ADULTICIDAL ACTIVITY AND TOXICITY OF EXTRACTIVES FROM *TECLEA TRICHOCARPA* AGAINST ADULT MAIZE WEEVIL (*SITOPHILUS ZEAMAIS*)

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Abstract
With a growing world population and increased affluence leading to demand for more and higher quality foods, and given environmental problems such as soil degradation, water scarcity, and biodiversity loss, new and innovative solutions are required to minimize food losses caused by pests. Organic solvent extracts and thereof isolated compounds of Teclea trichocarpa Eng. were evaluated for adulticidal activity against maize weevil, Sitophilus zeamais Motchulsky, and for brine shrimp, Artemia salina, lethality. Hexane extract of the leaves of T. trichocarpa displayed mild brine shrimp toxicity (LD_{50} =153.2 µg/ml), while the other extracts showed no significant toxicity (LD_{50}> 240 µg/ml). Both hexane and dichloromethane extracts of leaves of T. trichocarpa showed dose dependent mean percentage adulticidal activity. At 600 and 800 ppm these extracts, respectively, were comparable to the positive control, actellic super, a synthetic pesticide which is in the market today. Considering the cost, increasing incidence of pesticide resistance and environmental concerns posed by synthetic pesticides, several pressures have accelerated the search for more environmentally and toxicologically safe, more selective and efficacious pesticides. Results discussed with regard to the use of the plant extractives as suitable and sustainable alternative to synthetic insecticide in maize grain storage and could be incorporated in integrated pest management.

Keywords: brine shrimp; *Teclea trichocarpa*; adulticidal activity; maize weevil, *Sitophilus zeamais*

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1. INTRODUCTION

Efficient production of good quality food grains is a big challenge to mankind. A variety of techniques have been applied to meet the challenge. One of the aspects is to improve efficiency in grain production and post harvest practices to ensure that food losses are minimized if not eliminated and that the grains produced is of good quality and safe for human consumption. Tropical countries suffer severe losses of stored food products due to pests. This is partly attributed to conducive climatic conditions. Apart from other causes of food losses like crop diseases and weeds, pre- and post-harvest pests are responsible for ~40% of Africa's food losses (Mandava, 1985). Prophylactic methods have not constrained the pests to acceptable levels. Synthetic pesticides have been used against post-harvest pests. However, the persistence, resistance, the cost and availability of these conventional insecticide and potential health hazard both to the consumers and to the environment have necessitated continued use of local plant products.

Traditional methods involves admixture with local plant materials as repellents, sunning and use of wood ash (Mutambuki *et al*., 1989). Although these botanicals have been in use since time immemorial their efficacy, safety and their active principles deserve more attention (Balandrin *et al*., 1985). Plants have been screened for repellency, and antifeedant against maize weevil, and various classes of the natural products been identified to be responsible for the activity such as terpenoids, flavonoids, flavones, alkaloids and essential oils. (Hassanali and Lwande, 1989; Hassanali *et al.*, 1990; Lwande *et al.,* 1983, Ndungu *et al.,* 1999 and Bekele *et al.,* 1996).

Teclea trichocarpa is reported to be used by traditional healers belonging to the Akamba tribe for malaria treatment and as anthelmintic, while the Giriama tribe of Kenya steam the leaves and inhale the vapour as a cure for fever (Watt and Breyer-Brandwijk, 1962). The plant bark has been shown to have antifeedant activity against the African armyworm, *Spodoptera exempta* (Lwande *et al.,* 1983). The leaves were reported to possess antiprotozoa activities against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Leishmania donovani*. (Muriithi *et al.,* 2002; Mwangi *et al.,* 2010). The leaves and stem bark of *T. trichocarpa* is also traditionally used to control maize weevil by Keiyo community living in the Rift Valley, Kenya. This study aimed at evaluating the pesticidal activity and toxicity of extractives from *T. trichocarpa* against adult maize weevil (*Sitophilus zeamais*) and a strategy of improving food security in the communities.

2. MATERIAL AND METHODS

Melting points were determined on an electro thermal melting point apparatus and expressed in degree centigrade (^oC) and were uncorrected. IR spectra were taken in KBr pellets and recorded on a Shimazdu (model FT-IR-8400 CE) with absorption given in wave numbers (cm^{-1}). NMR spectra were recorded on a Bruker DPX- 400 NMR. The spectra were recorded in $CDCl_3$ as the solvent and TMS as the internal standard. The chemical shifts reported in δ (ppm) units relative to TMS signal. TLC was performed on aluminium sheets pre-coated with silica gel 60 F_{254} (Merck) with a 0.2 mm layer thickness, Preparative TLC was done using normal phase silica gel (F_{254} Merck) pre-coated on aluminium plate (20 x 20 cm) and a layer thickness of 0.25 mm. Spots on chromatograms were examined under UV light (254 and 366 nm), and by anisaldehyde and dragendorff's visualization reagents. VLC column were packed with thin layer chromatography silica gel 60 (6-35 microns mesh, ASTM) and column chromatography on silica gel 60 (0.040-0.063 mm 230-400 mesh, Merck). Solvents were laboratory grade and were obtained from BDH, Nairobi and were double distilled before use.

2.1. Plant Materials

The leaves of *T. trichocarpa* (Rutaceae) (2.0 kg) were collected from Siroch also in Keiyo District in rift Valley, Kenya. The samples of *T. trichocarpa* were authenticated by a taxonomist at the National Museums of Kenya in Nairobi, Kenya and given voucher specimen number SKM/JKUAT/002/006. The leaves were dried in the shade, and ground into powdered material using a grinding mill (Christy and Norris Ltd, England). The powdered plant material were hermetically sealed in polythene bags and stored in a refrigerator at 4^oC in the dark until the time of extraction.

2.2 Extraction, Fractionation and Isolation

The air-dried, powdered leaves of *T. trichocarpa* (2.0 kg), were extracted sequentially with 7.5 litres each of hexane, dichloromethane (CH_2Cl_2) ethyl acetate and methanol exhaustively at room temperature. Each extract was concentrated under reduced pressure at 45^oC. The yields and percentage yields of the extracts are presented in Table 1. The extracts were screened for toxicity using brine shrimp.

Leaves of *T. trichocarpa* yielded a yellow paste of hexane extract (25.0 g) and a green paste of dichloromethane extracts (48.5 g). These were subjected to vacuum liquid chromatography (VLC) separation on silica gel 60 each at a time, eluted with *n*-hexane with increasing amount of CH_2Cl_2 and later increasing amount of methanol in CH_2Cl_2 up to 1:5.Fifty five and 65 fractions were collected, respectively, from TLC analysis similar fractions pooled together. UV active spots on TLC were considered for further separation. From the hexane extract of *T. trichocarpa*, fraction (31-37) 2128.9 mg that eluted with *n*-hexane: CH_2Cl_2 (1:4) was further chromatographed on sephadex and eluted with a mixture of CH_2Cl_2 and Methanol (1:1) to give 32 sub-fractions, sub fraction 31-32 on

crystallization in methanol afforded α-amyrin [**6**] (32.7 mg). Fractions 34-38 (8034.1 mg) that eluted with 2:3 (*n*-Hexane: CH_2Cl_2) was loaded onto VLC and eluted with hexane and increasing amount of CH_2Cl_2 and then increasing amount of methanol. Twenty-eight sub fractions were obtained from which fraction 13-21 was further chromatographed on silica gel and eluted with 2:3 (*n*-Hexane: CH_2Cl_2) this yielded β-sitosterol [**5**].

From VLC of the CH_2Cl_2 extract, fraction 34-40 (1014 mg) was loaded onto sephadex column and eluted with CH_2Cl_2: methanol (1:1) to give 16 sub fractions. Sub fraction 3-4 was subjected to column chromatography and eluted with ethyl acetate: CH_2Cl_2 (1:3). This afforded 38.1 mg of melicopicine [**1**]. Sub Fraction 5-18 showed UV active spots, column chromatography of this fraction eluted with CH_2Cl_2: ethyl acetate (2:1) mixture gave 25 fractions from which sub fractions 9-11, 12-15 and 21-25 were further subjected to chromatographic separation. Sub fractions 9-11 were subjected to preparative thin layer chromatography, this afforded skimmianine [**4**] (22.2 mg), sub fractions 21-25 was subjected to preparative thin layer chromatography. This afforded two compounds Melicopicine [**1**] (64.8 mg), and normelicopicine [**2**] (46.9 mg). Fraction 41-42 from VLC was subjected to column chromatography and eluted with ethyl acetate: CH_2Cl_2 (1:9). The sub fraction (5-8) that eluted with CH_2Cl_2: ethyl acetate (1:1) afforded yellow needle like compound, arborinine [**3**] (99.0 mg) on partitioning between methanol and CH_2Cl_2 (2:1).

2.3 Toxicity Testing Against the Brine Shrimp

The hatching brine shrimp eggs, *Artemia salina* leach were hatched in artificial seawater prepared by dissolving 38 g of sea salt (Sigma chemicals Co., UK) in 1 litre of distilled water. After 48 hrs incubation at room temperature (25^{o}C), the larvae (nauplii) were attracted to one side of the vessel with a light source and collected with pipette. Nauplii were separated from eggs by aliquoting them three times in small beakers containing seawater.

The bioactivity of the extracts was monitored by the brine shrimp lethality test (Meyer *et al*., 1982). Samples were dissolved in dimethylsulphoxide (DMSO) and diluted with artificial sea salt water so that final concentration of DMSO did not exceed 0.05%. Fifty microlitres of sea salt water was placed in all the 96-well microtitre plates. Fifty microlitres of 4000 ppm of the plant extract was placed in the row one and a two-fold dilution carried out down the column. The last row left with sea salt water and DMSO only served as the drug free control. Hundred microlitres of suspension of nauplii containing 10 larvae was added into each well and incubated for 24 h. the plates were then examined under a microscope (12.5X and the number of dead napulii in each well counted and recorded. Lethality concentrations fifties (LC_{50} values) for each assay were calculated by taking average of three experiments using a Finney Probit analysis program on an IBM computer (McLaughlin *et al*., 1991).

2.4 *Sitophilus zeamais* Culture

Adult *Sitophilus zeamais* was obtained from a laboratory colony reared under ambient conditions with natural photoperiods on untreated (insecticide-free) whole maize grains obtained and maintained at National Agricultural Research Laboratories (NARL), Nairobi, Kenya.

One hundred *S. zeamais of* mixed sexes were introduced into 2 litre glass jars containing 400 g weevil susceptible maize grains following the methods of Bekele and Hassanali (2001). The mouths of the jars were then covered with nylon mesh held in place with rubber bands. Freshly emerged adults of *S. zeamais* were then used for the experiments (Asawalam and Emosairue, 2006).

Adulticidal Assessments

Bioassay tests were carried in the laboratory to determine the efficacy of the botanicals under different dosage levels against *S. zeamais*. Three doses of each plant extracts, were used as treatment to assess adulticidal activity against maize weevil. For pure compounds and

blend mixtures the concentrations were double, equal and half that of positive control (Actellic super). A synthetic insecticide Actellic super 2% dust at 0.05 % w/w and untreated maize grains were included as positive and negative controls, respectively.

The test samples were mixed with talc thoroughly and the dust were admixed with 50 g of maize held in jam jars covered with ventilated lids. To ensure a thorough admixture, the grain was put in plastic jam jar, dust applied and top lid replaced. The grain was then swirled within the jar until a proper admixture was realized. Twenty, 5-day old *S. zeamais* adults were introduced into treated and untreated maize grains and confined by perforated lids placed over muslin cloth that was held in place by a rubber band.

The design of the experiment was Completely Randomized Design (CRD) with three replications. The treatments were kept on at room temperature for seven days before mortality was assessed. Percentage mean mortality for *S. zeamais* was recorded after seven days exposure period as described by Bekele *et al*., (1996).

Data were subjected to analysis of variance (ANOVA) procedure (SAS, 2000) and significantly different (P>0.05) means were separated by using Tukey's studentised range (HSD) test.

3. RESULTS AND DISCUSSION

The potential of using the *T. trichocarpa* extracts and the constituent components of extracts as protectant for stored maize grains against maize weevil, and toxicity against brine shrimp, were the main objectives for this study. On extraction with various organic solvents the yields were as shown in Table 1.

The percentage yields of hexane extract was lower than the other extracts, with thrice and twelve fold percentage yield of DCM and methanol extracts, respectively. These extracts were subjected to brine shrimp lethality test and adulticidal test against maize weevil before embarking on fractionation of the crude extract.

Table 1. Percentage yield of *T. trichocarpa* organic extracts

Extract	hexane	CH_2Cl_2	EtOAc	MeOH
Yields (g)	26.0	52.4	41.6	199.2
Percentage yields (%)	1.3	2.6	2.1	10

3.1 The Toxicity Assay

The hexane, dichloromethane, ethyl acetate and methanol crude extracts of *T. trichocarpa* were tested for their toxicity against brine shrimp lethality assay. The results are shown in Table 2. The hexane extracts of *T. trichocarpa* leaves with LD_{50} values of 153.2 µg/ml was considered active, while CH_2Cl_2, EtOAc and MeOH extracts showed mild toxicity against brine shrimp (Table 2). Since a crude sample is considered active up to a concentration of 240 µg/ml (Meyer *et al*., 1982); and brine shrimp test is an indicator of toxicity, various pharmacological actions, and pesticidal effects (Meyer *et al*., 1982), it was deduced that both hexane and CH_2Cl_2 extracts of *T. trichocarpa* had greater potential as insecticide.

Table 2. The mean LD_{50} values ± s.d. for the *T. trichocarpa* leaves organic crude extracts screened against brine shrimp (*Artemia salina*, leach).

Plants extract	LD_{50}
Hexane extract	153.2 ± 1.0
DCM (CH_2Cl_2) extract	279.9 ± 0.7
EtOAc extract	416.1± 0.9
MeOH extract	567.8 ±1.8

3.2 Adulticidal Screening

From the adulticidal assay against maize weevil (*S. zeamais*) the methanol and ethyl acetate extracts showed no activity. The crude extracts (hexane and dichloromethane) of *T. trichocarpa* were therefore subjected to further adulticidal test against maize weevil (*S. zeamais*). The effects of different doses of hexane and CH_2Cl_2 extracts on maize weevil after seven days were determined and LD_{50} values computed and the results are summarized in Table 3.

Table 3. Percent mortality of adult *S. zeamais* on maize grains treated with different concentrations of hexane and CH_2Cl_2 crude extracts from *T. trichocarpa* leaves against maize weevil (*S. zeamais*).

Plants extract	100 ppm	200 ppm	400 ppm	600 ppm	800 ppm
Hexane	25.0 ± 5.0 b	75.0 ± 5.0 b	75.0 ± 5.0 b	100.0 ± 0.0 a	100.0 ± 0.0 a
DCM, (CH_2Cl_2)	25.0 ± 5.0 b	40.0 ± 0.0 d	45.0 ± 0.0 d	70.0± 10.0 b	95.0 ± 5.0 a
EtOAc	25.0 ± 5.0 b	50.0 ± 0.0 d	60.0 ± 5.0 c	70.0± 10.0 b	87.0 ± 5.0 a
Actellic super	75.0 ± 5.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Negative control	5.0 ± 5.0 c				

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test).

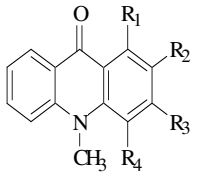
From the results in Table 3, it is evident that adulticidal activities are dose dependent for both organic extracts. The most active extracts, with the highest mean adulticidal activity at almost all doses being hexane extracts showing 75% adulticidal at a concentration of 200 ppm and 100% adulticidal from 600 ppm, at which was comparable to the positive control at 95% confidence level; at 200 ppm, actellic super, a synthetic pesticide at the recommended rate of 0.05%, which is in the market today.

The fact that, the crude extracts at high concentration had significant mean percentage adulticidal against maize weevil is interesting and led support to the traditional use of this plant material as grain protectant against destructive pests. Both extracts represents an attractive candidate for field evaluation as a protectant of stored maize. It is also expected that, the crude plant extract could offer suitable and sustainable alternative to synthetic pesticide. However, conclusive recommendation of their use can only be made after exhaustive analysis of the effect of the crude on the quality of grain and safety.

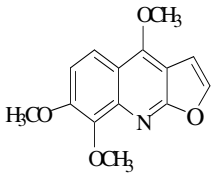
Adoption of these natural plant products could improve efficiency in post-harvest practices as a strategy of providing people with sufficient and healthy food in an ecologically sustainable manner. Being natural, protectants from plant materials would be easily degraded by biological factors, and cases of pollution and poisoning would be reduced. Improving grain storage would mean less hunger, improved nutrition for mankind, a higher standard of living and a sounder economy for the nation.

Examining Tables 2 and 3, the brine shrimp lethality and adulticidal activity results for the crude extracts, respectively, the hexane extracts of *T. trichocarpa* showed higher toxicity as well as adulticidal activity against maize. It was evident that toxicity against brine shrimp may be a basis of deducing an active adulticidal extract, similarly blending hexane and CH_2Cl_2 crude extract lowered activity showing antagonistic effect. For this reason, hexane and CH_2Cl_2 crude extract was fractionated and pure compounds isolated.

The TLC profile of *T. trichocarpa* revealed the presence of several UV active and fluorescing compounds in the crude extracts. Chromatographic separation of the hexane and dichloromethane extracts afforded two terpenoids (α-amyrin and β–sitosterol) and four alkaloids; melicopicine, arborinine, normelicopicine (acridone alkaloids) and skimmianine (furoquinoline alkaloid).
The structures of the compounds were characterized and identified by their IR., ^{1}H NMR and ^{13}C NMR, and comparing with data of authentic samples α-amyrin (Mahato and Kundu, 1994), arborinine (Bergenthal *et al*., 1979), melicopicine (Rasoanaivo *et al*., 1999), normelicopicine (Muriithi *et al*., 2002), skimmianine and β–sitosterol (Knight, 1974) .



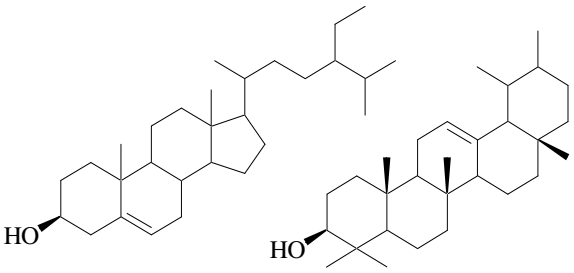
1 $R_1, R_2, R_3, R_4 = OCH_3$ Melicopicine



4 Skimmianine

2 $R_1 = OH, R_2, R_3, R_4 = OCH_3$ Normelicopicine

3 $R_1 = OH, R_2, R_3 = OCH_3, R_4 = H$ Arborinine



5. 3β–sitosterol

6. α-amyrin

The six compounds thus isolated from hexane and CH_2Cl_2 extracts were tested against maize weevil (adulticidal) at different doses. Actellic super, a synthetic insecticide, was used as positive control and no treatment was included as the negative control. Results are summarized in Table 4.

From the results in Table 4, the mean percentage adulticidal was dose dependent. However, all the compounds showed low activities at both 0.1 and 0.05 w/w, being between 10% to 22% at 0.1% w/w when compared to actellic super. The adulticidal activity of the three-acridone alkaloids melicopicine [**1**], normelicopicine [**2**] and arborinine [**3**] were noted to be low with the mortality being between 10% to 22% at 0.1% w/w of the compound. Comparing the two terpenoids, 3β–sitosterol [**5**] showed higher activity (12.5±2.5) than α-amyrin [**6**] (5.0±0.0) at 0.05 w/w and were significantly different ($p < 0.05$). Although the two compounds share a common biosynthetic pathway, the difference in activity may be attributed to their structural difference. β–sitosterol has also been reported to show weak feeding inhibitory activities against the larvae of *Chilo partellus* [26] (Tsanuo, 1992). This compound could be a better protectant against destructive pests due to its feeding inhibitory and adulticidal activities.

Table 4: Mean percentage adulticidal ± s.d. of isolated compounds from *T. trichocarpa* against maize weevil.

Compounds	Mean percentage adulticidal at different concentration in w/w	
	0.1 w/w	0.05 w/w
Melicopicine [**1**]	12.5 ± 2.5 c	2.5 ± 2.5 c
Normelicopicine [**2**]	15.0 ± 0.0 a	7.5 ± 2.5 ac
Arborinine [**3**]	22.5 ± 2.5 a	10.0 ± 0.0 a
Skimmianine [**4**]	17.5 ± 2.5 a	7.5 ± 2.5 ac
β–Sitosterol [**5**]	20.0 ± 0.0 a	12.5 ± 2.5 a
α–Amyrin [**6**]	10.0 ± 5.0 ac	5.0 ± 0.0 c
Actellic super	95.0 ± 0.0 b	87.5 ± 2.5 b
Negative control	5.0 ± 0.0 c	

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence

The isolated compounds were less active than the crude extracts, from which they were isolated, an indication of possible loss of synergism in the isolation process. In order to ascertain these observations, pure isolated compounds were blended in the same ratio and subjected to adulticidal test. The adulticidal assay results at different dosage of thereof blended mixture of isolated compounds; actellic super (positive control) and drug free (negative control) are summarized in Table 5.

Table 5: Mean percentage adulticidal ± s.d. of the blended compounds from *Teclea trichocarpa* against maize weevil.

Compounds	Mean percentage adulticidal at different concentration in w/w	
	0.1 w/w	0.05 w/w
Skimmianine/ Arborinine	20.0 ± 0.0 c	12.5 ± 2.5 a
α-Amyrin/ Normelicopicine	17.5 ± 2.5 a	10.0 ± 5.0 a
Arborinine /Melicopicine	20.0 ± 0.0 a	15.0 ± 5.0 a
α-Amyrin/ Arborinine	22.5 ± 2.5 c	17.5 ± 2.5 a
3□–sitosterol/ Arborinine	17.5 ± 2.5 a	12.5 ± 2.5 a
Actellic super	95.0 ± 0.0 b	87.5 ± 2.5 b
Negative control	5.0 ± 0.0 c	

Key: Mean values with the same letters within the same column are not significantly different at 95% confidence level (Tukey's studentized test).

From the results in Table 5, it is evident that the adulticidal activities are concentration dependent. However, comparing these results with those presented in Table 4, mixture of α-amyrin/ normelicopicine, and skimmianine/ arborinine, at higher concentration showed higher activity than corresponding pure compounds, implying some synergism. Whether this implies, a mixture of terpenoids and alkaloids or different types of alkaloids are more effective remains to be investigated. Arborinine/normelicopicine and β–sitosterol/ arborinine mixtures showed lower activity than corresponding pure compounds, implying there was loss of activity (antagonist). Mixture of α-amyrin and arborinine did not show significant change in activity at high concentration but at 0.05% w/w there was increased activity, implying synergism is in play.

Although all the test mixtures used were in the ratio of 1: 1, their occurrence in the crude extracts of the plant is not in these ratios hence their effects could differ. Similarly, the isolated compounds were not the only compounds present in the crude extracts as evidenced from TLC analysis and therefore, it is evident adulticidal activity is caused by additive effect of most constituent components with different levels of activity.

4. CONCLUSION

The study has shown that hexane and CH_2Cl_2 extracts of *T. trichocarpa* displayed higher toxicity against brine shrimp as well as adulticidal activity against maize weevil. The results provide a scientific rationale for the use of *T. trichocarpa* in post-harvest protection. There is, therefore, a good promise to use of this botanical pesticide as alternative to the synthetic pesticide, Actellic super 2% dust.

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