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Spectroscopic and Chromatographic Characterization of *Anacardium occidentale* nut Shell Extract and its Enzyme Responses in *Periplaneta americana* (Cockroach)

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Abstract

Synthetic pesticides induce resistance in target insects, and toxicity in untargeted organisms. There is scarce information on the enzyme mechanisms involved in mortality response of *Periplaneta americana* to *Anacardium occidentale* nut shell extract (AONSE). The present study characterized the chemical constituents of AONSE, and investigated its *in-vitro* effects on selected enzymes of *P. americana*. The AONSE was subjected to Fourier-Transform Infrared (FT-IR) spectroscopy, ultraviolet (UV) spectroscopy, High-performance liquid chromatography (HPLC), Gas chromatography (GC) and Gas Chromatography-Flame ionization detector (GC-FID). Two portions of de-winged adult *Periplaneta americana* were separately homogenized with phosphate buffer and Tris-HCl buffer, followed by cold centrifugation. *In-vitro* effects of AONSE (concentrations 10 - 80 µg/ml) on catalase, superoxide dismutase (SOD), Glutathione -S- transferase (GST), acetylcholinesterase (AChE) and carboxylesterase (CE) in the homogenates were determined spectrophotometrically. Cypermethrin (CYP) and chlorpyrifos (CPF) were used as standard insecticides. The FT-IR spectrum of AONSE shows five prominent peaks at 1017.6cm^{-1}(ether), 1449.9 cm^{-1}(aromatic ring), 2834.6 cm^{-1}(methyl stretching), 2946.5 cm^{1}(methyl bending) and 3324.8 cm^{-1}(carboxylic group), while UV spectroscopy shows maximum absorption between 211.4 and 246.4 nm. The HPLC result reveals presence of three flavonoids including catechin, rutin, and quercetin. The GC analysis shows that AONSE predominantly contains phenolics and alkenes, while GC-FID spectrum shows major constituents as cardol (18.10%), cardanol (8.30%), beta-sitosterol (9.09%), lutein (10.38%) and anacardic acid (39.05%).The AONSE, CYP and CPF significantly ($P < 0.05$) reduced the activities of catalase and AChE, while AONSE alone increased SOD and GST activities in *P.americana*. The AONSE and CYP significantly reduced Carboxylesterase activity relative to control. The present study reveals that *A. occidentale* nut shell extract is rich in catechin, rutin, quercetin, cardol, cardanol, beta-sitosterol, lutein and anacardic acid, and could induce oxidative stress, and inhibit activities of cholinergic and carboxylesterase enzymes in *Periplaneta americana*.

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Chemical elucidation,Anacardium occidentale, Periplaneta americana, antioxidant enzymes, esterases

Introduction

Anacardium occidentale L (Cashew) is a tropical and subtropical plant belonging to the family Anacardiaceae, the genus Anacardium Linn, and the species *Anacardium occidentale* Linn var. nanum (Trevisan *et al.*, 2006; Santos *et al.*, 2007; Adeigbe *et al.*, 2015). Cashew tree is a branched, an evergreen tree with a height between six

meters (6 m) and twelve meters (12 m), and diameter between four meters (4 m) and twelve meters (12 m). Cashew trees are widely distributed in regions of 0–1000 m altitude with average annual temperature between 17°C and 38°C, and average annual rainfall between 500 mm and 3,500 mm (Orwa *et al.*, 2009). Cashew originated from Latin America, specifically Northeastern Brazil (Ohler, 1979; Adeigbe *et al.*, 2015). It was introduced by the Portuguese explorers to the tropical Asia and Africa from where it spread to other parts of the world. In Nigeria, commercial Cashew planting started in the mid-1950s in places such as Udi, Ogbe, Mbala, Oji, Iwo, Eruwa and Upper Ogun as documented by Akinwale and Esan, (1989) and Asogwa *et al.*, (2009). Studies have shown that the main producers of Cashew include Ghana, Cote d'Ivore, Brazil, Guinea Bissau, India, Nigeria, Vietnam, Benin Republic, Mozambique, Sri Lanka, Philippine, and Tanzania (Orwa *et al.*, 2009; Asogwa *et al.*, 2009; Hammed *et al.*, 2011). A report by Asogwa *et al.*, (2009) revealed that Cashew nut has served as food and industrial raw materials, as well as, a good source of income and foreign exchange in many parts of the world.

The nut and apple of Cashew are the two morphological parts of interest, with the nut being referred to as the true fruit, and the apple as the false fruit. The Cashew nut is kidney-shaped with an exterior, hard shell and interior white kernel (Aliyu, 2012). The apple is a hard, pear-shaped, and green fruit which may be red, yellow, or orange when matured (MacLeod and Troconis, 1982). Cashew nut is rich in many elements such as manganese, potassium, copper, iron, magnesium, zinc, selenium as well as macronutrients like protein, carbohydrate and fats (Blomhoff *et al.*, 2006).

Cashew juice and its pulp have been documented to have a high level of vitamin C (Eca *et al.*, 2015; Silva *et al.*, 2017). It has been reported that Cashew nut shell oil is a mixture of 70% anacardic acid (a salicylic acid analog, and a strong skin irritant), 18% cardol, and 5% cardanol. The two latter compounds are caustic phenolic substances that readily polymerize, and are useful for making epoxy resins, varnishes, and many high-tech materials that can withstand high temperatures, such as brake linings (Orwa *et al.*, 2009; Buxton *et al.*, 2017). Due to the presence of several compounds in Cashew plant, the leaves and stem bark have been employed in traditional medicine, particularly against bacterial infections, fever, high blood pressure and high blood sugar (Konan and Bacchi, 2007; Brandao *et al.*, 2008; Dahake, 2009; Olife *et al.*, 2013). It has been reported that, nearly all the parts of Cashew tree are reported to have ethno-medicinal importance (Emelike and Ebere, 2015).

The cashew nut shell liquid has been used as a good source of agrochemicals, varnishes, paints, surface coatings (Nyirenda *et al.*, 2021). Furthermore, *A. occidentale* plant extracts have shown several biological properties including anti-genotoxic and anti-mutagenic (Melo-Cavalcante *et al.*, 2011), antioxidant, anti-inflammatory, anticancer and antimicrobial (Salehi *et al.*, 2020), and antifungal (Jebapritha and Karpagam, 2017) properties. The juice of the Cashew apple has been reported to reduce the levels of insulin and low-density (LDL) and elevate high-density lipoprotein (HDL) in individuals with type-2 diabetes mellitus (Darvish *et al.*, 2019). Studies carried out by Oladejo *et al.*, (2016); Acero (2018) and Adeleke *et al.*, (2021) have revealed the insecticidal potential of Cashew nut shell liquid in insect pests. In addition, the liquid of Cashew nut shell inhibits acetylcholine esterase, and triggers membrane perturbation (Stasuik and Kozubek, 2008).

The objective of the study was to chemically characterize *Anacardium occidentale* nut shell (AONSE) extract, and investigate its *in-vitro* effects on some antioxidant and esterase enzymes in Cockroach (*Americana periplaneta*). This study targets the possible exploitation of Cashew nut shell extract as an insecticide, alternative to currently used synthetic insecticides.

Materials and Methods

Collection and Extraction of *Anacardium occidentale* (Cashew) nut shell

Cashew Nuts were bought from WAZO market, Ogbomosho, Oyo state, Nigeria in May, 2019. The nuts were de-shelled, and the shells were air-dried for three weeks at the room temperature. The shells were then pulverized using electric grinder, and then subjected to Soxhlet extraction (dissolving 25 g of pulverized nut shell in 250ml of methanol). The extract obtained was concentrated using rotary evaporator and then subjected to oven drying at 40^{o}C, to obtain an oily *Anacardium occidentale* nut shell extract (AONSE), which was stored under refrigeration until use.

Fourier-Transform Infrared (FT-IR) and Ultraviolet (UV) spectroscopy

The FT-IR analysis of AONSE was run on a Cary 630 FTIR spectrophotometer (Agilent). The wavelength was

expressed in reciprocal centimeter (cm^{-1}). The spectral data in both analyses were compared with literature data.

The UV analysis of AONSE was carried out using a UV-1800 series spectrophotometer (Shimadzu) at the wavelength of 340 nm.

High-performance liquid chromatography (HPLC) Analysis

The flavonoid contents of AONSE was determined using an isocratic HPLC (Mumbai machine) profiling at a flow rate of 0.5 mL/min. Exactly 25 mg of the extract was dissolved in the mobile phase (acetonitrile and methanol-80:20, v/v), and the injection volume was 20µL. The C18 (4.5 x 250 mm x 5µm) column was maintained at the room temperature and the eluent was detected at 210nm with a run time of 30 minutes. The peaks were compared with the standard available in the NIST 11 library.

Gas chromatography (GC) and Gas Chromatography-Flame ionization detection (GC-FID) Analyses

The AONSE was analyzed using GC machine, Clarus 500 Perkin Elmer (Auto system XL). The instrument was set to an initial temperature was maintained at 110°C for 2 minutes, while the Oven temperature was maintained at 280^0C for 9 minutes.

The GC-FID identification of compounds in AONSE 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, injector and detector temperatures were 50°C, 220°C and 270°C, respectively, while the oven was maintained at 240^0C at the rate of 10^0C/min, with a holding time of 2 minutes.

Constituents were identified by comparing the mass spectra with the standard available in the NIST 11 library. The peak area of each constituent was used to estimate the percentage composition.

Collection and homogenization of *Periplaneta americana* (Cockroach)

Adult *Periplaneta americana* (50) was collected from a dark cupboard in March 2019 at a residence in Ogbomoso, Oyo state, Nigeria. It was identified by Dr. *A. olayioye* at the Department of Crop and Environmental Protection, Faculty of Agricultural Science, LAUTECH, Ogbomoso. The insects were de-winged and divided into two groups. One group was homogenized using phosphate Buffer (pH 7.4) and the other group with Tris-HCl buffer (pH 7.8). The insect homogenates were centrifuged using a refrigerated centrifuge ((HITACHI model)) at 10000x g for 5 mins. The supernatants were collected and kept at 4^0C until used for biochemical analysis.

Determination of Total Protein in insect Homogenates

The total protein of homogenates of *Periplaneta americana* (Cockroach) was determined spectrophotometrically using the Biuret method described by Lowry *et al.*, 1951.

Determination of Catalase activity

Catalase activities in the cockroach homogenates were estimated according to the method of Aebi (1984) with modification. The reaction mixture contained 4.0 ml of hydrogen peroxide solution (0.2 M), 5.0 ml of Phosphate buffer (0.01 M, pH 7.0) and 1.0 ml of properly diluted insect homogenates (obtained with Phosphate buffer).

An aliquot of 0.3 ml each of ANOSE, Cypermethrin and Chlorpyrifos at concentrations10, 20, 30, 40, 50, 60, 70 and 80µg/ml was added separately to the reaction mixture at the room temperature. The control mixture contained neither AONSE nor standard insecticides. An aliquot of 1.0 ml of the reaction mixture was blown into 2 ml of dichromate/acetic acid reagent at 60 seconds intervals. Absorbance was taken at 240nm at an interval of 60 seconds for 180 seconds. Catalase activity was calculated as shown below:

$$H_2O_2 \text{remained} = \frac{\text{Change in absorbance/min}}{0.171}$$

H_2O_2 consumed = 800 - H_2O_2remaned

$K_o = H_2O_2$ Consumed
Catalase activity = K0/mg protein

Catalase activity = Unit/mg protein

Determination of Superoxide dismutase activity

The superoxide dismutase (SOD) activity in cockroach homogenate was estimated according to the method

described by Misra and Fridovich (1971), with modifications. Briefly, a mixture of 0.2 ml diluted insect homogenate(obtained with Phosphate buffer) and 2.5 ml carbonate buffer (0.05M, pH 10.2) was allowed to equilibrate in the spectrophotometer. Each mixture was treated separately with 0.2 ml of AONSE, Cypermethrin and Chlorpyrifos at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml, followed by addition of 0.3ml of freshly prepared adrenaline (0.3M). The absorbance was read at 480 nm at an interval of 30 seconds for 150 seconds. The control mixture contained neither AONSE nor standard insecticides. The SOD activity was expressed as Units/mg protein. Given the Molar extinction of SOD at 480nm (ε_{480nm}) to be 525 $M^{-1}cm^{-1}$, SOD activity was calculated using the formula below:

SOD Activity = (Absorbance x Volume of mixture) / (ε_{480nm} x Sample volume x mg protein)

SOD activity = Unit/mg protein

Determination of Glutathione-S-transferase activity

Glutathione-S-transferase (GST) activity of insect homogenates was assayed according to the method of Habig *et al.*, (1974) with modification. The reaction mixture contained 2.79 ml of phosphate buffer (0.1 M, pH 6.5), 30 µl of GSH (0.1 M), 30 µl of insect homogenate (obtained with Phosphate buffer) and 150 µl of CDNB (20 mM). An aliquot of 0.3 ml each of AONSE, Cypermethrin and Chlorpyrifos at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml was then added separately to the mixture. The blank contained 30 µl of GSH, 150 µl of CDNB and 2.82 ml of phosphate buffer (pH 6.5). The reaction was carried out at 31^{0}C for 60 seconds, and absorbance was taken 340 nm. The extinction coefficient of CDNB was taken as 9.6mM^{-1} cm^{-1}. The GST activity was calculated as below:

$$\text{GST specific activity} = \frac{\text{OD/min}}{9.6} \times \frac{1}{0.03\text{ml/ mg protein}}$$

GST specific activity = µM Conjugate/min/mg protein

Determination of acetylcholinesterase Activity

Acetylcholinesterase (AChE) activity in insect homogenate was estimated according to the methods described by Ellman (1961), and Nachmansohn and Neumann (1975) with modification. Reaction mixture contained 2.6 ml phosphate buffer (0.1M, pH 7.4), 0.1 ml Dithionitrobenzoic acid (DTNB) and 0.4 ml insect homogenate (obtained with Phosphate buffer). An aliquot of 0.3 ml each of AONSE, Cypermethrin and Chlorpyrifos at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml was added to the mixture. Then 0.1 ml of Acetylthiocholine iodide solution was added, as substrate, to initiate the reaction. The control mixture contained neither AONSE nor the standard insecticides. Acetylcholinesterase activity was determined spectrophotometrically by taking absorbance at 412 nm at an interval of 2 minutes for 10 minutes. Given the molar extinction as 1.361x $mmol^{-1}$ xmm^{-1}, AChE activity was calculated as below:

$$\text{AChE activity} = \frac{\text{Change in absorbance x Total reaction volume}}{\text{Time x sample volume x molar extinction}}$$

AChE activity = U/mg protein

Determination of carboxylesterase Activity

Carboxylesterase (CE) activity in insect homogenates was determined using the method of Clement and Erhardt (1990) with modification. Cockroach was homogenized in ice-cold Tris-HCl buffer (0.1 M, pH 7.8 with 1 % Triton X-100 at 25^{0}C) using a tissue homogenizer. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 *x g* for 5 minutes at 4 ^{0}C to obtain supernatant. An aliquot of 0.5 ml of diluted supernatant (1:10 dilution) of insect homogenate was added to 2 ml of the working buffer (0.1 M Tris-HCl, pH 7.8, containing 2 mM EDTA at 25 ^{0}C). An aliquot of 0.3 ml each of AONSE, Cypermethrin and Chlorpyrifos at concentrations 10, 20, 30, 40, 50, 60, 70 and 80µg/ml was then added. The mixture was incubated for 10minutes at 37 ^{0}C followed by adding 0.2 ml of paranitrophenyl acetate (50 mM) (in acetone) to initiate the reaction. The absorbance was measured spectrophotometrically at 405nm, at an interval of 60 seconds for 180 seconds. The blank reagent contained 2.0 ml of working buffer and 0.2 ml paranitrophenyl acetate. Para-nitrophenol standard curve was prepared to calculate the activity of Carboxylesterase enzyme, expressed as mM/min/ml protein.

Statistical Analysis

Values were expressed as mean ± SD. Differences in the mean values were estimated statistically by one-way analysis of variance (ANOVA) by using the Statistical Package for Social Sciences (SPSS) software for

Windows version 10.0 (USA). Values were considered to be significant at $P < 0.05$.

Results and Discussion

TheFT-IR spectrum of the AONSE shows five prominent peaks at 1017.6cm^{-1}(ether), 1449.9 cm^{-1}(aromatic ring), 2834.6 cm^{-1}(methyl stretching), 2946.5 cm^{1}(methyl bending) and 3324.8 cm^{-1}(carboxylic group) as shown in figure 1. The UV spectroscopy of the extract shows maximum absorption at a wavelength range between 211.4 and 246.4 (Figure 2). The HPLC chromatogram (Figure 3) of AONSE shows the presence of three flavonoids including catechin, rutin and quercetin, with their retention times as 1.915, 2.516 and 5.593 minutes, respectively. The GC spectroscopy (Figure 4) of AONSE shows major compounds suspected to be eugenol/phenolic monoene (40.89%), phenolic alkene(13.28%),Caryophylene (9.08%) and allyl compounds (12.89%), corresponding to peaks 17, 18, 22 and 27, respectively. phenolics and alkenes. The GC-FID spectrum (Figure 5) showsthe major compounds to be cardol (18.10%), cardanol (8.30%), beta-sitosterol (9.09%), lutein (10.38%) and anacardic acid (39.05%). The result in table 1 indicates that AONSE, CYP and CPF significantly ($p < 0.05$) reduced catalase activity in the homogenates of *P. americana* across the concentrations compared with control treatment. The activity of SOD was significantly lowered at nearly all the concentrations by CYP and CPF, while AONSE reduced it at high concentrations compared with the control (Table 2).

Investigation of the effects on the GST activity in the insect shows, that AONSE elevated the activity in a concentration-dependent manner. However, CYP and CPF significantly reduced the activity nearly at all the concentrations relative to the control treatment (Figure 6). The result in figure 7 reveals that the in-vitro activity of AChE in *P. americana* was significantly ($p < 0.05$) reduced by AONSE, CYP and CPF compared with control at all the concentrations. The result in figure 8 shows the effects on the in-vitro carboxylesterase activity. The AONSE and CYP significantly ($p < 0.05$) reduced the activity across the concentrations (10 – 80 µg/ml) against the control. However, CPF increased the activity at all the concentrations used in the study compared with the control.

For several years, synthetic insecticides have been used in the management and control of insects, both on field and store. However, these insecticides have been noticed with certain short-comings such as inducing pest resistance, affecting untargeted beneficial animals and humans, food poisoning, and environmental pollution (Tapondju *et al.,* 2002). In the recent times, the global concern has been towards development of bio-based insecticides with potency, biosafety and ecological friendliness (Asogwa *et al.,* 2012; Buxton *et al.,* 2017; Acero, 2018). The present study has investigated the chemical constituents of *Anacardium occidentale* nut shell extract and the possible in-vitro effects on some antioxidant and esterase enzymes in *Periplaneta americana* (Cockroach), aiming at the possibility of this extract becoming a suitable and safe alternative to synthetic insecticides.

The FT-IR analysis of AONSE exhibited five prominent wave numbers, including 1017.6cm^{-1}, 1449.9 cm^{-1}, 2834.6 cm$^{-1,,}$ 2946.5 cm^{1} and 3324.8 cm^{-1}. As reported by Fessenden and Fessenden (1986); Halilu *et al.,* (2013); Bulus *et al.,* (2011) and Hossain and Ismail (2013), these peaks correspond to ether bond, aromatic ring or aromatic methyl group, methyl stretching, methyl bending and carboxylic group or aliphatic hydroxyl group, respectively (Figure 1). The AONSE shows maximum UV absorption between 211.4 and 246.4 (Figure 2). Compounds containing carbonyl functional group have been reported to show UV absorption at 211 nm (Bulus *et al.,* 2011).

The HPLC chromatogram (Figure 3) shows that the AONSE contains three flavonoids, including catechin, rutin and quercetin. Flavonoids are important phenolic compounds with several biological properties. For instance, Mendki *et al.,* (2005) reported that glycosides of kaempferol and quercetin exhibited significant toxicity against *Callosobruchus chinenesis*. In another study by Rosa *et al.,* (2017), it was demonstrated that the flavonoid-rich extract of *Gliricidia sepium* leaves showed an insecticidal activity against Coffee Mealybugs (*Planococcus citiri*). Furthermore, a recent study by Hay *et al.,* (2020) showed an insecticidal potential of a mixture of kaempferol, genistein and daidzein on *Trichoplusia ni*, a lepidopteran host of soybean plant.

Table.1 In-vitro effects of AONSE, CYP and CPF on Catalase activity in *Periplaneta Americana*

Concentrations (µg/ml)	Catalase activity (U/mg protein)		
	AONSE	CYP	CPF
10	1.20 ± 0.14#	0.64 ± 0.04#	1.25 ± 0.07#
20	1.10 ± 0.14#	0.32 ± 0.01#	1.90 ± 0.14#
30	1.45 ± 0.21#	1.10 ± 0.14#	0.34 ± 0.01#
40	0.25 ± 0.07#	0.08 ± 0.11#	4.05 ± 0.07*
50	0.67 ± 0.01#	0.59 ± 0.11#	2.20 ± 0.14#
60	0.69 ± 0.04#	0.63 ± 0.04#	0.99 ± 0.01#
70	0.37 ± 0.06#	0.32 ± 0.02#	0.32 ± 0.01#
80	0.64 ± 0.04#	0.63 ± 0.05#	0.31 ± 0.03#
Control	2.30 ± 0.14		

Data expressed as Mean ± Standard deviation (Values obtained in duplicates)
***** = significantly higher than control (P<0.05)
# = significantly lower than control (P<0.05)
AONSE: *Anacardium occidentale*nut shell extract; CYP: Cypermethrin; CPF: Chlorpyrifos

Table.2 In-vitro effects of AONSE, CYP and CPF on Superoxide dismutase activity in *Periplaneta Americana*

Concentrations (µg/ml)	Superoxide dismutase activity (U/mg protein)		
	AONSE	CYP	CPF
10	8.9 ± 0.06*	1.00 ± 0.01#	2.06 ± 0.08#
20	12.95 ± 0.07*	7.01 ± 0.01*	1.00 ± 0.01#
30	10.01 ± 0.01*	2.06 ± 0.08#	0.71 ± 0.01#
40	8.76 ± 0.11*	1.25 ± 0.07#	0.92 ± 0.03#
50	4.06 ± 0.08#	1.01 ± 0.01#	1.06 ± 0.08#
60	4.23 ± 0.36#	2.06 ± 0.08#	0.96 ± 0.06#
70	0.30 ± 0.06#	5.00 ± 0.01*	2.03 ± 0.04#
80	4.01 ± 0.01#	1.95 ± 0.07#	0.56 ± 0.08#
Control	4.61 ± 0.07		

Data expressed as Mean ± Standard deviation (Values obtained in duplicates)
***** = significantly higher than control (P<0.05)
# = significantly lower than control (P<0.05)
AONSE: *Anacardium occidentale*nut shell extract; CYP: Cypermethrin; CPF: Chlorpyrifos

Fig.1 FT-IR Spectrum of *Anacardium occidentale* nut shell extract

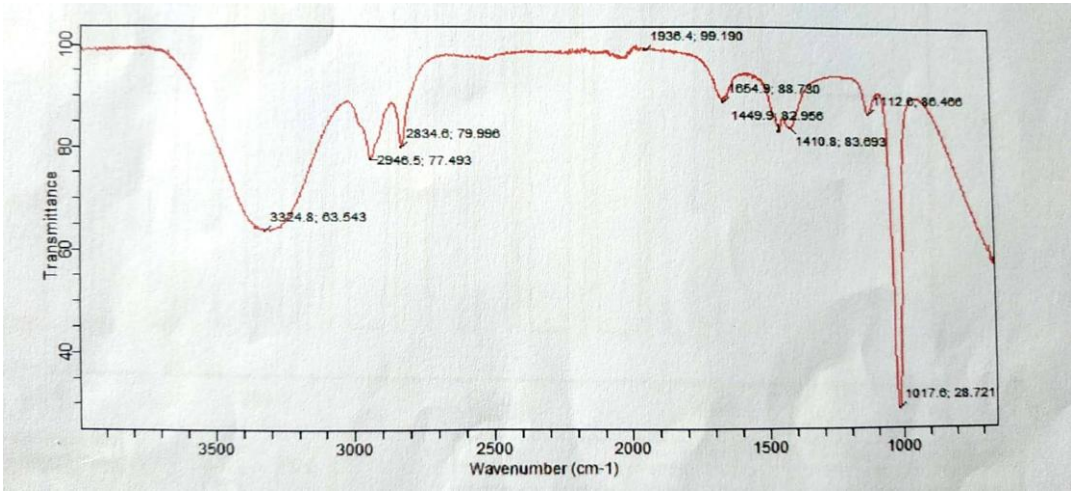


Fig.2 UV Spectrum of *Anacardium occidentale* nut shell extract

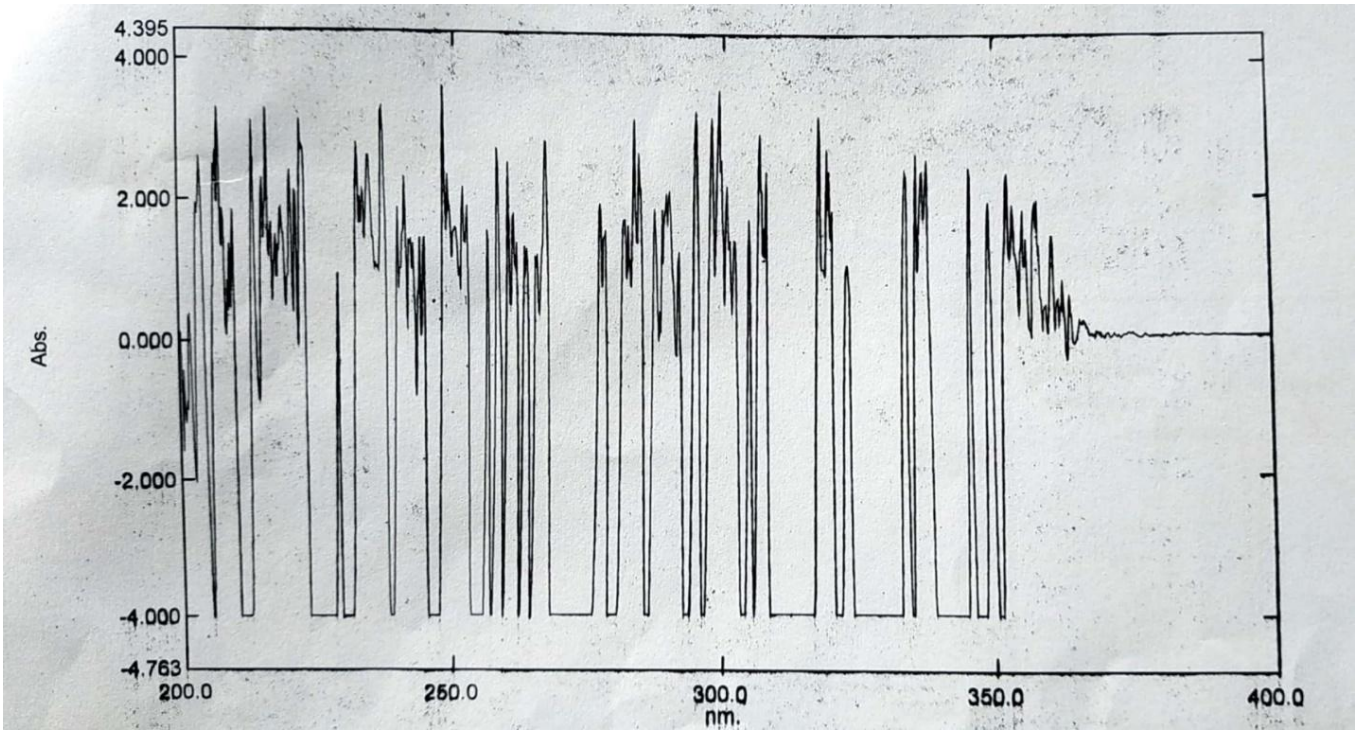


Fig.3 HPLC Chromatogram of flavonoids contents of *Anacardium occidentale* nut shell extract

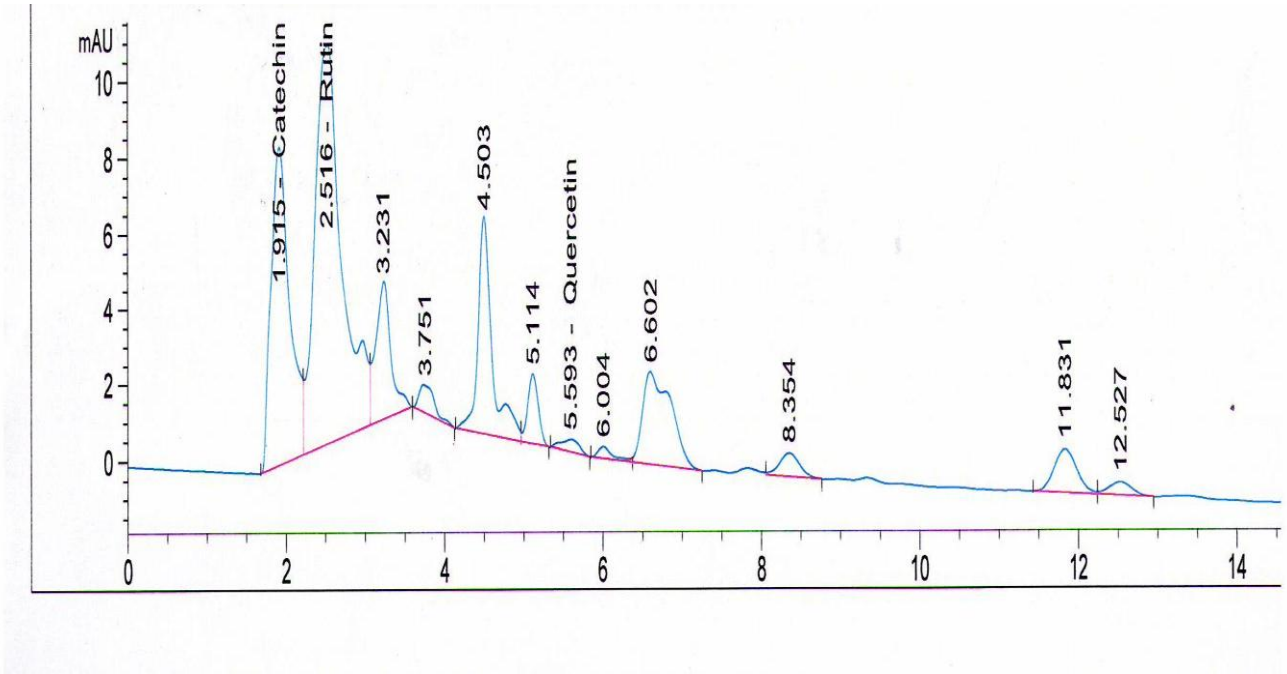


Fig.4 Gas Chromatogram (GC) of *Anacardium occidentale* nut shell extract

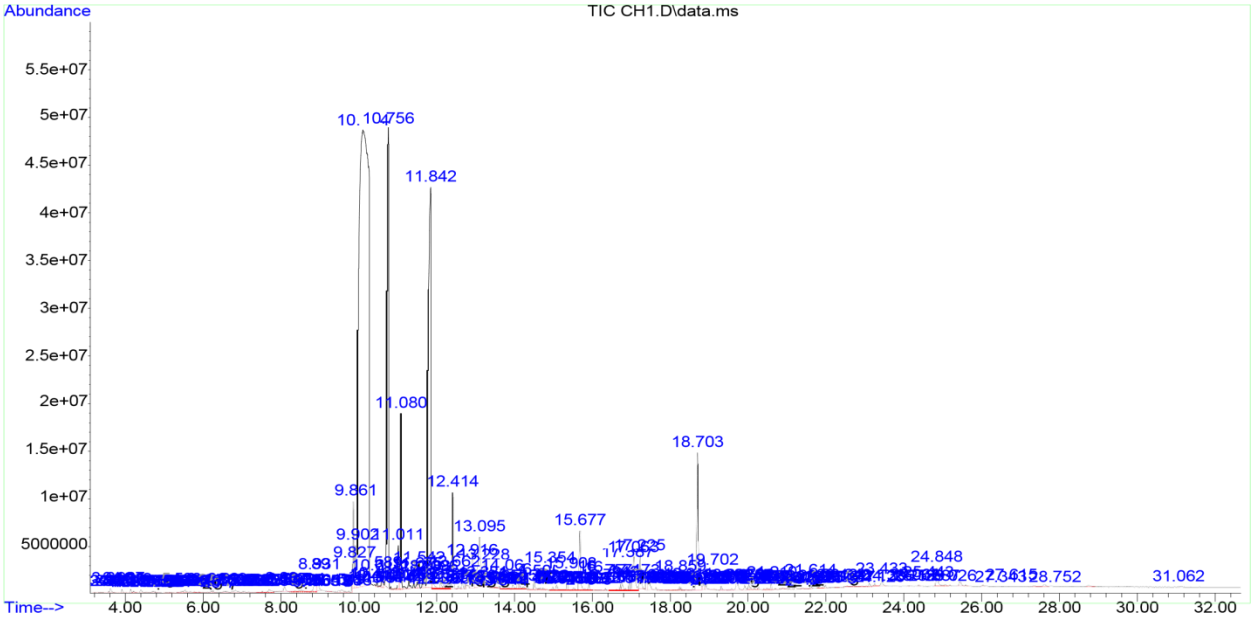


Fig.5 Gas Chromatography-Flame ionization detector (GC-FID) chromatogram of *Anacardium occidentale* nut shell extract

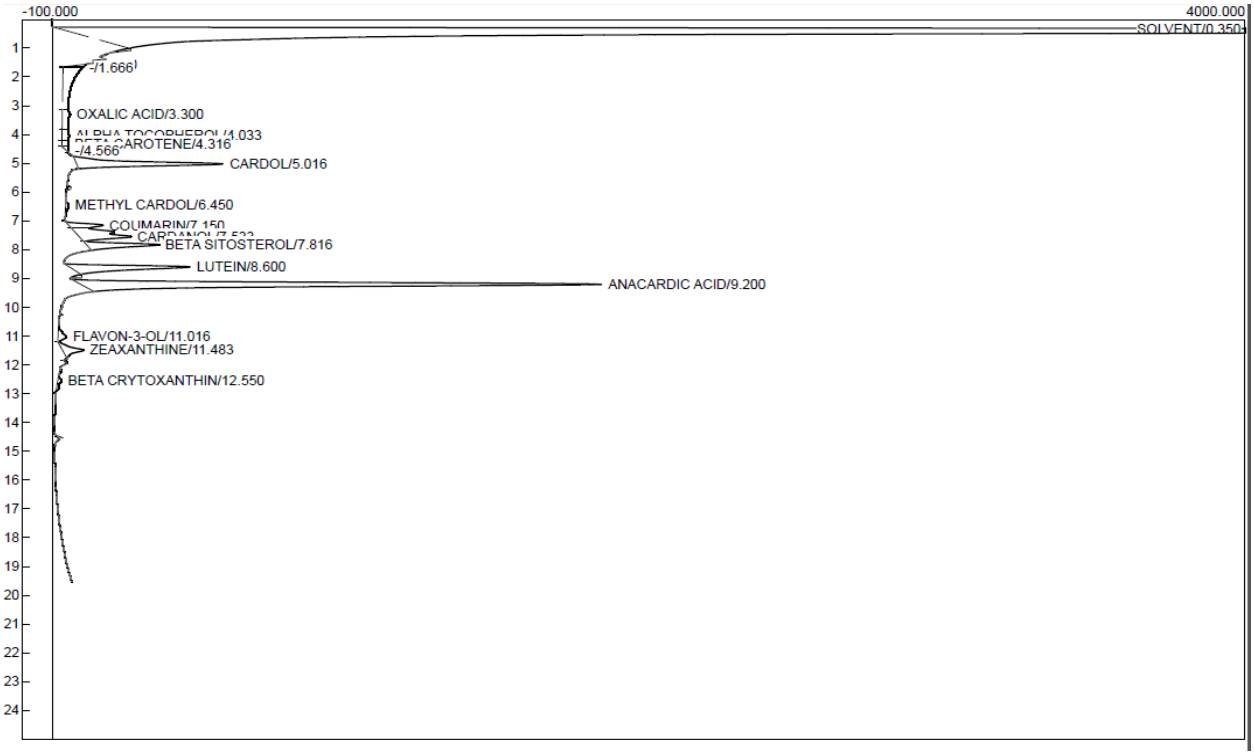
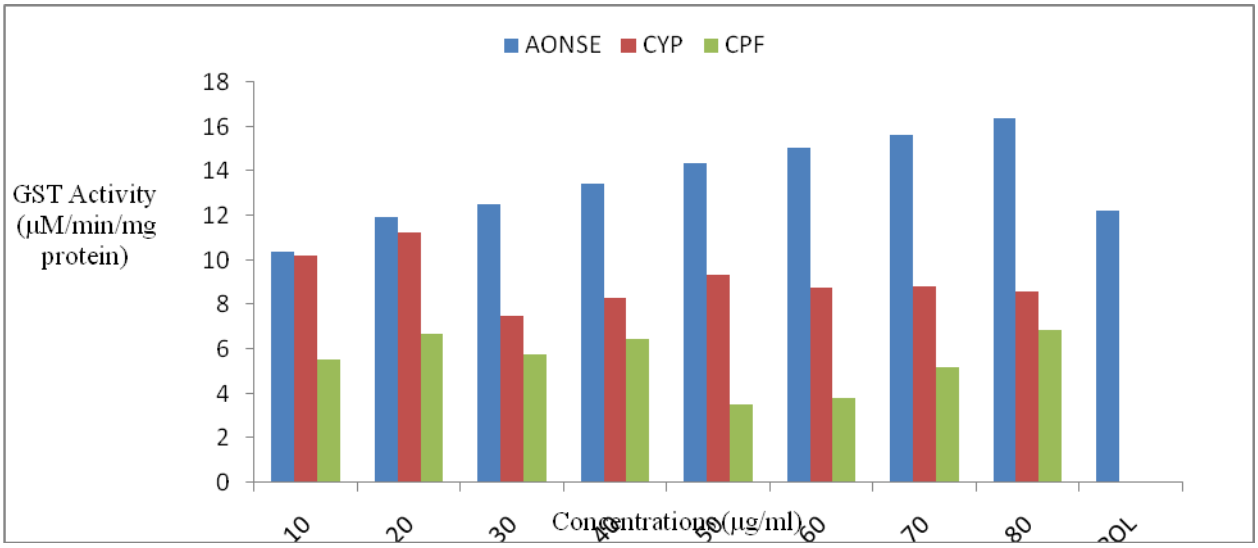
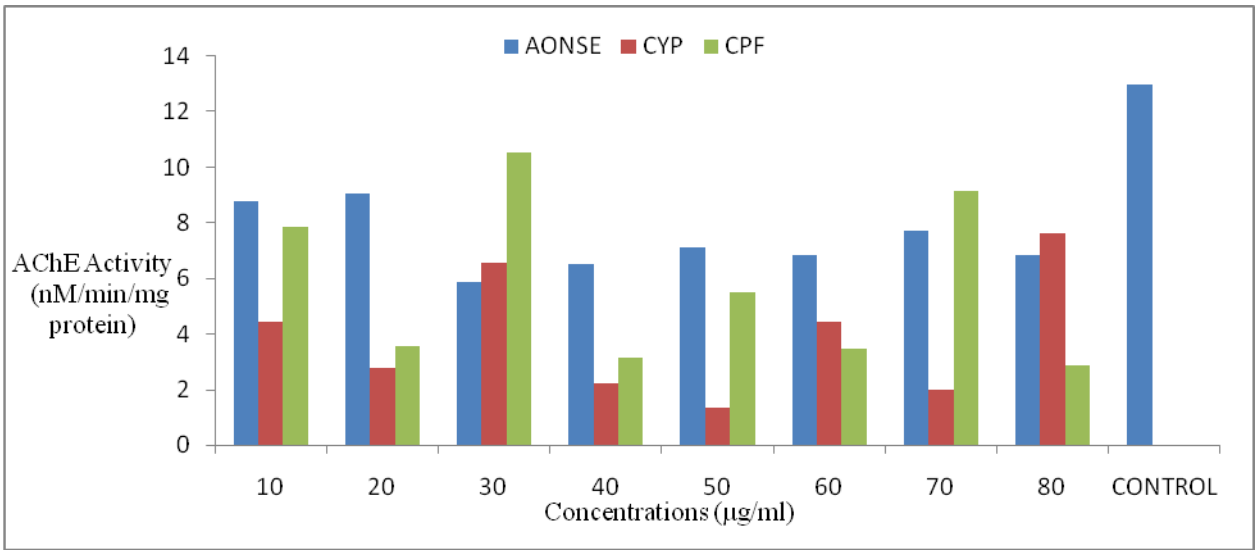


Fig.6 In-vitro effects of AONSE, CYP and CPF on glutathione-S-transferase activity in *Periplaneta americana*



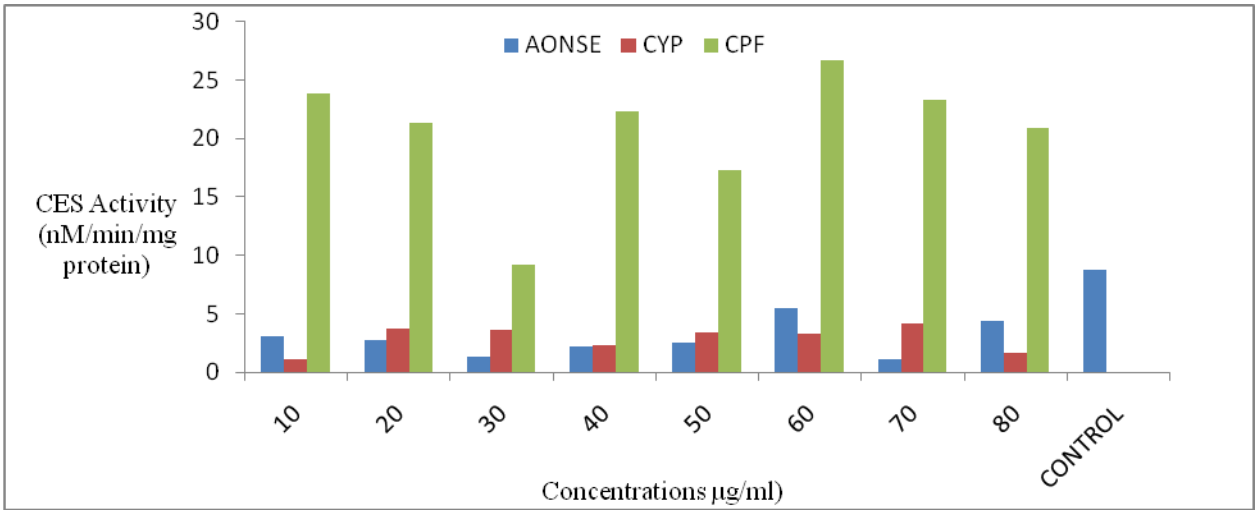
Data expressed as Mean ± Standard deviation (Values obtained in duplicates)
AONSE: *Anacardium occidentale*nut shell extract; CYP: Cypermethrin; CPF: Chlorpyrifos

Fig.7 In-vitro effects of AONSE, CYP and CPF on Acetylcholinesterase activity in *Periplaneta americana*



Data expressed as Mean ± Standard deviation (Values obtained in duplicates)
AONSE: *Anacardium occidentale*nut shell extract; CYP: Cypermethrin; CPF: Chlorpyrifos

Fig.8 In-vitro effects of AONSE, CYP and CPF on Carboxylesterase activity in *Periplaneta americana*



Data expressed as Mean ± Standard deviation (Values obtained in duplicates)
AONSE: *Anacardium occidentale*nut shell extract; CYP: Cypermethrin; CPF: Chlorpyrifos

The mechanisms involved in the roles of flavonoids in plant-insect interactions have been reported to be species specific instead of being a broad spectrum pattern (Simmonds, 2003). Figure 4 shows the GC spectrum of AONSE with four major compounds suspected to be eugenol/phenolic monoene, phenolic alkene, Caryophylene and allyl compounds. However, figure 5 depicts the GC-FID spectrum with five major constituents, including cardol, cardanol, beta-sitosterol, lutein and anacardic acid. Studies by Gómez-Caravaca (2010) and Almeida *et al.,* (2019) indicated the presence of anacardic acid, cardol, cardanol, and 2-methylcardol as the four main constituents in cashew nut shell liquid.

However, their finding was in accordance with that of the present study, in that anacardic acid has been reported as being the highest in the two studies. Natural cashew nut shell liquid (CNSL), which is obtained by cold solvent extraction, majorly contains anacardic acid. However, in the technical CNSL, cardanol forms a major component due to decarboxylation of anacardic acid to cardanol on heating (Risfaheri *et al.,* 2009). The larvicidal activities of both Cardanol and Cardol against *Aedes aegypti* have been reported by Paiva *et al.,* (2017). It has been documented by Oliviera *et al.,* (2011) that the unsaturated side chain of 15 carbon atoms of anacardic acid strongly enhances high larvicidal activity of this compound, while cardol with tri-unsaturated alkyl chain exert high insecticidal activity. Andrade *et al.,* (2011) has reported that the unsaturated long side chain on cardanol could be associated with the high activity of this compound.

The *in-vitro* responses of some antioxidant enzymes to AONSE were investigated in the present study. Studies have shown that the antioxidant system in insects involves complex mechanisms (Rajapakse and Walter, 2007; Wu *et al.,* 2011). Our finding in this study reveals that catalase activity was lowered by AONSE in *P. americana* at all the concentrations, similar to CYP and CPF (Table 1). A reduction in catalase activity has been associated with accumulation of hydrogen peroxide and Malondialdehyde, which may lead to oxidative modification of biomolecules in the cellular system (Dordevic *et al.,* 2021). A similar investigation revealed that cashew nut shell extract reduced catalase activity in *Acanthoscelides obstectus*, while the activity was elevated in Zonocerus variegatus (Adeleke *et al.,* 2021). The effects of AONSE and the standard insecticides on *in-vitro* SOD activity are presented in table 2. The activity of SOD was reduced at most concentrations by CYP and CPF, while AONSE reduced it at high concentrations. Superoxide dismutase is responsible for dismutation of superoxide anion radical, which becomes accumulated in cells when the enzyme activity is reduced, causing interactions capable of inducing oxidative stress and toxicity (Velioglu *et al.,* 1998). The reduction in the activities of both catalase and SOD by AONSE signals possible induction of oxidative stress and death of *P. americana*.

Glutathione-S-transferases (GSTs) enzymes are a family of detoxification enzymes which act by making toxicants more polar/hydrophilic and thus less toxic to the insect. Elevated GST enzyme activity has been reported to be

due to high transcriptional rate or gene amplification, and not via qualitative modifications of the enzyme (Ranson *et al.*, 2001; Enayati *et al.*, 2005). Figure 6 indicates that AONSE elevated the *in-vitro* GST activity in a concentration-dependent manner, while the activity was lowered by both CYP and CPF across the concentrations. Studies have demonstrated elevated levels of detoxification enzymes in resistant strains relative to the susceptible ones (Barros *et al.*, 2013; Wang *et al.*, 2014). In accordance with the finding in the present study, we had earlier reported that cashew nut shell extract induced the *in-vitro* activities of GSTs in *Acanthoscelides obstectus* and *Zonocerus variegatus* (Adeleke *et al.*, 2021). The elevated activity of GST found in the present study, has indicated increased detoxification of the extract, which can be a possible mechanism of resistance and protection in *P. americana*.

In the cell, acetylcholinesterase (AChE) is mainly involved in synaptic transmission by catalyzing acetylcholine hydrolysis. However, AChE has also been found in non-cholinergic and non-neuronal cells (Soreq and Seidman 2001), in which they regulate growth, reproduction and cell differentiation, cell adhesion (Revuelta *et al.*, 2009; Small *et al.*, 2009; Lu *et al.*, 2012; Mondal *et al.*, 2018). Figure 7 indicates that AONSE, reduced the *in- vitro* AChE activity in *P. americana* in a similar manner with CYP and CPF, compared with control at all the concentrations. One of our earlier studies using *A. obstectus* and *Zonocerus variegatus* revealed that CNSL inhibited acetylcholinesterase activity *in-vitro*. A recent study has demonstrated that AChE contributes to apoptosis in insects (Knnor *et al.*, 2020).

Carboxylesterases (CEs) form a major class of detoxifying enzymes in insects responsible for resistance through sequestration and metabolism of insecticides (Grigoraki *et al.*, 2015; Grigoraki *et al.*, 2017; Feng and Liu, 2020). Studies have shown that insect resistance involving carboxylesterase (CE) occurs through transcriptional up-regulation and overexpression of the multiple genes encoding this enzyme, causing rapid sequestration, hydrolysis and excretion of insecticides (Field and Blackman, 2003; Wheelock *et al.*, 2005; Fuentes-Contreras *et al.*, 2013; Demkovich *et al.*, 2015). In the present study, AONSE and CYP reduced CE activity, while CPF elevated it in P. americana (Figure 8). The ability of AONSE to reduce in-vitro CE activity, as observed in the present study, indicates that the resistance mechanism in the insect could be compromised by the extract. A similar investigation by Adeleke *et al.*, (2021) using bean weevil and grasshopper showed that cashew nut extract also reduced the activity of carboxylesterase in the insects.

A. occidentale nut shell extract contains catechin, rutin, quercetin, cardol, cardanol, beta-sitosterol, lutein and anacardic acid. In addition, these compounds could induce oxidative stress, and inhibit cholinergic and carboxylesterase enzymes in *Periplaneta americana*, indicating a toxic effect of the extract in this insect pest.

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Conflicts of Interest

There was no conflict of interest between the authors of manuscript.

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