The Inhibition of Hepatic and Renal Glucuronidation of \( p \)-Nitrophenol and 4-Methylumbelliferone by Oil Palm Empty Fruit Bunch Lignin and Its Main Oxidation Compounds

Norliyana Mohamad Salleh 1,2, Sabariah Ismail 1,2, Mohamad Nasir Mohamad Ibrahim 3

1Centre for Drug Research, Universiti Sains Malaysia, 2Centre for Herbal Standardization, Sains@USM, Bayan Lepas, 3School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia

Submitted: 01-11-2015 Revised: 08-02-2016 Published: 07-04-2017

ABSTRACT

Background: In order to develop oil palm empty fruit bunch (EFB) lignin as a nutraceutical and health supplement, the investigation of its potential in interacting with other drugs via inhibition of drug-metabolizing enzymes (DMEs) would ensure product safety. Objective: The study was aimed to investigate the in vitro effect of oil palm EFB lignin and its main oxidation compounds on phase II DME UDP-glucuronosyltransferases (UGTs) in rat liver and kidney microsomes. Materials and Methods: The \( p \)-nitrophenol (\( p \)-NP) and 4-methylumbelliferone (4-MU) were employed as probe substrates in glucuronidation assays. The effect of soda oil palm EFB lignin on \( V_{\text{max}} \), \( K_{\text{m}} \), \( CL_{\text{int}} \), \( K_{\text{i}} \) and mode of inhibition of 4-MU glucuronidation in RLM was also determined. Results: The inhibitory potency of oil palm EFB lignin for both \( p \)-NP and 4-MU glucuronidation in rat liver microsome (RLM) and rat kidneys microsomes (RKM) was found to be in the rank order of soda > kraft > organosolv. However, the inhibitory potency of its main oxidation compounds were in the rank order of vanillin > syringaldehyde > \( p \)-hydroxybenzaldehyde. Soda oil palm EFB lignin exhibited mixed-type inhibition against 4-MU glucuronidation in RLM, showing the change in apparent \( V_{\text{max}} \) and with only a minor effect on \( K_{\text{m}} \) compared with control.

Conclusions: The findings showed that effect of oil palm EFB lignin on both \( p \)-NP and 4-MU glucuronidation in RLM and RKM was enhanced by the presence of vanillin as well as flavonoids. Kinetic study showed that soda oil palm EFB lignin exhibited strong inhibition on UGT activity in RLM with mixed-type inhibition mode.

Key words: In vitro, oil palm EFB lignin, 4-MU glucuronidation, \( p \)-NP glucuronidation, UDP-glucuronosyltransferase, vanillin

SUMMARY

- The inhibitory potential of oil palm EFB lignin extracts for \( p \)-NP and 4-MU glucuronidation in RLM and RKM can be listed in the following rank order: soda > kraft > organosolv
- The inhibitory potential of oil palm EFB lignin main oxidation compounds for \( p \)-NP and 4-MU glucuronidation in RLM and RKM can be listed in the following rank order: vanillin > syringaldehyde > \( p \)-hydroxybenzaldehyde
- Results suggested that the effect of oil palm EFB lignin on \( p \)-NP and 4-MU glucuronidation activity in both RLM and RKM was enhanced by the presence of vanillin as well as total flavonoid content
- Results also suggested that oil palm EFB lignin may inhibit glucuronidation of substrate by UGT enzymes, especially UGT1A6, particularly in rat liver

INTRODUCTION

The renewable resource namely lignocellulosic (waste from oil palm empty fruit bunch [EFB]) as a raw material in the pulp and paper industry has been investigated by many researchers due to its high \( \alpha \)-cellulose content.[1,2] Through the alternative and its potential in the future pulp and paper industry, it can decrease the deforestation rate in the country. The alkaline pulping process such as soda and kraft pulping is known as potential pulping process for non-woody materials.[3,4] Both of these pulping processes function to liberate lignin, hemicelluloses, and cellulose to enhance the quality of the pulp.[2]
The main difference of soda pulping compared with the kraft pulping is the sulfur-free medium of the cooking liquor. Soda lignin is sulfur-free; therefore, its chemical composition is closer to natural lignin compared with kraft lignin. While the organic pulping process such as organosolv pulping is also one of the most promising alternatives to existing pulping technologies, lignin is separated via solubilization from organosolv pulping process. Solubilization makes it possible to obtain a less-modified lignin. The properties of organosolv lignin differ from other technical lignins. The major features are low molecular weight and high chemical purity.

Lignin is a major cell wall component in plants. Lignin provides support for the plant, contributes to the transport of nutrients and water and protects against microorganisms. Lignin is an amorphous polymer built up by oxidative coupling of three major phenylpropanoid units namely trans-p-coumaryl alcohol, trans-coniferyl alcohol, and trans-sinapyl alcohol. The phenylpropanoids form a randomized structure in a three-dimensional network inside the cell wall. The phenylpropane units are commonly classified into three main types that differ in the amount of methoxyl groups, namely p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin. These basic units are primarily attached to different types of aryl-aryl ether type and have more than 10 different types of linkages.

Besides producing biomaterials products, lignin that can be extracted from any lignocellulosic waste such as oil palm EFB, sugar cane, wood and others, has potential use in pharmaceutical and food product industry such as emulsifier, antidiarrheal drug, and natural antioxidant. The development of oil palm EFB lignin for its application in pharmaceuticals and food industries promises an additional value to lignin. However, before oil palm EFB lignin can be developed as a nutraceutical and health supplement, the investigation of its potential in interacting with other drugs or pharmaceutical agents must be carried out to ensure product safety. This is because many dietary polyphenolic compounds undergo extensive biotransformation including oxidation, reduction, methylation, sulfation, glucuronidation, and microbial degradation. Therefore, oil palm EFB lignin, which is composed of polyphenol compounds, would have the potential to be metabolized by the same drug-metabolizing enzymes (DMEs) as other drugs or pharmaceutical agents. Therefore, it could result in food-drug interaction and may cause adverse side effects.

DMEs are a diverse group of proteins that are responsible for metabolizing a vast array of xenobiotic chemicals, including drugs, carcinogens, pesticides, pollutants and food toxicants, as well as endogenous compounds such as steroids, prostaglandins and bile acids. Generally, DMEs eliminate foreign compounds (xenobiotics) and endogenous substances by increasing solubility through the functionalization processes in phase I and/or conjugation reactions in phase II.

The glucuronidation reaction is the most important detoxification pathway of the phase II drug metabolism in human and animals. Conjugation of suitable functional group present on a substrate with UDP-glucuronic acid (UDPGA) as a cofactor and catalyzed by the enzyme UDP-glucuronosyltransferase (UGT) is responsible for the elimination pathway of many xenobiotics such as drugs, chemical carcinogens, and endogenous compounds such as bilirubin, steroid hormones, and fat-soluble vitamins in humans and animals. In addition, glucuronidation facilitates excretion of these compounds and also the products of phase I metabolism as their hydrophilic conjugates in urine and bile, although a limited number of glucuronides possess biological activity.

Given the importance of glucuronidation conjugation as a detoxification and metabolic pathway for numerous xenobiotics and endogenous compounds and the development of oil palm EFB lignin for pharmaceuticals and food industries applications, there is growing interest in the study of the potential for its interaction effect on UGT enzymes activity. Therefore, the main aim of this study was to determine the in vitro effect of oil palm EFB lignin (soda, kraft, and organosolv) and its main oxidation compounds (vanillin, syringaldehyde, and p-hydroxybenzaldehyde) on UGT enzymes activity in rats by using p-nitrophenol (p-NP) and 4-methylumbellifere (4-MU) as probe substrates. 4-MU and p-NP are known to be metabolized by multiple UGT isoforms and also represent convenient probes for comparing the glucuronidation activity and kinetics of tissues and recombinant enzymes. Rat liver microsomes (RLM) and rat kidneys microsomes (RKM) were used as sources of the UGT enzymes. Liver is known as the major "metabolic clearing house" for both endogenous and xenobiotics compounds. However, UGT enzymes activity also appears to have a distinct pattern of extrahepatic expression, particularly in the kidney.

Therefore, it is especially necessary to examine the effect of oil palm EFB lignin on both hepatic and renal UGT enzymes activity in rats. This study was carried out to determine the IC50 values for oil palm EFB lignin and its main oxidation compounds on liver and kidney UGTs activity in rats by in vitro study. Further, the maximal velocity of reaction (Vmax), Michaelis-Menten constant (Km) value, intrinsic clearance (CLint), inhibition constant (K_i) and mode inhibition in an inhibitor concentration-dependent were determined for the inhibitor that showed the lowest IC50 value among all oil palm EFB lignin.

MATERIALS AND METHODS

Materials

The raw material used in this study consisted of oil palm EFB long fiber supplied by Sabutek (M) Sdn. Bhd., Teluk Intan, Malaysia, a local company specializing in recycling of oil palm lignocellulosic wastes. The EFB fiber was pulped in a 10-L stainless steel rotary digester unit together with 20% w/v NaOH (cooking liquor) for 3 h at a maximum cooking temperature of 170°C. The ratio of cooking liquor to EFB fiber was 2:8 (v/w). Prior to the pulping process, the fiber was soaked in water for 2 days to remove dirt.

Soda, kraft, and organosolv pulping process

For kraft pulping, the EFB fiber was pulped in a 10-L stainless steel rotary digester unit together with 19% of active alkali and 25% of sulfur with water-to-fiber ratio of 8. The digester was heated from room temperature to 170°C for 1 h and continued at 170°C for 2 h. For soda pulping, 25% of active alkali with no percentage of sulfur was applied under similar conditions. For organosolv pulping pretreatment process, a 1.0-L glass-lined pressure Parr reactor with Parr 4842 temperature controller (Parr Instrument Company, Moline, IL) was employed. A mass of 22.0 g of EFB (dry mass) was treated with ethanol/water mixture in a volume ratio of 65:35 with sulfuric acid as the catalyst. The solid-to-liquid ratio used was 1:8. The reactor was heated at room temperature to 190°C for 1 h and continued at 190°C for 45 min.

Preparation of oil palm EFB soda, kraft, and organosolv lignin

The soda and kraft lignin were precipitated from concentrated black liquor by acidifying them to pH 2.0 using a calculated amount of 20% (v/v) sulfuric acid. Both precipitates were filtered and washed with distilled water at pH 2.0, which was prepared using the same acid as in the earlier step. The soda and kraft lignin were dried in an oven at 55°C for 24 h. The preparation of organosolv OPEFB lignin was carried out by washing the pretreated EFB with warm ethanol/water (8:1, 3 × 50.0 mL).
The washes were combined and 3 volumes of water were added to precipitate the ethanol organosolv lignin, which was collected by centrifugation and air dried.

**Purification of oil palm EFB lignin**

The lignin sample was purified by extracting it in the soxhlet apparatus for 6 h with n-pentane to remove wax and lipids. The precipitate was filtered and washed with pH 2 water to remove the excess n-pentane and nonlignin phenolic compounds that may remain in the black liquor. The purified lignin was then dried further in the oven at 45°C for another 24 h before storage in plastic bottles for further analysis.

**Animal sources**

Male Sprague-Dawley rats (150-200 g) were obtained from the Animal House of Universiti Sains Malaysia (USM). The rats were maintained under controlled temperature (25 ± 2°C), 12-h light/12-h dark conditions for 1 week before the start of the experiments. They were provided with water and food ad libitum. Animals were maintained and handled according to the recommendations of the USM Ethical Committee, which approved the design of the experiments. Six rats were obtained and remain untreated. The rats were sacrificed by cervical dislocation and the liver and kidneys were taken out for microsomes preparation.

**Preparation of rat liver and kidneys microsomes**

Microsomes were prepared as described by Gibson and Skett.[31] Rat livers and kidneys were removed immediately after sacrifice and rinsed with ice-cooled distilled water followed by ice-cooled 67 mM potassium phosphate buffer (pH 7.4), blotted dry, and weighed. Isolated rats liver samples were homogenized in 3 volumes of 67 mM potassium phosphate buffer (pH 7.4) containing 1.15% potassium chloride using a Potter-Elvehjem homogenizer. After centrifugation of the homogenate fraction at 12,500g for 20 min at 4°C, the resultant supernatant was decanted to ultracentrifuge tubes (Optiseal™) and centrifuged at 100,000 g for 60 min in a Optima™ TLX refrigerated ultracentrifuge (Beckman Coulter, Inc., USA). The supernatant contains microsomal and soluble cytosolic fraction. The microsomal pellet obtained was reconstituted and each was resuspended in 300 µL of 67 mM potassium phosphate buffer with 1.15% (v/v) KCl and 20% (v/v) glycerol. The pooled microsomes were homogenated again to mix the solution. The supernatants were kept at −80°C until use.

**Determination of protein content**

Protein concentration of the RLM and RKM were determined by Lowry’s method using bovine serum albumin (BSA) as the standard,[32] with slight modification according to Pomory.[33] The determination of protein content was performed in triplicates.

**Kinetic analysis for p-NP glucuronidation assay**

Substrate saturation experiments were conducted to generate the rate of reaction for determining the apparent substrate $K_m$ and $V_{max}$ values for RLM and RKM assay. The $K_m$ and $V_{max}$ values were determined by nonlinear regression analysis of the p-NP-glucuronide formation data using seven different substrate (p-NP) concentrations (0-3,000 µM). Data points were fitted to the Michaelis-Menten model using Prism 6.0 (Graphpad Software, San Diego, CA).

**Inhibition of p-NP glucuronidation assay**

The method for inhibition of p-NP glucuronidation assay was obtained from the work published by Bock et al.,[34] with modification by Azizi et al.,[35] and was optimized for the current study. The incubation conditions were optimized with respect to time of incubation, microsomal protein concentration, and Triton X-100 concentration. Briefly, the incubation mixture in a total volume of 0.20 mL containing 5 mg mL⁻¹ RLM or RKM was pre-incubated with 0.25 v/v % Triton X-100 (0.125 v/v % Triton X-100 for RKM assay) on ice for 5 min. A volume of 20 µL of 1 M Tris-HCl (pH 7.4), 20 µL of 50 mM MgCl₂, and 20 µL of 5 mM p-NP were added to the mixture. The volume was made up to 0.2 mL with distilled water. The reaction was initiated by adding 20 µL of 30 mM UDPGA. The incubation was carried out in a shaking water bath (Stuart® SBS40) at 37°C for 30 min for both RLM and RKM assays. The reaction was terminated with the addition of 200 µL trichloroacetic acid (TCA) (20 v/v %). The mixture was vortexed vigorously for 2 min and 200 µL of aliquots in each tube was taken out into a new tube. Then, 0.5 M of NaOH was added and left for 10 min. Absorbance was measured at 405 nm using a microplate reader (Plate CHAMELEON™ multitechnology plate reader, 425-106). Screening experiments were conducted to generate IC₅₀ values by incubating p-NP at the estimated $K_m$ value in the presence of various concentrations of positive inhibitor (diclofenac), oil palm EFB lignin (soda, kraft, and organosolv) and its main oxidation compounds (vanillin, syringaldehyde, and p-hydroxybenzaldehyde).

The amount of the substrates glucuronidated was calculated by subtracting the known concentration of p-NP in a blank tube with the concentration of the unreacted p-NP in the reaction tubes. Consequently, the value of the glucuronilated substrate was divided with the protein concentration used and the incubation time. The equation is illustrated below:

**Kinetic analysis for 4-MU glucuronidation assay**

The $K_m$ and $V_{max}$ values were determined by nonlinear regression analysis of the 4-MU-glucuronide (4-MUG) formation data using different substrate (4-MU) concentrations (0-10 mM). Data points were fitted to the Michaelis-Menten model using Prism 6.0 (Graphpad Software).

**Inhibition of 4-MU glucuronidation assay**

The method for inhibition of 4-MU glucuronidation assay was adopted from the work published by Hanioka et al.[36] and optimized for the current study. The incubation conditions were optimized with respect to time of incubation, microsomal protein concentration, and Triton X-100 concentration. The incubation mixture containing a total volume of 0.25 mL (0.1 mg mL⁻¹ for liver or 0.125 mg mL⁻¹ for kidney) microsomal protein was pre-incubated with Triton X-100 (0.1 µg mL⁻¹ protein for RLM or 0.2 µg mL⁻¹ protein for RKM) on ice for 5 min. Then, 5 µL of 2.5 M Tris-HCl (pH 7.4), 10 µL of 250 mM MgCl₂, and 12.5 µL of 2 mM 4-MU were added into the incubation mixture. The volume was increased to 0.25 mL with distilled water. Control group was replaced with equivalent volume of distilled water instead of test inhibitor samples. After pre-incubulation for 5 minutes in a shaking water bath (Stuart® SBS40) at 37°C, the reaction was initiated by adding 12.5 µL of 100 mM UDPGA and the mixture was further incubated in a shaking bath at 37°C for 15 min for both RLM and RKM assays. Screening experiments were conducted to generate IC₅₀ values by incubating 4-MU at the estimated $K_m$ value in the presence of various concentrations of positive inhibitor (diclofenac), potential inhibitors, oil palm EFB lignin (soda, kraft, and organosolv) and its main oxidation compounds (vanillin, syringaldehyde, and p-hydroxybenzaldehyde).

The reaction was terminated with the addition of 100 µL TCA (20 v/v %). The mixture was vortexed and placed on ice for 10 min and centrifuged at 2,000 rpm at 4°C for 10 min in order to coagulate the protein. The supernatant fraction (10 µL) was injected into the high-performance liquid chromatography (HPLC) system according to the method that has previously been validated.[37]
The metabolite formed were quantified using a calibration curve of 4-MUG. The concentration of 4-MUG was divided with incubation time and protein concentration used to obtain the UGT-specific activity. The UGT-specific activity was calculated using equation (1).

Further, the inhibition constant ($K_i$) was determined for the inhibitor with the lowest IC$_{50}$ value among all oil palm EFB lignin extracts. In such cases, concentration of 4-MU (0.1-10 mM) with RLM and a range of concentrations of soda oil palm EFB lignin (0.25, 5, and 10 µg mL$^{-1}$) were used for the construction of Dixon plots and estimation of $K_i$ values.

Determination of 4-MUG

HPLC determination of 4-MUG was performed using a method by Lewis et al.,[37] with a slight modification. The method was validated earlier by Haron.[38] Ten microliter of the filtrate was injected into the HPLC system (Shimadzu) equipped with reversed-phase Gemini NX C18 column ( particle size 5 µm, 150 mm × 4.6 mm i.d.). The column was kept at 37°C. The eluent was monitored with a UV detector at 316 nm with gradient elution at a flow rate of 1 mL min$^{-1}$. The 4-MUG standard solutions were freshly prepared for each experiment with concentration ranges of 5-100 µM.

RESULTS

The pNP-glucuronide and 4-MUG formations in RLM and RKM were best explained by Michaelis-Menten kinetics. For p-NP glucuronidation, the $K_m$ and $V_{max}$ values were 860.50 ± 86.81 µM and 43.04 ± 1.64 nmol min$^{-1}$ mg$^{-1}$, respectively, for RLM and 857.70 ± 68.30 µM and 9.320 ± 1.11 nmol min$^{-1}$ mg$^{-1}$, respectively, for RKM. For 4-MU glucuronidation, the $K_m$ and $V_{max}$ values were 0.104 ± 0.020 mM and 27.87 ± 1.08 nmol min$^{-1}$ mg$^{-1}$, respectively, for RLM and 0.101 ± 0.006 mM and 12.01 ± 0.12 nmol min$^{-1}$ mg$^{-1}$, respectively, for RKM. Further, the inhibition effects of positive inhibitor (diclofenac), oil palm EFB lignin, and its main oxidation compounds on p-NP and 4-MU glucuronidation activity assays by RLM and RKM were performed at p-NP and 4-MU concentrations that are close to the apparent $K_m$ value.

The effect of oil palm EFB lignin and its main oxidation compounds on p-NP glucuronidation

The inhibitory effects of soda, kraft, and organosolv oil palm EFB lignin on p-NP glucuronidation in RLM were screened and the half maximal inhibitory concentration (IC$_{50}$), which is the effective concentration of inhibitor, was compared with positive inhibitor (diclofenac) in RLM and is presented in Figure 1. Results showed that soda, kraft, and organosolv oil palm EFB lignin started to show significant inhibition ($P < 0.05$) by RKM at concentration of 10.0 µg mL$^{-1}$ onward. In order to examine the contribution of oxidation compounds of oil palm EFB lignin on UGT enzymes activity, the IC$_{50}$ values of three main oxidation compounds (vanillin, syringaldehyde, and p-hydroxybenzaldehyde) were subsequently determined. The concentration of each oxidation compound in different types of oil palm EFB lignin has been investigated previously.[40] The study has identified and quantified eight compounds from alkaline nitrobenzene oxidation process of oil palm EFB lignin. Among them, syringaldehyde was found to have the highest percentage in all three types of oil palm EFB lignin, followed by vanillin and p-hydroxybenzaldehyde.[Table 1]. The calculated IC$_{50}$ values of vanillin, syringaldehyde, and p-hydroxybenzaldehyde were compared with that of positive inhibitor (diclofenac) for RLM and RKM.[Figures 3 and Figure 4] An accurate IC$_{50}$ value for p-hydroxybenzaldehyde could not be determined because maximum inhibition (>50% inhibition) did not occur at the highest dose investigated.

Vanillin and syringaldehyde started to show significant inhibition ($P < 0.05$) for RLM at concentration of 0.01 µM onward, while, p-hydroxybenzaldehyde started to show significant inhibition ($P < 0.05$) at concentration of 1.0 µg mL$^{-1}$ onward. In RKM, vanillin started to show significant inhibition ($P < 0.05$) at concentration of 100.0 µM onward and syringaldehyde started to show significant inhibition ($P < 0.05$) at concentration of 10.0 µM onward.

The effect of oil palm EFB lignin and its main oxidation compounds on 4-MU glucuronidation

The formation of 4-MUG generated by the incubation of 4-MU with RLM was investigated in the presence of oil palm EFB lignin (soda, kraft, and organosolv) and the calculated IC$_{50}$ values of the three types of oil palm EFB lignin were compared with positive inhibitor (diclofenac) in RKM and are presented in Figure 2. Results showed that soda, kraft, and organosolv oil palm EFB lignin started to show significant inhibition ($P < 0.05$) by RKM at concentration of 10.0 µg mL$^{-1}$ onward.

In order to examine the contribution of oxidation compounds of oil palm EFB lignin on UGT enzymes activity, the IC$_{50}$ values of three main oxidation compounds (vanillin, syringaldehyde, and p-hydroxybenzaldehyde) were subsequently determined. The concentration of each oxidation compound in different types of oil palm EFB lignin in this study as a positive inhibitor and showed a good inhibition of p-NP glucuronidation activity assays by RLM and RKM were performed at p-NP and 4-MU concentrations that are close to the apparent $K_m$ value.
Figure 1: Inhibition of p-NP glucuronidation in RML by (a) soda, (b) kraft (c), and organosolv oil palm EFB lignin compared with positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for five replicate incubations (n = 5). Error bars represent two-sided SD. The goodness-of-fit R² value was 0.9 or higher.

Figure 2: Inhibition of p-NP glucuronidation in RKM by (a) soda, (b) kraft, and (c) organosolv oil palm EFB lignin compared with positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for five replicate incubations (n = 5). Error bars represent two-sided SD. The goodness-of-fit R² value was 0.9 or higher.
NORLIYANA MOHAMAD SALLEH, et al.: Effects of EFB

**Figure 3**: Inhibition of p-NP glucuronidation in RLM by (a) vanillin, (b) syringaldehyde, and (c) p-hydroxybenzaldehyde compared with positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for five replicate incubations ($n = 5$). Error bars represent two-sided SD. The goodness-of-fit $R^2$ value was 0.9 or higher.

**Figure 4**: Inhibition of p-NP glucuronidation in RKM by (a) vanillin and (b) syringaldehyde compared with positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for five replicate incubations ($n = 5$). Error bars represent two-sided SD. The goodness-of-fit $R^2$ value was 0.9 or higher.
Effects of EFB glucuronidation study. A summary of the IC\textsubscript{50} results presented in Table 3.}

Diclofenac was also chosen as a positive inhibitor for 4-MU (diclofenac) in RLM, as represented in Figure 5. of oil palm EFB lignin were compared with that of positive inhibitor (diclofenac) in RLM, as represented in Figure 5. Diclofenac was also chosen as a positive inhibitor for 4-MU glucuronidation study. A summary of the IC\textsubscript{50} results presented in Table 3 illustrates the differential effects of oil palm EFB lignin on 4-MU glucuronidation. Three different extracts of oil palm EFB lignin exhibited mixed-type inhibition against 4-MU glucuronidation in RLM, showing the change of apparent \( V_{\text{max}} \) with only a minor change effect on \( K_s \) compared with the control [Table 4]. The \( K_s \) value is 7.55 ± 0.78 \( \mu \text{g mL}^{-1} \).

The effect of soda oil palm efb lignin on michaelis-menten kinetics of 4-mu glucuronidation in rlm

Since soda oil palm EFB lignin showed the most potent inhibition for UGT activity [Table 2 and Table 3], the in vitro effect of soda oil palm EFB lignin on maximal velocity of reaction (\( V_{\text{max}} \)), Michaelis-Menten constant (\( K_s \)) value, and intrinsic clearance (CL\textsubscript{int}) of 4-MU glucuronidation in RLM was investigated. The \( K_s \), \( V_{\text{max}} \), CL\textsubscript{int}, and \( K_s \) values are given in Table 4, while the Lineweaver-Burk plots and secondary plots showing the graphical data for interpretation of inhibition mode are given in Figures 9 and 10, respectively. Based on the analysis, soda oil palm EFB lignin exhibited mixed-type inhibition against 4-MU glucuronidation in RLM, showing the change of apparent \( V_{\text{max}} \) with only a minor change effect on \( K_s \) compared with the control [Table 4]. The \( K_s \) value is 7.55 ± 0.78 \( \mu \text{g mL}^{-1} \).

**DISCUSSION**

In this study, we have investigated the inhibitory effect of three types of oil palm EFB lignin extracts (soda, kraft, and organosolv) and its main oxidation compounds (vanillin, kraft, and organosolv) on p-NP and 4-MU glucuronidation. Three different extracts of oil palm EFB lignin were investigated because all of these extracts have their own potential in pharmaceutical and food industry. Our data showed that all oil palm EFB extracts exhibited differential inhibitory effect on p-NP glucuronidation either in RLM or RKM. However, oil palm EFB lignin showed a more potent inhibition of UGT activity in RLM compared with RKM. In both RLM and RKM, the inhibitory potency for the extracts was found to be listed in the rank order of soda > kraft > organosolv. Previous study by Mohd Salleh et al.\textsuperscript{[40]} reported that the flavonoid content in oil palm EFB lignin was found to be listed in the rank order of soda > kraft > organosolv. Therefore, the data showed that the inhibitory pattern of p-NP UGT activity in both RLM and RKM correlated well with total flavonoid content.

For 4-MU glucuronidation study, oil palm EFB lignin also showed a more potent inhibition of UGT activity in RLM compared with RKM. The in vitro study using 4-MU as a probe substrate for UGT activity showed that the inhibitory potency for 4-MU glucuronidation in both RLM and RKM can be listed in the rank order of soda > kraft > organosolv. The effect of oil palm EFB lignin on 4-MU glucuronidation in both RLM and RKM also correlated well with flavonoids content of oil palm EFB lignin. Therefore, both studies (the effect of oil palm EFB lignin on p-NP and 4-MU glucuronidation) suggest that flavonoids might be responsible for the observed inhibition. The ability of flavonoids as the main constituent

**Table 3**: Inhibition of 4-methylumbelliferone glucuronidation in RLM and RKM by oil palm EFB lignin and its main oxidation compounds. Data are expressed as the best-fit IC\textsubscript{50} values ± SD for three replicates (n=3).

| Compound                        | IC\textsubscript{50} Values | RLM | RKM |
|---------------------------------|-----------------------------|-----|-----|
|                                 | µg mL\(^{-1}\)              | µg mL\(^{-1}\) | µg mL\(^{-1}\) | µg mL\(^{-1}\) |
| Diclofenac                      | 15.520 ± 1.627              | 33.480 ± 1.715 |
| Soda oil palm EFB lignin        | 6.018 ± 1.231               | 11.400 ± 1.429 |
| Kraft oil palm EFB lignin       | 9.558 ± 1.225               | 29.310 ± 1.161 |
| Organosolv oil palm EFB lignin | 40.20 ± 1.150               | 46.360 ± 1.122 |
|                                | µM                          | µM |
| Diclofenac                      | 48.785 ± 5.114              | 105.240 ± 5.391 |
| Vanillin                        | 6.121 ± 1.611               | 16.100 ± 1.754 |
| Syringaldehyde                  | 742.300 ± 1.213             | 898.700 ± 1.217 |
| p-hydroxybenzaldehyde          | > 1000                      | > 1000 |

of oil palm EFB lignin were compared with that of positive inhibitor (diclofenac) in RLM, as represented in Figure 5.

Diclofenac was also chosen as a positive inhibitor for 4-MU glucuronidation study. A summary of the IC\textsubscript{50} results presented in Table 3 illustrates the differential effects of oil palm EFB lignin on 4-MU glucuronidation in RLM and RKM compared with diclofenac. Based on this result, diclofenac inhibited 4-MU glucuronidation in RLM with IC\textsubscript{50} values of 21.14 ± 1.32 µM, 1.6 times more potent than in RKM on this result, diclofenac inhibited 4-MU glucuronidation in RLM with IC\textsubscript{50} values of 33.48 ± 1.715 µM. Further, the formation of 4-MUG generated by the incubation of 4-MU in RLM and RKM was also investigated in the presence of three main oxidation compounds in oil palm EFB lignin (vanillin, syringaldehyde, and p-hydroxybenzaldehyde) and the calculated IC\textsubscript{50} values compared with positive inhibitor (diclofenac) in RLM and RKM are presented in Figure 7 and Figure 8.

As shown in Figure 7, vanillin and syringaldehyde started to show significant inhibition (\( P < 0.05 \)) by RLM at concentration of 0.1 µM onward. While, p-hydroxybenzaldehyde started to show significant inhibition (\( P < 0.05 \)) by RLM at concentration of 1.0 µM onward. The same observation was also found for RKM [Figure 8]. Vanillin and syringaldehyde started to show significant inhibition (\( P < 0.05 \)) by RKM at concentration of 0.1 µM onward, while p-hydroxybenzaldehyde started to show significant inhibition (\( P < 0.05 \)) by RKM at concentration of 1.0 µM onward.

| Compound | IC\textsubscript{50} values | \( V_{\text{max}} \) (nmol min\(^{-1}\) mg\(^{-1}\)) | CL\textsubscript{int} (µL min\(^{-1}\) mg\(^{-1}\)) |
|----------|-----------------------------|-----------------------------|-----------------------------|
| Soda oil palm EFB lignin concentration (µg mL\(^{-1}\)) | \( K_s \) (mM) | \( V_{\text{max}} \) | CL\textsubscript{int} |
| 0       | 0.104 ± 0.020               | 27.870 ± 1.075             | 269.464 ± 2.887           |
| 2.5     | 0.160 ± 0.026             | 19.110 ± 0.741*           | 119.551 ± 2.887          |
| 5.0     | 0.026*                     | 15.920 ± 0.660*           | 13.546*                  |
| 10.0    | 0.122 ± 0.026             | 13.150 ± 0.396*           | 96.516 ± 16.602*         |
|         | 0.149 ± 0.020             | 13.546 ± 0.660*           | 96.516 ± 16.602*         |

Table 4: Kinetics parameters for 4-MU glucuronidation in concentration-dependents of soda oil palm EFB lignin. Data are expressed as the values ± SD for three replicates (n=3). The statistical analysis was conducted using one-way ANOVA and Dunnet test. *\( p < 0.05 \) vs. control (0 µg mL\(^{-1}\) of soda oil palm EFB lignin).
Figure 5: Inhibition of 4-MU glucuronidation in RLM by (a) soda, (b) kraft, and (c) organosolv oil palm EFB lignin compared with positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for three replicate incubations ($n = 3$). Error bars represent two-sided SD. The goodness-of-fit $R^2$ value was 0.9 or higher.

Figure 6: Inhibition of 4-MU glucuronidation in RKM by (a) soda, (b) kraft, and (c) organosolv compared with positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for three replicate incubations ($n = 3$). Error bars represent two-sided SD. The goodness-of-fit $R^2$ value was 0.9 or higher.
Figure 7: Inhibition of 4-MU glucuronidation in rat liver microsomes by (a) vanillin and (b) syringaldehyde compared with positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for three replicate incubations \((n = 3)\). Error bars represent two-sided SD. The goodness-of-fit \(R^2\) value was 0.9 or higher.

Figure 8: Inhibition of 4-MU glucuronidation in RKM by (a) vanillin and (b) syringaldehyde compared to positive inhibitor (diclofenac). Each data point represents the means of UGT relative activity ± SD for three replicate incubations \((n = 3)\). Error bars represent two-sided SD. The goodness-of-fit \(R^2\) value was 0.9 or higher.
Figure 9: Lineweaver-Burk plots of inhibition of UGT-catalyzed 4-MU glucuronidation by soda oil palm EFB lignin. Each data point represents an average of triplicate measurements ($n = 3$).

Figure 10: Secondary plots of UGT activity using the slopes of the primary Lineweaver-Burk plots vs. the concentrations of soda oil palm EFB lignin. Each data point represents an average of triplicate measurements ($n = 3$).
that could modulate UGT activity in vitro has been reviewed by Moon et al.\textsuperscript{[41]} Flavonoids are present in many dietary supplements that were derived from natural plants including Ginkgo biloba, soy isoflavones, tea, red wine, and lignin.\textsuperscript{[40]} The effects of flavonoids on enzyme activity are generally dependent on the concentrations of flavonoids present and also different flavonoids ingested.\textsuperscript{[41]} Other research studies on the effect of natural plants such as Ginkgo biloba\textsuperscript{[42]} and Mitragyna speciosa\textsuperscript{[39]} on UGT enzymes activity also indicated that flavonoids content was responsible for the inhibition effect.

Incubations with RLM exhibited 1.1-1.5-fold more potent inhibition of p-NP glucuronide formation than with RKM by oil palm EFB lignin. However, incubation with RLM exhibited 2.7-4.0-fold more potent inhibition of p-NP glucuronide formation than in RKM by oil palm EFB lignin main oxidation compounds [Table 2]. This difference in inhibition potency of liver and kidney suggests that both of them have differentially expressed UGT enzymes in liver and kidney;\textsuperscript{[33,34]} thus contributing to different glucuronidation capacities between liver and kidneys. Incubations with RLM exhibited 1.2-3.1-fold more potent inhibition of 4-MUG formation than in RKM by oil palm EFB lignin [Table 3]. However, the incubation with RLM exhibited 2.6- and 1.2-fold more potent inhibition of 4-MU glucuronidation than in RKM by vanillin and syringaldehyde, respectively [Table 3]. In both RLM and RKM, p-hydroxybenzaldehyde showed no notable effect of inhibition for 4-MU glucuronidation (IC\textsubscript{50} > 1,000 μM).

This difference in inhibition potency of liver and kidney microsomes supported the fact that the expression levels of UGT enzymes in liver microsomes are different compared with kidney microsomes.\textsuperscript{[40-48]} In addition, p-NP and 4-MU are planar phenol derivatives. These compounds have been reported to be mainly conjugated by UGT1A6 isoforms in human and rat;\textsuperscript{[36]} although some studies have reported that they are also metabolized by multiple recombinant UGT isoforms.\textsuperscript{[39,40]} Further, it has been suggested that the higher specific activity in RLM compared with RKM is due to the fact that the enzyme activity of UGT1A6 is higher in liver than in kidneys.\textsuperscript{[36-39]}

The inhibitory effect of three main oxidation compounds of oil palm EFB lignin (vanillin, syringaldehyde, and p-hydroxybenzaldehyde) on p-NP and 4-MU glucuronidation in both RLM and RKM was also investigated in order to examine the relative role, if any, of each compound in the inhibitory effect observed in different oil palm EFB lignin extracts. Our previous study\textsuperscript{[40]} showed that syringaldehyde was the predominant compound in all three types of oil palm EFB lignin, followed by vanillin and p-hydroxybenzaldehyde [Table 1]. Meanwhile, as can be seen in Tables 2 and 3, based on the IC\textsubscript{50} values, vanillin highly inhibits UGT activity of both p-NP and 4-MU, whereas syringaldehyde has only weak inhibitory effect and p-hydroxybenzaldehyde did not show any notable effect on the activity. Therefore, although syringaldehyde content was the highest percentage in all extracts,\textsuperscript{[40]} it can be concluded that syringaldehyde did not exert a major influence on the inhibitory potency of oil palm EFB lignin on both p-NP and 4-MU glucuronidation. Specifically with 4-MU UGT inhibition in RLM, the IC\textsubscript{50} values of syringaldehyde is 120-fold higher in comparison with vanillin.

Results also suggested that the effect of oil palm EFB lignin on p-NP and 4-MU glucuronidation activity in both RLM and RKM was enhanced by the presence of vanillin in all three types of oil palm EFB lignin, as well as the total flavonoid content. Vanillin, which is a food-flavoring agent utilized widely in foods, beverages, drugs, and perfumes, was also found as one of the main oxidation compounds in oil palm EFB lignin.\textsuperscript{[40-34]} Previous study has investigated the UGT conjugation pathway of vanillin by liver microsomes from human, dog, rat, mice, minipig, and cynomolgus monkey.\textsuperscript{[33]} The study showed that vanillin was efficiently glucuronidated by liver microsomes obtained from various species. However, the inhibitory effect of vanillin on UGT DMEs is not documented to date. In summary, this study suggested that the inhibitory effect of oil palm EFB lignin on p-NP and 4-MU glucuronidation can be highly attributed to the presence of vanillin in all three types of oil palm EFB lignin.

Further, the effect of 4-MU glucuronidation by soda oil palm EFB lignin in RLM on K\textsubscript{m}, V\textsubscript{max}, CL\textsubscript{int}, and K\textsubscript{i} values and inhibition mode was investigated because it is the most potent inhibitor among all oil palm EFB lignin compounds investigated. Moreover, the HPLC method for the determination of 4-MUG in the 4-MU glucuronidation assay is more specific and sensitive compared with spectrophotometric method that was used on p-NP glucuronidation. The Michaelis-Menten constant (K\textsubscript{m}) showed the lowest value in 4-MU glucuronidation in RLM control compared with 4-MU glucuronidation in the presence of soda oil palm EFB lignin. The kinetic analysis results showed that 4-MU glucuronidation in RLM without soda oil palm EFB lignin had the highest affinity compared with others. Therefore, it is suggested that the 4-MU glucuronidation activity in RLM may be decreased in the presence of soda oil palm EFB lignin, by an increase in its K\textsubscript{m} value, and a decrease in V\textsubscript{max} value.

The determination of intrinsic clearance (CL\textsubscript{int}) for drug candidates in the early discovery stage is a common practice in the pharmaceutical industry.\textsuperscript{[46-48]} The intrinsic clearance can be defined as the ability of the liver to remove drug in the absence of flow limitations and binding to cells or proteins in the blood. The in vitro CL\textsubscript{int} may be derived from enzymes’ kinetic data such as V\textsubscript{max}/K\textsubscript{m}. From the result in Table 4, the CL\textsubscript{int} value is decreased as the concentration of soda oil palm EFB lignin in 4-MU glucuronidation is increased. The lowest CL\textsubscript{int} value for 4-MU glucuronidation in RLM with highest concentration of soda oil palm EFB lignin suggests that drugs that undergo the 4-MU glucuronidation may be cleared less efficiently by liver.\textsuperscript{[61]} Therefore, soda oil palm EFB lignin exhibited strong inhibition on UGT activity in RLM with mixed-type inhibition mode.

The mechanism of mixed-type inhibition by the plant extracts with regards to UGT enzymes is also shown by many herbs and plant extracts.\textsuperscript{[63,64]} Mohamed and Frye\textsuperscript{[49]} reported that the incubation of ginkgo flavonoids, that is, quercetin (in human liver and intestine microsome) and kaempferol (in human intestine microsome) exhibited mixed type of inhibition toward mycophenolic acid (MPA) glucuronidation. Zainal Abidin et al.\textsuperscript{[44]} reported that the incubation of Andrographis paniculata extracts and its active constituent (neandrographolide) exhibited mixed mode of inhibition toward p-NP glucuronidation in UGT1A1.

CONCLUSION

The effect of oil palm EFB lignin and its main oxidation compounds on p-NP and 4-MU glucuronidation were successfully investigated in this study. Based on the data, the inhibitory potential of the investigated extracts for p-NP and 4-MU glucuronidation in RLM and RKM can be listed in the following rank order: soda > kraft > organosolv. For compounds, the rank order is vanillin > syringaldehyde > p-hydroxybenzaldehyde. Results suggested that the effect of oil palm EFB lignin on p-NP and 4-MU glucuronidation activity in both RLM and RKM was enhanced by the presence of vanillin as well as the total flavonoid content. Based on our findings, oil palm EFB lignin may inhibit glucuronidation of substrate by UGT enzymes especially UGT1A6 particularly in rat liver. These findings suggest interactions of oil palm EFB lignin with UGT1A6 substrates are possible. However, future clinical studies are warranted to evaluate the in vivo pharmacokinetic relevance of these interactions.
Financial support and sponsorship
Research University Research Grant (304/PKIMIA/815097), Ministry of Education, Malaysia for the My PhD scholarship.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Khoo KC, Lee TW. Pulp and paper from oil palm. APPITA 1991;44:385-88.
2. Wai Rosli WD, Law KN, Valade JL. Chemical pulping of oil palm empty fruit bunches. Cell Chem Technol 1998;32:133-43.
3. Garcia A, Toledoano A, Serrano L, Eguíes I, González M, Marín F. Characterization of lignins obtained by selective precipitation. Sep Purif Technol 2009;68:193-8.
4. Feng Z, Álen R. Soda-AQ pulping of reed canary grass. Ind Crop Prod 2001;14:31-9.
5. Gullichsen J, Fagenholm C-J. Chemical pulping. In: Gullichsen J, Paulapuro H, editors. Papermaking science and technology. Fapet Oy 2000.
6. Nafid A, Hunekeler D, Kaper P. Sulfur-free lignins from alkaline pulping tested in mortar for use as mortar additives. Bioresour Technol 2002;84:49-55.
7. Wörmeyer K, Ingram T, Saake B, Brunner G, Smimova G. Comparison of different pretreatment methods for lignocellulosic materials. Part II: Influence of pretreatment on the properties of raw straw lignin. Bioresour Technol 2011;102:415-64.
8. Buranov AU, Maza G. Lignin in straw of herbaceous crops. Ind Crop Prod 2008;28:237-59.
9. Lewis NG, Yamamoto E. Lignin: occurrence, biogenesis and biodegradation. Annu Rev Plant Physiol Plant Mol Biol 2008;41:459-96.
10. Brunov G, Lundquist K, Gellerstedt G. Analytical methods in wood chemistry pulping and papermaking. In: Sjöstöm E, Álen R, editors. Lignin. New York: Springer Verlag; 1999;77-124.
11. Mohamed Ibrahim MN. Application of lignin extracted from oil palm empty fruit bunch as an emulsifier for solid food products. Paper presented at the 30th International Conference on Science and Technology 2010; August 5-6 Budapest: Hungary 2010.
12. Mitjans M, Garcia L, Marrero E, Vinardell MP. Study of Ligned-A, an antioxidant drug based on lignin, on rat small intestine enzyme activity and morphometry. J Vet Pharmacol Ther 2001;24:349-61.
13. Dohhne T, Telysheva G, Jurkane V, Vestrus U. Characterization of the radical scavenging activity of lignins-natural antioxidants. Bioresour Technol 2004;95:309-17.
14. Mitjans M, Vinardell MP. Biological activity and health benefits of lignans and lignins. Trends Biotech 2005;11:55-62.
15. Pan X, Kada JF, Ehsa K, Gilkes N, Saddler JN. Organosolv ethanol lignin from hybrid poplar as a radical scavenger: relationship between lignin structure, extraction conditions, and antioxidant activity. J Agric Food Chem 2006;54:5806-13.
16. Ugartondo V, Mitjans M, Vinardell MP. Comparative antioxidant and cytotoxic effects of lignins from different sources. Bioresour Technol 2008;99:6893-7.
17. Lambert JD, Yang CS. Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. Mutat Res Fund Mol Mech Mut 2003;523-524:201-213.
18. Hu M, Chen J, Lin H. Metabolism of flavonoids via enteric recycling: mechanistic studies of disposition of apigenin in the CaCO-2 cell culture model. J Pharmacol Exp Ther 2003;307:314-21.
19. Honig PK, Wortham DC, Zamani K, Conner DP, Mullin JC, Cantilena LR. Terfenadine-ketoconazole interaction. Pharmacokinetic and electrocardiographic consequences. JAMA 1993;269:1513-8.
20. Soronen JM, Herbi-drug, food-drug, nutrient-drug, and drug-drug interactions: mechanisms involved and their medical implications. J Altern Complement Med 2005;20:187-10.
21. Brandt H, Myberg O, Rundlof T, Arvidsson A-K, Brenning G. Adverse effects of artificial caffeine and theophylline on clinical, animal, and in vitro studies. Planta Med 2005;71:111-21.
22. Gill KL, Houston JB, Galetin A. Characterization of in vitro glucuronidation clearance of a range of drugs in human kidney microsomes: comparison with liver and intestinal glucuronidation and impact of albumin. Drug Metab Dispos 2012;40:925-35.
23. Wang J, Evans AM, Knights KM, Miners JO. Differential disposition of intra-renal generated and preformed glucuronidates: studies with 4-methylumbelliferone and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. Drug Metab Dispos 2004;32:413-23.
24. Naharka N, Jhin H, Tanaka-Kagawa T, Nishimura T, Ando M. Determination of UDP glucuronosyltransferase (UGT) activities in male Sprague-Dawley rats. Drug Metab Drug Interact 2013;28:95-105.
25. Haniika N, Jhin H, Tanaka-Kagawa T, Nishimura T, Ando M. UDP glucuronosyltransferase UGT1A6 activity in human and rat liver microsomes by HPLC with UV detection. J Pharm Biomed Anal 2001;25:65-75.
26. Lewis BC, Mackenzie Pl, Elliot DJ, Burchell B, Basker CR, Miners JO. Amino terminal domains of human UDP-glucuronosyltransferases (UGT) 2B7 and 2B15 associated with substrate selectivity and autoactivation. Biochem Pharmacol 2007;73:1462-73.
27. Hanover H. Comparison of inhibition potentials of Mitragynine, 7-hydroxymitragynine and several selected drugs of abuse on glucuronidation by rat and human liver microsomes and human recombinant UGT isoforms. Pharm M. Thesis, Centre for Drug Research, Universiti Sains Malaysia, Pulau Pinang, Malaysia; 2014.
28. Udbasichat V, Mackenze PI, Guo XH, Gardner-Stephen D, Galetin A, Houston J, et al. Human UDP-glucuronosyltransferases: isomere selectivity and kinetic of 4-methylumbellifere and 1-naphthol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. Drug Metab Dispos 2004;32:413-23.
29. Mohamed ME, Fiyen RE. Effects of herbal supplements on drug glucuronidation. Review of clinical, animal, and in vitro studies. Planta Med 2011;77:011-21.
30. Mohamed ME, Fiyen RE. Effects of herbal supplements on drug glucuronidation. Review of clinical, animal, and in vitro studies. Planta Med 2011;77:011-21.
31. Gill KL, Huntington JB, Galetin A. Characterization of in vitro glucuronidation clearance of a range of drugs in human kidney microsomes: comparison with liver and intestinal glucuronidation and impact of albumin. Drug Metab Dispos 2012;40:925-35.
32. Wang J, Evans AM, Knights KM, Miners JO. Differential disposition of intra-renal generated and preformed glucuronidates: studies with 4-methylumbelliferone and 4-methylumbelliferone glucuronide in the filtering and nonfiltering isolated perfused rat kidney. J Pharm Pharmacol 2011;63:507-14.
33. Tukey RH, Snassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression and disease. Annu Rev Pharmacol Toxicol 2000;40:581-618.
34. Gagnais P, Miners JO, Brennan JS, Thomas A, Knights KM. Human renal cortical and medullary UDP-glucuronosyltransferases (UGT1A): immunohistochemical localization of UGT2B7 and UGT1A enzymes and kinetic characterization of Snaproxen glucuronidation. J Pharmacol Exp Ther 2007;323:422-30.
35. Ohnso S, Nakaji S. Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in human tissues by real-time reverse transcriptase-polymerase chain reaction. Drug Metab Dispos 2009;37:32-40.
48. Court MH, Duan SX, Guillemette C, Journault K, Krishnaswamy S, Von Molite LL, et al. Stereoselective conjugation of oxazepam by human UDP-glucuronosyltransferases (UGTs): S-oxazepam is glucuronidated by UGT2B15, while R-oxazepam is glucuronidated by UGT2B7 and UGT1A9. Drug Metab Dispos 2002;30:1257-65.

49. Tsoutsikos P, Miners JO, Stapleton A, Thomas A, Sallustio BC, Knights KM. Evidence that unsaturated fatty acids are potent inhibitors of renal UDP-glucuronosyltransferases (UGT): kinetic studies using human kidney cortical microsomes and recombinant UGT1A9 and UGT2B7. Biochem Pharmacol 2004;67:191-9.

50. Münzel PA, Brück M, Bock KW. Tissue-specific constitutive and inducible expression of rat phenol UDP-glucuronosyltransferase. Biochem Pharmacol 1994;47:1445-8.

51. Ikushiro S-I, Emi Y, Iyanagi T. Identification and analysis of drug-responsive expression of UDP-glucuronosyltransferase family 1 (UGT1) isozyme in rat hepatic microsomes using anti-peptide antibodies. Arch Biochem Biophys 1995;324:267-72.

52. Münzel PA, Bookjans G, Mehner G, Lehmküster T, Bock KW. Tissue-specific 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible expression of human UDP-glucuronosyltransferase UGT1A6. Arch Biochem Biophys 1996;335:205-10.

53. Strassburg CP, Manns MP, Tukey RH. Expression of the UDP-glucuronosyltransferase 1A locus in human colon: identification and characterization of the novel extrahepatic UGT1A9. J Biol Chem 1998;273:8719-26.

54. Mohamad Ibrahim MN, Nor Nadiah MY, Norliyana MS, Sipaut CS, Shuib S. Separation of vanillin from oil palm empty fruit bunch lignin. CLEAN 2008;36:287-91.

55. Yu J, Han JC, Hua LM, Gao YJ. In vitro characterization of glucuronidation of vanillin: identification of human UDP-glucuronosyltransferases and species differences. Phytother Res 2013;27:1392-7.

56. Houston JB. Utility of in vitro drug metabolism data in predicting in vivo metabolic clearance. Biochem Pharmacol 1994;47:1469-79.

57. Lave T, Dupin S, Schmitt C, Chou RC, Jaekc D, Coassolo P. Integration of in vitro data into algometric scaling to predict hepatic metabolic clearance in man: application to 10 extensively metabolized drugs. J Pharm Sci 1997;86:584-90.

58. Obach RS. Nonspecific binding to microsomes: impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine, and propranolol. Drug Metab Dispos 1997;25:1359-69.

59. Lin JH, Chiba M, Balani SK, Chen IW, Kwei GY, Vastag KJ, et al. Species differences in the pharmacokinetics and metabolism of indinavir, a potent human immunodeficiency virus protease inhibitor. Drug Metab Dispos 1996;24:1111-20.

60. Tan E, Pang S. Sulfation is the rate limiting in the futile cycling between estrone and estrone sulfate in enriched perportal and perivenous rat hepatocytes. Drug Metab Dispos 2001;29:335-46.

61. Griffin SJ, Houston JB. Comparison of fresh and cryopreserved rat hepatocyte suspensions for the prediction of in vitro intrinsic clearance. Drug Metab Dispos 2004;32:552-8.

62. Soars MG, Riley R, Findlay KA, Coffey MJ, Burchell B. Evidence for significant differences in microsomal drug glucuronidation by canine and human liver and kidney. Drug Metabol Dispos 2001;29:121-6.

63. Mohamed ME, Frye RF. Inhibition of intestinal and hepatic glucuronidation of mycophenolic acid by Ginkgo biloba extract and flavonoids. Drug Metab Dispos 2010;38:270-6.

64. Zainal Abidin S, Liew WL, Ismail S, Chan KL, Mahmoud R. Inhibitory effects of active constituents and extracts of Andrographis paniculata on UGT1A1, UGT1A4 and UGT2B7 enzyme activity. Int J Pharm Pharm Sci 2014;6:58-66.