Inhibitory Effect of Selected Malaysian Herbal Plants on Glutathione S-transferase Activity

M.S.M. Zabri Tan, M.R. Ab Halim, S. Ismail, F. Mustaffa
N.I. Mohd Ali and R. Mahmud
Centre of Drug Research, Universiti Sains Malaysia, 11800 Minden Penang, Malaysia
School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden Penang, Malaysia

Abstract: Many compounds from plants have been found to play an active role in inhibition and induction of GST activity. This study aims to evaluate the capability of five commonly used medicinal plants in Malaysia to inhibit the glutathione S-transferases (GST) activity in vitro. The ability of the plant extracts to inhibit GST activity was examined on rat liver cytosolic fraction and was analyzed using ultraviolet (UV) absorbance at 340 nm. When 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate, tannic acid exerted inhibition with IC₅₀ value of 6.18 μg mL⁻¹. The methanol extracts of Orthosiphon stamineus and Cinnamomum iners demonstrated the highest inhibitory activity against GST activity showing IC₅₀ values of 35.20±8.72 and 35.55±3.84 μg mL⁻¹. It is noted that, at 250 μg mL⁻¹ C. iners exhibited 1.3 more inhibition activity than tannic acid. The different parts of Croton argyrum plant were also evaluated on the GST inhibitory potential. Comparing the inhibition abilities of each part of Croton argyrum in decreasing order are leaves > roots > stems with IC₅₀ values of 40.42, 57.88 and 143.80 μg mL⁻¹, respectively. The data may suggest potential use of Orthosiphon stamineus and Cinnamomum iners as herbal medicines with GST inhibitory effect.

Keywords: Glutathione S-transferases inhibition, Croton argyrum, Curcuma xanthorrhiza, Eurycoma longifolia, Orthosiphon stamineus, Cinnamomum iners

INTRODUCTION

Herbal medicines which are utilized traditionally to cure illness may interact with human cytochrome P450s (Hanapi et al., 2010; Sharif, 2003) and with the drug metabolizing enzyme such as glutathione S-transferases (GST’s) (Azizi et al., 2010; Coruh et al., 2007; Zhang and Wong, 1997). Indeed, focus on the interaction of the herbal compound with drug metabolizing enzymes has received increased attention. The CYP (phase I) and GST (phase II) catalytic enzyme play crucial role in drug metabolism pathway in mammals. Both enzymes determine the pharmacological and toxicological properties of the ingested drugs.

GST is a phase II enzyme that accounts for multifunctional role in the cell defense system against electrophilic compounds (Ploemen et al., 1994). GSTs also exhibit antioxidant properties due to their selenium-independent glutathione peroxidase activity (Widersten and Mannervik, 1995). GSTs are known to be associated with the cell’s resistance to chemotherapy (Burg and Mulder, 2002), antibiotics (Area et al., 1988) and insecticides (Fourmier et al., 1992). Therefore, researchers are continuously investigating the evaluations of GST inhibitory potential.

Resistance of tumor cell to electrophilic xenobiotics such as alkylating agent after cancer chemotherapy treatment has been linked with the over-expression of certain GST isoenzyme (Burg and Mulder, 2002). GST is involved in the detoxification and metabolism of various anticancer drugs such as chloramphenicol (Hayes and Pulford, 1995). Consequently, the effort to detoxify the drug during continuous chemotherapy treatment leads to the increase in the GST level (Tew, 1994). Thus, the plant exhibiting inhibitory potential on the GST activity will be crucial in increasing the efficiency of cancer chemotherapeutics agent (Athar et al., 2007). Moreover, there have been wide applications of the natural occurring GST inhibitors on treating the Alzheimer’s disease (Lovell et al., 1998; Sultana and Butterfield, 2004) and Parkinson (Kiyohara et al., 2010; Menegon et al., 1998) diseases.

The present study focuses in evaluating the rat liver glutathione S-transferases (GSTs) inhibitory potential by five local plants namely Croton argyrum, Curcuma xanthorrhiza, Eurycoma longifolia, Orthosiphon stamineus and Cinnamomum iners (Table 1).

Curcuma xanthorrhiza is a traditional herb which originated from the ginger family (Zingiberaceae). This plant is used for various purposes from treatment of liver
related disease to heart and joint disorders. Xanthorizol is identified as the major constituent in the essential oil of this plant (Cheah et al., 2009; Devaraj et al., 2010).

Croton argyranthus or known as “merkolan” by the locals is commonly grown in Borneo, Malaysia (Yusoff et al., 2010). Study noted that the leaves, stems (Horgen et al., 2001) and root (Norizan et al., 2007) possess medical properties. Both are used by the locals to stop purging and diarrhea.

Literature search on Cinnamomum iners revealed that the bitterness of this plant is exploited by the locals to relieve fever, to treat digestive system related problem and to cure appetite related illness (Pengelly, 2004). Phuthdhawong et al. (2007) reported on the antioxidant activity of C. iners. Furthermore this plant exhibits analgesic activity mediated peripherally and proved to be non-toxic for consumption (Mustafah et al., 2010).

Tongkat Ali, or scientifically known as Eurycoma longifolia is a popular sought after herbal remedy for vast illness treatments and as additional supplements. Originating from the family of Simaroubaceae, some parts of the plants have known to possess antimalarial activity and plasmocidal activities (Chan et al., 2004; Noor-Rain et al., 2007; Wernsdorfer et al., 2009). It is also commonly prescribed in traditional medicine for sexual insufficiency (Wahab et al., 2010).

Orthosiphon stamineus Benth. has gained its popularity as one of the most popular traditional folk medicines because of its anti-fungal and anti-diuretic properties (Olah et al., 2003). It is also served as beverages for treatment of kidney, urinary tract diseases, gout (Wright et al., 2007) and diabetes mellitus (Awale et al., 2003a, b).

The high medicinal values of the mentioned plants have generated our interest to investigate their inhibition on GST activity that might be beneficial for treating GST related disease.

MATERIALS AND METHODS

Chemicals: 1-chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), cupric sulphate (CuSO₄), Folin–Ciocalteau’s phenol reagent, sodium potassium tartrate and sodium carbonate (Na₂CO₃) were obtained from Sigma Chemicals Company (USA). Tartric acid that was used as the positive control was purchased from HmbG Chemical (German). Propane-1, 2-diol and Tween-80 were purchased from Fischer Scientific (UK). The powder of dipotassium hydrogen phosphate was obtained from Riedel-de Haen (Germany). Potassium chloride (KCl) was acquired from BDH Chemical (UK). All the reagents and organic solvents used throughout this project were of analytical grade. This project was conducted from December 2009 to July 2010 at Centre for Drug Research, Universiti Sains Malaysia, Malaysia.

Plant materials: the laboratory of Centre prepared The Curcuma xanthorrhiza, Cinnamomum iners and Croton argyranthus extracts for Drug Research, Universiti Sains Malaysia (USM). Eurycoma longifolia and Orthosiphon stamineus extracts were obtained from Prof. Chan Kit Lam and Prof. Zahri Ismail respectively from the School of Pharmaceutical Sciences, Universiti Sains Malaysia. The purity of the extracts was verified by comparing the physical and spectroscopic data.

Animal: Fifteen Sprague Dawley rats weighing from 150 to 200 g provided by Animal House of Universiti Sains Malaysia were used in the study. The rats were housed in a temperature controlled room at 22±2°C under a 12 h light/12 h dark cycle for one week prior to sacrifice. The animals were fed with water and food ad libitum.

Preparation of rat cytosolic fraction: All the rats were anaesthetized with diethyl ether and followed by cervical dislocation. The liver was removed from each rat immediately after the sacrifice. The livers were washed with ice-cooled water and potassium phosphate buffer (pH 7.4). Furthermore, the samples were suspended in three volumes of phosphate buffer (pH 7.4) and were homogenized using Potter-Elvehjem homogenizer (Azizi et al., 2010). The homogenate were then centrifuged at 12500xg for 20 min. All subsequent steps were carried out at 4°C. The resulting supernatant was further centrifuged at 100,000xg for 60 min. The supernatants obtained were the cytosolic fraction. The total protein content of each cytosol sample was determined using Lowry method (Pomory, 2008). All preparations were stored at -80°C until used.

Determination of GST Activity in various plant extracts: In vitro GST activity was determined as described previously by Habig et al. (1974) with certain
modifications (Azizi et al., 2010). The experiments were conducted at room temperature. The mixture consisted of 0.1 M potassium phosphate buffer at pH 6.5, 30 mM of CDNB as the substrate, 30 mM GSH and GST enzymes (0.125 mg mL⁻¹). Rat liver cytosolic fractions were prepared and used as the GST enzyme source to determine GSH conjugation towards GST activity. The concentrations of plant extracts and tannic acid ranging from 0.01-1000 and 0.01-250 μg mL⁻¹ were used. The concentration of all plant extracts ranged from 0.01-1000 μg mL⁻¹ except for Croton argyranthum leave extract which ranged from 0.01-250 μg mL⁻¹. The reason for not determining the activity above 250 μg mL⁻¹ was because maximum absorbance was reached at concentration higher than that. Five replicates were used for each measurement. The enzyme activity was detected in vitro through the measurement of the conjugation activity with CDNB at 340 nm using Plate CHAMELEON™ (Hidex Oy, Finland) for 5 min. The specific GST activity for each plant extract was measured based on the formation of GSHI conjugate with CDNB.

Statistical analysis: All experiments were carried out in 5 replicates and expressed as the Mean±SD. All the computation analysis was carried out using GraphPad Prism® 5 software.

RESULTS AND DISCUSSION

All the values were obtained graphically by non-linear regression analysis of the remaining enzyme activity (unit mg⁻¹) versus the logarithm of natural product concentration (μg mL⁻¹). Their inhibitory effects on GST activity were shown in Fig. 1 and 2. The IC₉₀ values were obtained from extracts showing more than 50% inhibition (Appiah-Opong et al., 2007). Concentration of extracts for 50% inhibitory effect on GST activity was summarized in Table 2.

Of all the plant extracts tested result shows that Croton argyranthum, Orthosiphon stamineus and Cinnamomum iners equally inhibit the GST activity relative to a known inhibitor, tannic acid. However, Curcuma xanthorrhiza and Eurycoma longifolia extracts showed negligible inhibition. Figure 1 shows plant exhibiting strong inhibition with more than 50% inhibition on GST activity. Figure 2 represents weak inhibition of plant extracts towards GST activity. Inhibition activity of all the plant extracts on Fig. 1 coincides with the IC₉₀ value from Table 2 with all the plant extracts expressed IC₉₀ values except for Curcuma xanthorrhiza and Eurycoma longifolia. Table 2 summarizes the IC₉₀ values of all the extracts from the inhibition result.

Table 2: IC₉₀ values (μg mL⁻¹) for rat liver cytosolic GSTs for various plant extracts

<table>
<thead>
<tr>
<th>Plants</th>
<th>Parts</th>
<th>IC₉₀ value (μg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Croton argyranthum ethanolic extracts</td>
<td>Leaves</td>
<td>40.42±6.63</td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>57.38±5.65</td>
</tr>
<tr>
<td>Curcuma xanthorrhiza aqueous extracts</td>
<td>Stems</td>
<td>143.89±33.35</td>
</tr>
<tr>
<td>Orthosiphon stamineus methanolic extracts</td>
<td>Leaves</td>
<td>35.55±3.84</td>
</tr>
<tr>
<td>Eurycoma longifolia aqueous extracts</td>
<td>Roots</td>
<td>ND</td>
</tr>
<tr>
<td>Cinnamomum iners methanolic extracts</td>
<td>Leaves</td>
<td>35.20±8.72</td>
</tr>
<tr>
<td>Tannic Acid (Positive Control)</td>
<td>Powder</td>
<td>6.18±1.03</td>
</tr>
</tbody>
</table>

All values are Mean±SD for n = 5 determination. ND (not determined) due to percentage of inhibition being less than 50%.

Fig. 1: The inhibitory effect on rat cytosolic GST activity with increasing concentration of varied plant extracts. Assays were performed in 5 replicate for each sample.

Fig. 2: Effect on rat cytosolic GST activity with increasing concentration of Curcuma xanthorrhiza and Eurycoma longifolia. Assays were performed in 5 replicate for each sample.

Based on Fig. 1, at 50 μg mL⁻¹, C. iners showed 1.04 times higher inhibition activity relative to tannic acid. At still higher concentration range of 50 to 250 μg mL⁻¹, Cinnamomum iners extract exhibited much higher inhibitory potential relative to tannic acid. At the high concentration of 250 μg mL⁻¹, C. iners is 1.30 times more
than tannic acid in inhibitory activity. Meanwhile, 
*Croton argyrum* bark had showed the lowest inhibition activity at the same concentration range. Figure 2 demonstrated the comparable inhibition activity between *Eurycoma longifolia* root and *Curcuma xanthorrhiza* rhizome. However, both of them are weaker inhibitor compared to tannic acid. To date, there are no published reports in *in vitro* GST inhibitions by all the selected plants used in this experiment.

Tannic acid was used as the positive control and showed an IC50 value of 6.18±1.03 μM. CDNB is a common substrate used in GSTs activity assays. Jemth and Mannervik (1997), however, reported that certain GST isoenzymes demonstrate very low activity towards CDNB. Tannic acid which is a hydrolysable type of tannin (Robbers et al., 1996) has been commonly used as a positive control due to the non competitive inhibition towards CDNB and competitive inhibition on GSH characteristics (Zhang and Das, 1994). The author also proved that tannic acid is a potent inhibitor amongst other polyphenol tested. Tannin is a type of polyphenol that is involved in the inhibition or induction of some enzymes (Chung et al., 1998). Zhang and Wong (1997) revealed that plant polyphenols are responsible for the inhibition of GST activity of cancer cells.

Methanolic extracts of *Cinnamomum iners* and *Orthosiphon stamineus* are the most effective GST inhibitors on rat liver cytosolic GST with IC50 values of 35.20±8.72 and 35.55±3.84 μg mL^-1^ (Table 2), respectively. Judging on the low IC50 values of both extracts, the data might correlate them as potent inhibitors of GST in vivo. The inhibitory potential on GST activity can be associated with the concentration of polyphenols content (Das et al., 1984) and total flavanoid content (Ghazali and Waring, 1999). This property proposed that these chemicals may possess important pharmacological and toxicological effects (Middleton et al., 2000). Corresponding to this fact is the presence of flavonoid and polyphenolic compounds detected in both plants (Table 1) (Malterud et al., 1989; Mustaffa et al., 2010; Olah et al., 2003) that might have contributed to the strong inhibitory effect on GST activity.

Gringauz (1997) also reported that *Orthosiphon stamineus* (OS) contain high amount of tripenoids and flavonoids. Furthermore, the methanolic extract of OS is found to possess anti-tumor activity through enhancement of anti-proliferative effect on cancer cell (Salib et al., 2009a). Salib et al. (2009b) also reported on the *in vitro* antiangiogenic activity of the OS methanolic extract. Meanwhile the antioxidant activity of *Cinnamomum iners* is largely associated with its polyphenolic content (Pang et al., 2009). Their team also reported on the involvement of *Cinnamomum iners* in signaling pathway by inhibiting the proliferation of various cell lines. In agreement with this, Mustaffa et al. (2010) also reported on the high antioxidant capacity of the standardized leave methanolic extract of *Cinnamomum iners*. When the IC50 values of the plants were compared to the IC50 value obtained from tannic acid, all the plants show weaker inhibition than tannic acid.

*Croton argyrum* or *Croton argyratus* has yet to receive huge attention from scientists judging on the lack of literature on this plant. Noor-Rain et al. (2007) reported on antiplasmodial activities of this plant towards *P. falciparum*. In addition, the isolated compounds of *Croton argyrum* such as goniothalamin (Norizan et al., 2007) from the root exhibited anti-cancer properties against a range of human tumor and animal cell lines (Umar-Tsafu et al., 2004). Besides plant extracts, essential oils are also been screened for their potential usage. The lowest IC50 value (40.42±6.63 μg mL^-1^) can be observed (Table 2) on leaf extract compare to other extracts of this plant indicating that the leaf is a better GST inhibitor. The stem of *Croton argyrum* showed 50% inhibition at concentration of 143.80±33.35 μg mL^-1^ (Table 2) representing the lowest inhibition on GST activity.

It was proposed that *E. longifolia* possesses high medicinal values due to the possession of wide range of quassinoids (Table 1) (Jiwajinda et al., 2002). However, the direct effect of quassinoids on GST activity has not been proved yet. Although methanol extract of *Curcuma xanthorrhiza* has been reported to possess cancer chemopreventive potential (Park et al., 2008), the author did not specify the exact compound that contributed to such effect. Alone, xanthorrhizol exhibits inhibition on the tumor nodules in the lung tissue (Choi et al., 2004). Meanwhile, it is reported that combination of both xanthorrhizol and curcumin (from the rhizome of *Curcuma longa*) inhibit the proliferation of cancer cells (Cheah et al., 2009). Present data showed no inhibition on GST activity in the root extract of *E. longifolia* and rhizome of *Curcuma xanthorrhiza* (Table 2).

The present results might substantiate the potential use of *Orthosiphon stamineus* and *Cinnamomum iners* extracts for drug discovery and development as adjuvant in chemotherapy. They can be subjected to further study on the drug development for cancer and other diseases which may cause an over expression of GSTs.

CONCLUSION

The GST inhibition abilities of 5 plant extracts have been compared relatively to tannic acid; extracts from *Cinnamomum iners* proved a more potent GST inhibitors compared to the other local plant extracts studied. Meanwhile, the stem of *Croton argyrum* is the least effective GST inhibitor. Following this encouraging
finding, further studies in the determination of the major constituents from *Cinnamomum iners* plant extract in contributing to the GST inhibitory effect are essential to fully understand its GST inhibition mechanism.

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**REFERENCES**


