60 ml), K_2CO_3 (0.06mole, 8.29g) and TBAI (0.005mole, 1.85g) in three neck round bottom flask. The reaction mixture was reflux for 5 hours to yield desired product **2b**. The workup procedure was according to general procedure. The product was obtained in yellow oils. The reaction was completed and gives percentage yield 49.8%. IR^{KBr} 3073, 3048, 2960, 2936, 2874, 1674, 1602, 1575, 1509, 1467, 1420, 1358, 1308, 1255, 1173, 1116, 1067, 1025, 1006, 956, 907, 875cm^{-1}.