Chemical constituents of water hyacinth (*Eichhornia crassipes*) methanol leaf extract and its effect on selected enzymes of *Periplaneta americana*

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Abstract

*Eichhornia crassipes* (water hyacinth) possesses several biological properties. *Periplaneta americana* (cockroach) is a household insect pest that can disseminate pathogens. The present study analyses the chemical constituents of water hyacinth leaf methanol extract and its *in vitro* effects on antioxidant and esterase enzymes in cockroaches. Water hyacinth methanol extract (WHME) was subjected to gas chromatography-flame ionization detection (GC-FID). Crude enzyme preparations were obtained from nymph and adult cockroaches. *In vitro* effects of different concentrations (10–80 μg/ml) of WHME on superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), acetylcholinesterase (AChE), and carboxylesterase (CE) in crude enzyme preparations were spectrophotometrically measured using cypermethrin (CYP) and chlorpyrifos (CPF) as reference insecticides. The GC-FID chromatogram of WHME showed the presence of gallic acid, para-hydroxybenzoic acid, eritosin, gentisic acid, catechin, protocatechuric acid, quercetin, and eugenol. The WHME and CPF reduced catalase in adults and SOD in nymphs, while CYP and CPF reduced SOD activity in the adults of *P. americana*. GST was significantly increased by WHME in both nymph and adult cockroaches. AChE was reduced by WHME, CYP, and CPF in nymphs, while CE was increased by WHME in nymphs and adults as compared to the control. In conclusion, our study indicates a high phenolic content in the water hyacinth leaf methanol extract, which increased the activities of the antioxidant enzymes AChE and CE in *P. americana*. The results also show that the efficacy and the mode by which the extracts could induce toxicity depend on the growth stage of the cockroach.

Key words: *Eichhornia crassipes*, spectroscopy, chromatography, *Periplaneta americana*, antioxidant enzymes, esterases

Introduction

The adult American cockroach (*Periplaneta americana*) is a large species of approximately 34–53 mm long. It is reddish brown with substantial variations in the light and dark patterns on the protum. The nymphs typically complete the developmental stages in approximately 2 years by undergoing a series of molts (Brenner and Kramer, 2019). Cockroach is perhaps the most cosmopolitan peridomestic pest species, which is believed to have spread from tropical Africa to North America (Brenner and Kramer, 2019). A high population of cockroaches may adversely affect human health through the contamination of food with their excreta, biting, and dissemination of pathogens, thereby inducing allergies, physiological stress, wounds, and blisters (Pomes et al., 2007; Bonnefoy et al., 2008; Ozdemir, 2014; Brenner and Kramer, 2019). Apart from their health impact, cockroaches are known to be destructive as they feed on books, leather, wallpaper paste, and even household furniture (Anonymous, 2004). They are among the most difficult household pests to control. Integrated methods of control involve the use of mechanical, physical, chemical, and/or biological means (Christopher, 2002; Mullen and Durden, 2002; Piper and Antoneli, 2007; Brenner and Kramer, 2019). The most effective method is chemical control which may be in the form of surface spray,
space spray, and insecticide baits (Ogunleye, 2010). However, chemical pesticides may be highly hazardous to human health, increase insect resistance, and cause general ecological problems; therefore, they need to be replaced with eco-friendly alternatives (Anchor Pest Control, 2008). Plant-derived insecticides could serve as suitable alternatives as plants contain a wide variety of bioactive compounds that protect them against herbivorous insects (Ennan et al., 1998; Tholl, 2006). In addition, plant-derived extracts and essential oils have been proven to be effective, are easily biodegradable, and are considered to be safe and cheap alternative sources of new insecticides (Bhat et al., 2012; Yu et al., 2012; Dhivya et al., 2017; Stevenson et al., 2017).

Synthetic insecticides are reported to affect untargeted animal species through environmental pollution, thus causing resurgence and pesticide resistance in the targeted insects. The use of alternative strategies based on botanical agents has therefore become a highly desired approach (Valicentre et al., 2010; Lenora and Senthilkumar, 2017; Acero, 2018). Many of the chemical insecticides act through mechanisms that involve inhibition of acetyl cholinesterase (AChE) and other molecular and cellular targets through overproduction of reactive oxygen species (ROS) (Zhu and Clark, 1997; Milatovic et al., 2006; Lionetto et al., 2011). Similar mechanisms of insecticidal toxicity have been suggested for certain botanical pesticides, as reported by Isman (2006), Lima (2006), Pavela and Benelli (2016), and Adeleke et al. (2019a, b).

Plants contain several secondary metabolites such as alkaloids, terpenes, glucosinolates, and phenolics, which prevent insects and microbial pests from attacking them (Freedman and Beattie, 2008). *Eichhornia crassipes* (water hyacinth) is a free-floating aquatic plant of the Pontederiaceae family (Xie et al., 2010; Annie et al., 2015). This plant is indigenous to Amazonia in Brazil (Barrett and Forno, 1982) and has spread to other places such as India, Egypt, Java, and Australia (Gopal, 1987) as well as Central South America, Venezuela, and the Caribbean Islands (Edward and Musil, 1975). The plant is known for its invasive nature (Villamagna and Murphy, 2010) and has been reported to contain bioactive constituents including stigmasterol, campesterol, β-sitosterol (Goswami et al., 1983), and naringenin (Bido et al., 2010) as well as alkaloids, flavonoids, sterols, anthraquinones (Jayanthi et al., 2011; Lalitha et al., 2012), and terpenoids (Shanab et al., 2010). Studies have revealed that water hyacinth has antimicrobial (Jayanthi and Lalitha, 2013), anti-inflammatory (Jayanthi et al., 2013), antitumor (Ali et al., 2009), antioxidant (Liu et al., 2010), and wound healing properties (Ali et al., 2010). A few studies have shown the insecticidal properties of water hyacinth extracts against some crop and household insects (Hassan, 2013; Annie et al., 2015; Chaudhary et al., 2017). The present study was designed to investigate the chemical constituents of methanol extract of water hyacinth leaf and its possible in vitro effects on some antioxidant and esterase enzymes in nymph and adult *P. americana*.

**Materials and methods**

**Chemicals**

Acetylthiocholine iodide, para-nitrophenyl acetate, 4-nitrophenol, and adrenaline (Sigma) were purchased from Germany, while Triton X-100, ethylenediamine tetraacetic acid (EDTA), and dithionitrobenzoic acid (DTNB) were purchased from the British Drug House Chemical Limited (Poole, UK). Two commercial insecticides, namely cypermethrin (CYP) and chlorpyrifos (CPF) (Sichuan Leschan Fuhua Tongda Agrochemical Technology Co. Ltd, China), were purchased from the TJP Agrochemical store in Ogbomoso, Oyo state, Nigeria. Other chemicals were of analytical grades and the purest quality available.

**Collection and extraction of water hyacinth** (*E. crassipes*)

The leaves of water hyacinth (*E. crassipes*) were collected at Olubere River, Ogbomoso (Oyo state, Nigeria) in March 2019. The greenish-yellow leaves were air dried at room temperature for approximately 3 weeks and then pulverized to obtain coarse powder. The powder was subjected to Soxhlet extraction using methanol to obtain the water hyacinth methanol extract (WHME). The extract was concentrated using a rotary evaporator and then subjected to oven drying at 40°C to obtain a dry powder. The concentrated extract was kept in an airtight container at 4°C until use.

**Chemical analysis of methanol extract of water hyacinth**

**Ultraviolet (UV) and Fourier transform-infrared (FTIR) spectroscopic analyses**

The UV spectra of WHME were obtained on a UV-1800 series spectrophotometer (Shimadzu) at the wavelength of 340 nm, while the IR spectra were obtained on
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A Cary 630 FTIR spectrophotometer (Agilent). The wavelength was expressed in reciprocal centimetre (cm⁻¹). The spectral data in both analyses were compared with literature data.

Gas chromatography-flame ionization detection (GC-FID)

GC-FID identification of compounds in the WHME was performed on HP SERIES II (5890) coupled to a flame ionization detector. Nitrogen was used as the carrier gas at the flow rate of 20 ml/min and hydrogen/compressed air was used as the combustion gas at the flow rate of 45 ml/min. The initial, and the injector and detector temperatures were 50°C, 220°C and 270°C, respectively, while the oven temperature was programmed to 240°C at the rate of 10°C/min with a holding time of 2 min. Chemical constituents were identified by comparing the mass spectra with the standard available in the NIST 11 library. The percentage composition of each constituent was estimated from the peak area of the chromatogram.

Collection and homogenization of insect (P. americana)

Forty nymph and adult cockroaches (P. americana) were collected separately from a dark cupboard at a residence in Ogbomoso (Oyo state, Nigeria) in March 2019. The adult insects were de-winged, and both nymphs and adults were divided into two groups (20 insects each). To determine the carboxylesterase (CE) activity, the first group was homogenized using phosphate buffer solution (pH 7.4), while the second group was homogenized with Tris-HCl buffer solution (pH 7.8). The insect homogenates were then centrifuged using a refrigerated centrifuge at 10,000 × g for 10 min at 4°C. The supernatants were collected and kept at 4°C until analysis.

Quantitative determination of the total protein content in P. americana homogenates

The total protein content in the nymph and adult P. americana homogenates was estimated spectrophotometrically at 546 nm as described by Lowry et al. (1951).

Enzyme analysis

Superoxide dismutase (SOD) activity

The activity of superoxide dismutase (SOD) in insects was estimated as described by Misra and Fridovich (1975). Briefly, an aliquot of 0.2 ml of the diluted insect homogenate (crude enzyme preparation) was added to 2.5 ml of 0.05M carbonate buffer solution (pH 10.2) for equilibration in the spectrophotometer. Then, 0.2 ml of WHME, CYP and CPF each was separately added at the following concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml in triplicates. The reaction was then initiated by adding 0.3 ml of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The absorbance at 480 nm was spectrophotometrically monitored every 30 s for 150 s. The SOD activity was calculated using the equation below by considering molar extinction of SOD at 480 nm as 525 M⁻¹·cm⁻¹.

SOD activity [Unit/mg protein] = \( \frac{\text{absorbance} \times \text{volume of mixture}}{\epsilon_{480 \text{ nm}} \times \text{sample vol} \times \text{mg protein}} \)

Molar extinction of SOD at 480 nm (\( \epsilon_{480 \text{ nm}} \)) = 525 M⁻¹·cm⁻¹

Catalase activity

The method described by Aebi (1984) was used to determine the catalase activity in the insect homogenates. The assay mixture contained 4 ml of hydrogen peroxide solution (0.2 M) and 5 ml of phosphate buffer (0.01 M, pH 7.0) in a 10 ml flat bottom flask. An aliquot of 1.0 ml of appropriately diluted crude enzyme preparation (homogenate) was mixed with the reaction mixture by a gentle swirling motion, and 0.3 ml of WHME, CYP and CPF was added separately at the following concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml in triplicates. The reaction was run at room temperature. An aliquot of 1 ml of the reaction mixture was blown into 2 ml of dichromate/acetic acid reagent at 60 s intervals. The changes in absorbance were monitored for 180 s at an interval of 60 s at 240 nm wavelength. The enzyme activity was calculated using the formula given below:

\[ \text{H}_{2}\text{O}_{2} \text{ consumed} = \frac{\text{H}_{2}\text{O}_{2} \text{ remained}}{0.171 \times \text{change in absorbance per minute}} \]

\[ \text{K}_0 = \text{H}_{2}\text{O}_{2} \text{ consumed} \]

\[ \text{Catalase activity} = \frac{\text{K}_0}{\text{mg protein}} \]

Glutathione-S-transferase (GST) activity

Glutathione-S-transferase (GST) activity was determined according to the method described by Habig et al.
G.E. Adeleke et al. (1974). The reaction mixture contained 30 μl of GSH (0.1 M), 150 μl of 1-chloro-2,4-dinitrobenzene (CDNB) (20 mM), 1.5 ml of phosphate buffer (0.1 M, pH 6.5), 50 μl of crude enzyme preparation and 30 μl of WHME, CYP and CPF (separately added at the concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml) added in a sequential order. The blank contained 30 μl of GSH, 150 μl of CDNB and 1.5 ml of phosphate buffer (pH 6.5). The reaction was allowed to run for 60 s at 31°C before the absorbance was read spectrophotometrically against the blank at 340 nm. All analyses were performed in triplicates for each sample analysed. The GST activity was calculated using the formula given below by considering the extinction coefficient of CDNB as 9.6 mM⁻¹·cm⁻¹.

GST specific activity [μM conjugate/min/mg protein] = \frac{OD_{\text{min}}}{9.6} \times \frac{1}{0.03 \text{ ml/mg protein}}

**AChE Activity**

The method described by Nachmanshon and Neumann (1975) and Ellman et al. (1961) was used to measure the activity of AChE. Briefly, 2.6 ml of phosphate buffer (0.1 M, pH 7.4), 0.1 ml of Ellman’s reagent, 5,5′-dithio-bis 2-nitrobenzoic acid (DTNB), 0.4 ml of the crude enzyme preparation (insect homogenate), and 0.3 ml of WHME, CYP and CPF each (at the concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml) were added to the reaction tubes. An aliquot of 0.1 ml of acetylthiocholine iodide solution was added to the reaction mixture as the substrate for the enzyme. The change in absorbance at 412 nm was read for 10 min at intervals of 2 min. The AChE activity was calculated using the formula given below by assuming the molar extinction to be 1.361 mmol⁻¹·mm⁻¹.

AChE activity [U/mg protein] = \frac{\Delta A \times \text{total reaction volume}}{\text{time} \times \text{sample volume} \times \text{molar extinction}}

CE Activity

The activity of CE enzyme was determined using the method described by Clement and Erhardt (1990), with some modifications. Each of the 20 nymph and de-winged adult cockroaches were homogenized in ice-cold Tris-HCl buffer (0.1 M Tris-HCl, pH 7.8 with 1% Triton X-100) using a tissue homogenizer. The homogenate was centrifuged in a refrigerated centrifuge at 10 000 × g for 10 min at 4°C. The supernatant was diluted with distilled water in 1:10 ratio. The reaction mixture contained 0.5 ml of diluted crude enzyme preparation (supernatant) and 2 ml of the working buffer (0.1 M Tris HCl, pH 7.8, containing 2 mM EDTA at 25°C), followed by 0.3 ml of WHME, CYP and CPF each (at the concentrations of 10, 20, 30, 40, 50, 60, 70 and 80 μg/ml). The mixture was incubated at 37°C for 10 min, and the reaction was initiated by adding 0.2 ml of 50 mM paranitrophenyl acetate (in acetone) as a substrate. The change in absorbance was measured spectrophotometrically at 405 nm at an interval of 1 min for 5 min. The blank reagent contained 2 ml working buffer and 0.2 ml paranitrophenyl acetate. A standard solution of paranitrophenol (50 mM) was used to evaluate the CE activity by using the following formula:

Carboxylesterase activity = \frac{\text{absorbance of sample}}{\Delta \text{absorbance of standard}} \times \text{concentration standard}

Carboxylesterase activity = mM/min/mg protein

**Statistical analysis**

All values are expressed as mean ± SD. Differences in the mean values were analysed statistically by one-way analysis of variance (ANOVA) by using SPSS and were considered to be significant at P < 0.05.

**Results**

**Spectral and chromatographic analyses**

This study conducted IR and UV spectroscopy of the WHME to detect various compounds present in it. As shown in Figure 1, the IR exhibited five prominent peaks at 1015.7 cm⁻¹, 1457.4 cm⁻¹, 1647.5 cm⁻¹, 2927.8 cm⁻¹ and 3324.8 cm⁻¹, while as shown in Figure 2, the UV spectrum exhibited maximal absorption at the wavelength range of 223–228 nm. The GC-FID chromatogram of WHME (Fig. 3) shows 10 different compounds with their respective retention times and percentage peak areas as follows: gallic acid (9.133 min, 9.73%), para-hydroxybenzoic acid (10.983 min, 7.23%), katekin (11.283 min, 4.95%), eritosin (11.550 min, 5.32%), gentisic acid (12.350 min, 12.59%), catechin (13.416 min, 7.31%), protocatechuic acid (14.233 min, 9.31%), kuersetin (15.216 min, 8.94%), quercetin (15.866 min, 28.52%) and eugenol (17.150 min, 6.10%).
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Antioxidant enzymes

Table 1 shows the effects of the WHME, CYP and CPF on the catalase activities in both nymph and adult P. americana. The result showed a significant increase ($P < 0.05$) in catalase activities following in vitro treatment with WHME, CYP and CPF in nymph P. americana when compared with that in the control. The catalase activity in the WHME-treated adult insect homogenate decreased significantly at 10 μg/ml (1.11 ± 0.13), 30 μg/ml (1.00 ± 0.01), 70 μg/ml (1.30 ± 0.01) and 80 μg/ml (1.26 ± 0.06), but only at 60 μg/ml (0.99 ± 0.01) and 70 μg/ml (1.20 ± 0.01) for CPF. In contrast, the activity was significantly reduced by CYP at all the concentrations tested when compared with that in the control (Table 1). The activity of SOD was significantly reduced ($P < 0.05$) by WHME at 30 μg/ml (0.99 ± 0.01), 40 μg/ml (1.99 ± 0.01), 70 μg/ml (0.99 ± 0.01) and 80 μg/ml (0.94 ± 0.09) and by CPF only at 60 μg/ml (1.96 ± 0.06) and 80 μg/ml (0.99 ± 0.01). CPF significantly reduced ($P < 0.05$) SOD activity at all concentrations tested, except at 10 μg/ml (2.95 ± 0.07) where the activity increased significantly ($P < 0.05$) in the nymphs (Table 2). However, in the adult cockroaches, WHME significantly elevated SOD activity, while both CYP and CPF lowered the enzyme activity across all concentrations tested, as shown in Table 2. Figure 4 and Figure 5 show the activities of GST in both nymph and adult cockroaches. WHME significantly increased ($P < 0.05$) the GST activity (ranging from 18.5 ± 2.21 to 22.42 ± 2.11 and 14.85 ± 1.10 to 15.61 ± 2.01 for nymph and adult cockroaches, respectively), while CPF significantly reduced ($P < 0.05$) the activity at all the concentrations tested (10–80 μg/ml). On the other hand, CYP was observed to significantly reduce the GST activity only at certain concentrations in both nymph and adult cockroaches when compared with the control (12.82 ± 0.97).

Esterase enzymes

A study of the AChE activity showed that WHME (4.00 ± 0.03 to 11.20 ± 0.12), CYP (0.8 ± 0.01 to 17.6 ± 1.63) and CPF (1.00 ± 0.01 to 5.10 ± 0.46) significantly reduced ($P < 0.05$) the activity at all the concentrations tested in the nymph P. americana when compared with the control treatment (21.70 ± 2.33) (Fig. 6). Figure 7 shows that WHME exerted no significant effect ($P > 0.05$) on the AChE activity in the adult insect. Furthermore, although CYP reduced the AChE activity, CPF increased it at all the concentrations (10–80 μg/ml) used in adults when compared with the control treatment (Fig. 7). The activity of CE was increased by both WHME and CPF at nearly all the concentrations tested, whereas CYP reduced it at nearly all the concentrations tested in nymph P. americana when compared with the control treatment (Fig. 8). The CE activity in adults was significantly increased ($P < 0.05$) by WHME at all the concentrations, except at 40 μg/ml (6.51 ± 0.14), 50 μg/ml (5.90 ± 0.11), 60 μg/ml (5.31 ± 0.12) and 80 μg/ml (5.70 ± 0.09) relative to control (5.93 ± 0.11) (Fig. 9). The treatment with CYP significantly reduced the CE activity at the concentrations of 10–60 μg/ml, while CPF increased the activity at all the concentrations tested in the study, as shown in Figure 9.
Fig. 3. Gas chromatographic chromatogram of water hyacinth methanol extract (figure shows gallic acid, p-hydroxybenzoic acid, eritosin, gentisic acid, catechin, protocatechuic acid, kuersetin, quercetin and eugenol which depicts nine prominent peaks with retention time 9.133 min, 10.986 min, 11.550 min, 12.350 min, 13.416 min, 14.233 min, 15.216 min, 15.866 min, 17.150 min respectively)

**Discussion**

Several secondary metabolites of plant origin function as protective chemicals against insects and pathogens that prey on such plants. These compounds have structural diversity and complex biological functions (Dhivya et al., 2017). In the present study, various functional groups present in WHME were elucidated by IR and UV spectroscopy. The results of the IR of WHME show five notable peaks at 1015.7 cm$^{-1}$, 1457.4 cm$^{-1}$, 1647.5 cm$^{-1}$, 2927.8 cm$^{-1}$ and 3324.8 cm$^{-1}$. According to the IR spectral studies by Fessenden and Fessenden (1986), Osman et al. (2010), Hossain and Ismail (2013) and Adeleke et al. (2019a), the functional groups in the extract included ether (1015.7 cm$^{-1}$), aromatic or aryl ring (1457.4 cm$^{-1}$), carbonyl (1647.5 cm$^{-1}$), methyl bident (2927.8 cm$^{-1}$) and aliphatic hydroxyl or carboxylic acid (3324.8 cm$^{-1}$) groups. Furthermore, in the present study, the UV data showed maximum absorption by the extract between 223 and 228 nm; this indicates the presence of carbonyl group, as reported by Bulus et al. (2011). The GC-FID chromatogram of WHME showed 10 different compounds, namely gallic acid (9.73%), para-hydroxybenzoic acid (7.23%), eritosin (5.32%), gentisic acid (12.59%), catechin (7.31%), protocatechuic acid (9.31%), kuersetin (8.94%), quercetin (28.52%) and eugenol (6.10%). In GC-MS studies by Lenora et al. (2016) and Lenora and Senthikumar (2017), water hyacinth was reported to contain certain chemical agents such as docosane, nonacosane, camarolide and erucyclamide, which have been found to possess insecti-
Chemical constituents of water hyacinth (Eichhornia crassipes) methanol leaf extract and its effect on selected enzymes

GST activity [µm conjugate/min/mg protein]:

<table>
<thead>
<tr>
<th>Concentration [g/ml]</th>
<th>WHME</th>
<th>CYP</th>
<th>CPF</th>
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<tbody>
<tr>
<td>10</td>
<td>1.01 ± 0.01 *</td>
<td>1.25 ± 0.07 *</td>
<td>4.55 ± 0.07 *</td>
</tr>
<tr>
<td>20</td>
<td>1.25 ± 0.07 *</td>
<td>0.34 ± 0.01 *</td>
<td>9.55 ± 0.07 *</td>
</tr>
<tr>
<td>30</td>
<td>3.55 ± 0.07 *</td>
<td>2.33 ± 0.04 *</td>
<td>10.20 ± 0.57 *</td>
</tr>
<tr>
<td>40</td>
<td>0.66 ± 0.01</td>
<td>0.99 ± 0.01 *</td>
<td>7.55 ± 0.07 *</td>
</tr>
<tr>
<td>50</td>
<td>3.55 ± 0.07 *</td>
<td>1.33 ± 0.04 *</td>
<td>5.65 ± 0.07 *</td>
</tr>
<tr>
<td>60</td>
<td>0.69 ± 0.04</td>
<td>0.95 ± 0.08 *</td>
<td>4.99 ± 0.01 *</td>
</tr>
<tr>
<td>70</td>
<td>2.95 ± 0.07 *</td>
<td>1.29 ± 0.01 *</td>
<td>3.97 ± 0.04 *</td>
</tr>
<tr>
<td>80</td>
<td>2.20 ± 0.01 *</td>
<td>0.99 ± 0.01 *</td>
<td>7.23 ± 0.11 *</td>
</tr>
<tr>
<td>Control</td>
<td>0.56 ± 0.01</td>
<td>1.54 ± 0.01</td>
<td></td>
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</tbody>
</table>

Table 1. Effects of water hyacinth (Eichhornia crassipes) methanol extract, cypermethrin, and chlorpyrifos on catalase enzyme activities in nymph and adult Periplaneta americana

<table>
<thead>
<tr>
<th>Concentration [µg/ml]</th>
<th>Catalase activity × 10⁻³ [U/mg protein]</th>
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</thead>
<tbody>
<tr>
<td>WHME</td>
<td>CYP</td>
</tr>
<tr>
<td>nymph Periplaneta americana</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.01 ± 0.01 *</td>
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<tr>
<td>20</td>
<td>1.25 ± 0.07 *</td>
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<tr>
<td>30</td>
<td>3.55 ± 0.07 *</td>
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<tr>
<td>40</td>
<td>0.66 ± 0.01</td>
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<tr>
<td>50</td>
<td>3.55 ± 0.07 *</td>
</tr>
<tr>
<td>60</td>
<td>0.69 ± 0.04</td>
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<tr>
<td>70</td>
<td>2.95 ± 0.07 *</td>
</tr>
<tr>
<td>80</td>
<td>2.20 ± 0.01 *</td>
</tr>
<tr>
<td>Control</td>
<td>0.56 ± 0.01</td>
</tr>
</tbody>
</table>

adult Periplaneta americana |          |              |
| WHME                  | CYP          | CPF          |
| 1.11 ± 0.13 *         | 0.61 ± 0.07 * | 1.30 ± 0.01  |
| 1.55 ± 0.07           | 0.34 ± 0.01 * | 2.65 ± 0.07 * |
| 1.00 ± 0.01           | 1.01 ± 0.01   | 1.75 ± 0.07  |
| 2.01 ± 0.01           | 0.76 ± 0.06   | 3.95 ± 0.07 * |
| 1.60 ± 0.01           | 0.71 ± 0.07   | 2.38 ± 0.11 * |
| 3.25 ± 0.07 *         | 0.63 ± 0.05   | 0.99 ± 0.01 * |
| 1.30 ± 0.01           | 0.34 ± 0.01   | 1.20 ± 0.01 * |
| 1.26 ± 0.06 *         | 0.67 ± 0.01   | 0.94 ± 0.08 * |

Mean ± SD: * – significantly higher compared to control (P < 0.05), # – significantly lower compared to control (P < 0.05); values expressed as in mean ± standard deviation; WHME – water hyacinth methanol extract; CYP – cypermethrin; CPF – chlorpyrifos

Fig. 4. Effects of WHME, CYP, CPF on Gluthatione-s-transferase activity in nymph cockroach (Periplanata americana). Mean ± SD: * – significantly higher compared to control (P < 0.05), # – significantly lower compared to control (P < 0.05); values expressed as in mean ± standard deviation; WHME – water hyacinth methanol extract; CYP – cypermethrin; CPF – chlorpyrifos

Our findings from the present study showed that WHME is significantly rich in phenolic compounds. Some phenolics are reported to be prooxidant in insects through their oxidation to hydrogen peroxide and organic peroxides in the gut of insects (Barbehenn et al., 2005), thus showing their significant role as insect antifeedants (War et al., 2012). An earlier report by Nappi et al. (2004) indicated the presence of prophenoloxidase (PPO) enzyme in the foregut of insects, which is capable of metabolic oxidation and subsequent detoxification of phenolic compounds ingested by these insects. A further investigation by Wu et al. (2015) suggested that plant phenolics only become toxic in insects when there is a loss of the PPO activity due to mutation or the level of phenolics exceeds the metabolic capacity of the enzyme. Although protocatechuic acid has been reported to show an in vitro antioxidant activity (Li et al., 2011), gallic acid and catechin have been shown to exert toxicity in Spodoptera frugiperda (Alves et al., 2014). Studies using quercetin have demonstrated a developmental or growth delay in Aedes aegypti (L) mosquito and mealworm beetle larvae (Gikonyo et al., 1998; Sosa et al., 2000), and an increased mortality in the wooly apple aphid Eriosoma lanigerum (Ateyyat et al.,
Fig. 5. Effects of water hyacinth methanol extract, cypermethrin, chlorpyrifos on Glutathione-S-transferase enzyme activities in adult *Periplaneta americana*. Mean ± SD: * – significantly higher compared to control (*P* < 0.05), # – significantly lower compared to control (*P* < 0.05); values expressed as in mean ± standard deviation; WHME – water hyacinth methanol extract; CYP – cypermethrin; CPF – chlorpyrifos

Table 2. Effects of water hyacinth (*Eichhornia crassipes*) methanol extract, cypermethrin, and chlorpyrifos on superoxide dismutase enzyme activities in nymph and adult *Periplaneta americana*

<table>
<thead>
<tr>
<th>Concentration [μg/ml]</th>
<th>Superoxide dismutase activity ×10⁻² [U/mg protein]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nymph <em>Periplaneta americana</em></td>
</tr>
<tr>
<td></td>
<td>WHME</td>
</tr>
<tr>
<td>10</td>
<td>11.95±0.70*</td>
</tr>
<tr>
<td>20</td>
<td>3.94±0.07*</td>
</tr>
<tr>
<td>30</td>
<td>0.99±0.01#</td>
</tr>
<tr>
<td>40</td>
<td>1.99±0.01*</td>
</tr>
<tr>
<td>50</td>
<td>5.91±0.13*</td>
</tr>
<tr>
<td>60</td>
<td>5.56±0.63*</td>
</tr>
<tr>
<td>70</td>
<td>0.99±0.01#</td>
</tr>
<tr>
<td>80</td>
<td>0.94±0.09#</td>
</tr>
</tbody>
</table>

Mean ± SD: * – significantly higher compared to control (*P* < 0.05), # – significantly lower compared to control (*P* < 0.05); values expressed as in mean ± standard deviation; WHME – water hyacinth methanol extract; CYP – cypermethrin; CPF – chlorpyrifos

2012) and the melon fruit fly *Bactrocera cucurbitae* (Sharma and Sohal, 2013). Eugenol, which was identified as another constituent of WHME in the present study, has been reported to be a natural constituent of the essential oil of several plants (El-Matti et al., 2016). This compound has been shown to exert termicidal (Xie et al., 2015), acaricidal (Ribeiro et al., 2016) and fungicidal (Koeduka et al., 2014) effects. Eugenol-rich essential oils were also reported to possess insecticidal properties against *Acanthoscelides obtectus, P. americana* and *Sitophilus granarius* (Viteri-Jumbo et al., 2014; Polatoglu and Karatoc, 2016), meal worm beetle (Martinez et al., 2018) and cowpea weevil (*Callosobruchus maculatus*) (Armijos et al., 2019). In the present study, these compounds might be responsible for the insecticidal activity exhibited by WHME.

Pesticides stimulate the generation of ROS in insects, resulting in damage to biomolecules and biological acti-
Chemical constituents of water hyacinth (Eichhornia crassipes) methanol leaf extract and its effect on selected enzymes

Fig. 6. Effects of WHME, CYP, CPF on acetylcholinesterase activity on Nymph cockroach (Periplaneta americana). Mean ± SD: * – significantly higher compared to control ($P < 0.05$), # – significantly lower compared to control ($P < 0.05$); values expressed as mean ± standard deviation; WHME – water hyacinth methanol extract; CYP – cypermethrin; CPF – chlorpyrifos

Fig. 7. Effects of water hyacinth methanol extract, cypermethrin, chlorpyrifos on acetylcholinesterase enzyme activities in adult Periplaneta americana. Values expressed as mean ± standard deviation; * – significantly higher compared to control ($P < 0.05$); # – significantly lower compared to control ($P < 0.05$); WHME – water hyacinth methanol extract; AChE – acetylcholinesterase

Fig. 7. Effects of water hyacinth methanol extract, cypermethrin, chlorpyrifos on acetylcholinesterase enzyme activities in adult Periplaneta americana. Values expressed as mean ± standard deviation; * – significantly higher compared to control ($P < 0.05$); # – significantly lower compared to control ($P < 0.05$); WHME – water hyacinth methanol extract; AChE – acetylcholinesterase

Activities (Narendra et al., 2007; Zhu et al., 2016). This damage may trigger transcriptional disruption and down-regulation of antioxidant enzyme-coding genes, resulting in the mortality of the insects (Abolaji et al., 2015, 2016). Our study of the catalase and SOD activities showed that WHME, CYP and CPF significantly increased the catalase activity in nymph $P. americana$, while this activity was reduced in the adult insect. The SOD activity was reduced by all the three agents in the nymph cockroach, whereas in the adult form, it was substantially increased by WHME but reduced by both CYP and CPF. In living cells, the first line of the antioxidant defence is the SOD enzyme that catalyses the dismutation of superoxide anion to hydrogen peroxide, which is finally decomposed to water and molecular oxygen (Mates et al., 1999; Koslov and Weidinger, 2015). Previous studies have indicated complexity in the nature of the antioxidant enzymatic system through which insects protect themselves from the toxic effects of ROS generated from plant toxins and insecticides (Felton and Summer, 1995; Rajapakse and Walter, 2007). High activities of the SOD and catalase have been implicated in the detoxification of ROS in Cimex lectularius (Mamidala et al., 2012) and Anopheles arabiensis (Nardini et al., 2013), thus enhancing the innate mechanism of resistance to ROS-generating pesticides in these insects. One of our earlier studies (Adeleke et al., 2019a) demonstrated that CYP, CPF and seed kernel extract of castor inhibit SOD and catalase in two local species of flea beetle, namely Podagrica sjsdfet and Podagrica
uniforma. The induction of the SOD activity coupled with the inhibition of the catalase activity by WHME in the adult P. americana, as observed in this investigation, may lead to the accumulation of hydrogen peroxide and the subsequent generation of oxidative stress in the insect. A study by Dhivy et al. (2017) indicated that antioxidant and detoxifying enzymes play significant roles in protecting tissues against oxidative damage. GSTs are a group of phase II detoxifying enzymes that catalyse the conjugation of several exogenous and endogenous electrophilic compounds with reduced glutathione to protect cellular macromolecules against oxidative stress-inducing agents (Edwards et al., 2000; Waczuk et al., 2019). The activity of the GST enzyme was significantly increased by WHME at all the tested concentrations (10–80 μg/ml) in both nymphs and adults. However, both CYP and CPF were found to significantly reduce the GST activity in both nymphs and adults when compared with that in the control group. The increase observed in the GST activity indicates possible innate resistance of cockroach to the extract. Although the present study showed that GST activities were reduced by
CYP and CPF in both nymph and adult cockroaches, a previous investigation by Sookrung et al. (2018) indicated insecticide resistance in cockroaches due to the increased detoxifying activity of GST. The opposing activities of SOD and catalase coupled with an enhanced GST activity following the treatment with WHME indicate a complexity in the antioxidant defence system of the investigated insect, as supported by the findings of Felch and Summer (1995) and Rajapakse and Walter (2007). The findings thus far suggest that the involvement of the antioxidant defence mechanism in cockroaches could protect them from oxidative stress-inducing agents, and this factor may be responsible for the wide spread of these insects in many household environments.

AChE, a cholinergic enzyme, degrades acetylcholine to form acetate and choline after a synaptic transmission (Dvir et al., 2010). In the present study, the AChE enzyme activity in the nymph cockroach was observed to be significantly reduced by WHME, CYP and CPF. However, in the adult cockroaches, although CYP reduced the AChE activity, CPF increased it significantly, while WHME showed no significant effect. Because the nymph stage is an early stage of development, the reduction in the AChE activity due to the three agents indicates a low production level of the enzyme and thus a high susceptibility of the cholinergic system at the nymph stage, which may not be the case at the adult stage of the insect. When mammals are exposed to carbamates and organophosphates, the AChE enzyme undergoes irreversible inhibition, resulting in an unending excitation and contraction of the muscle (Cochran, 2011). Studies have shown the activity of AChE in many insects, including mosquitoes (Kim and Lee, 2013), flea beetles (Adeleke et al., 2019a) and grasshopper (Adeleke et al., 2019b). In insects, the acetylcholine-binding nicotinic receptor is a major target for many toxicants that affect the central nervous system (Waczuk et al., 2019). A recent study (Saad et al., 2018) using *Sitophilus oryzae* reported that eugenol, one of the compounds identified in the extract used for this study, could readily penetrate the cuticle of this insect to inhibit the activities of AChE and adenosine triphosphatases (ATPases). In both nymph and adult *P. americana*, WHME and CPF significantly increased the in vitro activity of the CE enzyme, whereas CYP reduced the activity of this enzyme when compared with the control. A study by Jackson et al. (2010) indicated the hydrolytic role of the CE enzyme in the resistance and protection of insects against organophosphate insecticides. However, the enzyme has been reported to be inactive in detoxifying deltamethrin (pyrethroid) and carbaryl insecticides applied against *Locusta migratoria*, the migratory locust (Zhang et al., 2013). The upregulation of the genes encoding AChE and CE enzymes has been reported to correlate positively with the resistance of ticks to pyrethroids and organophosphates (Cossio-Bayugar et al., 2009). Our earlier investigation using a local species of flea beetles, namely *Podagrica sjostedti*, showed that CPF (IC50 = 2.65 μg/ml) and CYP (IC50 = 2.37 μg/ml) inhibited the in vitro activity of the CE enzyme (Adeleke et al., 2019a). The present study thus suggests that the elevated activities of both AChE and CE enzymes in adult cockroaches could be due to the high levels of production of these esterases. The induction of these enzymes may enhance resistance, which could be a possible mechanism underlying the wide spread of *P. americana* in homes and offices.

**Conclusion**

The results of the present study showed that WHME might contain some insecticidal properties, although with lower efficacy than that of the commercial insecticides CYP and CPF. Our study showed that the water hyacinth leaf methanol extract contains a high level of phenolics which may induce the activities of the antioxidant enzymes, AChE and CE in *P. americana*. The present study thus suggested that the efficacy and the mode by which the extracts could induce toxicity depend on the growth stage of the cockroach.

**References**


