Bisdesmosidic Saponins from *Securidaca longepedunculata* Roots: Evaluation of Deterrency and Toxicity to Coleopteran Storage Pests

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Powdered dry root bark of *Securidaca longepedunculata* was mixed with maize and cowpea and effectively reduced the numbers of *Sitophilus zeamais* and *Callosobruchus maculatus* emerging from these commodities, respectively, more than 9 months after treatment. This effect was reciprocated in grain treated with a methanol extract of the root bark, indicating that compounds were present that were oviposition deterrents or directly toxic to the adults or larvae. Two new bisdesmosidic saponins, 3-β-α-D-glucopyranosyl-28-(α-L-arabinopyranosyl(1 → 3)-β-D-oxylypyranosyl(1 → 4)[β-D-apiofuranosyl(1 → 3)]-α-L-rhamnopyranosyl(1 → 2)-[4-O-(4-methoxyxanthenyl-β-D-fucopyranosyl)][)-medicagenic acid (securidacaside A) and 3-β-α-D-glucopyranosyl-28-(α-L-arabinopyranosyl(1 → 3)-β-D-oxylypyranosyl(1 → 4)-[β-D-apiofuranosyl(1 → 3)]-α-L-rhamnopyranosyl(1 → 2)-[4-O-(3,4,5-trimethoxy-(E)-cinnamoyl-β-D-fucopyranosyl)][)-medicagenic acid (securidacaside B), were isolated from the methanol extract of the roots of *S. longepedunculata* and characterized by spectroscopic methods. Securidacaside A, which occurred as (E)- and (Z)-regioisomers, showed deterrency and toxicity toward *C. maculatus* and *S. zeamais* and could contribute to the biological activity of the methanol extract. The potential to optimize the use of this plant for stored product protection using water extracts, which would be appropriate technology for target farmers, is discussed.

**KEYWORDS:** Securidaca; saponins; oviposition deterrent; Sitophilus; Callosobruchus; bruchid

INTRODUCTION

Despite the commercial difficulties associated with the registration of plant compounds as agrochemicals, interest in pesticidal plants continues to grow (1–2). In the developed world, this is linked to increasing demand for organic produce, for which plant derived products are acceptable in pest control, despite examples such as rotenone having well-known mammalian toxicity (3). Effective alternatives to synthetic pesticides, however, are often a necessity rather than a choice for small-scale farmers in sub-Saharan Africa. This is because synthetic pesticides can be expensive, are often adulterated, are increasingly ineffective owing to pest resistance, and may be difficult to access reliably (4). At best, pesticidal plants provide low-cost, safer, and environmentally benign alternatives to synthetic pesticides.

Understanding why pesticidal plants are effective may facilitate the optimization of their use and therefore increase agricultural productivity, particularly among some of the world’s poorest farmers (5). In this respect, we have investigated *Securidaca longepedunculata* Fresen. (Polygalaceae), a widespread tree of tropical African savannah, especially of Miombo and Caesalpinioideae woodland. This species has a wide variety of indigenous uses including the protection of stored grain from weevil damage (6–7). The activity is reportedly associated with nonpolar compounds in the roots (4, 8). Compounds identified from *Securidaca longepedunculata* previously include methyl salicylate (9), tannins (10), sucrose derivatives (11), phenolics (12) saponins (13, 14), xanthones (15–17), and alkaloids (18, 19). The aim of the present study was to evaluate the effect of the powdered root bark and methanol extracts of the root bark of this species against the Coleopteran stored product pests, *Sitophilus zeamais* Motchulsky and *Callosobruchus maculatus* F., up to 9 months after treatment of the stored commodity, and identify and elucidate structures of compounds responsible for the effects.

MATERIALS AND METHODS

**Reagents.** Methanol (HPLC grade) and acetic acid (HPLC grade) were obtained from Merck (U.K.). All other chemicals were of analytical grade. Deionized water was obtained from an in-house Milli-Q Plus System (Millipore, Inc., Billerica, MA) at 18.2 MΩ.

**Plant Material Extraction.** *S. longepedunculata* roots were collected from Tamale in North Ghana (Royal Botanic Gardens, Kew ref
BI-14863). Roots were ground to a fine powder (50 g) (KIKA mill, Janke & Kunkel GmbH & Co. KG, Germany) and extracted in methanol (150 mL) for 24 h at room temperature (22 °C), filtered, and evaporated to dryness under reduced pressure.

**HPLC Analysis and Compound Isolation.** The dried MeOH extract of *S. longepedunculata* (5 g) was redissolved in MeOH and analyzed by HPLC (Waters 600E pump and 996 PDA detector). The column used was a 250 mm × 4.0 mm i.d., 5 μm, LiChrospher 100 RP-18 (Merck), with a gradient elution program based on A = MeOH and B = 2% AcOH; A = 100% at t = 0 min, A = 70% at t = 26 min, and A = 100% at t = 40 min; column temperature 30 °C and flow rate of 1 mL/min. Chromatograms were extracted at 310 nm. Two compounds of interest, eluting at tR = 28.3 (1) and 29.3 (2) min were collected by repetitive isolation yielding 4.5 mg and 2.0 mg, respectively.

**Sugar Analysis.** Acid hydrolysis of 1 and 2 was carried out by dissolving approximately 1.0 mg of each in 2 mL of dioxan/2M HCl (1:1) and heating at 100 °C for 1 h. The hydrolysate was transferred to a 7 mL vial and dried under a stream of N2 on a heating block at 40 °C. The absolute configurations of the constituent monosaccharides of 1 and 2 released by acid hydrolysis were determined by GC-MS analysis of their trimethylsilylated thiazolidine derivatives, which were prepared using the method of Ito et al. (20). Conditions for GC were as follows: capillary column, DB-5MS (30 m × 0.25 mm × 0.25 μm), oven temperature program, 180–300 °C/min; injection temperature, 350 °C; carrier gas, He at 1 mL/min. Both 1 and 2 gave α-apiose, α-xyllose, α-arabinose, α-rhamnose, α-fucose, and α-glucose at tR = 0.25, 1.18 (27-CH3), 0.94 (28-CH3), 1.63 (29-CH3), 1.18 (28-CH3), 0.92 (25-CH3), 0.94 (3, 28-CH3), 1.61 (25-CH3), 1.25 (25-CH3), 2.07, 1.63 (25-CH3), 2.84 (dd, J = 14.0, 4.7 Hz, H-18), 1.75, 1.16 (25-CH3), 1.41, 1.25 (25-CH3), 1.77, 1.61 (25-CH3), 1.36 (24-CH3), 1.24 (25-CH3), 0.81 (26-CH3), 1.18 (27-CH3), 0.92 (29-CH3), 0.94 (30-CH3). 13C NMR data for the sugar moieties can be seen in Table 1.

**Insect Culturing and Handling.** Strains of *S. zeamais* and *C. maculatus* from West Africa were used. Insect cultures were maintained (*S. zeamais* on maize and *C. maculatus* on cowpea) in 2.5 L glass jars in a controlled temperature and humidity (CTH) room at 27 ± 5 °C, RH 60 ± 5%, and 12:12 light/dark. *C. maculatus* is a highly mobile and fragile insect compared to *S. zeamais*; therefore, counting and transfer of *C. maculatus* to the test commodities was carried out with the aid of a low vacuum suction pump (Charles Austen model DATC, United Kingdom) and a glass aspirator. A rubber tube was attached to the glass aspirator for flexibility and to allow easy collection of the bruchids in the 2.5 L culturing jars. *S. zeamais* were handled by sieving the cultures using 710 μm sieves (Philip Harris Scientific, London) and with feather-light forceps.

**Preparation of Known Age Insects.** To prepare insects of known age (7–14 day old *S. zeamais* and 1–7 day old *C. maculatus*), subcultures of both insect species were prepared in their respective commodities. To prepare treatments, examination of the cultures was carried out on maize and *S. zeamais* for three weeks after the initial setup, whereas *C. maculatus* adults were removed after 10 days due to their shorter adult life span of 7–10 days. After four (*S. zeamais*) and three (*C. maculatus*) weeks, respectively, the cultures were checked daily for the F1 emergence. To collect adults, the cultures were either sieved (*S. zeamais*) or adults collected using an aspirator (*C. maculatus*) at the appropriate time.

**Test Commodities.** Whole organic cowpea (Canterbury Wholefoods, Canterbury, Kent, U.K.) and whole organic maize (Gillet and Cook Ltd., Faversham, Kent, U.K.) were frozen at −20 °C for one week to kill any existing infestation and then stored at 4 °C to prevent further infestation. Three weeks prior to use, commodities were equilibrated to experimental conditions in a controlled temperature and humidity room at 27 ± 5 °C and 60 ± 5% RH 12:12 light/dark.

**Effect of *S. longepedunculata* Root Bark Powder and Methanol Extract of the Root Bark on the Emergence of *S. zeamais* and *C. maculatus* in the F1 Generation.** Pre-equilibrated commodity (100 g) was placed in glass jars (250 mL), and after treatment and infestation, the jars were sealed with black filter paper lids and molten wax, and maintained at 27 ± 5 °C, 60 ± 5% RH, 12:12 light/dark. Powdered *S. longepedunculata* root (0.5 g) was admixed into 10 jars for each test insect at intervals of three months for a period of nine months (40 treatments plus 40 untreated controls). The concentration of crude powdered material was identified by African farmer practice as well as a series of experiments where a range of concentrations was evaluated previously (8). A second, similar experiment was set up in which each commodity was treated at the same time intervals but spraying 100 g commodity was treated at the same time intervals but spraying 100 g of 250 mL jar. Commodity treated with methanol only was used as the control. After 9 months, 40 known-age adult insects of each species were introduced into jars containing their respective commodity. The number of live adult insects of the two species was recorded after 5 weeks (C. maculatus on cowpea) or 8 weeks (*S. zeamais* on maize).
of live adults in each commodity was analyzed using ANOVA. The differences between the number of live insects in the treatments and controls were compared at $\alpha = 0.05$ using the LSD test in the SPSS 10 statistical package.

**Table 1.** $^1$H and $^{13}$C NMR Spectroscopic Data for the Glycosidic Moieties of Saponins 1 and 2 (CD$_3$OD, 30 $^\circ$C)

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**Effect of the Methanol Extract of S. longepedunculata Root on Adult Mortality and F1 Emergence of S. zeamais and C. maculatus on Maize and Cowpea, respectively.** The methanol extract of S. longepedunculata root (5 mL; 2.5% w/v) was admixed with 100 g of either
maize or cowpea equilibrated as described above and dried for 3 h in a fume hood at 22 °C, and placed into 250 mL test jars. Methanol only treated grain was used as a control. Forty known-age unsexed adult insects of each species were introduced into jars containing their respective commodity with C. maculatus on maize and S. zeamais on cowpea. The experiment was maintained at 27 ± 5 °C, 60 ± 5% RH, and 12:12 light/dark, and the commodity in each jar was sieved (aspirated in the case of C. maculatus) every week for a period of eight weeks, and the number of live adults in each replicate in each week was recorded. The live adults in each replicate were replaced in their relevant jar in each week, and the dead ones were collected separately. The total number of dead adults in each commodity was also recorded weekly for eight weeks. The data were analyzed using ANOVA, and the means were subjected to the LSD test at α = 0.05.

Differences in the number of eggs, hatched eggs, and F1 adults between the treatments and the control were tested for significance at α = 0.05.

Identification of Securidaca Saponins 1 and 2. Structures for two saponins isolated from the MeOH extract of S. longepedunculata roots were determined by MS, NMR, and sugar analysis (Figure 1). Complete assignment of all proton and carbon resonances in the NMR spectra of I was achieved using COSY, TOCSY, HSQC, and HMBC data (Table 1 and Materials and Methods). Among the characteristic resonances in the 1H NMR spectrum were those for six quaternary methyl groups at δH 0.82, 0.92, 0.94, 1.18, 1.26, and 1.36 (all 3H, s), correlated in the HSQC spectrum with δC 18.0, 33.6, 24.2, 26.4, 17.5, and 14.4, respectively. Also of note was the characteristic resonance at δH 4.31 (1H, m, δC 123.6 by HSQC) corresponding to H-12 of a olean-12-ene triterpenoid skeleton. Six anomeric proton resonances were observed at δH 4.39, 4.47, 4.60, 5.25, 5.40, and 5.46, correlated in the HSQC spectrum with δC 104.4, 106.7, 105.0, 111.9, 95.1, and 101.4, respectively. These preliminary data suggested that I was a triterpenoid saponin with six sugars. The structure of the triterpenoid moiety was confirmed to be that of medicagenic acid (2β,3β-dihydroxysterol-12-ene-23,28-dioic acid), on the basis of correlations observed in the HMBC spectrum and good agreement between the 13C NMR assignments (Materials and Methods) and literature values acquired in the same solvent (21, 22).

Acid hydrolysis of I followed by determination of the absolute configurations of the constituent monosaccharides confirmed the presence of β-Api, α-Ara, α-Fuc, β-Glc, α-Rha, and β-Xyl. 1H and 13C NMR assignments for the six sugar residues were obtained using a combination of COSY, TOCSY (τm = 60 ms), HSQC, and HMBC data, using the anomeric proton resonances as starting points (Table 1). The sets of assignments were identified with specific sugars (β-Api, α-Ara, α-Fuc, β-Glc, α-Rha, and β-Xyl) on the basis of coupling constant data for 1H NMR resonances, where resolved, and characteristic 1H and 13C NMR chemical shift values (21–23). The two deoxyhexose residues were readily distinguished, as the full set of J values obtained for one of them (H-1 at δH 5.40, δJ1,2 = 8.1, δJ2,3 = 9.9, δJ2,3 = 3.5, δJ3,4 = 1.1, δJ5,6 = 6.4 Hz) was consistent with those of β-Fucp (21, 22). The second deoxyhexose (H-1 at δH 5.46, δJ1,2 = 1.5, δJ5,6 = 6.3 Hz) was thus α-Rhap. The single hexose residue was β-Glc (H-1 at δH 4.39, δJ1,2 = 7.6 Hz), for which the 13C NMR assignments were comparable to literature values (22).

For the three pentose residues, a combination of J values and chemical shift data (Table 1) differentiated β-Api/β-Ara (H-1 at δH 5.25, δJ1,2 = 3.8 Hz, δJ1,2 = 1.19, δJ4,5 = 9.6 Hz) from α-Arap (H-1 at δH 4.47, δJ1,2 = 7.0 Hz) and β-Xyl/β-Fuc (H-1 at δH 4.60, δJ1,2 = 7.6 Hz) (21, 22).

Analysis of HMBC connectivities between sugar residues and the medicagenic acid aglycone indicated that I was a bisdesmoside. At C-3, correlations were observed between H-3 of the aglycone (δH 4.08) and C-1 of Glc (δC 111.9), and from H-1 of Glc (δH 4.39) to C-3 (δC 86.4). In a site selective ROE experiment, H-1 of Glc (δH 4.39) correlated to both H-2 (δH 4.26) and H-3 (δH 4.08) of the aglycone. Thus, a β-Glc moiety was α-linked at C-3. The remaining sugars constituted a branched pentasaccharide O-linked at C-28. A correlation in the HMBC spectrum between the anomeric proton at δH 5.40 and C-28 (δC 178.1) defined the primary sugar as β-Fucp. The interglycosidic linkages of the pentasaccharide chain followed from correlations in the HMBC spectrum, in particular, H-1 of Rha to C-2 of Fuc (likewise, H-2 of Fuc to C-1 of Rha), H-1 of Api to C-3 of Rha (likewise H-3 of Rha to C-1 of Api), H-1 of Xyl to C-4 of Rha (likewise, H-4 of Rha to C-1 of Xyl), and H-1 of Ara to C-3 of Xyl (likewise, H-3 of Xyl to C-1 of Ara). The pentasaccharide O-linked at C-28 was, therefore, α-Arap-(1→3)-β-Xylp-(1→4)[β-Apip-(1→3)]-α-Rhap-(1→2)β-Fucp. In addition, the resonance of H-4 of Fuc was downfield shifted (δH 5.20) and correlated in the HMBC spectrum with the carbonyl group (δC 169.1) of a 4-methoxy-(E)-cinnamoyl moiety (Table 1). A second, minor set of resonances corresponding to a 4-methoxy-(Z)-cinnamoyl moiety was also noted in the 1H NMR spectrum. The ratio of (E)- to (Z)-forms was 5:3 by integration of proton intensities. Resonances
corresponding to a minor form could also be distinguished for H-1 to H-4 of the primary Fuc residue (Materials and Methods), most obviously at $\delta^H 5.37$ (d, $J = 8.2$ Hz, H-1) and $\delta^H 5.17$ (dd, $J = 3.4$, 1.1 Hz, H-4), both slightly upfield ($-0.03$ ppm) of those for the major (E)-form (Table 1). The resonance of Fuc H-4 (minor) correlated in the HMBC spectrum with the carbonyl carbon ($\delta^C 168.0$) of the 4-methoxy-(Z)-cinnamoyl moiety. Thus, saponin 1 was determined to be a 5:3 mixture of the (E)- and (Z)-regioisomers of 3-O-$\beta$-D-glucopyranosyl-28-O-(\alpha-L-arabinopyranosyl-(1 $\rightarrow$ 3)-\beta-D-xlyopyranosyl-(1 $\rightarrow$ 4)\beta-D-apiofuranosyl-(1 $\rightarrow$ 3)-\alpha-L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[4-O-(4-methoxycinnamoyl-\beta-D-fucopyranosyl)]-medicagenic acid (securidacside A). Confirmation of the molecular formula of C$_{73}$H$_{108}$O$_{33}$ for 1 was by HR-ESIMS.

The $^1$H NMR spectrum of 2 was similar to that of 1, except that resonances corresponding to three OMe groups were observed as two singlets at $\delta^H 3.80$ (3H) and 3.89 (2 $\times$ 3H). The aromatic region comprised only a 2H singlet at $\delta^H 6.98$ and two spin-coupled doublets at $\delta^H 7.68$ and 6.64 (both $J = 15.9$ Hz). These observations were consistent with the presence of a 3,4,5-trimethoxy-(E)-cinnamoyl moiety. Full analysis of a set of one-dimensional (1D) and two-dimensional (2D) NMR data sets comparable to those acquired for 1 indicated that 2 had the same triterpenoid aglycone (medicagenic acid) and glycosylation profile (Table 1 and Materials and Methods). Acid hydrolysis of 2 followed by determination of the absolute configurations of the constituent monosaccharides confirmed the presence of $\alpha$-Api, $\alpha$-Ara, $\alpha$-Fuc, $\alpha$-Glc, L-Rha, and D-Xyl. Thus, saponin 2 was 3-O-$\beta$-D-glucopyranosyl-28-O-(\alpha-L-arabinopyranosyl-(1 $\rightarrow$ 3)-\beta-D-xlyopyranosyl-(1 $\rightarrow$ 4)\beta-D-apiofuranosyl-(1 $\rightarrow$ 3)-\alpha-L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[4-O-(4-methoxycinnamoyl-\beta-D-fucopyranosyl)]-medicagenic acid (securidacside B). Confirmation of the molecular formula of C$_{75}$H$_{112}$O$_{35}$ for 2 was by HR-ESIMS.

Effect of S. longepedunculata Root Bark Powder and Methanol Extract of the Root Bark on the Emergence of S. zeamais and C. maculatus in the F1 Generation over Nine Months. The number of live adults emerging in the F1 generation was significantly lower from maize and cowpea that had been mixed with either S. longepedunculata root bark powder or sprayed with the methanol extract of the root bark than emerged from the methanol treated control (LSD, $p < 0.05$) across all treatment times (Figure 2). This amounted approximately to an 80% reduction in S. zeamais and 50% reduction in C. maculatus emergence and confirmed that the dry powdered root bark material, reportedly used by farmers in North Ghana to control...
storage pests (4), had a quantifiable preventative effect against stored product insect pest infestation of grain and that the effect was chemical rather than physical as reported for other materials (24). While a similar activity had been reported previously (8, 25), the present study demonstrates that active components were extractable in methanol and that the activity was effective for up to 9 months with treatment applied every 3 months, a treatment regime recommended for commercial alternatives such as Actellic Super dust. A volatile component of the root extract, methyl salicylate, is a known deterrent and also toxic to storage pests, although there is little trace of it after 3 months (8). The present work suggests that a different class of compound, saponins, can contribute to the protective effect of *S. longepedunculata* root bark.

**Effect of Methanol Extract of* S. longepedunculata* Root on Adult Mortality and F1 Emergence of* S. zeamais* and *C. maculatus*.** The number of *S. zeamais* and *C. maculatus* adults emerging in the F1 generation from their respective commodity treated with the methanol extract of *S. longepedunculata* roots was significantly lower than the number emerging from solvent controls during an 8 week period (Figure 3). LSD analysis of the results showed that the numbers of live *S. zeamais* increased significantly after the fifth week (LSD, \( p < 0.05 \), Figure 3) as would be expected for this species, while similarly, the number of *C. maculatus* adults increased significantly after the third week (LSD, \( p < 0.05 \), Figure 3). More importantly, significantly more adults emerged in the F1 generation on control grain, treated with methanol only, indicating that the methanol extract of *S. longepedunculata* roots contained compounds that reduced the F1 emergence of adults on the grain. The results did not show a delay in the development period of the F1 generation for either of the insect species, suggesting that the effect is either an oviposition deterrent or toxic to eggs or larvae, but not a development inhibitor.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of *S. longepedunculata* methanol extract treated commodities on live F1 emergence of *S. zeamais* and *C. maculatus*: mean (±SEM, \( n = 10 \)) number of live *S. zeamais* (a) and *C. maculatus* (b) adult insects recorded in commodity treated with the methanol extract of *S. longepedunculata* (0.125% w/w) over 8 weeks.

**Effect of Maize and Cowpea Treated with Securidacaside A (1) on the Feeding Behavior of *S. zeamais* and on the Oviposition, Hatching, and F1 Emergence of *C. maculatus*.** The number of damaged seeds in the maize treated with 1 was significantly lower than that of the control maize (t test, \( p < 0.05 \), Figure 4), and more dead adults were recorded on maize treated with 1 than on the control maize, which was equivalent to >95% damage, whereas in the treated seeds the damage was <35%. This indicated that securidacaside A (1) was either deterrent or toxic to *S. zeamais* adults and could explain, at least in part, the effect of the crude extracts against this insect and also the effect of the whole plant material when mixed into the grain. It was not possible, however, to determine whether the increased mortality in the treated grain was caused by starvation owing to the deterrent effect of 1 or whether this compound was toxic by consumption.

The number of oviposited and hatched eggs on the surface of cowpea seeds treated with 1 was significantly lower than that on the control seeds (Figure 5). The number of adults emerging in the F1 generation was also significantly lower in the treatment when compared to the control (t test, \( p < 0.05 \)) suggesting that 1 acts as an oviposition deterrent as well as being toxic to *C. maculatus* larvae as shown by the methanol extracts of the roots (Figure 5).
As with *S. zeamais*, this could explain, at least in part, the effect of the crude extracts against bruchids and also the protective effect of the whole plant material when used by farmers.

Previously, Taylor et al. (26) demonstrated that a saponin, dehydrosoyasaponin I, isolated from the seed of the pea, *Pisum sativum*, showed antifeedant activity against *S. zeamais*, an effect enhanced by the presence of three lysolecithin-type seed phospholipids. These lysolecithins were inactive when presented to the insects alone. Thus, there is evidence that saponins are deterrent compounds to *Sitophilus* spp., although this property was mitigated by lysolecithins (26).

The results presented here corroborate anecdotal reports on the practice by farmers in Ghana and Zambia in which the roots of *S. longepedunculata* are pounded and mixed into stored grain (4). The longevity of activity recorded in the present work is suitable for grain storage in the tropics by subsistence farmers (at least 9 months) since most depend upon their own crops for food, and thus, personal stores must last until the next crop is harvested. This is especially relevant in the resource poor agricultural environments of sub-Saharan Africa. The fact that the extract applied to the stored product was as effective as the dried root powder indicated that both contain active components. However, the use of the extract may offer a more sustainable and efficient application procedure than hand mixing coarsely powdered root bark. Although pounded roots of *S. longepedunculata* are effective when mixed into grain, ensuring that the roots are sufficiently well powdered to mix efficiently throughout the grain, is labor intensive and often wasteful. Furthermore, maize grains have a glassy surface, and the powdered plant material tends not to adhere to the surface, leaving many grains exposed.

Some farmers in Ghana have reported effective treatment of stored grain with water extracts from *S. longepedunculata* roots (4). Jayasekara et al. (25) showed that methyl salicylate, a volatile component of the methanol root extract, was toxic to *S. zeamais* and the grain borers *Rhyzopertha dominica* and *Prostephanus truncatus*. Methyl salicylate is poorly water-soluble, however, and unlikely to entirely account for the protective effect of water extracts of the roots. The reported activity of water extracts is also consistent with the presence of active saponin constituents, which are water-soluble. Thus, the use of water extraction of *S. longepedunculata* roots by farmers could optimize the use of this plant and thus reduce the amount required for treatment while facilitating the development of a more sustainable use of this increasingly scarce plant material. *S. longepedunculata* is not widely used in storage protection; therefore, there is scope for developing applications for this plant material in other parts of Africa, although optimization of its use and information about its safety is required before it is promoted widely. Belmain et al. (27) investigated the potential mammalian toxicity of several pesticidal plant species including *S. longepedunculata*. They reported that rats, which had been fed the root bark of *S. longepedunculata*, showed mild symptoms of toxicity but only at the highest concentrations (5% w/w) tested and concluded that since farmers intentionally remove both synthetic pesticidal dusts or pesticidal plant materials from stored grain by winnowing and washing with water they are highly unlikely to be exposed during consumption to toxic levels of material. In addition, the roots are consumed for traditional medicinal purposes, suggesting further that the consumption of small quantities is of very low risk (28).

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