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Insecticidal Activity of *Agarista salicifolia* against *Sitophilus zeamais* (Maize weevils)

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ABSTRACT: *Sitophilus zeamais* (maize weevil) is one of the most important pests of maize that cause significant deterioration of the quality and quantity of maize in storage. Ethiopia experiences an annual loss of 64% of its maize grain yield due to weevil infestations. Synthetic organophosphorus insecticides such as acetellic and malathion are still used as the primary means of controlling maize weevils in Ethiopia. However, the bulk use of these chemicals has a significant impact on human health and the environment. Recently, employing plants and their derivatives as pesticides has gained popularity as an appealing substitute in organic farming. *Agarista salicifolia* is one of the indigenous plants of Ethiopia, the chemical and biological activities of which are not exhaustively investigated. The leaves of the plant were extracted with ethanol and subjected to bioassay against maize weevils. The result displayed the effectiveness of the plant extract in controlling the weevil (above 80% mortality rate) at concentrations of 10 mg/mL in the laboratory at 28±3°C and RH 78±3%. The crude ethanol extract was then partitioned with solvents of different polarities: hexane, $CHCl_3$, ethyl acetate, and methanol. The hexane and chloroform fraction showed 100% mortality at 10 mg/L concentration within 3 h of contact. Following the bioassay result, the hexane extract (2 g), which showed a similar chromatogram on its TLC profile to the chloroform extract, was packed with silica gel and applied to silica gel chromatography. Elution was made using pet ether and pet ether: EtOAc mixture with a gradual increase of polarity. Three triterpene compounds (Lupeol, alpha amyrin, and β-sitosterol) together with one straight-chain alkane were isolated from the active fraction. Alpha amyrin was tested for its insecticidal activity and exhibited activity with 100% mortality of test weevils at 10 mg/mL.

Keywords: *Agarista salicifolia*, Insecticidal activity, Maize weevil, α-amyrin and lupeol.

INTRODUCTION

Insect pests are the main important factors for the qualitative and quantitative loss of grains during storage. Postharvest grain losses reach up to 40%, especially in developing countries (Moreira *et al.,* 2007). As frequently indicated in Ethiopia's crop yield assessment, large economic losses are caused by insect pests (Kalsa *et al*., 2019). Insects are the largest group of animal kingdom living species on the planet. These insect pests are a problem throughout the world, especially in developing countries like Ethiopia where there are no modernized farming practices and storage devices and highly reduced quality and quantity of grains both in the pre-harvest and post-harvest situations. Most farmers don't use modern storage devices and the grains can easily be damaged by insects like weevils, grain borers, beetles, grain moths, etc. These insects represent the major insect species of economic importance (Moreira *et al*., 2007).

Maize weevil (*Sitophilus zeamais* Motschulsky*)* is one of the most important pests of maize that cause significant deterioration of the quality and quantity of maize seed in storage (Waktole Sori, 2012). Reports showed that maize crop losses due to weevil range from 41 to 80% worldwide and average grain damage of 64% is reported in Ethiopia (Marid & Md Jamshed 2021). Even though the qualitative and quantitative production of maize has increased since the development of high-yielding hybrids, these varieties are reported to be highly susceptible to insect pest attacks (Marid & Md Jamshed 2021). Particularly maize weevil is the dominant and most important postharvest insect pest in all major growing areas of maize. Due to its economic importance, management of storage pests is very critical to minimize the qualitative and quantitative loss of maize grains. Different insect pest management strategies have been implemented so far to combat the loss of grains due to storage pests among which synthetic chemical, cultural/mechanical, and botanicalcontrolling approaches are very common.

Synthetic chemicals are the conventional way to control weevils and other post-harvest insects. These insecticides are either directly applied to grains or used for gas fumigation (Prates *et al.*, 1998). Synthetic insecticides are still the principal means of controlling

pests. This is because it is easy to apply, fast-acting, and in most instances can be relied on to control the pest. However, the residual effect on food and the environment, resistance development among insects, and the broad-spectrum nature of the chemical insecticides are some of the challenges in using these chemicals.

Mechanical controlling strategies such as sieving can be very helpful in separating the contaminant or insect from the flour. The separated insects may be destroyed by milling. The other mechanical approach is using fire or solar heat energy. Solar heating of maize grain placed on a black polyethylene sheet, and covered with a translucent plastic sheet for at least five sunny days caused significant mortality of maize weevil (Demmirew *et al.*, 2018). Similarly, oven heating or using fire is also an effective method to manage weevils (Demmirew *et al*., 2018).

Botanicals are becoming attractive alternatives for the management of weevils and other storage pests. Because allelochemicals produced by the secondary metabolism of plants have been considered a valuable source of new pest-control agents with improved qualities that are relatively safe for humans and other beneficial microorganisms (Ma *et al*., 2014).

A review of the past 20 years of plant protection research in Ethiopia revealed that several botanicals have been found to display insecticidal activities (Degu *et al*., 2020). However, the reported bioactivities were mainly based on crude extracts or direct plant parts which were not standardized.Some of these botanicals have also been investigated, extensively used, and commercialized in other countries. Neem has been well investigated for insecticidal properties and its constituents are characterized and extensively used. The leaf and seed powder of *Azadirachta indica* at 2% and 3% w/w was confirmed as an effective controlling botanical agent of *S. zeamais* infesting maize. *Tanacetum parthenium*, *Syzygium aromaticum,* and *Dennettia tripetala* were also found effective against damage by *S. zeamais* grains (Nwosu, 2018). Ethiopia is endowed with a huge genetic diversity of plants and, therefore, there is a huge potential to use plants as a source of insecticides to control insects. Therefore, the main aim of this study was to evaluate the insecticidal activity of *Agarista salicifolia* (katam in Amharic) against maize weevils and to isolate and characterize the active principle from the plant.

MATERIAL ANDMETHODS

A. Description of the Study Area

Dawuro zone is one of the zones in the southwest region of Ethiopia as indicated inthe map below (Fig. 1). The zone is a naturally gifted land with diverse topography, diverse climate, and varied ecology. It is home to a wide range of fauna and flora diversity in wildlife and botanical resources. Chebera-Churichura National Park is also found in the zone and is a natural habitat for many wildlives. Tarcha is the main administrative town of the zone which is located about 507 km Southwest of Addis Ababa. Out of the five weredas of the zone (Essera, Tocha, Loma, Gena Bosa, and Mareka),the plant collection was made from Tocha Wereda, Tuta kebele of the zone with a specific location at La 7° 5′7.19″ Lo 36° 59′51.2″ A 2532 m.

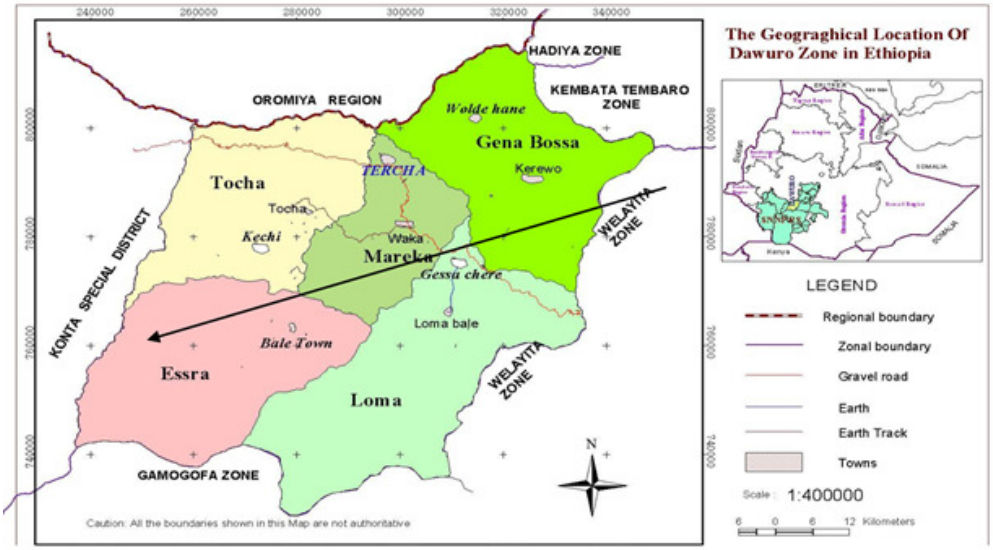


Fig. 1. Location of the study area in Dawuro Zone and the sampling site.

Extraction, purification, and structural elucidation of the insecticidal compounds were conducted at Addis Ababa Science and Technology University (ASTU). Bioactivity tests and formulation development were done at the Ethiopian Biotechnology Institute (EBTi).

B. Sample Collection

(i) Botanical samples collection. *Agarista salicifolia* Hook. f. (family of Ericaceae) (Fig. 2) is a shrub or small tree up to 8 m tall and found on steep, rocky slopes. This plant has wide biological activities like insecticidal (Haile, 2015), and bactericidal (Yemata & Fetene 2016).

The local people in Dawuro zone, Ethiopia, largely use *A. salicifolia* leaves as postharvest insect replants to prevent their grain damage during storage. Their strong recommendation to scientifically investigate the insecticidal activity of *A. salicifolia* against postharvest insects, its wide biological activity literature profile, and its abundant accessibility led us to investigate the insecticidal activity of the plant against maize weevils.

Agarista salicifolia (Comm. ex Lam.) Hook. f. leaf sample collection was done with responsibility for the accomplishment of the research in keeping with (IUCN, 1989) guidelines Version 1:4b. Botanical identification of the plant was undertaken using Ethiopian Flora book from volume 4-part 1 page 48 and by comparison with authenticated voucher specimens at the National Herbarium of Ethiopia (Addis Ababa University) and further confirmed by a senior taxonomic expert there. Finally, the identified sample voucher specimen with its number and labels (MA0124) was deposited there at the National Herbarium of Ethiopia and the Mini-Herbarium of Dawuro Historical and Cultural Museum.

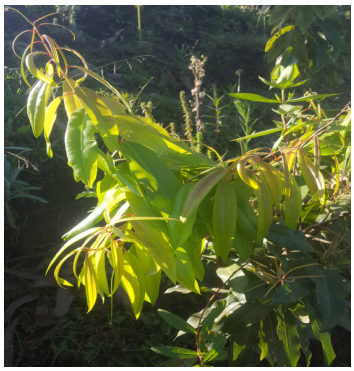


Fig. 2. *A. salicifolia* plant (Dawuro zone, Ethiopia).

(ii) Collecting and rearing of insects' understudy. Maize weevils (*Sitophilus zeamais*) were collected from the Bako Agricultural Research Center and reared at EBTi. Nurturing was made in their appropriate host grain and placed until used. This was used as a stock of insects during our study.

C. Preparation of botanicals for extraction

The preparation and extraction of the plant are done in a similar protocol to Zelalem follows in his dissertation (Gizachew, 2019). The leaves of *A. salicifolia* were air-dried for a week at room temperature and ground into a fine powder, then labeled and put into airtight plastic bags until used.Extraction of plant samples was done using cold extraction procedures (maceration). Powder of the plant (50 g) was soaked in a 500 mL Erlenmeyer flask containing ethanol (300 mL) for a duration of 72 h with occasional shaking. The extract was filtered using Whatman filter paper No.1 linked with a Buchner funnel. The solvent was removed by rotary evaporator under reduced pressure to obtain the crude extract (8 g) and the resulting crude extract was subjected to bioassay for its insecticidal activities against the target insect. Based on the biological activity result, the active crude ethanol extract was further partitioned with solvents of different polarities (hexane, $CHCl_3$, EtOAc, and MeOH) using vacuum liquid chromatography (VLC) (Khan *et al.*, 2017). Each solvent fraction was concentrated and subjected to bioassay.

D. Bioassay procedures

A laboratory bioassay was conducted to determine the mortality of insects under study. The mortality rate of insects is the main detrimental factor for the evaluation of the botanical understudy for its insecticidal activity. Insects were reared in the laboratory at 25°C and 75% humidity. The maize grains were washed with tap water, disinfected with 5% sodium hypochlorite, and allowed to dry. It was then used as a food medium for the bioassay. Under laboratory conditions, extracts (100 mg) were dissolved in 10 mL ethanol to prepare 10mg/mL test solution. Maize (6 g) was weighed and applied to Petri dishes. The maize was then mixed well with 1 mL test solution until all the surfaces of the grain were contaminated. Untreated maize grains were used as a negative control. Both the poisoned and untreated grains were allowed to air dry. After drying, 10 adults of maize weevils were added to their respective poisoned grain-containing Petri dishes (5 cm diameter). Each experiment was done in triplicate. The treated Petri-dishes having insects were kept at room temperature 25±0.5°C in a secured place with 75±5% relative humidity(RH) and 12 h photo phase (Moreira *et al.*, 2007). Insect mortality was recorded from 3 h of application time to 36 h after exposure to the poisoned maize and finally, the mortality rate was recorded based on the standard formula below and the result was analyzed with SAS. Insects were considered dead if they did not respond to probing by a blunt probe.

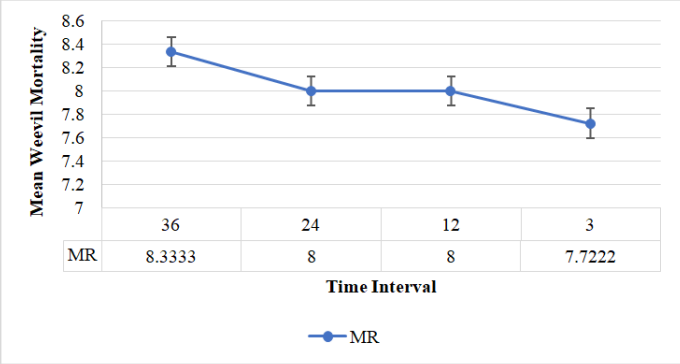
$$\text{Mortality rate} = 1 - \frac{\text{No of survivals in sample}}{\text{No of survivals in control}} * 100 \quad \text{.....Eq.1}$$

Statistical analysis. Data were analyzed by two-way analysis of variance (ANOVA) of SAS version 9.2 (Institute Inc., Cary, NC) and mean separation was done using LSD when significant differences were found at $P < 0.05$.

RESULTS AND DISCUSSION

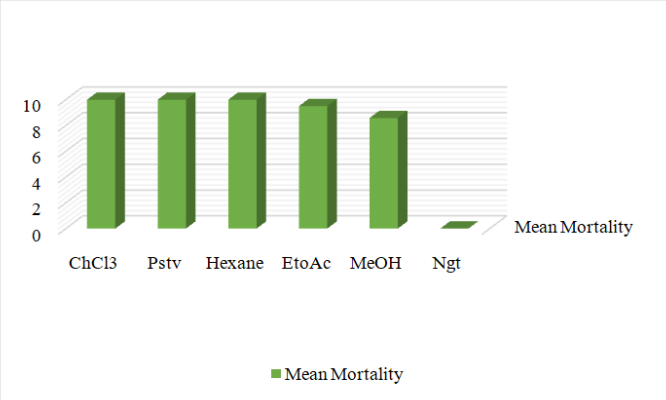
Ethanol extracts of *A. salicifolia* (10 mg/mL) showed an average mortality of 80% adult test weevils per Petri dish after 3 h of application (Fig. 3). The mortality rate of weevils increased as the application time interval increased from 3 to 36 hour. The mortality rate (90%) was recorded at 36 h time intervals with the application of ethanol extract. Malathion (5%), which was used as a positive control, showed 100% mortality of insects after 12 h contact time. Acetone, which was used as a negative control (vehicle), showed no effect on weevils. The hexane and $CHCl_3$ fractions of ethanol extracts of *A. salicifolia* exhibited the highest activity with a 100% mortality rate at concentrations of 10 mg/mL after 3 h

application time. Data were analyzed by two-way analysis of variance (ANOVA) of SAS version 9.2 (Institute Inc., Cary, NC), and mean separation was done using LSD when significant differences were found at $P < 0.05$. The toxicity of methanol extracts of *A. salicifolia* increased when the application time increased.



Means of the same letter are not significantly different

Fig. 3. The effect of ethanol extract of *A. salicifolia* leaves on mortality of maize weevils at 10 mg/mL concentration.



means of the same letter are not significantly different

Fig. 4. Average mortality rates of hexane, $CHCl_3$, EtOAc, and MeOH soluble portions of ethanol extracts of *A. Salicifolia.*

A. Bioassay-guided isolation of active compounds from A. salicifolia

Since the hexane partitioned part was active with a 100% mortality rate, two grams were adsorbed on silica gel (3 g) and applied over silica gel column chromatography packed with petroleum ether. Elution started with hexane (100%) and continued by increasing polarity slightly using the addition of ethyl acetate with the ratio of hexane: ethyl acetate (95:5, 90:10, 80:20, 70:30, and 60:40) and a total of 30 fractions were collected. These fractions were combined into 6 subfractions (fr1 180 mg, fr2 220 mg, fr3 70 mg, fr4 115 mg, fr5 125 mg, and fr6 175 mg) based on their TLC profile. The TLC analysis of the first fraction (Fr 1, 180 mg) displayed a single violet spot (Fig. 5) and this was submitted to NMR analysis. The compound was identified as straight-chain alkane which is labeled as compound 1.

The fifth fraction collected from the CC using hexane as eluent solvent was allowed to stay overnight on the bench. A white powder (25 mg) was precipitated out from the fifth fraction (fr5, 125 mg). TLC was developed for fraction 5 using hexane: EtOAc (4:1) solvent system and vanillin in H_2SO_4 as spraying agent and a single violet spot (RF 0.6) was observed as is shown below (Fig. 5). Based on the TLC evidence, fraction 5 was submitted to NMR analysis and identified to be a mixture of two triterpenes which are labeled as compounds **2** and **3**. The attempt to separate these mixtures of compounds was not successful due to similar RF values on its TLC development using different polarity combinations of the solvent system. These mixed compounds were isolated as a white solid (25 mg) from column chromatography using hexane as eluent.

The six fractions were dissolved in hexane: ethyl acetate (1:1) and applied on a small column packed with the same solvent ratio. Elution was made with solvent systems of hexane: ethyl acetate (1:1, 4:6, and 3:7). 16 fractions were collected and grouped into 5 parts following the TLC Profile. The second and third parts showed 1 major spot. These two parts were combined and weighted to yield 104 mg and adsorbed on silica gel for further purification. The adsorbed sample was applied to a column packed with PE. Elution made using solvent system PE: EtOAc (100%,

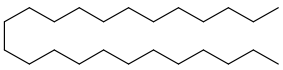
99:1, 95:5, 90:10). three combined fractions were collected and the second part was concentrated to afford 50 mg white powder labeled as compound **4**. The characterization of all isolated compounds follows the writing style of Zelalem Gizachew (Gizachew, 2019).

Characterization of Compound 1. The TLC profile of compound **1** was studied using a hexane: ethyl acetate (8:2) solvent system and vanillin in H_2SO_4 as a spraying agent. A single violet chromatogram (RF 0.8) was observed.

The ^{1}H-NMR spectrum of compound **1** showed a broad singlet between δ_H 1.27-1.33 which was due to many overlapping methylene protons (22 CH_2), which was also supported by the presence of an intense carbon signal in the ^{13}C-NMR spectrum at δ_C 29.75 and other signals at δ_C 22.79, 29.73, 31.63 and 31.97. All the five-carbon signals mentioned above are suggested to be methylene carbon signals from their direction in the DEPT-135 spectrum, all are pointing downward direction (negative signals). The more upfield multiplet signal in the ^{1}H-NMR spectrum at δ_H 0.91 was assigned to the terminal methyl group which was confirmed by the presence of carbon signal at δ_C 14.14 in ^{13}C-NMR and DEPT-135 spectra. The number of methylene groups was estimated by integrating each signal concerning the methyl carbons and the ^{1}H-NMR and ^{13}C-NMR spectra of compound **1** were in agreement with the literature value for tetracosane (Table 1) (Yamaji T, Saito T, Hayamizu K, Yanagisawa M, Yamamoto O(2010). ^{1}H and ^{13}C NMR spectra of tetracosane). This estimation has to be supported with MS spectroscopy to confirm whether compound **1** is tetracosane or not.

Table 1: 1H- and ^{13}C- NMR data comparison of compound 1 withliterature report for tetracosane.

Experimental Result of compound 1		Literature Report	
δ_H, mult	**δ_C (Position)**	**δ_H, mult**	**δ_C (Position)**
0.91 (m, 6H, H-1 & H-26)	14.14 (**C-1 & C-26**)	0.90 (m, 6H, H-1 & H-26)	14.1 (**C-1 & C-26**)
1.27-1.33 (m, 22H, H-2 to H-25)	22.79 (**C-2 & 25**)	1.26 (br s, 56H, H-2 to H-29)	22.76 (**C-2 & 25**)
	31.97 (**C-3 & C-24**)		32.02 (**C-3 & C-24**)
	31.63 (**C-4 & C-23**)		29.46 (**C-4 & C-23**)
	29.71 (**C-5 & C-22**)		29.61 (C-5 &C-22)
	29.75 (**C-6 to C-21**)		29.79 (**C-5 to C-22**)



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Characterization of compounds 2 and 3. The TLC profile of compound **1** was studied using a hexane: ethyl acetate (4:1) solvent system and vanillin in H_2SO_4 as a spraying agent. A single violet chromatogram with an RF value of 0.6 was observed as depicted below (Fig. 5).

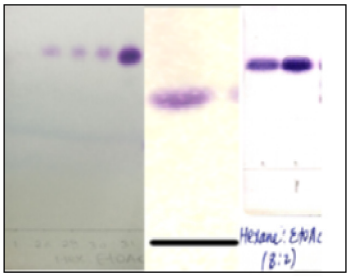


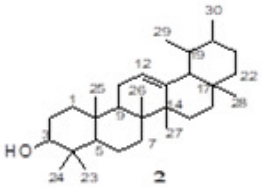
Fig. 5. The picture showed the TLC profile of compounds 1. 2 & 3 and 4 from left to right.

The IR spectrum of compounds **2** and **3** displayed an absorption band at 3386 cm^{-1} for O-H; a band at 3038 cm^{-1} for olefinic =C-H; a band at 1616 cm^{-1} for unconjugated olefinic C=C; a band at 2946 cm^{-1} and 2865 cm^{-1} for aliphatic -C-H.

The ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum displayed the most downfield signal appearing at δ 5.1 (1H: *t, J* = 3.2) is assigned for an olefinic methine proton (C-12). The other downfield signals appeared at δ 4.7 (1H: *d*) and δ 4.58 (1H, *t*) were assigned for the terminal exocyclic olefinic protons. The ^{1}H-NMR spectrum also exhibited signals at δ 3.2 (2H, *m*) which are assigned for two oxygenated protons. The ^{13}C-NMR (400MHz, $CDCl_3$) displayed downfield signals at δ 150.9 ppm and at δ 109.0 ppm which is a characteristic signal for lupine derivatives (compound **3**). This agrees with the ^{1}H-NMR spectrum signal that appeared at δ 4.7 (1H: *d*) and δ 4.58 (1H, *t*). The ^{13}C-NMR spectrum exhibited signals at δ 124.4 and 139.6 is evident for the presence of olefinic carbons which are assigned for C12 and C13 of compound **2**. The structures of compounds **2** and **3** were elucidated by comparing ^{1}H and ^{13}C-NMR data with literature values of similar reports (Table 2). The NMR data of compound **2** is in close agreement with the reported data for α-amyrin ((Vázquez *et al.*, 2011), and similarly, compound **3** agrees with the reported values of Lupeol (Srikanth *et al.*, 2015).

Table 2: ^{13}C NMR chemical shift values and literature report for α-amyrin and Lupeol.

Sr. No.	Experimental Results of compound 2&3				Literature Report (Srikanth *et al.*, 2015 ; Vázquez *et al.*, 2011)			
	^{13}C of 2	^{1}H of 2	^{13}C of 3	^{1}H of 3	amyrin	^{1}H amyrin	lupeol	^{1}H lupeol
1.	38.8		38.87		38.7		38.7	
2.	27.3		27.45		27.2		27.4	
3.	79.0	3.2 (1H)	78.99	3.2 (1H)	78.3	3.2 (1H)	79.0	3.2 (1H)
4.	38.9		38.71		38.7		38.7	
5.	55.2		55.30		55.2		55.3	
6.	18. 4		18.02		18.3		18.3	
7.	32.9		34.28		32.9		34.3	
8.	40.0		40.82		40.9		40.8	
9.	47.7		50.43		47.7		50.4	
10.	37.1		37.16		36.9		37.1	
11.	23.3		20.94		23.3		20.9	
12.	124.4	5.1 (1H)	25.13		124.3	5.2 (1H)	25.1	
13.	139.6		38.05		139.3		38.0	
14.	42.0		42.83		42.0		42.8	
15.	28.8		27.45		28.7		27.4	
16.	26.6		35.59		26.6		35.6	
17.	33.8		43.01		33.7		43.0	
18.	59.0		48.30		58.9		48.3	
19.	39.7		47.99		39.6		48.0	
20.	39.6		150.96		39.6		150.9	
21.	31.2		29.85		31.2		29.8	
22.	41.5		40.01		41.5		40.0	
23.	28.1		28.01		28.1		28.0	
24.	15.7		15.40		15.6		15.3	
25.	15.7		16.14		15.6		16.1	
26.	16.9		15.99		16.8		16.0	
27.	23.4		14.59		23.3		14.5	
28.	28.1		18.02		28.1		18.0	
29.	17.5		109.35	4.5 (1H), 4.7 (1H)	17.4		109.3	4.5 (2H)
30.	21.4		20.00		21.3		20.94	



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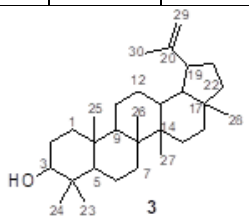

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α-Amyrin and lupeol were previously reported from different plant sources and used for various biological activities like exhibiting insecticidal, antimicrobial, and anti-inflammatory activities (Victor *et al*., 2017; Viet *et al.,* 2021).

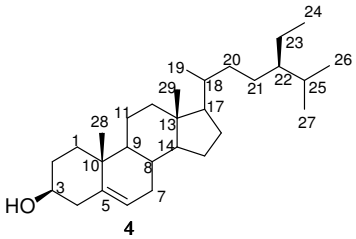
Compound 4. Compound **4** was obtained as a white solid recrystallized material (50 mg) from the six fractions of hexane extract. The compound melts at 133-136^{o}C. The TLC (Rf 0.53) developed using n-hexane and ethyl acetate (1:1) as a mobile phase was visualized after spraying with vanillin in H_2SO_4. The most downfield signal in the ^{1}H-NMR spectrum at δ 5.36 is accounted to one olefinic proton. On the other hand, the signal resonating at δ 3.53 is attributed to the presence of a proton on carbon-bearing oxygen as a multiplet for a proton corresponding to the proton connected to the C-3 hydroxyl group. The ^{1}H-NMR spectrum revealed the presence of 6 methyl groups of which three are methyl singlets at δ 0.82 (3H, *s*), 0.70 (3H, *s*), 1.03 (3H, *s*), and the other two are observed as methyl doublets at δ 0.86 (3H, *d*) and 0.93 (3H, *d*) and the remaining is methyl triplet at 0.84 (3H, *t*).

The ^{13}C-NMR and DEPT-135 spectra of compound **4** revealed the presence of 28 well-resolved signals and one overlapped signal a total of 29 signals for 29 carbon atoms. The signals include six methyls, eleven methylenes, nine methines, and three quaternaries. The ^{13}C-NMR spectrum showed downfield signals at δ 140.7 and 121.7 for olefinic carbons. In the ^{13}C-NMR, one methine-oxygenated signal was observed at δ 71.8. ^{1}H and ^{13}C-NMR data generated for compound **4** were consistent with the very well-known β-sitosterol. The generated data and literature report is shown below.

Table 3: ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) spectral data ($CDCl_3$) of compound 4 and NMR data (600 MHz, $CDCl_3$) reported for *β*-sitosterol (Ododo *et al.*, 2016).

^{13}C &^{1}H-NMR data of compound 4			Lit. value	^{13}C&	^{1}H-NMR data of Compound4		Lit. value
1	37.2		37.5	16	28.2		28.5
2	31.6		31.9	17	56.0		56.3
3	71.8	3.53 (1H, *m*)	72.0	18	36.1		36.3
4	42.3		42.5	19	19.4	0.93 (3H, *d*, 6.8)	19.2
5	140.7		140.9	20	33.5		34.2
6	121.7	5.37 (1H, *t*)	121.9	21	24.3		26.3
7	31.9		32.1	22	45.8		46.1
8	31.9		32.1	23	23.0		23.3
9	50.1		50.3	24	12.0	0.84 (3H, *t*, 7.2)	12.2
10	36.5		36.7	25	29.1		29.4
11	21.1		21.3	26	19.8	0.84 (3H, *d*, 6.8)	20.1
12	39.7		39.9	27	19.0	0.82 (3H, *d*, 6.8)	19.1
13	42.3		42.6	28	18.8	0.70 (3H, *s*)	19.0
14	56.7		56.9	29	11.8	1.03 (3H, *s*)	12.0
15	26.0		26.3				

Chemical shifts are reported in parts per million The data generated from the characterization of compound **4** agreed well with the literature report on *β*-sitosterol. Even though there is no former report for the isolation of *β*-sitosterol from leaves of *A. salicifolia*, it was previously reported from other different plant sources and is known for its anti-bacterial (Ododo *et al.*, 2016), anti-cancer (Novotny *et al.*, 2017), anti-inflammatory (Prieto and Recio 2006) activities.



4

B. Bioassay results of isolated compounds

Mixture compounds of α-amyrin and lupeol, β-sitosterol, tetracosane, hexane, and $CHCl_3$ extracts were tested against maize weevils. Each was dissolved in acetone and prepared as a 10 mg/mL concentrated ($CDCl_3$), ***J*** values are in Hertz. solution. It was then applied to Petri dishes containing 50 g of maize thoroughly mixed and allowed to stay open for an hour until the solvent (acetone) dried. Adult weevils (10) were added to each test Petri dish. The treatments were replicated three times.. The two mixture compounds (α-amyrin and lupeol) exhibited 100% mortality of insects after 12 h application time as depicted below. This activity might be the synergistic effect of the two compounds. Similarly, the hexane and $CHCl_3$ extracts showed 100% mortality.β-sitosterol showed 80% mortality after three hours of application time and there was no significant result difference with different time intervals. Tetracosane didn't show any significant biological activity against test weevils. Acetone was used as a negative control. Malathion 0.5 mg / G (W/W) was used as a positive control. There was no mortality recorded on the negative control but the positive control malathion killed all test insects. Generally, the nonpolar extracts of *A. salicifolia* exhibited interesting insecticidal activity against maize weevils. So, it is an attractive alternative for the management of weevils in household stores.

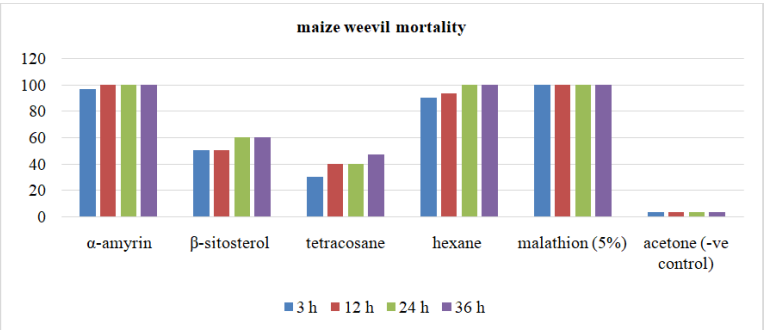


Fig. 6. Bioassay results of isolated compounds against maize weevil.

CONCLUSIONS

The ethanol extract of leaves of *A. salicifolia* exhibited promising insecticidal activity (above 80%) against maize weevils. Our bioassay-guided study confirmed that the nonpolar extracts of *A. salicifolia* leaves showed 100% weevil mortality which can be used as an alternative pest management strategy for smallholder farmers. Following the bioassay-guided fractionation, four compounds (tetracosane, α-amyrin, lupeol, and β-sitosterol) were isolated from the hexane fraction. Even

though α-amyrin and lupeol were isolated as a mixture, these compounds showed 100% mortality in maize weevils at 10 mg/mL concentration.

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