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Effect of reaction parameters on lipase-catalyzed synthesis of caffeic acid bornyl ester (CABE)

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ABSTRACT

Caffeic acid bornyl ester (CABE) is a rare natural product with high potential of biological and pharmacological properties. However, the chemical extraction and synthesis of CABE is inefficient, uneconomical and toxic for the environment and human consumption. In this study, the lipase-catalyzed synthesis of CABE using transesterification reaction is proposed because of its advantages over chemical synthesis. CABE was synthesized using different reaction parameters such as various types of enzymes, organic solvents, enzyme loading, reaction temperature and reaction time to investigate their effects on the lipase-catalyzed transesterification reaction. Novozym 435 produced the highest conversion for synthesis of CABE compared to Lipozyme TLIM and Lipozyme RMIM. Mixed solvents system (n-hexane:acetone, 80:20, %v/v) was found to be the best solvent for synthesis of CABE compared to isooctane, n-hexane, n-heptane and toluene. The highest CABE yield with 84% conversion was obtained after 48 hours with enzyme activity of 125 U at 40°C.

INTRODUCTION

Natural products have long been used to cure numerous diseases and illnesses in the history of mankind. Today, natural products (NPs) have indeed become one of the most essential sources of drug in the development of novel medicinal compounds for the treatment of various illnesses and diseases. Originate mainly from the biodiversity of flora and fauna, most of natural products are encoded to be bio-active with various beneficial biological activities, pharmacological properties, nutritional value, flavor and preservation activities (David *et al*., 2014; Amirkia and Heinrich, 2015).

Despite extensive application and interest in natural products as medicinal drugs, a large number of big pharmaceutical industries discontinued their natural product programs (David *et al*., 2014). A study by Kingston revealed that Food and Drug Administration (FDA) approval on new drugs have decreased significantly from year 1990 to 2010 (Kingston, 2011). As a result, a lot of pharmaceutical companies terminated their NPs discovery and development programs. The main reason to explain the termination of these programs is the application of chemical method in the extraction of lead compound directly from natural sources. This approach resulted in the difficulty to preserve biodiversity, difficulty in obtaining remarkable amount of lead compounds since they presence in very small quantity, hardness in harvesting the sources, complicated synthetic pathway resulted in low yield, prolong development times, problem in isolation and purification of the compound of interest, high toxicity of final products, funding issue and governments policies (David *et al*., 2014).

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Among natural products that have gained researchers interest in the last few years is the caffeic acid (3,4-dihydroxycinnamic acid) and its derivatives or analogs. Caffeic acid (CA) can be found widely in human diet and nature including wide variety of plants such coffee, wine, fruits and vegetables (Wang *et al*., 2008). Extensive studies on caffeic acid from the past few years had revealed their potential biological and pharmacological properties. Among significant properties of CA are antioxidant (Sroka and Cisowski, 2003), antibacterial (Tsou *et al*., 2000), anticancer, anti-inflammatory and antiviral activities (Wang *et al*., 2008). Due to the remarkable properties present, CA could serve as a potential lead compound for the development of new drug or new chemical entities.

Caffeic acid bornyl ester (CABE), a newly identified caffeic acid derivative is a naturally occuring compound found in *Valeriana wallichii* (*V. wallichii*) rhizomes. CABE have been identified as the active component in the treatment of Leishmaniasis, an infectious disease caused by protozoan parasites (Glaser *et al*., 2014). In addition, CABE have also been found to have potential anticancer property (Yang *et al*., 2014). However, the chemical extraction and synthesis is inefficient, uneconomical, time consuming (Wang *et al*., 2014) and toxic for human consumption. Up to now, few attempts have been made to efficiently synthesize CABE. Thus, biocatalysis was used as an alternative approach in this study to synthesize CABE.

Biocatalysis can be defined as the use of biocatalysts such as enzymes or whole cells in the industrial synthetic chemistry. It is one of the most popular approaches in synthesizing numerous industrial compounds in various industries such as food, pharmaceutical, agricultural and chemical industry (Johannes *et al*., 2006). Additionally, biocatalysis or enzyme-catalyzed reaction offers several advantages such as environment friendly, faster reaction time, high catalytic efficiency, mild reaction conditions and high substrate specificity or selectivity which in turn minimize byproducts formation, reduced use of protecting groups, easier separation and reduce environmental problem (Johannes *et al*., 2006; Lopez Giraldo *et al*., 2007).

There are several parameters affecting the enzyme-catalyzed reaction. The solvents used in the reaction must allow the activity of enzyme. For example, polar solvents resulted in lower activity of enzyme due to stripping of essential water layer on the surface of enzyme (Gupta, 1992). The different types of enzymes work best in specified chemical reaction and substrate and each of them has its own optimum temperature. Therefore, the best enzyme should be chosen and correct temperature for the reaction should be determined to avoid inactivation of enzyme at lower or higher values (Desnuelle and Savary, 1963; Garlapati *et al*., 2013). Therefore, the main objective of this study is to synthesize CABE via lipase-catalyzed transesterification reaction using selected reaction parameters such as various types of organic solvents, various immobilized enzymes, enzyme loading, temperature and reaction time to obtain the highest conversion.

MATERIALS AND METHODS

Materials:

All enzymes used were commercially available immobilized lipases. Novozym 435 (*Candida antarctica B* lipase immobilized on acrylic resin), Lipozyme TLIM (*Thermomyces lanuginosus* lipase immobilized on silica gel carrier) and Lipozyme RMIM (*Rhizomucor mehei* lipase immobilized on a macroporous ion-exchange resin) were purchased from Novo Nordisk (Bagsvaerd, Denmark). All chemicals are of analytical grade: isooctane and toluene (Fischer Scientific, Nepean), acetone, acetonitrile, n-hexane, and n-heptane (Merck, German), borneol (Thermo Fischer Scientific, USA), and ethyl caffeate (Enzo Life Sciences, New York). All chemicals were used without purification.

Methods:

1. Lipase-catalyzed synthesis of CABE:

Lipase-catalyzed transesterification for the synthesis of caffeic acid bornyl ester (CABE) was conducted according to the method by Jun *et al*. (2013) with little modification. The reaction was carried out in tightly closed container by dissolving 0.05 mmol ethyl caffeate (EC) and 0.1 mmol borneol in mixed solvents system, n-hexane:acetone (80:20, %v/v) up to 5 ml. The mixture was agitated for 30 minutes at 40°C and 200 rpm before addition of 50 mg (125 U) Novozym 435 and 12.5 µl water to start off the reaction. The reaction was monitored periodically by taking out 100 µl liquid samples from the reaction mixture. The liquid sample was then air dried to remove the solvents followed by addition of 1 ml acetonitrile prior to analysis. The samples were analyzed using High Pressure Liquid Chromatography (HPLC). The CABE yield was estimated by calculating the EC conversion as follow:

$$\text{EC Conversion} = \frac{\text{Consumptive molar amount of EC}}{\text{Initial molar amount of EC}} \times 100\%$$

2. The effect or reaction parameters on lipase-catalyzed synthesis of CABE:

The effect of reaction parameters on lipase-catalyzed transesterification reaction to produce CABE were conducted using general method such as in method number 1. The effect of various enzymes was conducted using three types of immobilized lipase which were Novozym 435, Lipozyme TLIM and Lipozyme RMIM. Various organic solvents were tested such as n-hexane:acetone (80:20, %v/v), isooctane, n-hexane, n-heptane and toluene to investigate their effects. The effect of enzyme loading was conducted from the range of 25 U to 250 U while the effect of reaction temperature was conducted from the range of 30°C to 45°C. The reaction time was carried out from 0 h to 72 h to obtain the highest conversion in lipase-catalyzed synthesis of CABE.

3. HPLC Analysis:

The samples were examined using HPLC (Shimadzu) equipped with ultra violet (UV) detector and Thermo Scientific C18 column. The determination of product was performed using acetonitrile and water as mobile phase (acetonitrile:water, 50:50, v/v) at a flow rate of 1 ml/min. The CABE produced were detected by ultraviolet (UV) detector at the wavelength of 290 nm.

RESULTS AND DISCUSSIONS

This section discusses the effect of various parameters on lipase-catalyzed synthesis of CABE using transesterification reaction. The parameters discussed here are the effect of various enzymes, the effects of various organic solvents, the effect of enzyme loading, reaction temperature and reaction time.

1. Effect of various enzymes:

The specificity of different types of enzymes on production of CABE was studied using three types of commercially available immobilized lipases which were Novozym 435, Lipozyme RMIM and Lipozyme TLIM (Fig. 2). Novozym 435 showed highest activity with 65 % conversion in 24 hours. The lowest yield of product was given by Lipozyme RMIM with only 32 % conversion. The different activity of different lipases is due to the specificity of certain types of lipases on the reaction they catalyze and specific substrate that they used (Desnuelle and Savary, 1963). In this study, Novozym 435 showed high specificity toward the synthesis of CABE which is a type of ester. This was supported by previous study in which Novozym 435 has been the most suitable lipase in most ester and transester synthesis (Yadav and Lathi, 2005; Pang *et al*., 2013). A study by Ha *et al*. (2012) on enzyme-catalyzed esterification for the synthesis of caffeic acid phenethyl ester (CAPE) also displayed that Novozym 435 exhibited the highest activity compared to other commercially immobilized lipase. Thus, Novozym 435 was used in all experiments.

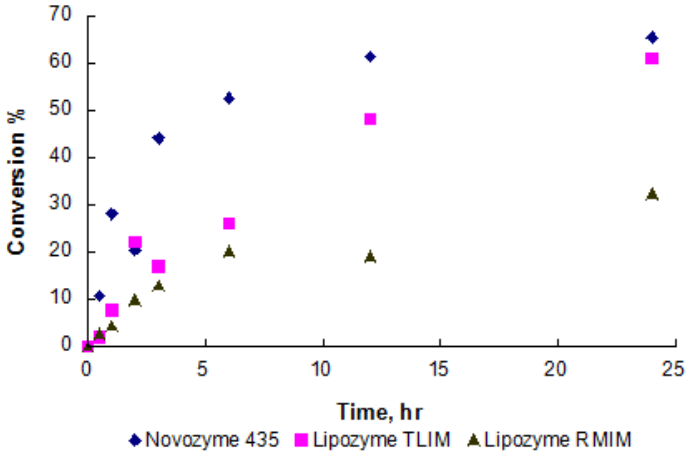

Fig. 1: Effect of various enzymes. Reaction conditions: EC, 0.05 mmol; Borneol, 0.1 mmol; Solvent, isooctane up to 5 ml; Enzyme unit activity, 500U (for all types of enzymes); Temperature, 40°C; Agitation speed, 200 rpm; Reaction time, 0-24h.

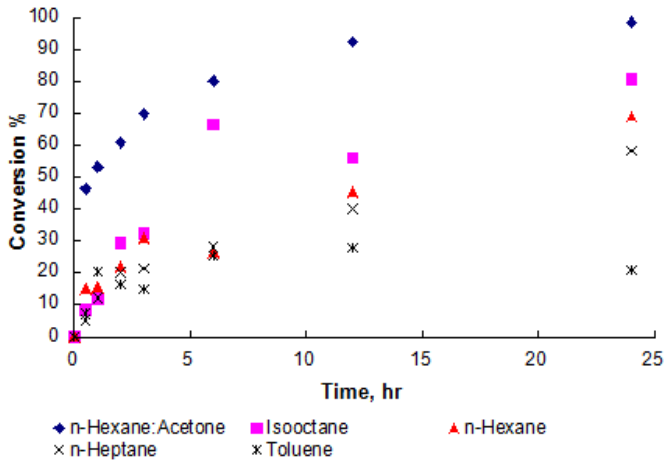

Fig. 2: Effect of various organic solvents. Reaction conditions: EC, 0.05 mmol; Borneol, 0.1 mmol; Solvent, all types of solvents up to 5 ml; Enzyme unit activity, 500U; Temperature, 40°C; Agitation speed, 200 rpm; Reaction time, 0-24h.

2. Effect of various organic solvents:

The selection of organic solvents system is crucial in determining the success of lipase-catalyzed reaction. The investigation on the effect of different organic solvents was carried out by using isooctane, toluene, n-hexane, n-heptane and a mixed solvents system, n-hexane:acetone (80:20, %v/v) while the other parameters were held constant. The highest production of CABE was obtained in the mixed solvents system, n-hexane:acetone (80:20, %v/v) with 98 % conversion followed by isooctane (80 %), n-hexane (77%), n-heptane (58%) and toluene

(20%) (Fig. 1). The effect of different solvents on enzymatic activity can be best correlated by the logarithm of partition coefficient (log P) of organic solvents. The higher the log P values, the solvents are more hydrophobic (non-polar) and thus result in better enzyme performance. In contrast, polar solvents with lower log P value result in enzyme inactivation. This is due to the fact that polar solvents such as acetonitrile and acetone are miscible in water and thus strip off the water layer around the enzyme (Gupta, 1992). Enzyme requires sufficient amount of water in order to retain its activity, structural integrity, stability and active site polarity (Sumbita, 2014). Therefore, non-polar solvents such as isooctane, n-hexane and n-heptane are preferred in most of enzymatic reaction. However, ethyl caffeate exhibits poor dissolution in these solvents. Since substrate solubility is important in obtaining high product yield, the mixed solvents system n-hexane:acetone (80:20, %v/v) were employed in this study. Hexane has high log P value (3.5) which is suitable for lipase-catalyzed reaction while acetone has very low log P value (-0.042) which could inactivate lipases. Thus, only small amount of acetone was used to completely dissolve EC before addition of n-hexane. As a result, the synthesis of CABE had been more efficient.

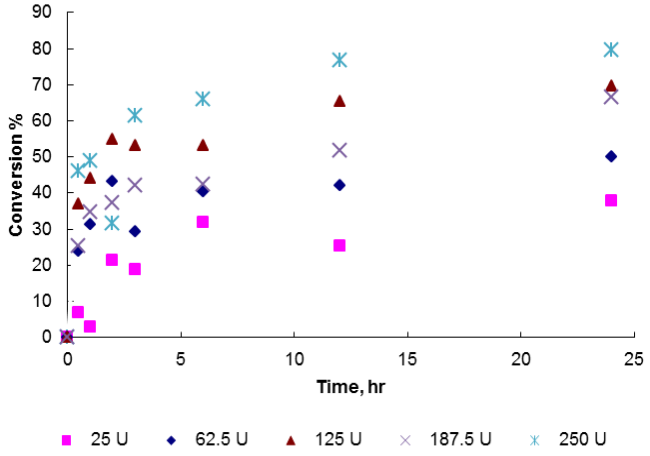

Fig. 3: Effect of enzyme loading. Reaction conditions: EC, 0.05 mmol; Borneol, 0.1 mmol; Solvent, n-hexane:acetone (80:20, %v/v) up to 5 ml; Enzyme unit activity, 25-250U; Temperature, 40°C; Agitation speed, 200 rpm; Reaction time, 0-24h.

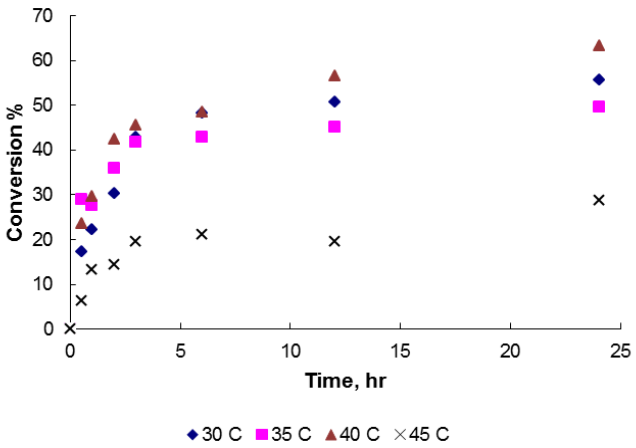

Fig. 4: Effect of reaction temperature. Reaction conditions: EC, 0.05 mmol; Borneol, 0.1 mmol; Solvent, n-hexane:acetone (80:20, %v/v) up to 5 ml; Enzyme unit activity, 125U; Temperature, 30-45°C; Agitation speed, 200 rpm; Reaction time, 0-24h.

3. Effect of enzyme loading:

The loading of enzyme in transesterification reaction was investigated using various activity of lipase Novozym 435 in the range of 25 to 250 U (10 to 100 mg). The yield of product has been significantly increased from 51 % (25 U) to 70 % (125U). However, further increased of the activity of enzyme from 187.5 U (67 %) to 250 U (80 %) has moderate effect on product conversion (Fig. 3). This is because further increased in the amount of enzyme higher than substrate concentration resulted in external mass transfer resistance (Yadav and Lathi, 2005) and enzyme saturation (Gu *et al*., 2014). The study on the effect of amount of enzyme used in the reaction is important for economical consideration in large scale industrial production (Garlapati *et al*., 2013). Although the highest product yield was obtained at lipase activity of 250 U, the increased was moderate. Therefore, 125 U (50 mg) of enzyme was selected to be used in all experiments for economical reason.

4. Effect of reaction temperature:

The correct reaction temperature is critical for enzymes to function optimally since different type of enzymes function at different optimum temperature. The increase of temperature up to enzyme's optimal condition speeds up the rate of the reaction. However, further increase of temperature after that resulted in enzyme denaturation or inactivation (Garlapati *et al*., 2013). The effect of different temperature on lipase-catalyzed transesterification of CABE was carried out in the temperature range from 30°C to 45°C (Fig. 4). The highest

CABE yield was obtained at 40°C. The percentage of CABE yield was increased from 30°C (56%) and 35°C (50%) to 40°C (64%) but decreased significantly at 45°C (29%). Therefore, the optimal temperature for lipase catalyzed synthesis of CABE is at 40°C which was used in all further experiments.

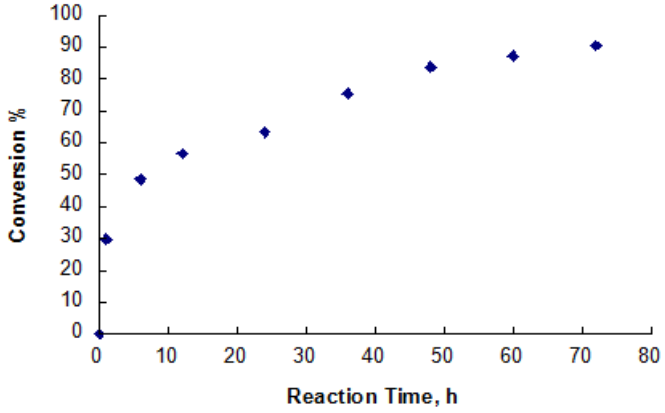

Fig. 5: Effect of reaction time. Reaction conditions: EC, 0.05 mmol; Borneol, 0.1 mmol; Solvent, n-hexane:acetone (80:20, %v/v) up to 5 ml; Enzyme unit activity, 125U; Temperature, 40°C; Agitation speed, 200 rpm; Reaction time, 0-72h.

5. Effect of reaction time:

A study on the effect of reaction time in lipase-catalyzed synthesis of CABE was conducted at various time intervals ranging from 0 to 72 h (Fig. 5). The reaction was conducted at the best conditions obtained using 0.05 mmol EC with 0.1 mmol borneol in 5 ml of n-hexane:acetone (80:20. %v/v). Then, 125 U of Novozym 435 was added followed by addition of 12.5 µl water at 40°C and 200 rpm. Results showed that the maximum CABE yield was obtained at 72 h with 91 % conversion. A rapid conversion was observed from 0 to 48 h and then moderately increased from 48 to 72 h. Since the increased in the percentage of CABE yield from 48 to 72 h was moderate, reaction time of 48 h with 84% conversion was selected to be used in lipase-catalyzed synthesis of CABE due to economic considerations. Previous study by Glaser *et al*. (2014) reported that only 19 % of CABE yield was obtained using chemical synthesis. This result indicated that lipase-catalyzed transesterification reaction is an efficient approach in synthesizing CABE compared to the chemical synthesis method with a higher percentage of conversion (84% at 48h). The use of chemical method to extract and synthesized CABE resulted in low yield and prolong development time (David *et al*., 2014). In contrast, the use of biocatalysis or enzyme-catalyzed reaction speeds up and increase efficiency of the reaction (Johannes *et al*., 2006). As a result, higher product yield was obtained in appropriate reaction time. The efficiency of lipase-catalyzed reaction had also been proved in previous studies. In a similar study on lipase-catalyzed synthesis of caffeic acid phenethyl ester (CAPE) by Ha *et al*. (2012) described that 92 % conversion was obtained in ionic liquids (ILs) in 48 h at optimized condition.

Conclusions:

Lipase-catalyzed synthesis is one of the most significant methods with several advantages in synthesizing useful industrial compound. In this study, CABE was successfully synthesized using lipase-catalyzed transesterification reaction. The investigation on the effect of reaction parameters was studied comprehensively as it is essential in obtaining the highest conversion for production of CABE. Among various organic solvents tested, the mixture of n-hexane:acetone (80:20, %v/v) produced the highest yield and Novozym 435 was found to be the most suitable enzyme. The highest CABE yield with 84% conversion was obtained with 125 U (50 mg) of Novozym 435 at 40°C after 48 h. The lipase-catalyzed synthesis method used in this study is mild and green compared to the chemical synthesis method. Thus, the application of this method in the synthesis of potential pharmaceutical product such as CABE should be highly considered.

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REFERENCES

Amirkia, V and M. Heinrich, 2015. Natural products and drug discovery: a survey of stakeholders in industry and academia. Frontiers in Pharmacology, 6: 237.

David, B., J.L. Wolfender and D.A. Dias, 2014. The pharmaceutical industry and natural products: historical status and new trends. Phytochemistry Reviews, 14(2): 299-315.

Desnuelle, P. and P. Savary, 1963. Specificities of lipases. Journal of Lipid Research, 4(4): 369-384.

Garlapati, V.K., R. Kant, A. Kumari, P. Mahapatra, P. Das and R. Banerjee, 2013. Lipase mediated transesterification of Simarouba glauca oil: a new feedstock for biodiesel production. Sustainable Chemical Processes, 1(1): 11.

Glaser, J., M. Schultheis, S. Hazra, B. Hazra, H. Moll, U. Schurigt and U, Holzgrabe, 2014. Antileishmanial lead structures from nature: Analysis of structure-activity relationships of a compound library derived from caffeic acid bornyl ester. Molecules, 19(2): 1394-1410.

Gu, S.S., J. Wang, X.B. Wei, H.S. Cui, X.Y. Wu and F.A. Wu, 2014. Enhancement of lipase-catalyzed synthesis of caffeic acid phenethyl ester in ionic liquid with DMSO co-solvent. Chinese Journal of Chemical Engineering, 22(11): 1314-1321.

Gupta, M.N., 1992. Enzyme function in organic solvents. European Journal of Biochemistry, 203: 25-32.

Ha, S.H., T.V. Anh, S.H. Lee and Y.M. Koo, 2012. Effect of ionic liquids on enzymatic synthesis of caffeic acid phenethyl ester. Bioprocess and Biosystems Engineering, 35(1-2): 235-240.

Johannes, T.W., M.R. Simurdiak and H. Zhao, 2006. Biocatalysis. Encyclopedia of Chemical Processing, 101-110.

Jun, W., L.I. Jing, Z. Leixia and G.U. Shuangshuang, 2013. Lipase-catalyzed synthesis of caffeic acid phenethyl ester in ionic liquids : effect of specific ions and reaction parameters. Chinese Journal of Chemical Engineering, 21(12): 1376-1385.

Kingston, D.G.I., 2011. Modern natural products drug discovery and its relevance to biodiversity conservation. Journal of Natural Products, 74(3): 496-511.

Lopez Giraldo, L.J., M. Laguerre, J. Lecomte, M.C. Figueroa-Espinoza, N. Barouh, B. Barea and P. Villeneuve, 2007. Lipase-catalyzed synthesis of chlorogenate fatty esters in solvent-free medium, 41: 721–726.

Pang, N., S.S. Gu, J. Wang, H.S. Cui, F.Q. Wang, X. Liu, X.Y. Zhao and F.A. Wu, 2013. A novel chemoenzymatic synthesis of propyl caffeate using lipase-catalyzed transesterification in ionic liquid. Bioresource Technology, 139: 337-342.

Sroka, Z. and W. Cisowski, 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology, 41: 753-758.

Sumbita, G., 2014. Enzymatic bioconversion in non-conventional media. Research Journal of Chemical Sciences, 4(11): 103-116.

Tsou, M.F., C.F. Hung, H.F. Lu, L.T. Wu, S.H. Chang, H.L. Chang, G.W. Chen and J.G. Chung, 2000. Effects of caffeic acid, chlorogenic acid and ferulic acid on growth and arylamine N-acetyltransferase activity in Shigella sonnei (group D). Microbios, 101: 37-46.

Wang, J., S.S. Gu, H.S. Cui, X.Y. Wu and F.A. Wu, 2014. A novel continuous flow biosynthesis of caffeic acid phenethyl ester from alkyl caffeate and phenethanol in a packed bed microreactor. Bioresource Technology, 158: 39-47.

Wang, L., K. Hsu, F. Hsu and S. Lin, 2008. Simultaneous determination of caffeic acid, ferulic acid and isoferulic acid in rabbit plasma by high performance liquid chromatography. Journal of Food and Drug Analysis, 16(1): 34-40.

Yadav, G.D. and P.S. Lathi, 2005. Lipase catalyzed transesterification of methyl acetoacetate with n-butanol. Journal of Molecular Catalysis B: Enzymatic, 32: 107-113.

Yang, C.B., W.J. Pei, J. Zhao, Y.Y. Cheng, X.H. Zheng and J.H. Rong, 2014. Bornyl caffeate induces apoptosis in human breast cancer MCF-7 cells via the ROS- and JNK-mediated pathways. Acta Pharmacologica Sinica, 35(1): 113-123.