Advances in Analytical Chemistry 2020, 10(2): 15-19
DOI: 10.5923/j.aac.20201002.01

Evaluation of *Warburgia ugandensis* Extracts and Compounds for Crop Protection against *Prostephanus truncates*

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Abstract Insect pests cause a significant loss of maize production in Africa. Maize weevil (*Sitophilus zeamais* Motchulsky) and the larger grain borer (*Prostephanus truncatus* Horn) are the most destructive insect pests of maize. Chemical control is the most commonly used and the most effective method at the farm level. However, some of the chemicals cause adverse effects to environment and humans. In addition, insecticides available in the market are expensive and mostly out of reach to smallholder farmers. The use of botanicals for pests and disease control is preferred because plant materials are non-toxic and are readily available. This study investigated the efficacy of extracts and compounds from *Warbugia ugandensis* leaves for control of *P. truncatus* infestation in stored maize. All the crude extracts exhibited repellent, toxicity and growth inhibition activities against *P. truncates.* The most active compounds were polygodial, warburganal, ugandensolide and mukaadial. The findings from this study show that extracts from *W. ugandensis* are effective in controlling the larger grain borer and therefore could be used to control the pest.

Keywords *Warbugia ugandensis,* Insect pests, *Prostephanus truncates*, Repellence, Mortality, Growth inhibition

1. Introduction

Insect pests cause significant losses of maize in Africa, reducing the 4.9 t/ha world average grain yield production to 1.5 t/ha average [1,2]. Infestation by post-harvest pests commences in the field but most damage occurs during storage [3]. Among pests of maize, beetles are the most important, with maize weevil (*Sitophilus zeamais* Motchulsky) and larger grain borer (*Prostephanus truncatus* Horn) being the major ones. The extensive tunneling in maize grain by pests allows the pests to convert maize grain into flour within a very short time [3]. Small-scale farmers are often forced to sell maize shortly after harvest to minimize losses during storage, thereby attract low prices and compromising food security at the house hold level. Technologies that can reduce yield losses from storage pest damage are necessary to increase maize production to cope with increasing demand for maize in Kenya.

Chemical control method is the most commonly used and most effective at the farm level. Pesticides such as organochlorines, organophosphates, carbamates, organoarsenicals and organothiocynates have been recommended to control the weevils [4]. However, some of the chemicals have adverse effects on environment and humans [5]. Banning some of the chemicals has lest just a few insecticidal alternatives for pest control operations. Furthermore, insecticides are expensive and mostly out of reach to smallholder farmers [6]. There is urgent need to develop safe alternatives that are of readily available, convenient to use and environmentally friendly [7].

Plant extracts contain secondary metabolites some of which inhibit the growth of pests and pathogenic microorganism [8-13]. The use of botanical for pests and disease control is preferred because they are safe and non-toxic to humans [14-19]. In addition, chances of pests and pathogens developing resistance to botanical pesticides are highly unlikely [20]. *Warbugia ugandensis* (Canellaceae) is traditionally used as a remedy for stomachache, constipation, toothache, malaria, sexually transmitted diseases, diarrhoea, cough and internal wounds/ulcers [21]. *Warburgia* species are characterized by the presence of drimane sesquiterpenes some of which have been reported to exhibit antibacterial, antifungal, insect antifeedant, insecticidal and molluscicidal activities [8,22,23].

The present study was conducted to investigate the efficacy of extracts and compounds of *W. ugandensis* in controlling of *P. truncatus* infestation in stored maize.

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Received: Oct. 15, 2020; Accepted: Nov. 5, 2020; Published: Nov. 15, 2020
Published online at http://journal.sapub.org/aac

2. Materials and Methods

2.1. Plant Materials

Leaves of *W. ugandensis* were collected along Nakuru Gilgil Highway near St. Mary's Hospital (latitude 0° 24' 42.49" S and longitude 36° 15' 10.59" E) in May 2014 and voucher specimen (2014/5/SAO/CHEMMK) was identified at the Kenya National Museum Herbarium after comparison with authentic samples. The plant materials were air dried at 24-28℃ until crispy. The dried leaves were pulverized and sieved through a 0.5 mm size mesh.

2.2. Extraction and Isolation of Compounds

Two kg of powdered leaves of *W. ugandensis* was cold extracted with organic solvents of varying polarities (n-hexane, ethyl acetate and methanol) sequentially by soaking in the solvents for seven days with occasional shaking. The mixture was filtered and concentrated using a rotary evaporator at reduced pressure to yield 20.2, 58.6 and 97.8 g of n-hexane, ethyl acetate and methanol extracts, respectively. The resultant extracts were stored at 4℃ for bioassays and phytochemical studies. *n*-Hexane and ethyl acetate extracts showed similar TLC profile and were combined for phytochemical isolation. The combined extract (50 g) was dissolved in a small amount of n-hexane – ethyl acetate mixture (1:1) and subjected to in silica gel for column chromatography using silica gel. Elution was done using n-hexane, n-hexane - ethyl acetate mixture, ethyl acetate and methanol to give 200 fractions (each 20 ml) whose compositions were monitored by TLC and those with similar profiles were combined to give seven pools labeled I -VII. Pool I, 3g, which was eluted with n-hexane did not show any major spot on TLC and was discarded. Pool II (7 g) was subjected to further column chromatography eluting with n-hexane: ethyl acetate (95:5, 9:1, 85:15 and 4:1) to give polygodial (**1**) 30 mg and warbuganal (**2**) 55 mg. Pool IV (5 g) on further fractionation with gradient n-hexane-ethyl acetate mixture (85:15, 4:1 and 7:3) gave polygodial (**1**) 35 mg and ugandensolide (**3**) 38 mg. Pool V (8 g) on further fractionation with n-hexane: ethyl acetate (4:1, 7:3 and 65:35) gave ugandensolide (**3**) 24 mg, ugandensidial (**4**) 42 mg and muzigadial (**5**) 75 mg. Pool VI (9 g) gave ugandensidial (**4**) 43 mg while Pool VII gave muzigadial (**5**) 15 mg and mukaadial (**6**) 72 mg.

2.3. Mass Rearing of *Prostephanus truncatus*

Adult weevils were obtained from infested maize grains purchased from a local market and from the stock, new generation was reared on dry pest susceptible maize grains [24]. Two hundred maize weevils of mixed sexes were introduced into a two liter glass jars containing 400 g weevil susceptible maize grains [25]. The mouths of the jars were then covered with nylon mesh held in place with rubber bands and the jars left undisturbed for 35 days for oviposition. Thereafter, all adults were removed through sieving and each jar was left undisturbed for another 35 days. Emerging adult insects were collected and kept in separate jars according to their age. Adults that emerged on same day were considered of the same age [26].

2.4. Repellency Test

The test was done according to [24] with some modifications. Transparent plastic tubings, 13 cm long x 1.3 cm diameter were used as test cylinders. Each test cylinder was plugged at one end with cotton ball containing solid crude extracts and compound isolated from the stem bark of *W. ugandensis* while the other end was plugged with clean cotton ball which served as control. Actellic dust was used as a positive control. Ten-three-day old unsexed test insects were introduced at the middle of each test cylinder through a hole at the middle portion of the cylinder (0.0 cm) and let to move in any direction of their choice with scoring of distance moved measured in cm using a ruler. The score time was 24 hours after exposure and all tests were done in triplicates.

2.5. Adult Mortality Test

Contact toxicity assay was done according to Ileke and Oni [27] with some modifications. Toxicity of the crude extracts and isolated compounds were tested against adult weevils. The test samples were mixed with talc thoroughly and the dust was admixed with 20 g of maize held in 12 cm high x 6.5 cm diameter glass jars covered with ventilated lids. To ensure a thorough admixture, the grain was put in 12 cm high x 6.5 cm diameter glass jars, dust applied and top lid replaced. The grain was then swirled within the jar until a proper admixture was realized [28]. Twenty-three-day old unsexed insect pairs were then introduced into each dish and exposed to treatments. Actellic dust was used as a positive control and all tests were done in three replicates. Maize weevils were considered dead when probed with sharp objects and there were no responses [27]. The number of dead insects in each vial was counted after 21 days after treatment to estimate maize weevil mortality as follows:

$$\% \text{ Mortality} = \frac{\text{Number of dead insects}}{\text{Total number of insect}} \times 100$$

Data on percentage adult weevil mortality were corrected using Abbott’s formula [29]: PT = (Po – Pc) / (100 - Pc)

Where PT = Corrected mortality (%); Po = Observed mortality (%); PC = Control mortality (%).

2.6. Growth Inhibition Assay

The test was done according to Ileke and Oni [27] with some modifications. 20 g of clean undamaged and uninfected corn grains were placed in 12 cm high x 6.5 cm diameter glass jars glass jars. Test materials in powder form were thoroughly mixed with the grains in each jar. Crude extracts and pure compounds were mixed with talc thoroughly before being applied to the grains [28]. A mixture of twenty-seven-day old unsexed maize weevils was introduced in each jar and covered with filter paper [26]. The female adults were allowed to oviposit on the seeds for 4 days. On day 5, all insects were removed from each container and the seeds returned to their respective containers. Progeny emergence (F1) was recorded at six weeks (42 days). The containers were sieved out and newly emerged adult weevils were counted [27]. At week six, the grains were reweighed and the percentage loss in weight was determined as follow:

$$\% \text{ Weight loss } = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

3. Results and Discussion

3.1. Phytochemical Studies

Fractionation of *n*-hexane and ethyl acetate extracts from *W. ugandensis* stem bark over silica gel column afforded six compounds (Figure 1) namely polygodial (**1**), warbuganal (**2**), ugandensolide (**3**), ugandensidial (**4**), muzigadial (**5**) and mukaadial (**6**) [22,30].

3.2. Repellent, Toxicity and Growth Inhibition Activities

Crude extracts (*n*-hexane, ethyl acetate and methanol) and compounds (polygodial (**1**), warbuganal (**2**), ugandensolide (**3**), ugandensidial (**4**), muzigadial (**5**) and mukaadial (**6**) isolated from the leaves of *W. ugandensis* were testes for repellence, toxicity and growth inhibition activities against the larger grain borer (Table 1). *n*-Hexane and ethyl acetate extracts (mean repellency = 5.8 & 5.1 cm, respectively) repelled the insects more than Actellic dust (mean repellency = 4.7 cm) which was used as a positive control. The repellence activity exhibited by the isolated compounds polygodial (**1**) and mukaadial (**6**) (mean repellency = 4.6 & 4.4 cm, respectively) were comparable to that of the standard.

In the insect toxicity test, *n*-hexane and ethyl acetate extracts killed 76 and 71% insects after exposure for 21 days while Actellic dust caused 100% death. Methanol extract was the least toxic to the insects among the crude extracts. For the pure compounds, the insects were most susceptible to polygodial (**1**) and warbuganal (**2**) which caused 64.3 and 61.7% deaths respectively. Mukaadial (**6**) and ugandensolide (**3**) exhibited moderate toxicity of 44.1 and 41.3% respectively.

In the growth inhibition test, the percentage adult emergences were 15.2, 19.4 and 31.1% in maize grains treated with *n*-hexane, ethyl acetate and methanol extracts respectively. Among the pure compounds, polygodial (**1**) and ugandensolide (**3**) exhibited significantly higher growth inhibition activity with percentage adult emergence of 11.4 and 12.5% respectively. Weight loss was least in the maize grains admixed with *n*-hexane and ethyl acetate extracts which had 7.4 and 13.4% weight loss respectively. For the pure compounds, polygodial (**1**) exhibited a significantly high protection with 14.8% weight loss.

The results from this study are in agreement with previous studies reported the efficacy of various plants extracts in management of grain storage insect pests [12,13]. Extract and compounds from *Warburgia* species have been reported to exhibit antifeedant and insecticidal activities [22]. Drimane sesquiterpenes which are the main compounds in *Warburgia* species have been reported to be the active principles [31,32,33]. Polygodial (1) showed antifeedant activity against the silver leaf whitefly *Bemisia tabaci* and the green peach aphid Myzus persicae [34]. It also showed insecticidal activity against the yellow fever mosquito *Aedes aegypti* [32] and black citrus aphid, *Toxoptera citricida* [35]. Ugandensidial had shown antifeedant activity against the yellow fever mosquito *Aedes aegypti* [32] while warburganal showed antifeedant activity on *Spodoptera exempta* [36]. Compounds 1-6 exhibited repellence, adult mortality and growth inhibition activities against Sitophilus zeamais [22].

Botanical insecticides affect insect physiology in many different ways and at various receptor sites. Drimane sesquiterpenes act by blocking the stimulatory effects of glucose and inositol on chemosensory receptor in cells located on the mouthparts of the insects [37].

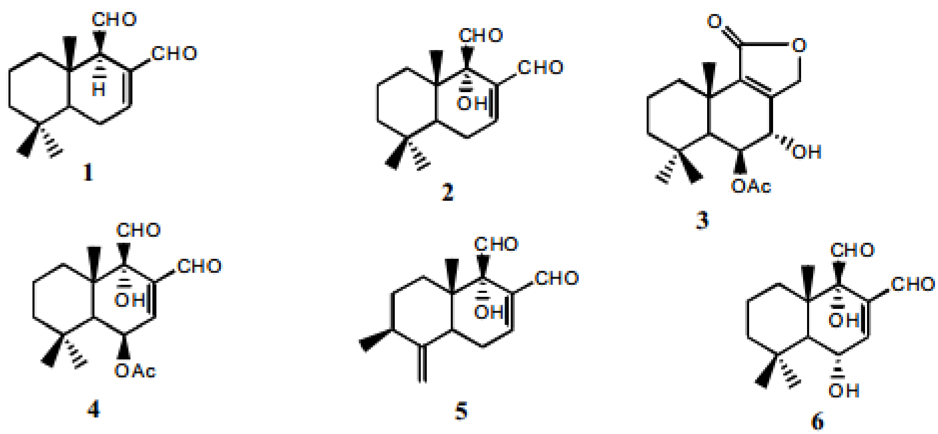

Figure 1. Structures of the tested compounds from *W. ugangensis* [22]

Table 1. Insecticidal Activities of Extracts and Compounds against *P. Truncates*

Test Materials (Powder)	Repellence*	% Mortality	% Adult Emergence	% Weight Loss
n-Hexane extract (50 mg)	5.8±0.1	76.3±2.9	15.2±0.7	7.4±0.5
Ethyl acetate extract (50 mg)	5.1±0.3	71.1±3.1	19.4±0.1	13.5±0.4
Methanol extract (50 mg)	2.3±0.5	48.4±7.2	31.1±0.4	23.1±0.3
Polygodial (**1**) (2 mg)	4.6±0.1	64.3±2.3	11.4±0.2	14.8±0.2
Warbuganal (**2**) (2 mg)	3.3±0.1	61.7±3.6	18.7±0.5	21.3±0.3
Ugandensolide (**3**) (2 mg)	2.6±0.3	41.3±5.2	12.5±0.3	19.2±0.2
Ugandensidial (**4**) (2 mg)	2.9±0.3	35.5±3.3	27.3±0.5	28.5±0.4
Muzigadial (**5**) (2 mg)	3.1±0.1	38.2±3.1	39.2±0.5	33.6±0.4
Mukaadial (**6**) (2 mg)	4.4±0.4	44.1±5.2	35.6±0.3	31.3±0.8
Actellic dust (2 mg)	4.7±0.3	100±0.0	0.0±0.0	0.0±0.0

* Repellence is distance (cm) of weevil from the center; Values are mean ± SD (n=3)

4. Conclusions

The insect repellence, mortality and growth inhibition activities tests with *W. ugandensis* have demonstrated that *n*-hexane and ethyl extract are effective in controlling larger grain borer which is one of the most important insects pets of maize grains. Among the isolated compounds, polygodial (**1**), warburganal (**2**), ugandensolide (**3**) and mukaadial (**6**) were the most potent for control of the insect.

ACKNOWLEDGEMENTS

The authors are grateful to the National Commission for Science, Technology and Innovation (NACOSTI) and Biosciences Eastern and Central Africa Network (BecANet) for financial support. We also acknowledge the assistance from Chemistry Department, Maseno University.

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