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Antioxidant, larvicidal and antiacetylcholinesterase activities of cashew nut shell liquid constituents

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ABSTRACT

Anacardic acid, cardanol and cardol, the main constituents of natural cashew nut shell liquid (CNSL), were obtained by solvent extraction and assayed for antioxidant, larvicidal and antiacetylcholinesterase activity. Their relative percent composition was obtained by HPLC analysis. Antioxidant activity was assessed using the DPPH and $ABTS^{\bullet+}$ tests, which showed cardanol as the most active, followed by cardol and anacardic acid. The three CNSL components demonstrated good larvicidal activity against *Aedes aegypti* (LC_{50} = 12.40 for anacardic acid, 10.22 for cardol and 14.45 for cardanol) and exhibited inhibition zones for acetylcholinesterase enzymes in the TLC test similar to carbachol, which was used as standard. Based on the results, these multipotent compounds represent promising agents in the control of *Ae. aegypti*, the main dengue vector in Brazil.

1. Introduction

The cashew culture is one of the main socio-economic activities of northeastern Brazil, region of where the majority of cashew nut production comes from (Maia, 1978). Cashew nut shell liquid (CNSL) represents approximately 25% of cashew nut weight and as a by-product from the cashew agribusiness, it is considered to have a very low income value (Mazzetto et al., 2009). Natural CNSL is constituted by phenolic compounds with a side chain of fifteen carbon atoms which contain one, two and three unsaturated bonds (Agostini-Costa et al., 2005) (Fig. 1). These are anacardic acid, which has in general a 70% yield, cardol with a 20% yield, cardanol with 5% and 5% of polymeric material. Anacardic acid is related to salicylic acid and presented several activities such as antibacterial against methicillin-resistant *Staphylococcus aureus* (Muroi and Kubo, 1996), anticancer, anti-inflammatory, and radiosensitization (Sung et al., 2008). The main constituent of technical CNSL produced by cashew nut processing industries is cardanol, which is obtained by anacardic acid decarboxylation. It can be used in the manufacturing processes of industrial products, such as cement, paint and varnish (Menon et al., 1985). Its main application is as a polymer (Paramashivappa et al., 2001). Cardol is a resorcinol type CNSL derivative. Due to their structure, similar to those of tocopherols, cardols present great interest from many point of views as biotechnological, biopharmaceutical and biomedical, as antimicrobial and antitumor agents, molluscicides and prostaglandin synthetase inhibitors (Tocco et al., 2009). These three compounds showed activity against insects and microorganisms and against antioxidant enzymes (Lomonaco et al., 2009; Trevisan et al., 2006). The mechanism of action of many insecticides is the inhibition of the enzyme acetylcholinesterase (Finkelstein et al., 2002) hence CNSL constituents may also control *Aedes aegypti* through this mechanism.

Ae. aegypti mosquitoes are widely dispersed in urban areas all over the world. This species is medically important because of its vector capacity for the four serotypes of dengue virus and yellow fever virus. Furthermore, its activity and complex diurnal habits present significant problems for frequent space applications of insecticides. Resistant populations of *Ae. aegypti* appeared as a consequence of the continued use of these products (Cavalcanti et al., 2004). Both the persistent effects of these insecticides on the environment and the inexistence of a dengue virus vaccine have

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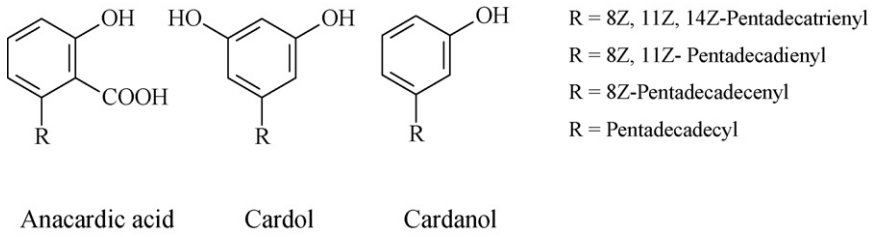

Fig. 1. Main cashew nut shell liquid constituents.

motivated the search for natural larvicide products. Many studies have indicated compounds of botanical origin with larvicidal activity (Cavalcanti et al., 2004; Morais et al., 2006).

The control of *Ae. aegypti* mosquito by using insecticides such as temephos, malathion and fenitrothion, constitutes the main strategy adopted by public health programs. Nevertheless, in Brazil (Macoris et al., 2003) and in several places of the world (Rawlins and Wan, 1995; Wirth and Georghiou, 1999) resistance to these conventional insecticides has been found. Absence of impact of aerial malathion treatment on *Ae. aegypti* was observed during a dengue outbreak in Kingston, Jamaica (Castle et al., 1999). Brazil has the greatest plant biodiversity of the world with 55,000 species in a total estimated between 350,000 and 550,000 (Sandes and Blasi, 2000). Therefore studies from plant extracts come with the expectation of finding substances with insecticidal and also selective properties to be used in future formulations of a commercial product.

CNSL was evaluated as a potential natural insecticide against termites, and demonstrated 100% mortality at concentrations of 6%, 8% and 10% (Asogwa et al., 2007). Recent research reveals the utilization of the technical CNSL component cardol as a new green larvicidal agent that can combat the spread of dengue (Lomonaco et al., 2009).

The antioxidant properties of the main constituents of CNSL were also evaluated. Anacardic acid was shown to be more active against either xanthine oxidase or superoxide dismutase (Trevisan et al., 2006). The antioxidant properties of phenols are due to their oxyreduction properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen eliminators (Guerra, 2001). The aim of this work was to assess the biological potential of CNSL constituents as natural larvicidal agents against *Ae. aegypti*, which is the major vector of dengue in Brazil, as acetylcholinesterase enzyme inhibitors and as free radical scavengers. The toxicity was also evaluated using the brine shrimp lethality test (BSLT) looking for a field application.

2. Methods

2.1. Reagents

Orcinol, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), quercetin and carbachol were purchased from Sigma, St. Louis, MO. Acetonitrile, hplc grade, was obtained from VETEC (Brazil). All other chemicals used were of analytical grade.

2.2. Plant material

Cashew nuts were obtained from early dwarf cashew trees (*Anacardium occidentale* L.) kept in the germplasm bank of the EMBRAPA-Tropical Agroindustry Experimental Station in Pacajus, Ceará, Brazil.

2.3. Extraction and separation of CNLS constituents

Natural CNLS was extracted with hexane in a sohxlet apparatus. CNLS constituents were obtained according to the following methodology developed by Paramashivappa et al. (2001). CNLS (100 g) was dissolved in 5% aqueous methanol, and 50 g of calcium hydroxide was slowly added to the resulting solution. The calcium anacardate precipitate (110 g) was washed with methanol, filtered in a Buchner funnel and dried under vacuum at 45–50 °C for 2 h. 11 M HCl (60 mL) was added to calcium anacardate (110 g) which was suspended in distilled water (440 mL), and stirred for 1 h. The resulting solution was extracted with ethyl acetate (2 × 100 mL) and the combined organic layers were washed with distilled water, dried with anhydrous Na_2SO_4 and evaporated. This process resulted in a 60 g yield of anacardic acid. 25% aqueous ammonia (200 mL) was added to the methanolic solution left over from the anacardic acid extraction and stirred for 15 min. The alkaline solution was extracted with a mixture of hexane/ethyl acetate (98:2) (3 × 100 mL), and the combined organic layers were washed with 5% HCl (100 mL) and distilled water (100 mL). The organic layer was dried under anhydrous sodium sulfate and concentrated resulting cardanol (0.9 g, 8.4%). The methanolic ammonia solution was extracted with ethyl acetate/hexane (80:20) (200 mL). The organic layer was washed with 5% HCl (100 mL), washed with distilled water (100 mL) and then dried over anhydrous sodium sulfate. This product was concentrated to yield cardol (20.4 g, 18.5%). The identity of cardanol and cardol was confirmed by HPLC (Fig. 2) and mass spectrometry.

2.4. HPLC analysis of CNSL constituents

The analysis was carried out using a Shimadzu SPD-10VP chromatograph, UV-VIS detector, which utilized a C-18 analytical column (25 cm × 4 mm), and a LC – 10AT pump. The mobile phase

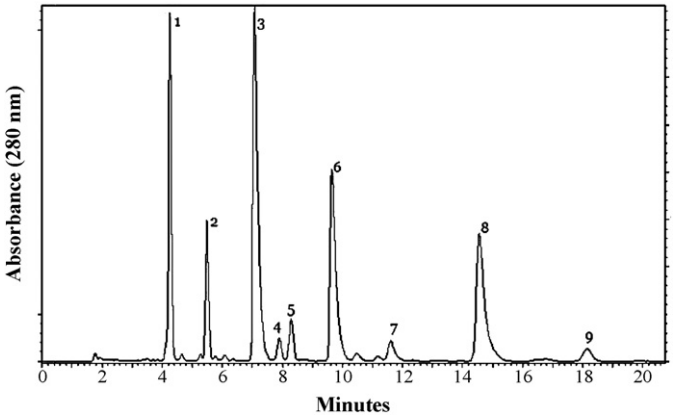

Fig. 2. Chromatogram of natural CNLS constituents cardol (1, 2, 4); cardanol (5, 7, 9) and anacardic acid (3, 6, 8).

consisted of acetonitrile–water–acetic acid (80:20:1), which was run in the isocratic mode phase at a wavelength of 280 nm.

2.5. Gas chromatography/mass spectrometry analysis of CNSL constituents

GC/MS was performed on a Hewlett-Packard 5971 instrument with the following components and conditions: dimethylpolysiloxane DB-1 coated fused silica capillary column (30 m × 0.25 mm); He (1 mL/min) carrier gas; 250 °C injector temperature and 200 °C detector temperature. The column temperature programming was 35–180 °C at 4 °C/min then 180–250 °C at 10 °C/min. Mass spectrometer operating conditions were 70 eV of ionization energy. Mass spectra were recorded from 40 to 450 m/z. The percent of area was obtained electronically from the GC–MS response without the use of an internal standard or correction factors.

2.6. Determination of anti-free radical activity of CNLS components

2.6.1. DPPH (1,1-diphenyl-2-picrylhydrazyl) method

According to the method developed by Yepez et al. (2002), 0.1 mL of sample methanolic solution (100 ppm, 1 mg/10 mL) was added to a test tube containing 3.9 mL of 6.5×10^{-5} M DPPH methanolic solution. To calculate the sample potential for DPPH inhibition in terms of percentage (IP %), the equation was used: $IP = A_{DPPH} - A_{sample}/A_{DPPH} \times 100$. The test was performed in triplicate, and the results were considered positive if the absorbance decreased with time (Brand-Williams et al., 1995). The inhibitory potential (%) was applied in Origin Pro 7.0 to calculate the medium inhibitory concentration (IC_{50}).

2.6.2. ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) method

The $ABTS^{\bullet+}$ radical solution was prepared from a reaction of 5 mL aqueous solution of 7 mM $ABTS^{\bullet+}$ (20 mg ABTS in 5.2 mL distilled water) and 88 mL of an aqueous solution of 140 mM potassium persulfate ($K_2S_2O_8$). This was prepared with 378 mg persulfate in 10 mL of distilled water. After storing the mixture in the dark for 16 h, 3.9 mL of $ABTS^{\bullet+}$ solution was added. After 6 min, 0.1 mL of the sample was read at an absorbance of 734 nm (Re et al., 1999).

2.7. Thin layer chromatography (TLC) bioassay detection for AChE inhibition

Inhibition of acetylcholinesterase enzyme (AChE) was evaluated by TLC in accordance with the methodology described by Elmann, which was later adapted by Rhee et al. (2001). This bioassay consists in the application of the sample to TLC plates and spraying the plate with Ellman's reagent, which was prepared by mixing 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and a buffer solution of acetylthiocholine iodide (ATCI). The TLC plate was subsequently sprayed with AChE enzyme (3 U/mL). After 3 min, enzyme inhibition is observed by the presence of white spots on the yellow plate. The TLC enzyme test is basically qualitative but is still significantly sensitive. The following solutions were prepared for this test: (1) 50 mM Tris/HCl pH 8 (buffer); (2) 50 mM Tris/HCl pH 8 containing 0.1% bovine serum albumin (BSA); (3) 1 mM Ellman's reagent; and (4) 1 mM ACTI. The lyophilized enzyme AChE was diluted in buffer solution (1) to prepare a 1000 U/mL enzyme solution. 5 μL aliquots of compounds 1–7 (4 mg/mL) were initially applied to TLC plates (DC-Alufolien, silica gel 60 F254, 0.2 mm Merck). The plate was then sprayed with solutions (3) and (4). After 3 min, which is the time necessary for the solution to completely dry, the plate was sprayed with AChE (3 U/mL). After approximately 10 min, the appearance of white spots was observed and their diameters were immediately measured. Carbachol was used as positive control.

2.8. Tests for larvicidal activity against Ae. aegypti

The tests were conducted in accordance with the methodology recommended by WHO (World Health Organization). 4.9 mL of filtered water containing twenty-five third-stage larvae of *Ae. aegypti* were added to beakers containing 15 mL of filtered water and 100 μL of sample in the desired concentration (30–1 ppm). The dilution of samples in water was performed using either ethanol, acetone or dimethylsulfoxide as a solvent. In all of the assays, the used solvents and water were maintained as a control. The tests of each sample concentration were repeated at least 4 times. The number of dead larvae was counted after 24 h. To determine the 50% lethal concentration (LC_{50}), data were analyzed using the probit plot mortality × concentration (ppm) in the statistical program SPSS (Statistical Product and Service Solutions) for Windows.

2.9. Brine shrimp lethality test (BSLT)

To determine the toxicity of CNLS constituents against *A. saline*, it was used the methodology described by Meyer et al. (1982) with some modifications. Test tubes were filled with 1.0 mL of sea water and 50 μL of dimethylsulfoxide. The tubes were placed in sonicator for 10 min and then, using a graduated pipette, 10 larvae were transferred to each test tube. The tube was filled with sea water to a total volume of 5 mL. After 24 h of contact with CNSL constituent solutions, the surviving larvae were counted. Larvae were considered dead if they remained immovable for more than 10 s after gentle agitation of the tube.

2.10. Statistical analysis

In all the experiments, compounds activity was expressed as means ± standard deviation (SD). The one-way analysis of variance (ANOVA) test was used to determine the statistical differences followed by Tukey's Multiple Comparison. The criterion for statistical significance was $P < 0.05$.

3. Results

The yields of constituents from natural CNLS were as follows: anacardic acids 62.9%, cardanols 6.99% and cardols 23.98%. The HPLC chromatogram of natural CNLS is shown in Fig. 2. The various constituents were separated and each peak identified by GC/MS, with peaks 1, 2 and 4 corresponding to monoene-, diene- and triene-cardol, peaks 3, 6 and 8 to monoene-, diene- and triene-anacardic acid and peaks 5, 7 and 9 to monoene-, diene- and triene-cardanol. Table 1 shows the areas of the peaks as they relate to the CNLS constituents.

The larvicidal activity against *Ae. aegypti* of the three components of CNLS are displayed in Table 2. Anacardic acid had a medium lethal concentration $LC_{50} = 12.40 \pm 0.10$, which is similar to the values found by Consoli et al. (1988) for this acid against the larvae of *Ae. fluviatilis* ($LC_{50} = 10$ ppm). Cardol showed $LC_{50} = 5.55 \pm 0.07$ and cardanol presented $LC_{50} = 8.20 \pm 0.15$. The three LC_{50} were statistically different and cardol was the most active followed by cardanol and anacardic acid. Temephos presents 100% mortality for *Ae. aegypti* at 3 ppm concentration.

Table 3 displays antioxidant activity of CNSL constituents and standards quercetin, BHT, and orcinol. In the DPPH test quercetin was the most active followed by BHT, orcinol, cardanol, cardol and anacardic acid. Using the radical $ABTS^{\bullet+}$ the most active compounds were quercetin, orcinol, cardanol and cardol, followed by BHT and

Table 1
Percentage composition of CNSL constituents by HPLC analysis.

Peak number	Constituent	Retention time (min)	Yield (%)
1	8Z, 11Z, 14Z-Pentadecatrienil-resorcinol	4.41	15.36
2	8Z, 11Z-Pentadecadienil-resorcinol	5.75	6.96
3	8Z, 11Z, 14Z-Pentadecatrienil-salicilic acid	7.48	28.00
4	8Z-Pentadecenil-resorcinol	8.43	1.66
5	8Z, 11Z, 14Z-Pentadecatrienil-phenol	8.83	2.96
6	8Z, 11Z-Pentadecadienil-salicilic acid	10.35	17.77
7	8Z, 11Z-Pentadecadienil-phenol	12.53	2.29
8	8Z-Pentadecenil-salicilic acid	15.93	17.13
9	8Z-Pentadecenil-phenol	20.03	1.74

Table 2
Larvicidal efficacy of CNLS constituents against *Aedes aegypti*.

CNLS constituents	Concentration ppm	Percent mortality	LC_{50} (ppm)	LC_{90} (ppm)
Anacardic acid	30	100	12.40 ± 0.10^a	21.15 ± 0.11^a
	15	71.5		
	5	35		
	1	13.5		
Cardol	10	100	5.55 ± 0.07^b	10.22 ± 0.02^b
	7.5	68		
	5	48		
	1	0		
Cardanol	10	86.5	8.20 ± 0.15^c	14.45 ± 0.03^c
	7.5	31.5		
	5	10		
	1	0		

LC_{50} = concentration that kills 50% of larvae. LC_{90} = concentration that kills 90% of larvae. Different small letters means significant differences in activity of compounds in the rows ($P < 0.05$).

Table 3
Antioxidant activity and antiacethylcholinesterase activity of CNLS constituents.

CNSL constituents	DPPH [a] IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)	AChE inhibition zone (cm)
Anacardic acid	4.41 ± 0.01^a	3.89 ± 0.09^a	0.5 ± 0.01^a
Cardol	3.57 ± 0.07^b	0.08 ± 0.01^b	1.2 ± 0.01^b
Cardanol	3.22 ± 0.07^c	0.06 ± 0.01^b	0.8 ± 0.01^c
Quercetin	0.09 ± 0.01^d	0.14 ± 0.03^b	
BHT	0.75 ± 0.07^e	0.35 ± 0.03^c	
Orcinol	1.04 ± 0.06^f	0.03 ± 0.01^b	
Carbachol			0.6 ± 0.01^a

Different small letters means significant differences in activity of compounds in the rows ($P < 0.05$).
[a] IC_{50} – median inhibitory concentration.

anacardic acid. In both tests anacardic acid was the least active antiradical compound.

In the AChE inhibition test, cardol (1.2 ± 0.10 cm) and cardanol (0.8 ± 0.10 cm) displays the inhibition zones larger than carbachol (0.6 cm $\pm$ 0.10), the standard used and anacardic acid (0.5 ± 0.10) (Table 3). Carbachol was used for comparison since it is a carbamate whose reaction mechanism with AChE is important because it is an isosteric analogue of acetylcholine (Rosenberry et al., 2008).

In the BSLT test (Table 4), the LC_{50} of CNLS constituents indicated the highest toxicity for anacardic acid (0.579), followed by cardol (0.616) and cardanol (6.447).

4. Discussion

The yields of constituents from natural CNLS are in agreement with previously reported data (Paramashivappa et al., 2001) and the analysis of each individual compound showed similar pattern of that reported by Trevisan et al. (2006). Triene components were present in higher proportion.

Comparing the antiradical activity among CNSL components, anacardic acid was the least active. According to Kubo et al. (2006), anacardic acid prevents the formation of superoxide radicals by inhibiting the enzyme xanthine oxidase but does not kidnaps reactive oxygen species. They concluded that the unsaturated side chain of 15 carbon atoms is more strongly associated with this activity when compared with the activity of salicylic acid (*ortho*-hydroxybenzoic acid). In a study that assessed the antioxidant activity of CNLS constituents against the enzymes xanthine oxidase and superoxide dismutase, Trevisan et al. (2006) found that the anacardic acids showed the highest activity, followed by cardol and cardanol.

The antioxidant mechanism for the inhibition of DPPH and ABTS free radicals probably differs from the enzymatic method. In the present analysis of anti-free radical activity, cardanol was the most active, followed by cardol and anacardic acid (as shown in Table 3). The antiradical activity of orcinol was also evaluated. This compound is a cardol analogue that differs in the length of its side chain and has only one carbon atom (methyl group). The fact that the strongest antiradical activity was exhibited by orcinol indicates that the side chain of 15 carbon atoms does not contribute to this activity.

The three components of CNLS had effective larvicidal activity against *Ae. aegypti* (Table 2). According to Cheng et al. (2003), essential oils or plant extracts with LC_{50} values <100 ppm should be considered active in larvicidal bioassays. Anacardic acid had $LC_{50} = 12.30 \pm 0.10$ ppm, which is similar to the values found by Consoli et al. (1988) for this acid against the larvae of *Ae. fluviatilis* ($LC_{50} = 10$ ppm). The insecticidal activity of sodium anacardate

Table 4
The mean LC_{50} and LC_{90} values ± S.D for CNSL constituents screened against brine shrimp larvae (*Artemia salina* Leach).

Compound concentration (μg/mL)	Number of dead larvae (n = 10)	% Mortalily	[a]LC_{50}
Cardanol			
100	10	100	
50	8.3 ± 0.57	83	6.447 ± 0.03[a]
10	6.3 ± 0.57	63	
5	3.3 ± 0.57	33	
1	1.6 ± 0.57	16	
Cardol			
10	10	100	
5	8.3 ± 0.57	83	0.616 ± 0.01[b]
1	5.3 ± 0.57	53	
0.5	3.6 ± 0.57	36	
0.1	1.3 ± 0.57	13	
Anacardic acid			
10	10	100	
5	8.3 ± 0.57	83	0.579 ± 0.01[b]
1	4.6 ± 0.57	46	
0.5	2.6 ± 0.57	26	
0.1	0	0	

Different small letters means statistical differences of compound activities at $P < 0.05$.
[a] Medium lethal concentration.

against *Ae. aegypti* 3rd instar larvae was also investigated showing [LC_{50}]=55.47 ± 3.0 μg/mL (Farias et al., 2009). Therefore the salt form of anacardic acid was less effective than anacardic acid.

Although cardol was proposed as a new green larvicide against *A. aegypti* (Lomonaco et al., 2009), it caused contact dermatitis in cashew nut workers (Diógenes et al., 1996). Because the larvicides are generally spread in the field and in water deposits, the use of cardol is not advisable. Cardanol had a relevant value (8.2 ± 0.15 ppm) and it did not demonstrated contact dermatitis so it was point out as the best CNSL constituent for a potential larvicide.

In Brazil dengue fever is endemic and temephos is widely used and applied by both private and public pest control in areas of standing water where the *Ae. aegypti* mosquito breeds in order to reduce the population of this disease-carrying insect. Resistance to temephos by *Ae. aegypti* has been seen in Brazil. The Brazilian *Ae. aegypti* resistance monitoring program detected temephos resistance in *Ae. aegypti* populations from several localities in the country since 1999 (Lima et al., 2003, 2006). Temephos was very lethal toward *Ae. aegypti* larvae with a LC_{50} = 0.025 μg/mL (Lima et al., 2006) and CNSL constituents were less active than temephos, but could be important models for the further development of new larvicides. There is no data on the effectiveness of these compounds in a field setting and the toxicity toward other organisms could be a problem in a field application. To investigate the action of CNSL over other nature organisms, the brine shrimp lethality test was used (Table 4).

Ecotoxicological analysis of cashew nut industry effluents, specifically two of its major phenolic components, cardol and cardanol indicated that these phenolic compounds were highly toxic under shrimp lethality assay conditions (Pimentel et al., 2009). Cardol had an LC_{50} of 0.56 mg/L and cardanol had an LC_{50} of 1.59 mg/L after 24 exposures. Cardol was significantly more toxic than cardanol ($P < 0.05$). For a comparison of ecotoxicological activities of main synthetic larvicides used in dengue control the pesticide toxicity index for freshwater aquatic organisms (PTI) was used. PTI for a particular sample is the sum of toxicity quotients (measured concentration divided by the median toxicity concentration from bioassays). The PTI for benthic organisms, where *A. salina* is classified, is for malathion LC_{50} = 0.012 ppm and for temephos LC_{50} = 0.031 ppm (Munn et al., 2006). Therefore, the medium lethal concentration of CNSL constituents is much less toxic than usual pesticides used in dengue control.

Comparing the antioxidant activity of compounds in Table 2 by DPPH test quercetin, a known natural antioxidant flavonoid (5,7,3′,5′-tetrahydroxy-flavonol) was the most active followed by BHT (synthetic antioxidant), orcinol (1,3-dihydroxy-5-methylbenzene) and among CNSL constituents cardanol, cardol and anacardic acid. Using ABTS radical, orcinol, cardol and cardanol were the most active followed by BHT and anacardic acid. CNSL constituents present similar antiradical activity as known antioxidants and cardanol and cardol were more active than anacardic acid. The antioxidant mechanism for the inhibition of DPPH and ABTS free radicals probably differs from the enzymatic method. Orcinol is a cardol analogue that differs in the length of its side chain and has only one carbon atom (methyl group). The fact that the strongest antiradical activity was exhibited by orcinol indicates that the side chain of 15 carbon atoms does not contribute to this activity.

In the anticholinesterase activity test, carbachol was used as positive control since it is a drug that binds and activates the acetylcholine receptor. Acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine at one of the highest known enzymatic rates. However, complete inactivation of AChE, which can occur with organophosphate chemical warfare agents, like temephos, leads to toxic accumulation of acetylcholine and failure of cholinergic synaptic transmission, with consequent deterioration of neuromuscular junctions, flaccid muscle paralysis, and seizures in the central nervous system (Rosenberry et al., 2008). The anticholinesterase activity of extracts from the leaves of *Triphasia trifolia*, which was studied out by Santos et al. (2008), showed that, aurapten, with an inhibition zone of 0.8 cm, was the most active of the coumarins isolated from *T. trifolia*. Therefore the anticholinesterase activity of CNSL constituents is relevant as compared with above results.

5. Conclusion

In the present work CNSL constituents can function as antioxidants, inhibitors of the enzyme acetylcholinesterase and larvicides against *Ae. aegypti*. Given the continual search for renewable and biodegradable sources of new medicinal products, the high yield of cardanol from cashew nut processing industries in Brazilian agribusiness represents an opportunity to increase the value of this by-product by developing green larvicidal compounds to be used in dengue control.

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