Chemical analysis of *Ricinus communis* L. (Euphorbiaceace) seed kernel extract and its in-vitro toxicity in two *Podagrica* Species (Coleoptera: Chrysomelidae)

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Abstract

Since synthetic pesticides are associated with many toxicological problems against untargeted subjects, pesticides from botanical sources become a better option. This study obtained *R. communis* seed kernel extract (RCSKE) through acidified aqueous extraction. The extract was screened using Infra red (IR) and Ultraviolet (UV) spectroscopy, High-Performance Liquid Chromatography (HPLC) and Cass Chromatography (GC) for bioactive agents. *In-vitro* effects of RCSKE, chlorpyrifos (CPF) and cypermethrin (CYPER-M) on superoxide dismutase (SOD), Catalase (CAT), acetylcholinesterase (AChE) and Carboxylesterase (CE) activities in *Podagrica sjosdteti* and *Podagrica uniforma* were determined spectrophotometrically. The IR and UV of RCSKE majorly depict the presence of aromatic ring, ethylenic bond, carbonyl bond, hydroxyl and carboxylic groups. The HPLC and GC majorly show the presence of ricinoleic acid, ricinine and ricin. The RCSKE, CYPER-M and CPF increased CAT activity in *P. sjosdteti*, but reduced it in *P. uniforma*. The RCSKE and CYPER-M significantly reduced SOD, and elevated AChE activities in *P. sjosdteti*. The IC_{50} values of RCSKE, CYPER-M and CPF against CE activity in *P. sjosdteti* were IC_{50} = 2.66 µg/ml, IC_{50} = 2.37µg/ml and IC_{50} = 2.65 µg/ml), respectively, while that of RCSKE against *P. uniforma* was IC_{50} = 2.49 µg/ml. This study suggests that the extract of *Ricinus communis* seed kernel contains bioactive substances, capable to inhibit the antioxidant, acetylcholinesterase and carboxylesterase enzymes in *Podagrica* species flea beetles, comparable to commercial pesticides, Cypermethrin and Chlorpyrifos.

Introduction

Ricinus communis L. (Castor) plant has been reported to originate from India, the southeastern Mediterranean Basin, and Africa, and has even become widely distributed throughout the tropics and even beyond (Sabina, 2009). In the tropics, the plant grows as a perennial plant with heights between 10 and 12 m, while in the temperate regions, it is an annual plant with heights between 1–3 m, as documented by Salihu et al. (2013).

A recent study has shown that Castor beans contain about 40 to 60% oil, which is highly rich in triglycerides, predominantly ricinolein (Azizat et al., 2016). Castor oil has been demonstrated to contain a mixture of saturated and unsaturated fatty acids, such as, ricinoleic acid, linoleic acid, stearic acid, palmitic acid, linolenic acid, ecosanoic acid and dihydroxystearic acid (Aplin and Eliseo, 1997; Gana *et al*., 2013). The oil is among the most sought-after oils of plant origin, mainly due to its various properties and applications, and has therefore gained world-wide increase in production, as established by NCRI (2014). Castor oil (CB) is widely used for several medicinal purposes and for making products like cosmetics, paints, lubricants and many others as reported by Ogunniyi (2006) and Worbs et al. (2011). However, certain clinical manifestations following CB toxicity have been reported such as oropharyngeal irritation, abdominal pain, vomiting and diarrhea bleeding

(Coopmann et al., 2009), and hypoglycemia and hemolysis (Balali-Mood and Moshri, 2015). Several studies have documented the presence of phytochemicals such as, alkaloids (Trease and Evans. 1989), cardiac glycosides, tannins and flavonoids (Edeoga et al., 2005), steroids, saponins and anthraquinones (Aziz et al., 2016) in castor bean oil. Castor seed kernel has been shown to contain a toxic glycoprotein, known as ricin (Azziz et al., 2016), which is a heterodimeric toxin, capable of ribosome-inactivation and fatal disruption of protein synthesis in mammalian species (Audi et al., 2005). This toxic glycoprotein enters animal cells via several processes, and the presence of a single ricin molecule in the cytosol could potentially kill the entire cell (Berman et al., 2011). In addition to ricin, castor also contains some other toxic agents such as ricinine (alkaloid) and castor bean allergen (CBA), which together with ricin, are not usually extracted in the oil (ICOA, 2013). Berman et al, (2011) have suggested that certain monoterpenoids and sesquiterpenoids present in castor leaves exhibit cytotoxicity against cancer cells, by inducing apoptosis via translocation of phosphatidyl serine to the external surface of the cancer cell membrane. This results in perturbation of mitochondrial membrane potential and eventual cell death. An investigation by Salihu et al. (2013) has demonstrated the potential of castor against the damaging activity of termites on *Mangifera indica* and *Pinus longifolia*, reporting significant weight loss in the woods. Furthermore, it has been reported that the various active substances in castor could potentially interfere with the life cycles of many insect pests, including *Callosobruchus chinensis* (Uspani et al., 2003), *Cosmopolites sordidus* (Tinzarra et al., 2006), *Spodoptera frugiperda* (Rossi et al., 2012) and *Musca domestica* (housefly) Singh and Kaur (2016).

The adult flea beetles are usually small in size ranging from 1.5 to 3.0 mm long. They damage the cotyledon and foliar leaves of crops (Lamb, 1984). Several genera of flea beetles, including *Diamphidia* spp. (Nuewinger, 1996), *Procalus* spp. (Jerez, 1999), *Blepharida* spp. (Furth and Lee, 2000), *Ophridia* spp. and *Asiophridia* spp. (Zhang and Yang, 2008), *Polycladia* spp. (Chaboo, 2011) and *Podagrica* spp. (Ojo et al., 2014), have been documented to be distributed in different localities, all over the world. In West Africa, the economic damage through defoliation of crops, such okra, by two species of flea beetles, *Podagrica uniforma* (Jac.) and *Podagrica sjostedti* (Jac.) (Coleoptera: Chrysomelidae) have been reported. The former has shiny brown elytra, while the latter has bluish-black elytra Odebiyi (1980), and Osisanya and Tayo (1981). A study carried out by Abebe (2015) has noted that a spray of foliar insecticides like sevin and endosulfan was effective against *Podagrica puncticolis*, resulting in increase in crop yield. In addition, the use of insecticides extracted from plants, such as neem, pawpaw and onion bulbs, have proved effective against *Podagrica sjosdteti* and *Podagrica uniforma* hosted by okra plant, as investigated by Ojo et al. (2014). The two species infest okra plants from germination up to the stage of fruition. They cause destruction of both the foliage and matured leaves, with loss of photosynthetic capacity (Fasunwon ant Banjo, 2010), as well as transmission of mosaic virus, which leads to 20-50% reduction in crop yield (Fajinmi and Fajinmi, 2010). We discovered that literature information on the toxicity of *R. communis* (castor) against the two local species of flea beetles, *Podagrica sjosdteti* and *Podagrica uniforma*, was actually scarce. The present study was therefore designed to chemically determine the bioactive agents in *R. communis* seed kernel extract (RCSKE) and evaluate the *in-vitro* effects on selected antioxidant and hydrolytic enzymes of the two species of *Podagrica* flea beetles.

Materials and Methods

Chemicals
Dithionitrobenzoic acid, Ethylenediamine tetraacetic acid (EDTA), para-nitrophenylacetate, para-nitrophenol, Triton-X-100, acetylthiocholine iodide, Adrenaline and Commercial pesticides (cypermethrin and chlorpyrifos). All other reagents were of good analytical grades.

Acidified aqueous extraction of Castor seed kernel
Fresh Fruits of Castor plant (*Ricinus Communis*) were collected from Lagbedu-Orile near Ogbomoso, Oyo state, Nigeria in March, 2018. The fruits were air-dried at room temperature for 3 weeks, depulped and then pulverized (261.95g) using mortar and pestle to obtain an oily pasty substance. The oily pasty substance was defatted using 1000 ml of n-Hexane at room temperature for 48 hours, followed by filtration through a linen cloth. The residue (Residue A), which was expected to contain Ricin, Ricinine and Ricinoleic acid, was subjected to acidified aqueous extraction described by Walker and Negis, (1958) with modifications. Briefly, the residue A was re-dissolved in 500 ml of distilled water in 1000 ml beaker and then acidified with concentrated HCl to obtain a mixture at pH 3.8. The resulting mixture was subjected to batch stirring for 24hours using a Magnetic Stirrer. The mixture was allowed to settle for 24hours at the room temperature, and then filtered through a linen cloth to obtain a filtrate (Filtrate A), and the residue (Residue B) was re-dissolved in 250 ml distil water in a 1000ml beaker and re-stirred for 2 hours, filtered to obtain filtrate B. Both filtrates A and B were pooled and allowed to settle for 24 hours, to allow the filtrate separate into two layers (a fluffy white upper layer and a lower clear colorless layer). The fluffy layer was removed by using a Pasteur's pipette into a beaker and labeled residue C.

The clear colorless liquid layer was precipitated by saturation with 5.69M solution of Sodium chloride, and then centrifuged at 3000 x g for 5 minutes. The topmost fluffy white layer was collected using a Pasteur's pipette and labeled as Residue D. The resulting liquid layer was further subjected to saturation with 2.35M solution of Na_2SO_4 and then centrifuged at 3000 x g for 5 minutes. The topmost fluffy white layer was collected and labeled Residue E. The Residues C, D, and E were pooled (pH adjusted to 8.0) and evaporated using a rotary evaporator and concentrated in an oven at 40-45^{0}c (Singh and Kaur, 2016) to obtain dry *Ricinus communis* seed kernel extract (RCSKE). The dry extract was kept in an air-tight bottle at 4 ^{0}c until use.

Ultraviolet (UV) and Infrared (IR) spectroscopic analyses
The UV -1800 series machine was used to analyze the light brown solid *Ricinus communis* seed kernel extract (RCSKE) at a wavelength of 340nm to obtain visible spectra. The IR spectral analysis of the *Ricinus communis* seed kernel extract (RCSKE) was done using an Agilent machine (USA), expressing the wavelength in reciprocal centimeter (cm^{-1}).

High-Performance Liquid Chromatographic (HPLC) analysis
An isocratic HPLC (Mumbai machine) profiling was carried out on the *Ricinus communis* seed kernel extract (RCSKE) at a flow rate of 0.5 mL/min. Exactly 25 mg of the extract was dissolved in the mobile phase containing a mixture of acetonitrile and methanol (80:20, v/v), and the injection volume was 20μL. The C_{18} (4.5 x 250 mm, 5μm) column was maintained at the room temperature and the eluent was detected at 210nm with a run time of 30 minutes.

Gas Chromatographic (GC) analysis
The extract was subjected to a Gas Chromatography-Flame Ionization Detector (GC-FID) machine (HP Series II- 5890). The carrier gas was Nitrogen at a flow rate of 22 ml/min and the air flow rate was 45 ml/min. The initial temperature was 50^{0}c. Using the peak areas from the chromatogram, the percentage composition of each constituent was calculated.

Collection and homogenization of insects
Fifty each of *Podagrica sjosdteti* and *Podagrica uniforma* were collected using a sweeping net on an okra plantation located at Obamoro village, Osun State, Nigeria. They were confirmed by Dr. Olayioye A. (Department of Crop and Environmental Protection, Faculty of Agricultural Science) and Professor Oyegoke O.O. (Entomology Unit, Department of Pure and Applied Biology), Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. Each of the two insect groups was divided into two portions (twenty-five insects each) and then de-winged. One portion each of the insect species was homogenized using phosphate buffer (pH 7.4) and centrifuged at 10000 x g for 10 minutes. The supernatant was kept at 4^{0}c until use. The second portion was processed for Carboxylesterase activity determination as described later in this work.

Biochemical Assays

Protein determination

The protein levels of the insect homogenates were determined according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

Determination of Superoxide dismutase activity
Activities of superoxide dismutase (SOD) in insect homogenates were determined by the method of Misra and Fridovich (1975). Briefly, an aliquot of 0.2 ml of the diluted insect homogenate was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. Then, 0.2 ml of RCSKE (15, 30, 45, 60, 75, 90 and 105μg/ml) followed by 0.3ml of freshly prepared 0.3M adrenaline (substrate) were added to the mixture. Similar concentrations of commercial Cypermethrin (Pyrethroid) and Chlorpyrifos (Organophosphate) were separately used as standards (positive controls) in place of the extract. The negative control was without RCSKE or the commercial pesticides. The absorbance was taken spectrophotometrically at 480nm every 30 seconds for 150 seconds, and enzyme activity was expressed as Units/mg protein.

Determination of Catalase activity
Catalase activities in the insect homogenates were determined by described by Aebi (1984). The assay is based on the ability of the enzyme to promote decomposition of hydrogen peroxide. 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 10ml flat bottom flask. A 1.0 ml of properly diluted enzyme preparation (insect homogenate) was rapidly mixed with the reaction

mixture by a gentle swirling motion and 0.3 ml of RCSKE (15, 30, 45, 60, 75, 90 and 105µg/ml) was added in separate test tubes at the room temperature. Similar varying concentrations of commercial Cypermethrin and Chlorpyrifos were separately used as standards (positive controls) in place of the extract. The negative control was without RCSKE or the commercial pesticides. A 1 ml portion of the reaction mixture was blown into 2 ml of dichromate acetic acid reagent at 60 seconds intervals Change in absorbance was monitored at 240nm for 180 seconds at an interval of 60 seconds, and the enzyme activity was expressed as units/mg protein.

Acetylcholinesterase Activity
Acetylcholinesterase (AChE) activities in insect homogenates were determined according to the methods described by Ellman, (1961), and Nachmansohn and Neumann, (1975) with modification. The reaction mixture contained 2.6 ml phosphate buffer (0.1M, pH 7.4), 0.1 ml Ellman's reagent (DTNB), 0.4 ml insect homogenate and 0.3 ml RCSKE (15, 30, 45, 60, 75, 90 and 105µg/ml). Then 0.1 ml of Acetylthiocholine iodide solution was added to the reaction mixture, as substrate to trigger the reaction. Similar varying concentrations of commercial Cypermethrin and Chlorpyrifos were separately used as standards (positive controls) in place of the extract. The negative control was without RCSKE or the commercial pesticides. The rate of acetylcholinesterase activity was measured spectrophotometrically by following the increase in intensity of the yellow product at 412nm for 10 mins at an interval of 2 mins.. Acetylcholinesterase activity was calculated using the formula below with the molar extinction of $1.361x$ $mmol^{-1}$ xmm^{-1}:

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

AChE activity = U/mg protein

Carboxylesterase Activity
Carboxylesterase (CE) activities in insect homogenates were determined using the method described by Clement and Erhardt (1990), with modifications, using paranitrophenyl acetate as a substrate for the enzyme. Insects were homogenized in ice-cold Tris-HCl buffer (0.1 M, pH 7.8 with 1 % Triton X-100 at 25^0C) using a tissue homogenizer. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 x g for 10mins at 4 ^0C. The supernatant was diluted with distilled water in ratio 1:10. An aliquot of 0.5 ml of diluted supernatant (crude enzyme preparation) was added to 2 ml of the working buffer (0.1 M Tris-HCl, pH 7.8, containing 2 mM EDTA at 25 ^0C) followed by 0.3 ml of RCSKE (15, 30, 45, 60, 75, 90 and 105µg/ml). The mixture was incubated at 37 ^0C for 10minutes and the reaction was started by adding 0.2 ml of 50 mM paranitrophenyl acetate (prepared in acetone) as a substrate. Similar varying concentrations of commercial Cypermethrin and Chlorpyrifos were separately used as standards (positive controls) in place of the extract. The negative control was without the extract or commercial pesticides. The change in absorbance was read spectrophotometrically at 405nm, at an interval of 1min for 5 minutes at 405nm. The blank reagent contained 2 ml working buffer and 0.2 ml paranitrophenyl acetate. A paranitrophenol standard curve was prepared to calculate Carboxylesterase activity, expressed as nmol/min/ml protein.

Statistical Analysis
All values (in duplicates) were expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was done to determine differences in treatments, while probit analysis (Annie et al., 2015) was done to evaluate the half maximal inhibitory concentration (IC_{50}) using SPSS software. Statistically significant values were taken at $p < 0.05$.

Results

Spectroscopy and Chromatography
The Infra red (IR) spectrum of *R. communis* seed kernel extract (RCSKE) showed ten notable peaks at 1082.8 cm^{-1}, 1161.1 cm^{-1}, 1239.3cm^{-1}, 1459.7 cm^{-1}, 1541.3 cm^{-1}, 1654.9 cm^{-1}, 2855.1 cm^{-1} 2926.0 cm^{-1}, 3013.6 cm^{-1} and 3289.4 cm^{-1}, as shown in Figure 1. The Ultra violet (UV) spectroscopy of the extract was found to depict absorptions at four different wavelengths at 222.5nm, 228.5nm, 258.5nm and 275.5nm (Figure 2). The HPLC spectrum of RCSKE (Figure 3) shows six prominent peaks with retention times of 1.309 min, 1.476 min, 1.804 min, 2.593 min, 3.252 min and 5.446 min. The GC chromatogram in figure 4 shows that the analysis of RCSKE exhibited nine different peaks (and their retention times), which include Ethyl ester (4.223min), Diethyl phthalate (4.783min), 1,4a-Lactone (5.000min), GIBB- 3-ENE-1 (5.2666min), Oleic acid (5.500min), Ricinoleic acid (7.783min), Ricinine (9.666min), Ricin (10.283min) and Octadecanoate (10.816min). Their respective peak areas (and % compositions) include 1394.5285mAU*S (6.27%), 423.9710mAU*S (1.91%), 361.1000mAU*S

(1.62%), 784.2020mAU*S (3.52%), 6081.5680mAU*S (27.33%), 1543.9470mAU*S (6.94%), 6743.4680 mAU*S (30.31%), 3710.8845mAU*S (16.68%) and 1206.9510mAU*S (5.42%).

Antioxidant enzymes

Table 1 has shown that at all the concentrations (15-105µg/ml) used in this study, in vitro catalase (CAT) activity was significantly ($p < 0.05$) increased by the RCSKE, Cypermethrin and Chlorpyrifos in *Podagrica sjosdteti*. However, the CAT activity was observed to be significantly reduced by these same agents in *Podagrica uniforma* compared to control. The result shown in table 2 depicts that at all the concentrations used in the study, the activity of superoxide dismutase (SOD) was significantly ($p < 0.05$) reduced by the RCSKE, Cypermethrin and Chlorpyrifos in *P. sjosdteti*. On the other hands, in *P. uniforma*, SOD activity was found to be significantly lowered by only RCSKE and Cypermethrin, while Chlorpyrifos effect was insignificant ($p > 0.05$).

Esterase enzymes

The present study also examined the effects of the extract, CYPER-M and CPF on the activities of Acetylcholinesterase (AChE) and Carboxylesterase (CE) in the two species of *Podagrica*, and the results are presented in figures 5, 6, 7 and 8. The result in figure 5 shows that RCSKE and CYPER-M significantly elevated ($p < 0.05$) AChE activity in *P. sjosdteti* at nearly all the concentrations (15-105 µg/ml) used compared with control. On the other hands, CPF was found to significantly reduce the AChE activity at all the concentrations in the same insect species relative to control. Figure 6 has shown that the *in vitro* AChE activity in *P. uniforma* was significantly increased ($p < 0.05$) at all the concentrations used for the study compared with the control. The result in figure 7 has revealed that RCSKE, CYPER-M and Chlorpyrifos significantly reduced ($p < 0.05$) the *in vitro* activity of Carboxylesterase enzyme in *P. sjosdteti* at all the concentrations used compared with control. Lastly, in figure 8, the activity of Carboxylesterase in *P. uniforma* was observed to be significantly reduced ($p < 0.05$) by treatment with the extract (RCSKE) at all the concentrations. However, on treatment with CYPER-M and CPF, the activity of the CE enzyme was slightly elevated compared with control. The result of   The probit analysis (Table 3) shows the individual potency of RCSKE, Cypermethrin and Chlorpyrifos on Carboxylesterase (CE) activity in the two *Podagrica* species. The IC_{50} values of the three agents were found to be 2.66 µg/ml, 2.37µg/ml and 2.65 µg/ml, respectively, for *P. sjosdteti*. However, for *P. uniforma*, the IC_{50} value of RCSKE was determined to be 2.49 µg/ml, while similar values for CYPER-M and CPF could not be determined.
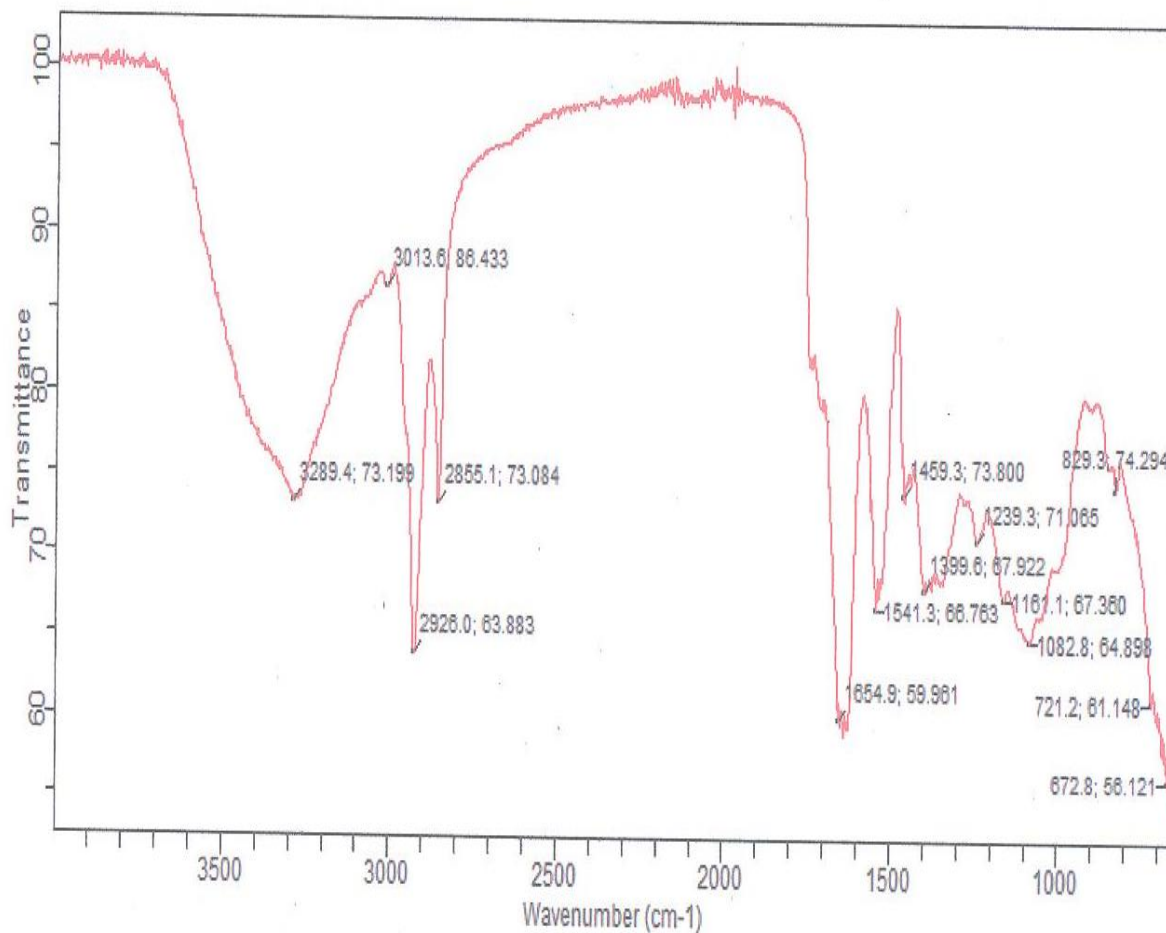
Figure 1. Infra red (IR) spectrum of *Ricinus communis* seed kernel extract (RCSKE)
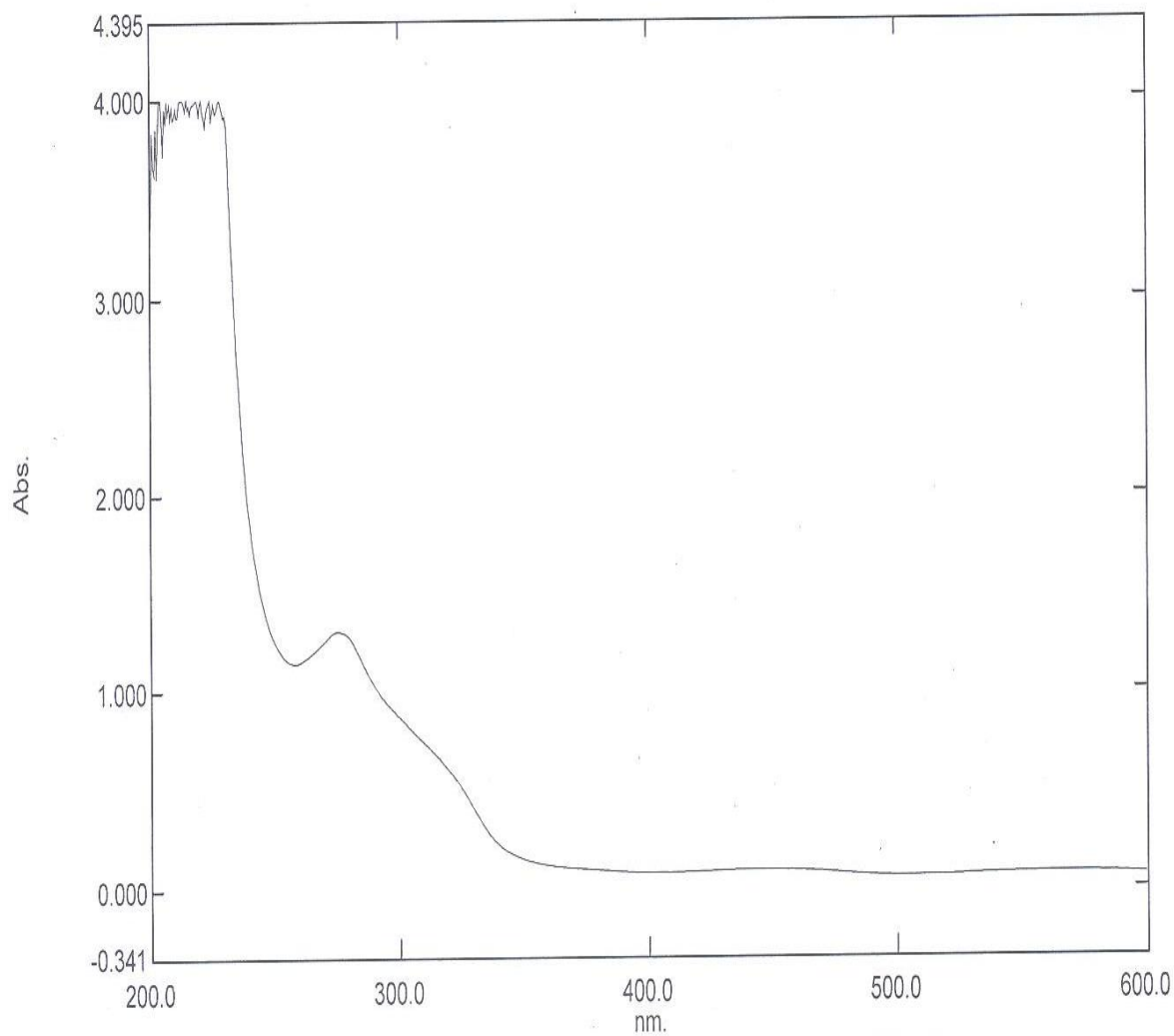
Figure 2. Ultra violet (UV) spectrum of *Ricinus communis* seed kernel extract (RCSKE)
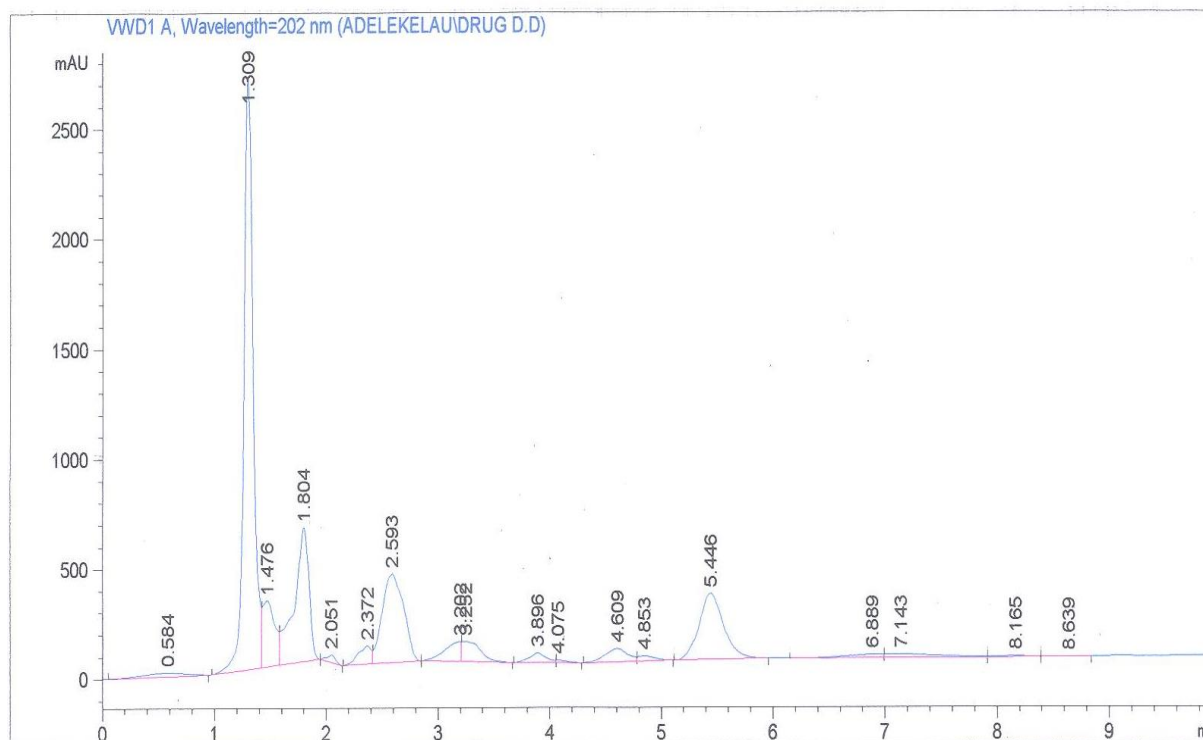
Figure 3. High Performance Liquid Chromatographic Chromatogram of *Ricinus communis* seed kernel extract (RCSKE)
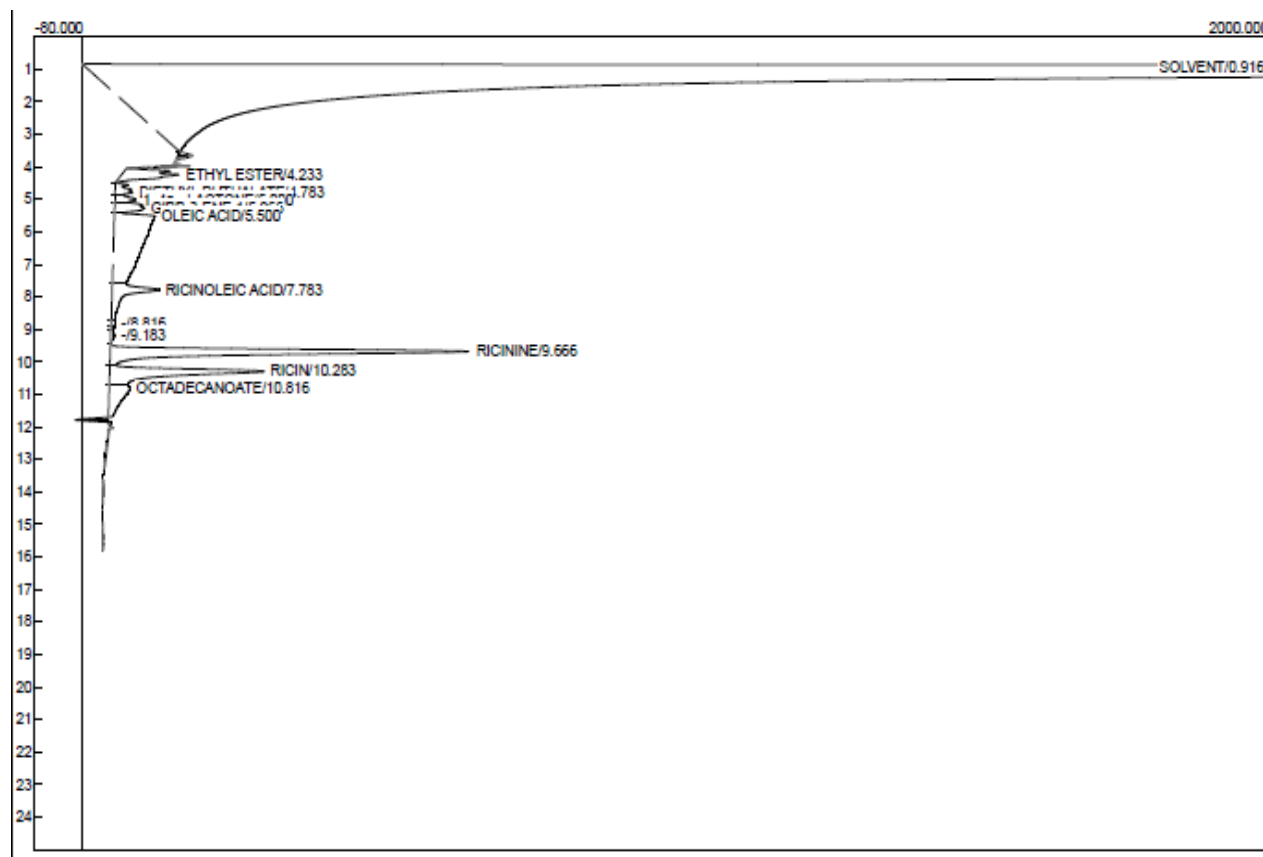
Fig. 4. Gas Chromatographic Chromatogram of *Ricinus communis* seed kernel extract (RCSKE)

Table 1. Effects of RCSKE, Cypermethrin and Chlorpyrifos on catalase activity in *Podagrica sjosdteti* and

Conc. (µg/ml)	Catalase activity (U/mg protein) in Podagrica sjosdteti			Catalase activity (U/mg protein) in Podagrica uniforma		
	CRSKE	Cypermethrin	Chlorpyrifos	CRSKE	Cypermethrin	Chlorpyrifos
15	2.48 ± 0.10[a]	1.42 ± 0.02[a]	2.94 ± 0.11[a]	4.83 ± 051	3.33 ± 0.40[b]	2.77 ± 0.13[b]
30	2.14 ± 0.08[a]	1.68 ± 0.10[a]	1.27 ± 0.21[a]	4.18 ±0.79[b]	3.35 ± 0.22[b]	3.71 ± 0.43[b]
45	2.28 ± 0.13[a]	1.41 ± 0.04[a]	2.68 ± 0.25[a]	4.74 ± 0.71	4.57 ± 0.44[b]	3.30 ± 0.07[b]
60	2.40 ±0.24[a]	1.27 ± 0.05[a]	1.89 ± 0.19[a]	3.40 ±0.88[b]	2.28 ± 0.35[b]	4.13 ± 0.81[b]
75	2.23 ± 0.19[a]	0.91 ± 0.06	1.29 ± 0.24[a]	2.00 ±0.31[b]	2.26 ± 0.19[b]	1.28 ± 0.48[b]
90	2.00 ± 0.06[a]	0.82 ± 0.07	0.73 ± 0.10	2.43 ±0.34[b]	2.66 ± 0.18[b]	2.80 ± 0.09[b]
105	1.92 ± 0.15[a]	1.50 ± 0.23[a]	0.41 ± 0.01	3.04 ±0.37[b]	1.78 ± 0.21[b]	1.69 ± 0.24[b]
Control	0.64 ± 0.07			5.67 ± 1.25		

Podagrica uniforma

Data expressed as Mean ± Standard deviation (values obtained in duplicates)

RCSKE-*Ricinus communis* seed kernel extract

CYPER-M- Cypermethrin

CPF- Chlorpyrifos

a- Statistically high compared to control

b- Statistically low compared to control

Table 2. Effects of RCSKE, Cypermethrin and Chlorpyrifos on superoxide dismutase (SOD) activity in

Conc. (µg/ml)	SOD activity (U/mg protein) in Podagrica sjosdteti			SOD activity (U/mg protein) in Podagrica uniforma		
	RCSKE	Cypermethrin	Chlorpyrifos	CRSKE	Cypermethrin	Chlorpyrifos
15	9.21 ± 2.20[b]	8.64 ± 1.54[b]	11.70 ±2.18[b]	5.50 ±0.98[b]	7.50 ± 1.55	6.60 ± 1.00
30	8.64 ± 2.01[b]	8.64 ± 2.17[b]	12.50 ±2.11[b]	4.95 ±2.26[b]	7.15 ± 1.68	6.60 ± 0.96
45	7.85 ± 2.10[b]	10.90 ± 3.22[b]	12.23 ±2.59[b]	5.05 ±0.93[b]	5.56 ± 1.05[b]	8.25 ± 1.89
60	8.64 ± 1.10[b]	10.20 ± 2.04[b]	8.14 ± 1.78[b]	4.50 ±0.81[b]	6.05 ± 1.01	7.15 ± 1.06
75	5.50 ± 0.91[b]	8.61 ± 2.71[b]	9.42 ± 1.95[b]	4.51 ±1.12[b]	6.60 ± 1.88	8.80 ± 1.06
90	9.40 ± 2.17[b]	12.60 ± 1.76[b]	13.30 ±4.59[b]	4.12 ±0.44[b]	2.20 ± 0.91[b]	7.70 ± 2.05
105	4.71 ± 0.78[b]	14. 10 ± 3.29	10.20 ±2.32[b]	3.85 ±0.17[b]	5.53 ± 2.03[b]	7.26 ± 1.25
Control	16.50 ± 3.11			7.15 ± 1.20		

Podagrica sjosdteti and *Podagrica uniforma*

Data expressed as Mean ± Standard deviation (values obtained in duplicates)

RCSKE-*Ricinus communis* seed kernel extract

CYPER-M- Cypermethrin

CPF- Chlorpyrifos

a- Statistically high compared to control
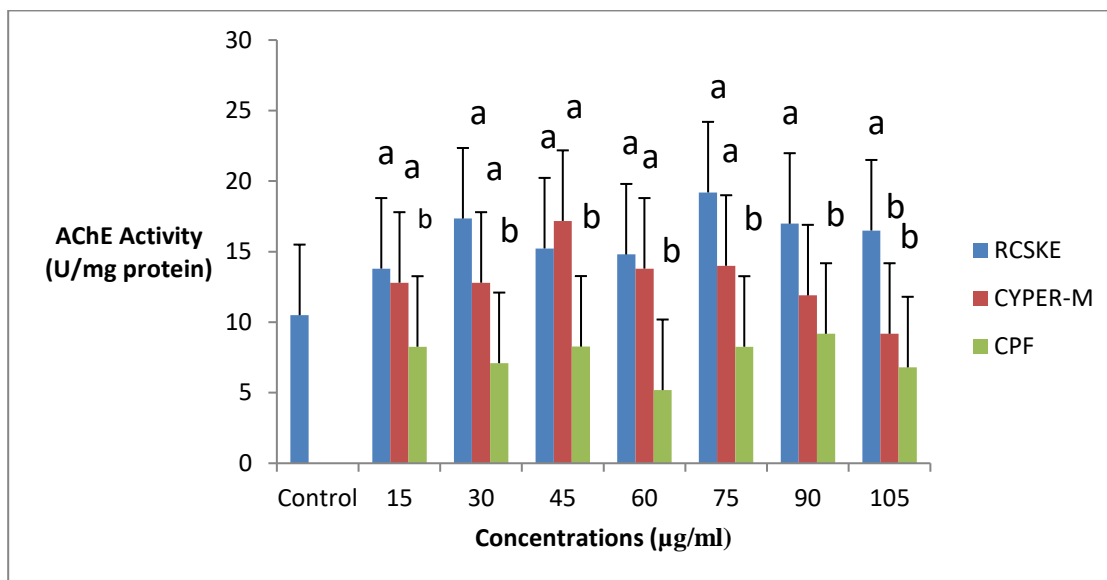
b- Statistically low compared to control

Fig 5. Effects of RCSKE, Cypermethrin and Chlorpyrifos on Acetylcholinesterase activity in *Podagrica sjosdteti*

Data expressed as Mean ± Standard deviation (values obtained in duplicates)
RCSKE-*Ricinus communis* seed kernel extract
CYPER-M- Cypermethrin
CPF- Chlorpyrifos
 a- Statistically high compared to control
 b- Statistically low compared to control

Fig. 6. Effects of RCSKE, Cypermethrin and Chlorpyrifos on Acetylcholinesterase activity in *Podagrica uniforma*

Data expressed as Mean ± Standard deviation (values obtained in duplicates)
RCSKE-*Ricinus communis* seed kernel extract
CYPER-M- Cypermethrin
CPF- Chlorpyrifos
 a- Statistically high compared to control
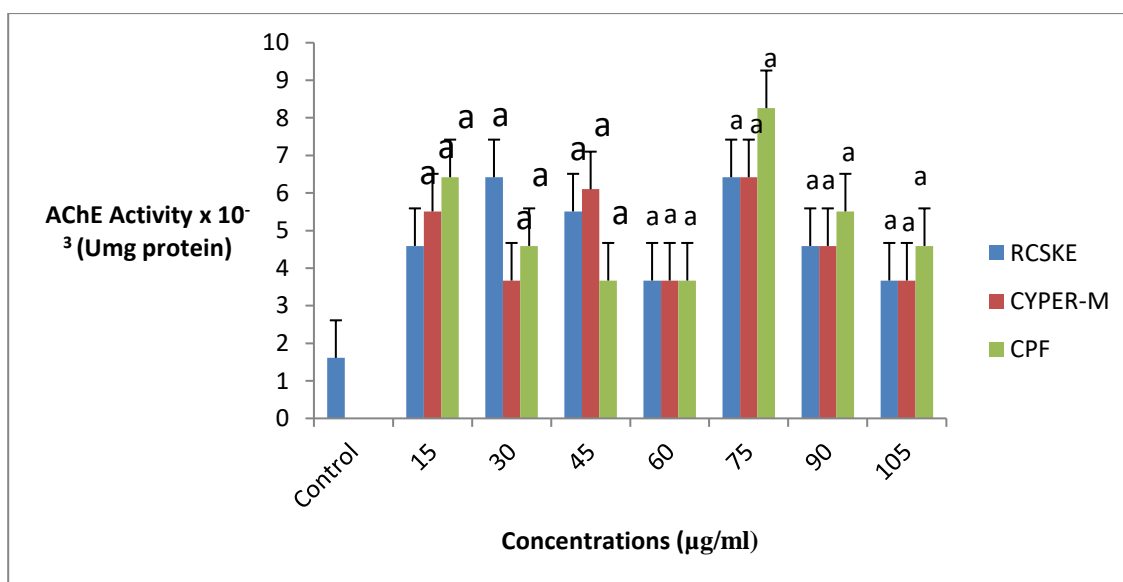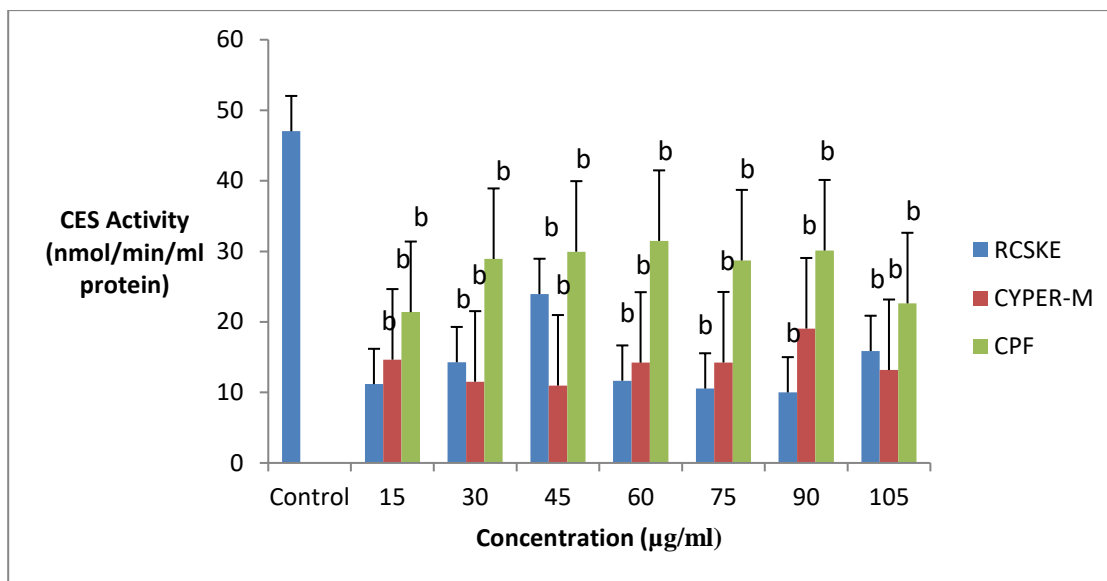
b- Statistically low compared to control

Fig 7. Effects of RCSKE, Cypermethrin and Chlorpyrifos on Carboxylesterase activity in *Podagrica sjosdteti*

Data expressed as Mean ± Standard deviation (values obtained in duplicates)
RCSKE-*Ricinus communis* seed kernel extract
CYPER-M- Cypermethrin
CPF- Chlorpyrifos
 a- Statistically high compared to control
 b- Statistically low compared to control

Fig 8. Effects of RCSKE, Cypermethrin and Chlorpyrifos on Carboxylesterase activity in *Podagrica uniforma*

Data expressed as Mean ± Standard deviation (values obtained in duplicates)
RCSKE-*Ricinus communis* seed kernel extract
CYPER-M- Cypermethrin
CPF- Chlorpyrifos

a- Statistically high compared to control
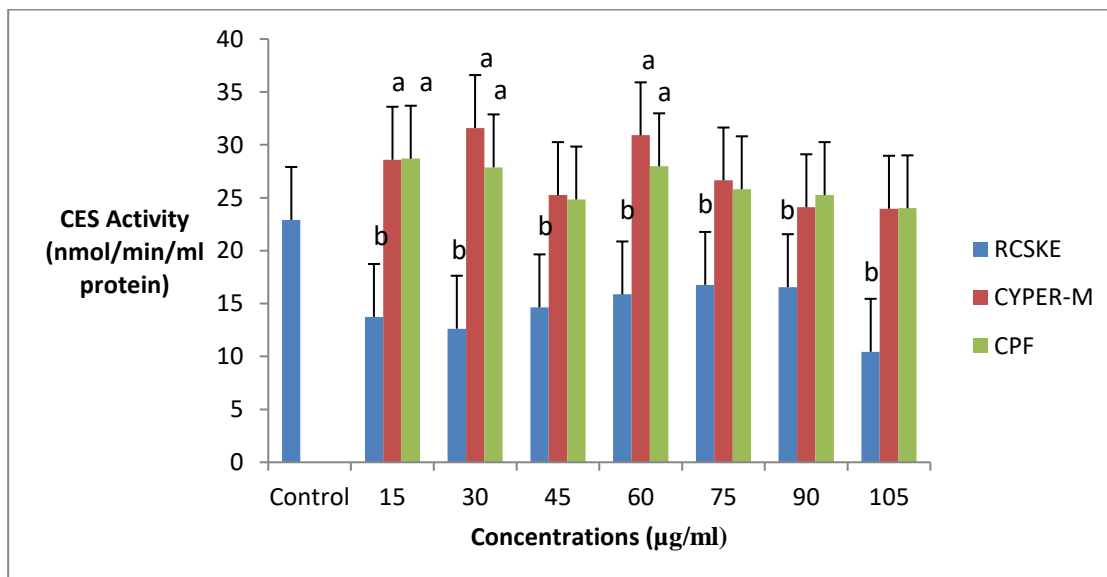b- Statistically low compared to control

Table 3. Inhibitory concentration-50 (IC$_{50}$) values of RCSKE, Cypermethrin and Chlorpyrifos on Carboxylesterase activities in the two *Podagrica* species

Treatments	*Podagrica sjosdteti*	*Podagrica uniforma*
	IC$_{50}$ values (µg/ml)	IC$_{50}$ values (µg/ml)
RCSKE	2.66	2.49
Cypermethrin	2.37	Indeterminate
Chlorpyrifos	2.65	Indeterminate

RCSKE- *Ricinus communis* seed kernel extract

Discussion

Over the years, synthetic pesticides have been applied in combating the menace of several insect pests, both on field and in stores. These synthetic compounds have been noted with negative effects on humans and other untargeted subjects in the environment. Several studies have therefore established the importance of pesticides from botanical sources, in controlling several field, store and household insect pests, due to their potency, safety and ecological friendliness (Lenora and Senthikumar, 2017; Buxton et al., 2017; Acero, 2018).

We analyzed RCSKE using both IR and UV spectroscopy in order to detect possible functional groups of compounds present in the extract. The Infra red (IR) spectrum of *R. communis* seed kernel extract (RCSKE) shows ten notable peaks at 1082.8 cm^{-1}, 1161.1 cm^{-1}, 1239.3cm^{-1}, 1459.7 cm^{-1}, 1541.3 cm^{-1}, 1654.9 cm^{-1}, 2855.1 cm^{-1} 2926.0 cm^{-1}, 3013.6 cm^{-1} and 3289.4 cm^{-1}. Comparing this result with the report of Fessenden and Fessenden (1986) and some others (Ayatollahi et al., 2011; Hossain and Ismail, 2013; Adeleke et al., 2018), the possible functional groups present in RCSKE include ether bond (1082.8 cm^{-1}), ester bond (1161.1 cm^{-1} and 1239.3cm^{-1}), aromatic or aryl ring (1459.7 cm^{-1} and 1541.3 cm^{-1}), asymmetric ethylenic double bond or carbonyl group (1654.9 cm^{-1}), methyl stretching or aliphatic C-H bond (2855.1 cm^{-1}), methyl bending (2926.0 cm^{-1}), hydroxyl or amine stretching (3013.6 cm^{-1}) and aliphatic hydroxyl or carboxylic acid group (3289.4 cm^{-1}). The Ultra violet (UV) spectroscopy of the extract was found to depict absorptions at four different wavelengths at 222.5nm, 228.5nm, 258.5nm and 275.5nm. Carbonyl group has been reported to absorb UV radiation maximally at around 211nm (Bulus et al., 2011), while conjugated double absorbs at around 257nm (Hossian and Ismail, 2013). The GC chromatogram of RCSKE exhibits nine different peaks, including ethyl ester, diethyl phthalate, 1,4a-Lactone, Gibb- 3-ene-1, oleic acid, ricinoleic acid, ricinine, ricin and octadecanoate. On using their respective peak areas from the GC chromatogram to calculate the percentage compositions in the extract, we found out that ricinine (30.31%) was the highest, followed by oleic acid (27.33%) and ricin (16.68%). It is worthwhile to suggest that the presence of ricinine in the extract may have been responsible for the ether, carbonyl and methyl groups (stretching and bending), while the aromatic ring may have been contributed by diethyl phthalate, as revealed from both the IR and GC results. Similarly, while the ester bond observed in IR spectrum of RCSKE in the present study is likely to be generated from ethyl ester, diethyl phthalate and octadecanoate, the ethylenic double bond may possibly be due to the presence of Gibb-3-ene-1, as revealed from the result of GC analysis. Furthermore, the carboxylic acid and amine groups shown from the IR analysis may have been generated from oleic and ricinoleic acids (fatty acids), and ricin (protein), respectively, as depicted in the GC chromatogram. Hussain et al. (2015) showed that a GC-MS analysis of methanol leaf extract of *R. communis* revealed the presence of n-hexadecanoic acid; octadecanoic acid; diethyl phthalate; ethyl ester; Gibb-3-ene-1, 10-dicarboxylic acid; 1,4a lactone and 2,4a, 7-trihydroxy-1-methyl-8-methylene. The UV absorption by RCSKE at around 222.5nm may indicate presence of carbonyl group (possibly generated from lactone and ricinine), while the absorption at around 258nm may suggest double bond supplied by Gibb-3-ene-1, as shown from the GC result. However, from the HPLC profile of RCSKE, six prominent peaks were observed, whose retention times are 1.309 min, 1.476 min, 1.804 min, 2.593 min, 3.252 min and 5.446 min. Certain studies have shown that the shoot part of castor plant is rich in substances like ricin, ricinine, *N*-demethylricinine and flavonoids (Kang et al., 1985). Although both ricin and ricinine have been noticed as potent antifeedant, insecticidal and repellent agents, ricinine has been reported to exhibit the highest level of insecticidal potential

(Kang et al., 1985; Sharma and Sharma, 1998). The chromatographic and spectroscopic analyses in this study have thus shown the presence of some possible insecticidal agents in the extract of castor seed kernel.

Oxidative stress develops from the imbalance between the reactive oxygen species (ROS) and antioxidant defense system in an organism, resulting in oxidation of macromolecules, including nucleic acids, proteins and lipids, which culminates in several events, such as, altered gene expression (Allen and Tresini, 2000), uncontrolled cell proliferation (Toyokuni, 2006), genomic or chromosome instability and genetic mutation (Ishikawa *et al*., 2008; Kumar *et al*., 2008).. Insects have been described to possess a complex network of enzymatic antioxidant systems protecting them against the deleterious effects of ROS generated during exposures to insecticides and phytotoxins (Felton and Summer, 1995; Rajapakse and Walter, 2007). Superoxide dismutase (SOD) and Catalase are two important antioxidant enzymes, responsible for mopping of ROS in insects. While SOD converts superoxide anion into hydrogen peroxide, Catalase converts the hydrogen peroxide into water and molecular oxygen (Aucoin *et al*., 1991; Koslov and Weidinger, 2015). A few studies have shown elevated activities and expression of SOD genes in insects after exposure to insecticides. A recent microarray study revealed elevated transcription of SOD enzymes in pesticide-resistant strains of *Anopheles arabiensis* (Nardini *et al*., 2013) and *Cimex lectularius* (Mamidala *et al*., 2012), suggesting the roles of these enzymes in the detoxification of intracellular toxins, which eventually contributes to the pesticide resistance in these insects. Studies have shown that some insects in the diptera group exhibited Catalase activity against oxidative stress-induced aging, and improvement of survival and fecundity (Das et al., 2001; Hector et al., 2011). In addition, inactivation or silencing of this enzyme in *Drosophila melanogaster* (Mackay and Bewley, 1989), *Musca domestica* (Allen et al., 1983), *Rhodnius prolixus* (Paes et al., 2001) and *Anopheles gambiae* (Magalhaes et al., 2008) resulted in ROS-induced high mortality in these insects. In our present investigation, we found out that RCSKE, CYPER-M and CPF significantly increased the *in vitro* activity of Catalase in *P. sjosdteti*, whereas the activity was reduced in *P. uniforma*. The RCSKE, CYPER-M and CPF were observed to significantly reduce the activity of SOD in *P. sjosdteti* at all the concentrations, whereas the enzyme activity in *P. uniforma* was reduced on treatment with only RCSKE and CYPER-M, with CPF having no significant effect. It could thus be suggested from this result, that although the activity of Catalase enzyme was induced by the three agents in *P. sjosdteti*, the inhibition of SOD activity may compromise proper dismutation of superoxide radicals to hydrogen peroxide, leading to oxidative stress in the insect. On the other hands, both Catalase and SOD were inhibited in *P. uniforma,* which could potentially lead to accumulation of hydrogen peroxide and oxidative stress in the insect. The finding thus far, may suggest insecticidal effects of the castor extract, CYPER-M and CPF in both *P. sjosdteti* and *P. uniforma*, via suppression of the antioxidant enzymes.

Lastly, we investigated the activities of Acetylcholinesterase (AChE) and Carboxylesterase (CEs) in the two *Podagrica* species. AChE is an important enzyme, catalyzing the hydrolysis of acetylcholine to choline and acetate at the synaptic junctions (Dvir et al.*,* 2010). In mammals, insecticides, like Organophosphates (e.g. Chlorpyrifos) and Carbamates, are irreversible inhibitors of AChE, causing continuous contraction of muscles, as acetylcholine floods the synapse (Cochran, 2011). The activity of AChE have been also identified in insects, such as *Drosophila melanogaster* (Fournier et al., 1989), Grasshopper (Weill et al., 2002) and mosquitoes (Kim et al., 2013), to mention a few. In the present study, RCSKE and Cypermethrin caused significant elevation in AChE activity in both *P. sjosdteti* and *P. uniforma*, which may result in a low level of ACh available for neuronal actions and subsequent adverse effect on muscular excitation. Similarly, the observed decrease in AChE activity by Chlorpyrifos in *P. uniforma* could cause a delayed ACh hydrolysis and its accumulation at the synapse, resulting also in uncontrolled muscular excitation in the insect. This finding therefore may suggest possible implications of RCSKE, Cypermethrin and Chlorpyrifos in causing toxicity in the neuronal system of the two flea beetles been investigated. CE enzymes on the other hands have been associated with the ability to confer substantial protection and resistance on insects through hydrolysis of Organophosphate insecticides, according to the report of Jackson et al. (2010). However, the present study has revealed that the castor extract (RCSKE), CYPER-M and Chlorpyrifos significantly inhibited the *in vitro* activity of CE in *P. sjosdteti*. This finding is quite consistent with the Probit analysis, which has shown that the IC_{50} value of RCSKE (2.66 µg/ml) was comparable to those of Cypermethrin (IC_{50} = 2.37 µg/ml) and Chlorpyrifos (IC_{50} = 2.65 µg/ml) in their inhibition against CE enzyme in *P. sjosdteti*. However, while CYPER-M and Chlorpyrifos increased CE activities in *P. uniforma*, (hence their IC_{50} values were indeterminable), RCSKE substantially reduced the activity (IC_{50} = 2.49 µg/ml) at all the concentrations used in the study. We can therefore conclude that the extract of *Ricinus communis* (Castor) seed kernel contains certain bioactive agents that could potentially inhibit the antioxidant and hydrolytic enzymes in *P. sjosdteti* and *P. uniforma*, flea beetles, in a manner comparable to the commercial pesticides, Cypermethrin and Chlorpyrifos.

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