Synthesis Of Curcumin Derivatives (2.5-Bis((E)-4-Hydroxy-3-Methoxy Benzylidine) Cyclopenta-1-On) from Vanillin and its Activity Test Against α-Glucosidase Enzymes

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Received: 5 Agustus 2021; Accepted: 23 Maret 2022; Published: 15 Juli 2022

Abstract

Synthesis of curcumin analogs (2.5-Bis((E)-4-Hydroxy-3-Methoxy Benzylidine) Cyclopenta-1-On) based on Claisen-Schmidt condensation using an acid catalyst and inhibition of glucosidase enzyme was carried out. The antidiabetic activity of curcumin analogs was carried out by the use of α-glucosidase enzyme from moldy rice (Oryza sativa). The curcumin analogs were synthesized by reacting the vanillin with cyclopentanone. The structure of all products was confirmed by FTIR, GC-MS, ¹H-¹³C-NMR spectrometers, and the activity enzyme. The results showed the curcumin analogs were yielded in 52.27%, respectively as yellowish-green solid. The inhibition test of the α-glucosidase enzyme showed that the curcumin analogs were the potential to inhibit the α-glucosidase enzyme with an inhibition percentage of about 94.26% in 5 mM.

Keywords: vanillin; cyclopentanone; curcumin; α-glucosidase enzymes

Introduction

Diabetes mellitus is a type of disease characterized by hyperglycemia (increased levels of blood sugar) which is continuous and varied. In the recent modern era, changes in lifestyle and diet make many people suffer from diabetes mellitus. Currently, more than 194 million people around the world have been afflicted with this disease and will have reached 333 million people by 2025, where developing countries are expected to experience more severe (Debjit et al., 2009). The number of people with diabetes mellitus were mostly those with type 2 diabetes mellitus (T2DM) (Lam et al., 2008).

The inhibition of the α-glucosidase enzyme can cause an inhibition of glucose absorption in patients with T2DM, to reduce the hyperglycemic state after eating. In humans’ digestion of dietary carbohydrates, the α-glucosidase enzyme which exists within the lysosomal membrane of instine epithelial cells functions as a breaker of glycoside bonds at the branching points of amylopectin and glycogen as well as a glucose producer.
Acarbose (Glucobay) and miglitol (Glyset) are inhibitors that are recently used clinically and can inhibit glycosidases such as α-glucosidase. According to Nampoothiri, these synthetic drugs can cause several side effects such as bloating, nausea, diarrhea and can increase diabetes complications (Nampoothiri et al., 2011). Recent studies have mostly focused on searching for analog active compounds that have activity toward α-glucosidase with low side effects and are derived from natural ingredients to obtain a product to cure T2DM (Ren et al., 2011).

*Curcuma Longa plant* which is commonly found in curcumin is one of the natural ingredients that can be used as a glucosidase inhibitor (Du et al., 2006). *Curcuma longa* Linn has been recommended as a traditional Chinese medicine to treat diabetic complications (Liang et al., 2009). The extract from the *Curcuma longa* Linn plant can also inhibit α-glucosidase activity which results in a decrease of high blood sugar levels (Lee et al., 2005).

Several curcumin analog compounds have been synthesized and examined to determine their activity on biological targets and to improve the pharmacological profile of their natural products such as increasing their selectivity, bioavailability, and stability. Therefore, the basic ingredients needed are benzaldehyde derivative compounds and ketone compounds in the form of both mono and di-ketone in terms of the disconnection approach to curcumin analog compounds. Both of these compounds can be obtained from essential oils through a semisynthetic method by applying natural ingredients that have been isolated and carried out in their synthetic step.

The main compounds in most essential oils contain aromatic compounds, one of which is vanillin which is the main product of a vanilla plant. However, the high price of natural vanillin, which reaches USD 9-11 per kg, has led to the emergence of alternative sources of other materials, that is synthetic vanillin compounds which have a similar chemical structure with isolated natural vanillin. Synthetic vanillin can be made from clove leaf oil, guaiacol, safrole, coniferin glucoside, and lignin (Rinasih, 1998). The easy manufacture of synthetic vanillin causes its price to be cheaper and its presence to be more abundant than natural vanillin. Chemical modification of synthetic vanillin is expected to increase its economic value, so it is necessary to do research (synthesis of curcumin analog compounds) which have activity as anti-diabetic compounds with vanillin as its base.

**Research Methodology**

**Materials**

The tools used are glassware, magnetic stirrer, *Ultrasonic bath* (Julabo USR3 35 kHz), analytical balance (Libor EB330 Shimadzu), vacuum desiccator, and *Electrothermal 9100*. The instruments applied for this study were infrared spectrophotometer (FTIR, Shimadzu Prestige-21), gas chromatography–mass spectrometer (GC-MS, Shimadzu QP-2010S), proton and carbon nuclear magnetic resonance spectrometer (NMR, 1H-(500 MHz) and 13C-(500 MHz), incubator, centrifuge, micropipette, and UV-Visible spectrophotometer (Shimadzu 1800-Series).

Meanwhile, the materials used have pre-analytical qualities from *Merck* which include vanillin, glacial acetic acid, hydrochloric acid, potassium bromate (KBrO₃), bromic acid 47% (HBrO), sodium thiosulfate (Na₂S₂O₃), methanol, absolute ethanol, chloroform, dichloromethane, cyclohexanone, cyclopentanone, potassium hydroxide, TLC plate (Silica Gel type 60 F₂₅₄), weathered rice, phosphate buffer, enzyme solution, nitrophenyl (NPG), ammonium sulfate, and sodium carbonate.

**Synthesizing Of Curcumin Analog**

The synthesis of vanillin-based curcumin analog as a source of benzaldehyde (10 mmol) was added with 5 mmol of cyclopentanone as a ketone source. The solution was then stirred at 25-30 °C until became homogeneous in a glacial acetic acid until the color changed from yellow to green. After that, the solution was added with concentrated hydrochloric acid drop by drop and stirred for two hours. The precipitate obtained was allowed to stand for two days.
and macerated with ethanol solvent, then dried in a vacuum desiccator. The results were weighed, and the compound structure was elucidated using FTIR spectrometer, direct-MS, $^1$H- and $^{13}$C-NMR.

Manufacturing Crude Extract of A-Glucosidase Enzyme

The weathered rice type IR 46 was turned into flour using a blender. Then 50 mL of cold 67 mM pH 6.8 phosphate buffer solution was added to a beaker glass. After that, the mixture was stirred with a magnetic stirrer with an ice bath for one hour and allowed to stand for 24 hours in the refrigerator. The mixture of weathered rice with the phosphate buffer solution was filtered using gauze to obtain the filtrate and residue. The obtained filtrate was centrifuged (8000 rpm, temperature 4°C) for 20 minutes and resulted in a supernatant whose $\alpha$-glucosidase activity would be examined.

Activity Test Method for A-Glucosidase Enzyme

The first step in testing the activity of the $\alpha$-glucosidase enzyme was to put 5mL of 67 mM phosphate buffer pH 6.8 and 0.2 mL enzyme solution into a test tube followed by mixing them at 37 °C. After that, 0.5 mL of 10 mM pNPG was added to the mixture and incubated for 20 minutes at 37 °C. The next step was to stop the reaction by taking 2 mL of the mixture and adding 8 mL of Na$_2$CO$_3$ 100 mM. Then, the absorbance of the solution was measured at a wavelength of 400 nm. The blank solution was produced in the same way by replacing the enzyme with mineral-free water. The enzyme activity was obtained from the formula in equation 1.1.

\[
\text{Unit/mL} = \frac{(A_{400\text{Unit}} - A_{400\text{Blank}})(10)(5.7)}{(18.3)(20)(2)(0.2)} 
\]  \hspace{1cm} (1.1)

Notes:
5.7 = volume of the reaction mixture
18.3 = millimolar extinction coefficient
20 = assay time

Purification of the A-Glucosidase Enzyme by Using Fractionation Method using Ammonium Sulfate

80 grams of sample flour was mixed into a 200 mL buffer solution with pH 6.0 to obtain a crude extract of the enzyme. This crude extract was then fractionated by mixing ammonium sulfate salt with a saturation level of 0-20 % and stirred using a magnetic stirrer in an ice bath and gradually added to the crude extract so that a precipitate and supernatant were gained. Then, the buffer with pH 6.0 was added until dissolved to separate the precipitate and supernatant. This precipitate was then referred to as fraction 1. Following this, the ammonium sulfate salt was added with a saturation level of 20-50% would be called fraction II, and a saturation level of 50-70% was named as fraction III to separate the supernatant after being left overnight. Then, the activity was tested on crude extract fractions I, II, III, and supernatant. To determine the value or amount of ammonium sulfate mixed into the saturation level, equation 1.2 was applied.

\[
\frac{53.55 - S_i}{120 - (0.03S_i)} \times \frac{\text{volume filtrat}}{1000} = \text{gram} \hspace{1cm} (1.2)
\]

Dialysis

The fraction that had the highest activity was then dialyzed (by changing the buffer every 2 hours), put into a cellophane membrane, and stirred using a magnetic stirrer for 9 hours at 4 °C. The cellophane membranes were immersed in mineral-free water at 70 °C for 5 minutes and immersed in 0.001 M EDTA solution containing 1% Na$_2$CO$_3$ for 1 hour. After that, the bag was rinsed with mineral-free water until clean and heated to 70 °C for 5 minutes. The optimum pH of an enzyme that had been
completely dialyzed was then determined by adding 5 mL of a solution with pH 4.5 into a test tube containing the enzyme. After all, the enzyme activity was determined.

**Determination of Km and Vmax**

The next step was to determine the enzyme kinetic parameters (Km and Vmax) based on a graph plot of the relationship between substrate concentration (S) and activity (V) for enzyme solutions whose optimum pH value had been already known.

The enzyme activity was tested according to procedure B to make a pNPG substrate solution with a concentration of 1-15 mM using the optimum pH buffer. Following this, a graph of the relation between 1/V and 1/S was made. From the graph, it can be seen that the values of Km and Vmax were based on equation 1.3, namely the Lineweaver-Burk as follows:

\[
\frac{1}{V} = \frac{K_m}{V_{\max}} \frac{1}{[S]} + \frac{1}{V_{\max}}
\]

\[
y = mx + c
\]

(1.3)

Inhibition activity test of α-glucosidase enzyme with curcumin analog compound

Standard solutions and curcumin derivative solutions were made with varying concentrations of 12.5; 10; 7.5; 5 and 2.5 mM. The percentage of inhibition was obtained from equation 1.4.

\[
\% \text{ inhibi} = \frac{(A_{\text{control}} - A_{\text{blank}}) - (A_{S1} - A_{S0})}{(A_{\text{control}} - A_{\text{blank}})} \times 100\%
\]

(1.4)

Note:

US1 = absorption of the system containing the sample and enzyme
AS0 = absorption of the system containing the sample without enzyme
Acontrol = absorption of the system containing the enzyme without sample
Abank = absorption of the system without containing enzymes and samples

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**Results and Discussion**

**Results of Synthesis of Curcumin Derivatives (2.5-Bis((E)-4-Hydroxy-3-Methoxy Benzylidene) Cyclopenta-1-On)**

Synthesis of curcumin compounds is generally carried out through reaction Claisen Schmidt. In this study, the synthesis of curcumin derivatives involved benzaldehyde, namely vanillin and ketone, namely cyclopentanone at room temperature in glacial acetate and HCl, the reaction equation can be seen in Figure 1.

![Figure 1. Reaction Equation of Vanillin-Based Curcumin Analog](image)

The result of the synthesis is a greenish-yellow solid. The results of TLC analysis using ethyl acetate eluent: dichloromethane (1:1) and observation at λ 254 nm showed the presence of two strains, namely the dark (V) reactant, and the yellow (K) product, and indicated an Rf difference.

The curcumin derivative compound of (2.5-Bis((E)-4-Hydroxy-3-Methoxy Benzylidene) Cyclopenta-1-On) was analyzed by using FTIR and resulting spectra which can be seen in Figure 2.
Figure 2. FTIR Spectra of Curcumin Analog

Several functional groups were detected, such as a hydroxyl group (OH) at the absorption of 3471 cm\(^{-1}\) and an aryl ether CO group at the absorption of 1211 cm\(^{-1}\). The wavenumbers at 1681 and 1620 cm\(^{-1}\) indicate the absorption of C=C alkenes (Konatham, Kumar and Pharma, 2010), and the wavenumbers 933 cm\(^{-1}\) indicate the trans alkene structure. Then, the sharper absorption of 1581 and 1512 cm\(^{-1}\) is characteristic of the absorption of -C=C- bonds in aromatic compounds.

The formation of curcumin derivatives (2,5-Bis((E)-4-Hydroxy-3-Methoxy Benzylidine) Cyclopenta-1-On) was confirmed using direct-MS, which can be seen in Figure 3.

Figure 3. Mass Spectra of Curcumin Analog

The direct-MS results show that the single peak formed has a molecular ion (M\(^{+}\)) as well as base peak, namely m/z 352 which corresponds to the molecular weight of the curcumin compound (2,5-Bis((E)-4-Hydroxy-3-Methoxy Benzylidine) Cyclopenta-1-On). Molecular ion (M\(^{+}\)) with m/z 352 releases radicals –OH and –OCH\(_3\) giving two fragments namely with m/z 335 and 321. Cleavage of larger aryl radicals gives fragments with m/z 215 and 137.

Further truth of the synthesized curcumin analog compound structure was proven by \(^1\)H-NMR, which can be seen in Figure 4.

Figure 4. Spectra \(^1\)H-NMR of Analog Curcumin

Spectra \(^1\)H-NMR showed 7 different proton signals and the results of the \(^1\)H-NMR spectra analysis are presented in Table 1. The proton uptake at –OH bound to the aromatic ring (δ 5.85 ppm) appeared with a singlet. Peak 1 at 3.02 ppm with a singlet of 2H is the absorption on the cyclopentanone ring. The chemical shift at 3.83 ppm has a singlet with the 3H integration being the absorption of three protons from the methoxy group.

Table 1. Analysis of Spectra \(^1\)H-NMR Analog Curcumin

| Peak | Chemical shift (δ, ppm) | Appearance, coupling constant (J, Hz) | Number and type of protons |
|------|-------------------------|--------------------------------------|---------------------------|
| 1    | 3.02                    | Singlet                             | 2H, –C-CH\(_2\)           |
| 2    | 3.83                    | Singlet                             | 3H, -OCH\(_3\)           |
| 3    | 5.85                    | Singlet                             | 1H, OH                    |
| 4    | 6.99                    | Doublet, J= 7.75                    | 1H, H-Ar                  |
| 5    | 7.10                    | Doublet, J= 7.75                    | 1H, H-Ar                  |
| 6    | 7.25                    | Doublet, J= 1.95                    | 1H, H-Ar                  |
| 7    | 7.53                    | Singlet                             | 1H, H\(_\beta\)          |

The structure of the curcumin derivative was further confirmed by \(^{13}\)C-NMR spectra (can be seen in Figure 5) and the results of the analysis are presented in Table 2.
Figure 5. Spectra $^{13}$C-NMR of Analog Curcumin

Spectra $^{13}$C-NMR showed that there were 11 carbon peaks according to the number of carbons present in the curcumin analog. At $\delta$ 26.75 ppm C atoms of the methoxy group were observed and several peaks were detected at $\delta$ 113.75, 115.24, 125.11, and 128.27 ppm. They were carbon uptake of C$_3$, C$_4$, C$_5$, and C$_6$ attached to an aromatic ring. Carbon 9 and 10 were observed at chemical shifts of 147.19 and 147.80 ppm. This shift was due to the presence of hydroxy and methoxy functional groups which have high electronegativity so they were deshielding. At $\delta$ 196.87 ppm, it is estimated as C=O carbonyl is located in the most deshielding due to the presence of O atoms which were directly bonded to C atoms.

Table 2. Analysis Results of $^{13}$C-NMR Analogue Curcumin

| Peak | Chemical shift (δ, ppm) | Carbon Types |
|------|------------------------|--------------|
| 1    | 26.75                  | C-H          |
| 2    | 55.88                  | -OCH$_3$     |
| 3    | 113.75                 | C$_{Ar}$     |
| 4    | 115.24                 | C$_{Ar}$     |
| 5    | 125.11                 | C$_{Ar}$     |
| 6    | 128.27                 | C$_{Ar}$     |
| 7    | 134.48                 | C-H          |
| 8    | 135.00                 | C-H          |
| 9    | 147.19                 | C$_{Ar}$OH   |
| 10   | 147.80                 | C$_{Ar}$-OCH$_3$ |
| 11   | 196.87                 | C=O          |

Enzyme Inhibition Test on A-Glucosidase making Crude Extract of A-Glucosidase Enzyme

The first step in making a crude extract of the enzyme was by crushing rice using a blender to get a texture that is easy to get out and into the liquid medium (buffer). To prevent the growth of microorganisms, rice flour was stored in the refrigerator. For the next step, a 67 mM phosphate buffer solution was added at pH 6.8 because the α-glucosidase enzyme worked optimally at that pH (Kita et al., 1991). According to Scopes, phosphate buffers have several advantages such as the pH range of pH 5 to 8.8 which is generally a physiological pH that is non-toxic to cells and the temperature changes do not affect pH drastically (Scopes, 2013).

Determination of The Optimum Buffer PH in The Crude Extract of The Enzyme

Each enzyme has a specific optimum pH and each enzyme has a different optimum pH. Therefore, it is necessary to determine the pH because pH is one of the variables that affect enzyme activity. Manufacturing the crude extract of α-glucosidase enzyme applied 67 mM phosphate buffer on pH 3.5; 4.0; 4.5; 5.0; 5.5; 6.0; 6.5; 7.0 and 7.5. The weathered rice was extracted at each of these pHs and the enzyme activity was tested. The greatest enzyme activity was found in crude enzyme extract pH 4.5 which was 0.0078 mU/ml (Table 3), equivalent to the liberation of 0.0234 μmol/minute of D-glucose from pNPG substrate.

Table 3. Data on α-glucosidase enzyme activity at several pH values

| pH  | Absorbance | Activity (mU/mL) |
|-----|------------|------------------|
| 3.5 | 0.0083     | 0.0032           |
| 4   | 0.0120     | 0.0047           |
| 4.5 | 0.0600     | 0.0078           |
| 5   | 0.0113     | 0.0044           |
| 5.5 | 0.0103     | 0.0040           |
| 6   | 0.0123     | 0.0048           |
| 6.5 | 0.0103     | 0.0040           |
| 7   | 0.0093     | 0.0036           |
| 7.5 | 0.0097     | 0.0038           |
Fractionation and Dialysis of α-Glucosidase Enzyme

Fractionation was carried out to fractionate crude extract of the enzyme which had optimum activity using graded fractionation which included ammonium sulfate salt. The ammonium sulfate salt was used to purify enzymes because of the strong interaction between the hygroscopic ammonium sulfate salt and water.

This study consisted of three fractions, namely fraction I with ammonium sulfate salt at a saturation level of 0-20%, fraction II with ammonium sulfate salt at a saturation level of 20-50%, and fraction III with ammonium sulfate salt at a saturation level of 50-70%. After the fractionation process, it obtained a precipitate which was then dialyzed to remove the remaining ammonium sulfate salt, small molecules, and impurities. Fractions I (0-20%), II (20-50%), and III (50-70%) were examined for their activities to determine which fraction had the highest activity. The fraction that had the highest activity would be next used for testing the inhibition of curcumin analog compounds toward the α-glucosidase enzyme.

Determination of the value of $K_m$ and $V_{max}$

The values that need to be investigated to determine the kinetic parameters of the enzyme are $K_m$ and $V_{max}$. $K_m$ is the substrate concentration when the enzyme reaction rate reaches $V_{max}$, while $V_{max}$ is the maximum speed for the enzyme to convert the substrate into a product. The α-glucosidase enzyme will show its activity if the substrate is successfully converted into $p$-nitrophenol and glucose. In this study, what was observed was the formation of $p$-nitrophenol by measuring the absorbance at a wavelength of 400 nm. The values of $K_m$ and $V_{max}$ were determined by measuring the activity of the α-glucosidase enzyme per minute [V] at the substrate concentration [S]. The graph of the relationship between $1/V$ and $1/S$ can be seen in Figure 7.

Figure 7. Graph of the relationship between $1/[S]$ to $1/[V]$

The relationship between substrate concentration (S) and enzyme activity was the basis for determining $K_m$ and $V_{max}$. The activity of the enzyme was proportional to the velocity (V) which described the speed of the α-glucosidase enzyme to hydrolyze the pNPG substrate to release 1 mol of D-glucose per minute at 37°C. According to Michaelis-Menten, the value of V continues to increase in line with the increase in substrate concentration [S] and will reach a state V where if [S] is increased again, the V will not increase anymore. This condition is said to have reached the maximum speed ($V_{max}$).
The Activity Test of α-Glucosidase Enzyme Inhibition with The Derivative Compound of Curcumin and Quercetin

The derivative compound of curcumin was tested for activity with a concentration of 2.5; 5.0; 7.5; 10.0; 12.5 mM. Table 4 showed that the vanillin-based analog of curcumin with cyclopentanone had lower absorption data at a concentration of 5 mM, which was 0.009 nm.

Table 4. Data on the absorption of the α-glucosidase enzyme with curcumin as a synthetic product result

| Concentration (mM) | Absorption of curcumin compound (nm) |
|--------------------|-------------------------------------|
| 2.5                | 0.046                               |
| 5                  | 0.009                               |
| 7.5                | 0.076                               |
| 10                 | 0.111                               |
| 12.5               | 0.269                               |

The lower the absorption value was shown, the higher the activity of the compound was produced. Du’s study stated that higher activity was obtained from the cyclopentanone-based curcumin analog (Du et al., 2006). Thus, this curcumin analog was compared with the positive control, which was quercetin. In some previous studies, quercetin could reduce the glucose contained within the blood by inducing streptozocin of diabetes (Vessal, Hemmati, and Vasei, 2003).

Table 5. Data on the percentage of inhibition of curcumin and quercetin analogs

| Concentration (mM) | % Inhibition |
|--------------------|--------------|
|                    | Curcumin analog | Quercetin |
| 2.5                | 70.70         | 49.68     |
| 5                  | 94.26         | 54.77     |
| 7.5                | 51.59         | 17.83     |
| 10                 | 29.29         | 71.97     |
| 12.5               | 27.09         | 67.36     |

The percentage value of inhibition was a parameter of curcumin activity in inhibiting the α-glucosidase enzyme. Thus, the higher the activity of the compound as an inhibitor was indicated by the higher % inhibition shown. The results of the enzyme activity showed that the curcumin analog showed a high percentage of inhibition (94.26%) at a concentration of 5 mM compared to quercetin (71.97%) at 10 mM concentration. These results indicate that the curcumin analog compound is quite potential in inhibiting the α-glucosidase enzyme since it can inhibit the α-glucosidase enzyme although with a small concentration.

Conclusions

The synthesis of curcumin analog was successfully synthesized by the cross aldol condensation reaction of vanillin base material with cyclopentanone using an acid catalyst and obtained a curcumin analog compound in the form of a yellowish-green solid with a yield of 52.27%. The curcumin derivative compounds have sufficient potency toward α-glucosidase enzyme with an inhibition percentage of 94.26% at 5 mM concentration.

References

Debjit, B., Chiranjib, B., Jitender, Y., & Chandira, M. R. (2009). Role of community pharmacist in management and prevention diabetic foot ulcer and infections. *Journal of Chemical and Pharmaceutical Research, 1*(1), 38–53.

Du, Z. Y., Liu, R. R., Shao, W. Y., Mao, X. P., Ma, L., Gu, L. Q., Huang, Z. S., & Chan, A. S. C. (2006). α-Glucosidase inhibition of natural curcuminoids and curcumin analogs. *European Journal of Medicinal Chemistry, 41*(2), 213–218. https://doi.org/10.1016/j.ejmech.2005.10.012

Kita, A., Matsui, H., Somoto, A., Kimura, A., Takata, M., & Chiba, S. (1991). Substrate specificity and subsite affinities of crystalline α-glucosidase from Aspergillus niger. *Agricultural and Biological Chemistry, 55*(9), 2327–2335. https://doi.org/10.1080/00021369.1991.10870952

Konatham, S., Kumar, P., & Pharma, J. A. (2010). Synthesis and Screening of Antidiabetic Activity of Some Novel Curcumin Analog. *International Journal of Pharma and Bio Sciences, 1*(2), 1–12.
Lam, S. H., Chen, J. M., Kang, C. J., Chen, C. H., & Lee, S. S. (2008). α-Glucosidase inhibitors from the seeds of Syagrus romanzoffiana. *Phytochemistry, 69*(5), 1173–1178. https://doi.org/10.1016/j.phytochem.2007.12.004

Lee, S. L., Huang, W. J., Lin, W. W., Lee, S. S., & Chen, C. H. (2005). Preparation and anti-inflammatory activities of diarylheptanoid and diarylheptylamine analogs. *Bioorganic and Medicinal Chemistry, 13*(22), 6175–6181. https://doi.org/10.1016/j.bmc.2005.06.058

Liang, R., Yuan, H., Xi, G., & Zhou, Q. (2009). Synthesis of wheat straw-g-poly(acrylic acid) superabsorbent composites and release of urea from it. *Carbohydrate Polymers, 77*(2), 181–187. https://doi.org/10.1016/j.carbpol.2008.12.018

Nampoothiri, S. V., Prathapan, A., Cherian, O. L., Raghu, K. G., Venugopalan, V. V., & Sundaresan, A. (2011). In vitro antioxidant and inhibitory potential of Terminalia bellirica and Emblica officinalis fruits against LDL oxidation and key enzymes linked to type 2 diabetes. *Food and Chemical Toxicology, 49*(1), 125–131. https://doi.org/10.1016/j.fct.2010.10.006

Ren, S., Xu, D., Pan, Z., Gao, Y., Jiang, Z., & Gao, Q. (2011). Two flavanone compounds from litchi (Litchi chinensis Sonn.) seeds, one previously unreported, and appraisal of their α-glucosidase inhibitory activities. *Food Chemistry, 127*(4), 1760–1763. https://doi.org/10.1016/j.foodchem.2011.02.054

Rinasih, I. (1998). *Sintesis Senyawa 3,4 Dimetoksi Benzaldehida dari Vanilin dalam Suasana Basa*. Universitas Gadjah Mada.

Scopes, R. (2013). Protein purification: principles and practice. *Springer Science & Business Media, 176*(1).

Vessal, M., Hemmati, M., & Vasei, M. (2003). Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology, 135*(3), 357–364. https://doi.org/10.1016/S1532-0456(03)00140-6