Preliminary studies on the characterization and toxicity of the cashew nut shell liquid in *Pomacea canaliculata* and *Artemia salina*

JOSE RENE L. MICOR¹, ELMER-RICO E. MOJICA¹, GERARD PAUL M. LEYSON², CHRISTINA A. PETRACHE² and CUSTER C. DEOCARIS³

Cashew nut shell liquid (CNSL) extracted by the Soxhlet method exhibited toxicity against the golden snail (*Pomacea canaliculata*) and brine shrimp (*Artemia salina*). Chemical tests and Infra-red (IR) spectroscopy indicated the presence of several phenolic compounds that may have contributed to these observed toxicities. The relationship between toxicity and structure of known CNSL phenolics is discussed in this paper.

KEYWORDS: cashew nut shell liquid, characterization, toxicity, golden snail, brine shrimp, *Pomacea canaliculata*, *Artemia salina*, Infra-red (IR) spectroscopy, Soxhlet method, proximate analysis, phenolic compounds, mollusccides.

¹Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna 4031, Philippines.
²Nuclear Biotechnology Laboratory, Biomedical Research Section, Philippine Nuclear Research Institute (PNRI), Commonwealth Avenue, Diliman, Quezon City 1101, Philippines.
³Gene Function Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan.

Received 12 April 2004;Accepted 06 June 2004.
©Rushing Water Publishers Ltd. 2004  Printed in the Philippines
MATERIALS AND METHODS

Extraction of CNSL. Cashew nuts were collected from a cashew nut plantation in Palawan (Philippines). Fifty grams of the cashew nut shells were chopped into small pieces and subjected to solvent extraction with 200 mL of hexane in the Soxhlet apparatus. Heating stopped when the hexane dripping down the flask became clear and the solvent was removed in vacuo with a rotary evaporator. CNSL extract was stored at room temperature and shielded from light.

Proximate analysis. Proximate analysis of the cashew nut followed the standard protocol of the Association of Official Analytical Chemists (AOAC 1993). The specific gravity of the cashew nut shell liquid was also determined using a 10-mL pycnometer.

Molluscicidal assay. Juvenile golden apple snails (12-18 mm) were collected from an open-field at the University of the Philippines Los Baños. The snails were placed in a 15-gallon aquarium equipped with an aerator. Tap water was used for conditioning the snails, which lasted for one week. The snails were fed with cabbage every two days and the aquarium was cleaned before the next feeding.

Molluscicidal assay was performed in three replicates for each treatment following the soaking method. Each 250 mL was covered with a wire screen to prevent the snails from escaping. Test solutions were prepared by weighing 20 mg of the solvent free extract necessary to prepare a 1000-ppm standard solution. A 0.5 mL absolute ethanol was added prior to dilution to disperse the extract in tap water. Ten young snails were used in each replicate for the bioassay using concentrations ranging from 10 to 1000 ppm. During the experiment, the snails were always in contact with the 150-200 mL of extract solution. Tap water and 0.5 mL absolute ethyl alcohol diluted in tap water were used in the controls. Mortality was observed after 24 and 48 hours of treatment.

RESULTS

Proximate analysis. Proximate analysis on the cashew showed very high crude fat in cashew nuts (Table 2). This could be due to the high oil content of the cashew nuts. A high amount of carbohydrates was also obtained.

Extraction. The CNSL extract appeared to be a brown viscous liquid that is immiscible with polar solvents like water and ethanol. The amount of CNSL extracted from the cashew shells ranged from 11 to 13% (weight/weight). Its specific gravity was 1.1951 ± 0.0051 g/mL.
Table 2. Proximate analysis of the cashew nuts.

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Fat (%)</td>
<td>42.00 ± 0.12</td>
</tr>
<tr>
<td>Crude Fiber (%)</td>
<td>1.36 ± 0.11</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>16.76 ± 0.32</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>5.11 ± 0.13</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>2.69 ± 0.10</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>32.09 ± 0.21</td>
</tr>
<tr>
<td>β-carotene (mg/100 g)</td>
<td>60.54 ± 0.69</td>
</tr>
<tr>
<td>Phosphorus (mg/100 g)</td>
<td>497.56 ± 6.24</td>
</tr>
</tbody>
</table>

**Toxicity.** Toxicity of CNSL to juvenile golden snails shows a dose-dependent pattern (Figure 1). The LC₅₀ of CNSL against *P. canaliculata* was 35.48 ppm at 24 hours and 13.75 ppm at 48 hours. No mortality was observed in the two controls used in the assay. Similarly, the toxicity of CNSL to *A. salina* is dose-dependent (Figure 2). CNSL was toxic to brine shrimps than its positive control, potassium chromate as indicated by the LC₅₀ of CNSL at 19.16 ppm which is lower than its positive control, potassium chromate with LC₅₀ of 20.19 ppm.

**Chemical analysis.** Results obtained from the chemical analysis of CNSL using different chemical tests are shown in Table 3. In addition, yellow sooty flame was observed upon ignition. IR spectrum of CNSL extract showed broad strong peaks at 3600-2800 nm and 1650-1600 nm, 1450 nm and 1200-1150 nm (Figure 3).

Table 3. Chemical tests on CNSL extract.

<table>
<thead>
<tr>
<th>Chemical Tests</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromination</td>
<td>+</td>
</tr>
<tr>
<td>Bromination (dark)</td>
<td>+</td>
</tr>
<tr>
<td>Baeyer’s test</td>
<td>+</td>
</tr>
<tr>
<td>Lucas test</td>
<td>-</td>
</tr>
<tr>
<td>Iodoform test</td>
<td>-</td>
</tr>
<tr>
<td>Tollen’s test</td>
<td>-</td>
</tr>
<tr>
<td>2-4-DNP test</td>
<td>-</td>
</tr>
<tr>
<td>FeCl₃ test</td>
<td>+</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Cashew nut shell liquid contains anacardic acid (71.7%), cardol (18.7%), cardanol (4.7%), novel phenol (2.7%) and two minor ingredients (2.2%) (Tyman & Morris 1967). Based on the IR spectra, it is possible that the local CNSL used in this study contained all the 16 phenolic compounds that have been isolated from cashew nut and its oil (Tyman 1979).
Figure 2. Brine shrimp (artemia salina) cytotoxicity test. Percentage mortality of brine shrimp was monitored after short-term, 24-hr exposure to CNSL. Vertical line on each bar represents standard deviation.

Cashew nut shell liquid toxicity

Figure 3. Infra-red (IR) spectra of cashew nut shell liquid.
The presence of phenolic substances in the extract was confirmed by the positive result with ferric chloride test. The positive reactions with the Baeyer's reagent and bromination suggested the presence of unsaturated bonds. Furthermore, the sooty yellow flame in the ignition test indicated the presence of aromatic compounds (Cheronis & Entrikin 1947). This was further supported by the IR spectra of the CNSL (Figure 3) which indicated the presence of C=C stretching, C-O stretching and ring stretching suggesting the presence of phenolic compounds with several side chains attached to it.

CNSL extract was found to be highly active against golden snail juveniles. This confirms the previous findings that CNSL, anacardic acid and hexane fractions are potential mollusccides against the aquatic snail Biomphalaria glabrata, one of the vectors of schistosomiasis (Kubo et al. 1986, Jurberg et al. 1995, Sullivan et al. 1982). More stable complexes and long-lived mollusccidal formulations of anacardic acid was obtained by complexing it with copper (II) (Mendes et al. 1990). Its mollusccidal activity on P. canaliculata could be due to the presence of the carboxyl group and the unsaturated side chains in compounds found in CNSL. These functional groups were responsible for the biological activity of anacardic acid against B. glabrata (Sullivan et al. 1982).

CNSL was also found toxic to A. salina, a useful preliminary test organism which showed strong correlation with costly cytotoxicity tests using human cancer cell lines (Carballo et al. 2002). Kubo et al. (1993) found that three anacardic acids and 13 other phenolics from the cashew apple were bioactive with BT-20 breast and HeLa cervical carcinomas. The highest cytotoxic activity was observed in the cardols, followed by anacardic acids and methylcardols. Cardols and anacardic acids from Ginkgo biloba L. also exhibit potent anti-tumor activity (Itokawa et al. 1987). However, this activity in mammals is very much different from that observed in microorganisms wherein the highest activity was shown by anacardic acid, followed by the cardols and methylcardols (Himejima & Kubo 1991).

Phenolic compounds isolated from cashew (either in its apple, nut, or nutshell) all contain a C15 alkyl side chain with up to three double bonds (Kubo et al. 1993). Previous studies on their mollusccidal and antibacterial properties (Kubo et al. 1986, Himejima & Kubo 1991) showed a general increase in their biological activities as the number of double bonds in the side chain increases. However, this was not observed in a cytotoxic study wherein phenolics with saturated C15 alkyl side chain were found to be less cytotoxic with unsaturated alkyl group (Kubo et al. 1993). The toxicity of the phenolic compounds could be due to their interaction with biological membranes. Phenolic compounds from rye grains interact with various biological and model (liposomal) membranes altering their structure and function (cited by Kozubek et al. 2001).

**CONCLUSION**

Results of this study showed that CNSL could be used as effective mollusccide against the pernicious golden snail. Moreover, the high toxicity of CNSL against brine shrimp indicates a promising potential of CNSL for anticancer therapy. Analysis using simple chemical tests and Infra-red (IR) spectroscopy indicated the presence of several phenolic compounds that may have contributed to these observed toxicities.

**LITERATURE CITED**


