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Purification and biochemical characterization of glutathione S-transferase from common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae)

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Abstract

Glutathione S-transferase (GST) was purified and isolated from adults of common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer, by using ammonium sulfate precipitation and affinity chromatography using Glutathione Sepharose 4B column. The purified enzyme appeared as a single band on SDS-PAGE with an apparent molecular weight of 28.4 kDa. GST was purified 39.27-fold with a yield of 12.37% and a specific activity of 30.08 U mg^{-1} protein from *A. pistaciae*. The optimum temperature and pH of the enzyme activity were 30 °C and 9.0, respectively. The K_m and K_{cat} values for GSH (Glutathione) substrate were also determined to be 0.44 mM and 152.9 s^{-1} and for CDNB (1-chloro-2,4-dinitrobenzene) substrate to be 0.33 mM and 207.7 s^{-1}, respectively. GST's activity was completely inhibited by the addition of EDTA, $ZnCl_2$, and SDS; however, partially inhibited by $CaCl_2$, $BaCl_2$, $CoCl_2$, KCl, $MnCl_2$, Urea, $MgCl_2$ and Hg_2Cl_2. The *in vitro* inhibition studies indicated that all kinds of conventional insecticides (*i.e.* imidacloprid, acetamiprid, phosalone, and amitraz) possessed inhibitory effects on the activity of purified GST. Our study broadens the biochemical information on *A. pistaciae*'s GST and this information will help us to understand the mechanisms of insecticide resistance in this key pest**.**

Key words: glutathione S-transferase; purification, *Aganosena pistaciae*, inhibitory effect

خالص‌سازی و تعیین ویژگی‌های بیوشیمیایی آنزیم گلوتاتیون اس- ترنسفراز از پسیل معمولی پسته *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae)

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چکیده

آنزیم گلوتاتیون اس- ترنسفراز (GST) از حشرات بالغ پسیل پسته، *Agonoscena pistaciae* Burckhardt and Lauterer به واسطه رسوب گذاری سولفات آمونیوم و کروماتوگرافی میل ترکیبی با بکارگیری ستون Glutathione Sepharose 4B

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جداسازی و خالص شد. آنزیم خالص شده بصورت تک باند در SDS-PAGE ظاهر شد و وزن مولکولی آنزیم ۲۸/٤ کیلو دالتون تخمین زده شد. آنزیم از پسیل پسته به صورت ۳۹/۲۷ برابر و با درصد بازیابی ۱۲/۳۷ خالص سازی شد و آنزیم خالص شده، فعالیت ویژه ۳۰/۰۸ $U\ mg^{-1}$ protein نشان داد. دما و pH بهینه فعالیت آنزیم به ترتیب ۳۰ درجه سلسیوس و ۹ به دست آمد. مقادیر K_m و K_{cat} برای سوبسترای GSH (Glutathione) به ترتیب ۰/٤٤ mM و ۱۵۲/۹ s^{-1} محاسبه شد و برای سوبسترای CDNB (1-chloro-2,4-dinitrobenzene) ۰/۳۳ mM و ۲۰۷/۷ s^{-1} به دست آمد. فعالیت آنزیم GST به طور کامل با اضافه کردن EDTA، $ZnCl_2$ و SDS متوقف شد، این در حالی است که $CaCl_2$، $BaCl_2$، $CoCl_2$، Hg_2Cl_2، $MgCl_2$، Urea، $MnCl_2$ و KCl به طور جزئی فعالیت آنزیم را متوقف کرد. مطالعات بازدارندگی ***in vitro*** نشان داد که تمام آفت‌کش‌های شیمیایی (از جمله: ایمیداکلوپرید، استامی پراید، فوزالون و آمیتراز) بر فعالیت آنزیم خالص شده GST اثر بازدارندگی دارند. نتایج مطالعه ما با گسترش اطلاعات بیوشیمیایی آنزیم GST حاصل از پسیل معمولی پسته، به درک مکانیسم های مقاومت در این آفت کلیدی کمک می کند.

واژه‌های کلیدی: گلوتاتیون اس- ترنسفراز، خالص سازی، *Aganosena pistaciae*، اثر بازدارندگی

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Introduction

The common pistachio psyllid (CPP), *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.: Psyllidae), is a destructive pest of pistachio trees in Iran. This pest distributed in all pistachio-producing regions of Iran such as Kerman, Khorasan, Semnan and Qazvin provinces. Both adults and nymphs reduced pistachio yield, as one of the strategic agricultural products in Iran, by sucking plant juices and producing large amounts of honey dew (Lababidi & Zebitz, 1995). Chemical control is still the predominant method for controlling of CPP in Iran and many insecticides belonging to different classes of insecticides are recommended by Iranian Plant Protection Organization (IPPO) for its control. Excessive application of these insecticides against CPP have serious problems including side effects on non-target organisms, sub-lethal effects on beneficial insects, entry of pesticide residue into the food chains and the emergence of resistant populations (Talebi *et al*., 2011). In the other hand, due to the short life cycle of CPP and its high reproduction, *A. pistaciae* shows a high potential for resistance development to insecticides (Alizadeh *et al*., 2011). That's why some resistant populations of CPP to synthetic insecticides was reported in Iran (Alizadeh *et al*., 2011; Talebi *et al*. 2001). The metabolic insecticide resistance mechanisms in this pest as other insects are associated with an increase in activity levels of general esterase, glutathione S-transferase (GST) and cytochrome P450 monooxygenases (MFO) (Alizadeh *et al*., 2011; Rodriguez *et al*., 2010).

GSTs (E.C. 2.5.1.18) are major detoxification enzymes in insects that show multifunction activity. These enzymes play crucial roles in detoxification of both insecticides and their metabolites in insects, acari and other living organisms (Li *et al*., 2009). In insects, GSTs play an important role in insect resistance to various insecticides such as organophosphorus (OP), carbamate and organochlorine insecticides (Oppernoorth *et al*., 1985). GSTs can detoxify insecticides by dehydrochlorination or by their conjugation with reduced glutathione (GSH) to produce more water soluble metabolites. The GSH conjugated insecticide is more excretable than the non-GSH conjugated insecticide (Enayati *et al*., 2005). Some researchers have been classified the GSTs based on their location within the cell as microsomal and cytosolic GSTs (Enayati *et al*., 2005; Morel *et al*., 2004).

The majority of studies on insect GSTs have focused on their role in detoxifying pesticides and plant allelochemicals and their involvement in oxidative stress responses (Fournier *et al*., 1992; Ranson

et al., 2001; Sawicki *et al*., 2003). However, little work has been done to date to study the purification, identification and expression of insect GSTs (Akkemica *et al*., 2012; Balakrishnan *et al*., 2018). So far, cytosolic GSTs have been purified from 12 insect species belonging to Homoptera, Lepidoptera, Diptera, Dictyoptera, and Hymenoptera (Shukor *et al*., 2014). But, no research exists addressing the purified GST properties form psyllids. The characterization of the insect GST is valuable research in order to do comparative investigations. So that understanding of GST's function is essential to develop new strategies for insecticide resistance management (IRM). In this study, the partial characterization of purified GST from CPP was investigated and also further inhibitory effects of some conventional insecticide on its activity were analyzed.

Material and methods

Chemicals

Bovine serum albumin (BSA), reduced glutathione (GSH), 1-choloro-2,4-dinitrobenzene (CDNB), Tris-HCl were purchased from Merck company (Germany). Glutathione Sepharose 4B was obtained from GE Healthcare Bio-Sciences (USA). Imidacloprid, phosalone, amitraz and acetamiprid (95 percent) were obtained from Shimikeshavarz Co. (Iran).

Crude enzyme preparation

Ten thousand CPP adults were homogenized in 1ml ice-cold sodium phosphate buffer (PBS 0.02 M; pH 7.0) and centrifuged at 5,000 ×g for 5 min at 4 °C. The pellets were discarded and the supernatant was centrifuged again at 4 °C for 15 min at 17500 ×g. The crushed solid ammonium sulfate was added to the sample at 4 °C to obtain 80% saturation. The precipitate was obtained by centrifugation of samples at 12000 ×g for 15 min at 4 °C. Then, the pellet was dissolved in 20 mM PBS (pH 7.0). The enzyme solution was dialyzed against the PBS buffer (pH 7.0), changing the buffer twice and centrifuged again. Finally, the supernatant was used as the crude enzyme for purification (Memarizadeh *et al*., 2013).

Purification of GST

GST from *A. pistaciae* was purified using Glutathione Sepharose 4B according to the protocol supplied by manufacture (GE Healthcare Bio-Sciences, NJ, USA). At first the gel bed was washed by 20 ml of PBS buffer (pH 7.3) to remove any preservative. When the column was equilibrated, 1 ml dialyzed sample was applied into the column. Then, the column was washed with PBS buffer to remove the unbounded proteins. After that, the GST was eluted with 10 ml of elution buffer (5 mM glutathione in 0.05 mM Tris-HCl, pH 8.0). The fraction containing GST (no, 6) was pooled for further analysis after dialyzing.

Determination of molecular weight

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using a 10 % (w/v) polyacrylamide gel by the method of Laemmli (1970) for determination of molecular weight. The molecular weight of the enzyme was estimated using the following standards: β-galactosidase (116 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), lactate dehydrogenase (35.5 kDa), restriction endonuclease Bsp 981 (25 kDa), and β-lactoglobulin (18.4 kDa).

Determination of enzyme activity

GST activity was determined by 1-chloro-2,4-dinitrobenzene (CDNB) and reduced GSH as substrates based on the method of Habig *et al.* (1974) modified by Mahdavimoghadam *et al*. (2012) in 96-well microplates. The total reaction volume per well of a 96-well microplate was 240 µl containing 80

µl CDNB and 100 µl GSH in 50 mM PBS buffer, pH 7.0, with a final concentration of 2 and 8.3 mM of CDNB and GSH, respectively. The conjugation of CDNB with GSH was measured without enzyme solution as control. The change in absorbance was measured continuously for 5 min at 340 nm and 30 °C in a microplate reader (Awareness Technology Inc, Stat Fax® 3200). Absorbance per minute was converted into specific activity (nmol CDNB conjugated/min/mg protein) by monitoring the rate of 2, 4-dinitrophenylglutathione formation at 340 nm with a molar extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ (Habig *et al.*, 1974).

Determination of protein concentration

Protein concentrations of the samples were estimated at 630 nm using bovine serum albumin (BSA) as standard (Bradford, 1976).

Effect of pH and temperature

The optimum pH was determined for CDNB conjugation with GSH by GST. Enzyme activity was determined at room temperature using universal buffer (acetate-phosphate-borate; 20 mM) adjusted to various pHs (pH 3.0 to 12.0) (Asadi *et al*., 2010). Also, the activity of GST was determined by incubating the reaction mixture at different temperatures ranging from 10 to 60 °C in 20 mM phosphate buffer, pH 7.0.

Thermal stability of GST

The thermal stability of the enzyme was established by incubating the enzyme in 20 mM phosphate buffer, pH 9.0 at 30, and 40 °C for a series of time intervals, then cooled on ice, and finally assayed for residual activity under conditions described above (Asadi *et al*., 2010).

Determination of kinetic parameters

The apparent Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) values of purified GSTs from *A. pistaciae* were determined for CDNB and GSH. The catalytic activity of the enzyme was investigated in the presence of different substrate concentrations (0.1–2.6 mM for CDNB or 2–20 mM for GSH). The GST activity was measured by the standard assay method mentioned above. The K_m and V_{max} of GST with varying concentrations of glutathione or CDNB at a fixed concentration of CDNB (1 mM) or glutathione (5 mM) were determined from the Lineweaver-Burk plots.

Enzyme inhibition

For investigating the inhibitory effects of different insecticides on GST, four conventional insecticides in controlling of CPP including imidacloprid, acetamiprid, phosalone and amitraz were selected. Stock solutions of the technical material of insecticides (95 percent) were prepared in acetone and diluted with 20 mM, pH 7.0, PBS buffer. To prevent the adverse effect of acetone on GST activity, the final concentration of acetone in the reaction mixture was lower than 1 %. 15 µl of the enzyme and 50 µl inhibitor solutions were incubated for 10 min at room temperature. Then CDNB and GSH mixture was added and the remaining activity was measured by the standard assay method mentioned above. Reaction without inhibitors was served as control. The median inhibition concentration (IC_{50}) values and the 95% confidence limits for each inhibitor were determined from using the POLO-PC computer program (LeOra Software, 1987).

Effects of metal ions and chemicals

The effects of metal ions and chemical reagents in 2.5 and 5 mM were determined on GST activity based on the preliminary tests. Conjugation activity was measured by the standard assay method mentioned above. The effects of various metal ions and chemical reagents on GST activity at different

concentrations (2.5 and 5 mM) were investigated, after pre-incubation of ions or reagents with the enzyme at room temperature; residual activity was measured by the above assay method. Enzyme activity determined in the absence of them was defined as 100 % activity.

Results

Purification of *A. Pistaciae*'s GST and determination of its molecular mass

The GST from *A. pistaciae* was successfully purified using affinity chromatography on glutathione Sepharose 4B. The chromatogram is shown in (Fig. 1). GST specific activity (CDNB conjugation) increased 39.27-fold after separation by GSH affinity chromatography from the crude homogenate. The specific activity of the GST was increased from 0.76 up to 30 µmol $min^{-1}mg^{-1}$ protein during purification steps (Table 1). The purified GST using glutathione Sepharose column showed high homogeneity and appeared as a single band on SDS-PAGE with a molecular weight of about 28.4 kDa (Fig. 2).

Table 1. Purification of GST, in which the GST comes from the whole body of *Agonoscena pistaciae*.

Step	Total activity (U)	Total Protein (mg)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Crude enzyme	1066.28	1391.86	0.766	100	1
Ammonium sulfate precipitation	188.61	101.5282	1.857	17.68	2.42
Glutathione Sepharose TM 4B	131.94	4.385	30.08	12.37	39.27

Kinetic properties of *A. Pistaciae*'s GST

The K_m and V_{max} values calculated from Lineweaver-Burk plots of enzyme-catalyzed reactions with varying concentrations of GSH (2-20 mM) or CDNB (0.1-2.4 mM) at a fixed concentration of CDNB (1 mM) or GSH (5 mM), respectively, are shown in Table 2. The K_m value of GST for CDNB was lower than that of GSH.

Table 2. The kinetic parameters of GST purified from *Agonoscena pistaciae*.

Substrate	K_m (mM)	V_{max} (mmol min^{-1} mg^{-1}protein)	k_{cat} (S^{-1})
CDNB	0.33±0.01	0.40±0.02	207.7±9.2
GSH	0.44±0.06	0.26±0.03	152.9±20.17

Determination of optimum pH and temperature

The effects of different temperatures and pH values on GST activity were measured. Maximum activity was obtained at pH 9.0 but it decreased on either side of this value. (Fig. 3). The temperature profile showed a typical bell-shaped curve with an optimum activity at a 30ºC. The enzyme preserved its activity at a broad range of temperatures, from 20 (~80 % of maximal activity) to 50ºC (~70 % of maximal activity) and a rapid loss of activity was observed at below and above these two temperatures (Fig. 4).

Thermostability of GST

Thermostability of GST was measured at around of its optimum temperature (Fig. 5). As shown in Fig. 5, the enzyme retained more than 90% of its original activity after 5 min of incubation at 30ºC, but it lost 20% of its original activity at 40°C. Furthermore, after 60 min of incubation of purified GST at 30 and 40ºC, more than 40% and 20% of original activity was remained, respectively. Generally, thermostability of GST at optimum temperature of enzyme activity (30ºC) is twice more than that of at 40ºC.

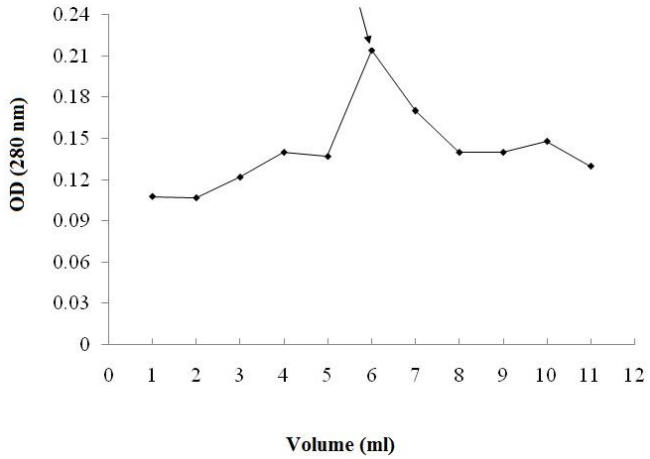

Fig. 1. The elution profiles of protein from *Agonoscena pstaciae* for GSH-affinity chromatography.

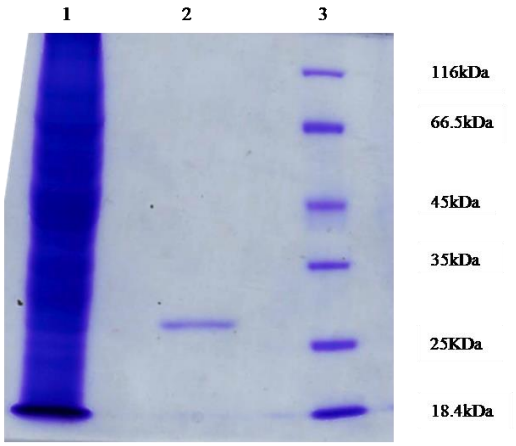

Fig. 2. SDS-PAGE photograph
lane 1, Ammonium sulfate precipitation; lane 2, purified enzyme from Glutathione Sepharose^{TM} 4B; Lane 3, standard molecular weight markers.

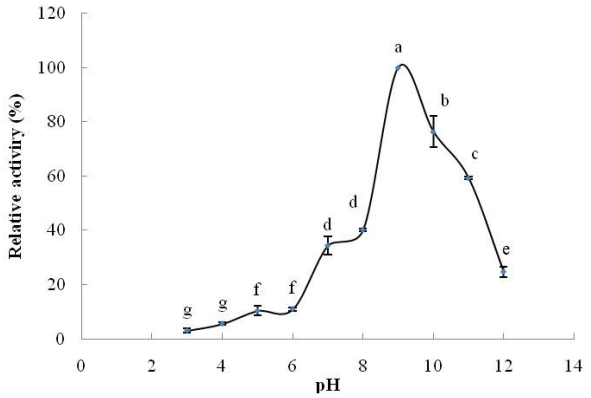

Fig. 3. Effect of pH on the activity of GST from *Agonoscena pistaciae*. Each value represents the mean ± SE of three independent experiments. The relative activity (%) was calculated as the percentage of GST activity in comparison with the maximum activity. Different letters indicate that the relative activity of enzymes is significantly different from each other by Tukey's test ($p < 0.05$).

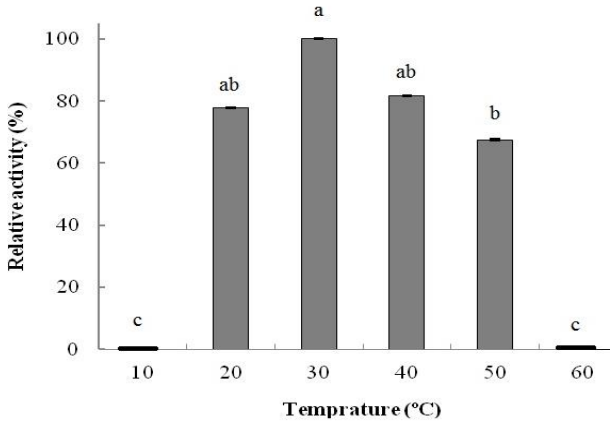

Fig. 4. The effect of temperatures on GST activity from *Agonoscena pistaciae*. The relative activity (%) was calculated as the percentage of GST activity in comparison with the maximum activity. Different letters indicate that the relative activity of enzymes is significantly different from each other by Tukey's test ($p < 0.05$).

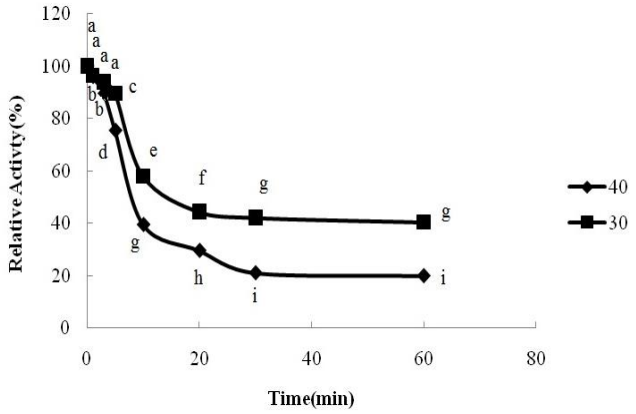

Fig. 5. Thermostability of purified GST from *Agonoscena pistaciae* at 30 and 40˚C. The relative activity (%) was calculated as the percentage of GST activity in comparison with the maximum activity.

In vitro inhibition of GSTs by insecticides

The inhibitory effects of four inhibitors on GST activity showed that all insecticides possessed significant inhibitory effects. The relative potency of insecticides was compared based on the IC_{50} values and their confidence limit. There was no significant difference among all tested compounds and phosalone was considered to be the most effective inhibitor (Table 3).

The effect of ions and chemicals

The effects of various metal ions and chemicals were determined on the activity of purified enzyme at two different concentrations (2.5 and 5 mM) (Table 4). The enzyme was inhibited by the addition of EDTA, $ZnCl_2$, and SDS. Partially inhibition of GST was occurred using $CaCl_2$, $BaCl_2$, $CoCl_2$, KCl, $MnCl_2$, Urea, $MgCl_2$ and Hg_2Cl_2.

Table 3. Effect of different compounds on the purified GST activity.

Chemical reagents (mM)		Relative activity (%)
Control (no ion)		100.0[a]
$BaCl_2$	2.5	41.4± 4.33[def]
	5	25.11±0.45[fgh]
KCl	2.5	8.54±0.82[hi]
	5	60.04±0.41[bc]
$MnCl_2$	2.5	11.52±0.99[hgi]
	5	70±0.45[b]
$CoCl_2$	2.5	15.15±0.24[hgi]
	5	21±0.67[fhgi]
EDTA	2.5	15.8± 0.08[hgi]
	5	0.0[i]
Hg_2Cl_2	2.5	16.99± 1.97[hgi]
	5	50.13±1.45[de]
$CaCl_2$	2.5	60.89± 2.65[cd]
	5	32.67± 1.48[efg]
$ZnCl_2$	2.5	51.1± 1.77[de]
	5	0.0[i]
$MgCl_2$	2.5	18± 0.61[fhgi]
	5	56± 2.13[d]
Urea	2.5	22.01±2.14[fgh]
	5	55.32±6.29[bc]
SDS	2.5	5.1±0.57[hi]
	5	0.0[i]

Different letters indicate that the relative activity of enzymes is significantly different from each other by using Tukey's test ($p < 0.05$).

Table 4. Inhibition parameters (IC_{50}) of different insecticides against purified GST from *Agonoscena pistaciae*.

Inhibitor	Slop	IC_{50}(mM) (95% confidence limit)	X^2(df)
Imidacloprid	1.98±0.2	26.02 (21.2-33.87)	4.4(3)
Phosalone	1.39±0.19	24.61(18.1-38.87)	4.89(3)
Amitraz	1.57±0.19	25.77 (21.62-32.06)	0.5(3)
Acetamiprid	2.1±0.21	27.23(23.72-32.12)	1.17(3)

Discussion

Up to now, there is a little information about GST properties in Psyllidae and recently, the rapid development of resistance to phosalone and imidacloprid by the *A. pistaciae* has been reported (Alizadeh *et al*., 2011). In our previous report, some phosalone-resistant CPP populations possessed higher GST activity compared to susceptible CPP and difference in GST activity was 3.14-fold (Alizadeh *et al*., 2011).

The results of the present study clearly demonstrate that the GST from the adult of *A. pistaciae* can be purified by ammonium sulfate precipitation and affinity chromatography, resulting in as much as 39.27-fold purification. The result indicates that by ammonium sulfate precipitation and affinity chromatography, the purification process is sufficient to obtain the purified enzyme. The specific activity of the purified GST from the *A. pistaciae* was 30.08 $\mu mol^{-1}min^{-1}$ mg^{-1} protein, which is higher than GST from *Anticarsiu gemmatalis* (Hübner) (Yu, 1989) and two syrphid flies (Vanhaelen *et al*., 2004). The purification yield can be affected by several factors, such as excessive amounts of the enzyme applied to each column, aging of the GSH-agarose and inactivation of the enzyme during chromatography (Yu, 1989). Thus, the recovery rates of *A. pistaciae*'s GST can be affected by the above factors.

CPP's GST sample obtained from ammonium sulfate precipitation still contained many impurities in SDS-PAGE and as shown in Figure 2, the GST was purified from the affinity chromatography and showed a single band on the gel. The molecular weight of GST purified from *A. pistaciae* is 28.4 kDa. The insect GSTs exist in multiple forms and nine isozymes were identified in midguts and fat bodies of insects (Yu, 1989). The molecular weights of GST purified from 35 invertebrate species have been mainly reported as 24.9 ± 3.1 kDa (Clark, 1989). The molecular weight of *A. pistaciae's* GST was near to those isolated from other pests, such as the *Spodoptera frugiperda* (J.E. Smith) (Yu, 1999), *Musca domestica* Linnaeus (Clark *et al*., 1984), *Drosophila melanogaster* Meigen, 1830 (Cochrane *et al*., 1987), and red flour beetle (Cohen, 1987). In contrast, the molecular weight of diamond back moth's GSTs was reported between 45-46 kDa (Cheng *et al*., 1983). Specific activity of purified GST by glutathione-agarose affinity chromatography from larvae of *Aedes albopictus* was calculated 196.0 ± 11 µmol/min/mg with a purification fold and yield of 28 and 69%, respectively. The purified GST appeared as a single band on SDS-PAGE with an apparent molecular weight of 23 kDa. (Shukor *et al*., 2014)

The kinetic parameters of GST from CPP were determined via Lineweaver-Burk plots and results presented in Table 2. Based on the Lineweaver-Burk plot, K_m and V_{max} values were calculated as 0.33 mM and 0.40 mmol min^{-1} mg^{-1} protein for GSH (5 mM) and CDNB substrates (0.1-2.4 mM), respectively. Furthermore, K_m and V_{max} values were calculated for GSH (2-20 mM) and CDNB (1 mM) as 0.44 mM and 0.26 mmol min^{-1} mg^{-1} protein, respectively. k_{cat} values were also determined as 152.9 s^{-1} for GSH and 207.7 s^{-1} for CDNB. Thus, these results showed that CDNB is a better substrate for the enzyme than GSH. The K_m and k_{cat} values determined in our study were similar to those obtained for GST from human erythrocytes (Awasthi and Singh, 1984) and bovine erythrocytes (Guvercin *et al*., 2008). The K_m of the GST was higher than those which were reported for some lepidopteran insects (Yu, 1989) and was most similar to GST3 from *Bulinus truncates* (Abdalla *et al*., 2006) and GST from *B. mori* (Hou *et al*., 2008). The K_m and V_{max} values for GST from *Taenia soliumis* were obtained as 2 mM and 77µM min^{-1} mg^{-1} when CDNB used as substrate, respectively (Plancarte *et al*., 2004).

The median inhibition concentrations (IC_{50}) of the conventional pesticide in control of CPB were calculated on GST of CPP (Table 4). In the present study, phosalone had the greatest inhibitory effect

on GST compared to acetamiprid, imidacloprid and amitraz. Ethacrynic acid showed the strong inhibitory effect on GST in *Liposcelis paeta* (Pearman) and IC_{50} values for ethacrynic acid were much lower than cypermethrin (Wu *et al*., 2009). Niu *et al.* (2011) showed that azocyclotin had the greatest inhibitory effect on GSTs from citrus red mite, *Panonychus citri* (McGregor) compared to ethacrynic acid and pyridaben. They concluded that azocyclotin could be used as an effective pesticide synergist for the control of pesticide resistance caused by GSTs.

The effects of pH and temperature on the activity of the GST were also investigated. GST of CPP showed maximum activity at slightly alkaline pH conditions. This optimal pH value observed for GST activity in our study was higher than those reported for other insects, which are around 7.0 (Grant and Matsumura, 1989; Reidy *et al*., 1990; Commandeur *et al*., 1995; Yu, 2002). Like most enzymatic reactions, as temperature increases the rate of 2, 4-dinitrophenylglutathione formation by CPP's GST will also increase. As shown in Figure 4.0, the reaction rate increases with temperature to a maximum level at 30°C, then abruptly declines with further increase of temperature due to denaturation of GST at temperatures above 30 °C. The optimum temperature for GST activity from *turkey liver* was reported at 50°C, while the optimum temperature for *B. mori's* GST (Abdalla *et al*., 2006) was corresponded at 25°C. Thermostability of purified GST from *A. pistaciae* at 30 and 40°C in different times showed that this enzyme was stable for 5 min (Fig. 5), but it lost 70% of its original activity after 20 min at 40°C. The GST from *Taenia solium* proved to be stable at 20°C and 37°C for 30 min (Hou *et al*., 2008). Since, GSTs are a large family of detoxification enzymes with a wide range of substrates; thus each isoform may require specific conditions of substrate concentrations, pH, temperature, and so on, for maximum activity (Commandeur *et al*., 1995; Yu, 2002).

Investigation on the sensitivities of GST from *A. pistaciae* to different metallic ions and chemicals showed that some chemicals and metallic ions such as Zn^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, SDS, and EDTA had inhibitory effects on GST. Wu *et al*. (2009) reported the same results on the purified GST from *L. paeta* and all tested metallic ions showed inhibitory action on GST from *L. paeta* except Ba^{2+}, which did not affect GST. The Zn^{2+}, Ca^{+2} and Mg^{2+} showed the activatory effect on many kinds of synthetase enzymes (34). But $Zn^{2+}$and Ca^{2+} worked oppositely in present study and case study of Wu *et al*. (2009). They concluded that metallic ions could combine with the sulfhydryl group of substrates (*i.e.* glutathione, CDNB and DCNB) and affected the further interaction with GST.

In summary, the current study has gathered some basic information on the biochemical and toxicological properties of GST from the very important pest of pistachio, *i.e. A. pistaciae*. The results of this research and further investigations will help us to understand the mechanisms of insecticide resistance in *A. pistaciae.*

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