INSECTICIDAL ACTIVITY OF ACETYL MORALDEHYDE AND AGAURIASTERONE FROM *AGAURIA SALICIFOLIA*.

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ABSTRACT. Bioassay guided chromatographic separation of a methanolic extract of *Agauria salicifollia* led to to the isolation of Acetylmoraldehyde (**1**) and Aguariasterone (**2**), whose structures were elucidated using UV, IR, NMR and melting points. Insecticidal activity was done using adult *Phaedon cochleariae* (Mustard beetle) at 1, 0.1, 0.01% concentration of 1.00 µg dose per insect of the compound. The compound **1** and **2** were found to be active with LD_{50} values of 0.04 and 0.15 µg/insect respectively.

KEY WORDS: *Aguaria salicifolia, Phaedon conhleariae,* Acetylmoraldhehyde,Agauriasterone.

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INTRODUCTION

Pests despite enomous expenditure on agrochemicals reportedly destroy approximately one third of the global food production each year, hence to protect our food supply from pests is a major worldwide problem [1]. To prevent economically unacceptable losses of yield and quality of agricultural crops caused by plant micro-organisms, especially insects and sometimes nematodes, snails and slugs, protection of crops with agrochemicals is necessary [2].

Currently synthetic pesticides are losing their effectiveness and to date, hundreds of pest species have developed resistance to at least one pesticide formula and a dozen or so species are immune to them all. Furthermore synthetic pesticides are often very persistent in soil and water (especially halogenated hydrocarbons) and can accumulate in the food chain hence threatening the health of entire ecosystem [3].

According to World Health Organization estimates, up to 20,000 people die of pesticides poisoning in the third world countries each year. This has sparked growing public concerns of undesirable effects of pesticides on human health and environment. These as led to the withdrawals of commercial pesticides, increasing the vulnerability of crops to pets and diseases [4]. Therefore there is need to develop alternative environmentally friendly, toxicologically safe and effective pesticides for the control of food pesticides. One way of developing safe insecticides is by screening the traditional botanical

pesticides. As part of on –going research on new insecticides, *Agauria salicifolia* (Ericaceae) was screened for insecticidal activity. The plant is known to be toxic to herbivores especially the goats. The roots and the bark of the stem are used to cure several ailments including snake bites, skin diseases, added to bait to kill rats and various ethnomedical uses in most of the African countries [5 and 6].

This study led to the isolation of Acetylmoraldehyde (**1**) and Aguariasterone (**2**) that were investigated for insecticidal activity against adult *Phaedon cochlearia*

EXPERIMENTAL

The melting points were determined using an electrothermal melting point apparatus with a thermometer range of 0 – 360 ^{0}C and was uncorrected. Infrared spectrums were run on a Nicolet Impact 410 FT-IR spectrometer with the compound in chloroform. ^{1}H and ^{13}C NMR analysis were done using a JOEL DRX500 (500-125.75 MHz) NMR spectrometer. The solvent used was deuteriated chloroform with TMS as the internal standard. Ultraviolet spectrums were run using a Shimadzu UV-160A UV-VIS spectrometer. The solvent used was chloroform. Electron impact mass measurements were recorded on a UG Autospec mass spectrometer at 70 ev. Chromatographic analysis done were HPLC using Gilson equipment and Peaks monitored using Holochrome UV detector set at 290 nm, analytical HPLC was done using synergi 4µ Hydro – RP 80A column (size 150 mm by 4.6 mm), Semi-preparative HPLC was done using Hichron KR 100 5C18 – 25074 column. Potentiometric chart recorder operating at 10 mV at a speed of 5 mm/sec. Dry column chromatography was carried out using 5-40 µm silica gel and flash chromatography was done using 220-440 mesh flash silica gel.

Phaedon coehleariae (mustard beetle) were used for insecticidal activity. The stem bark of *Agauria salicifolia* was collected from Uasin-Gishu district, 320 Km West of Nairobi, Kenya. The staff of Botany Department Herbarium, Moi University, where a voucher specimen was deposited, identified the plant.

Solvent extraction and chromatography.

The stem bark of the plant were chopped into small pieces, air dried at room temperature for three weeks .Then ground into a powder .The powder (1 kg)was soaked in cold methanol for 3 days to extract the compounds. The resulting brown filtrate was concentrated under reduced pressure using a rotatory evaporator to a dark brown semi-solid, 5 g of which was subjected to solvent partitioning using petroleum ether, ethyl acetate and methanol to obtain three solvent extracts and were subjected to insecticidal test. The concentrate of the insecticidal active petroleum ether fraction (500 mg) was chromatographed over silica gel, eluting under pressure with increasing amounts of ethyl acetate in petroleum ether. The eluted fractions were monitored by thin layer chromatography using petroleum ether and ethyl acetate (6:4) as developing solvent. Four fractions were obtained. The four fractions were subjected to insecticidal test and the most active fraction 2 (10 ml) was concentrated and further purified by flash

chromatography using increasing amount of dichloromethane in petroleum ether as eluting solvent. This lead to the isolation of **1** and **2,** which were re-crystallized using 20% of petroleum ether in dichloromethane

Insecticidal test

Insecticidal tests were done using adult Mustard beetle *(Phaedon cochleariae)*. The beetles were held on a sticky pad and 1 µg dose of the compounds was applied to underside using an Arnold micro-applicator. By varying the concentrations of the dose applied to 0.01, 0.1, and 1 % of 1µg/insects, the treatment was done in batches of 10 with 2 batches per concentrations. The treated beetles were kept in plastic Petri-dishes at 20 ^{0}C, and assessed for mortality after 48 hours.
The numbers of dead beetles were recorded and the mean mortality (%) for both **1** and **2** were calculated. LD_{50} values were obtained by data analysis using Polo orbit program.

RESULTS AND DISCUSSION

Structural elucidication

Acetyl moraldehyde (**1**) was isolated as clear needles, M.P. 275 ^{0}C with positive purple colouration with phosphomolybdic acid test for terpenes.
IR spectroscopic analysis showed absorptions at: 1730 cm^{-1} (aldehyde group), 1725 cm^{-1} (acetate group), 1620 and 830 cm^{-1} (olefenic group).
^{1}H- NMR spectroscopic analysis showed absorptions at: δ 9.4 ppm (aldehyde hydrogen), δ 1.0 – 0.7 ppm (six singlets for the seven methyl groups), δ 5.30 ppm (olefinic proton at C-19), δ 4.48 ppm (multiplet due to one proton)
^{13}C-NMR spectroscopic analysis showed absorptions that conformed to the reported values of similar compounds reported in literature [7].
EI Mass spectrum confirmed the molecular mass, It showed molecular ion at 482.6 (482.72 calculated) in accordance with the formula $C_{32}H_{50}O_3$.

Agauriasterone (**2**) was isolated as clear needles, M.P. 119 ^{0}C with positive purple colouration with phosphomolybdic acid test for terpenes.
UV spectroscopic analysis showed a peak at 246 nm (maximum).
IR spectroscopic analysis showed absorptions at: 1730 cm^{-1} (strong ketone absorption), 1620 and 830 cm^{-1} (double bond)
^{1}H-NMR spectroscopic analysis showed absorptions at: δ 5.01 (t, J = 6 Hz, one olefin proton), δ 2.01 – 2.5 (multiplets for 3 protons, CH_2-CO-CH), δ 0.73, 0.88, 1.20, 1.62, 1.6 (singlets), δ 0.93 (J = 6.4 Hz, two doublets), δ 1.02 (J = 6.5 Hz, doublets for the seven methyl groups).
^{13}C-NMR spectroscopic analysis showed absorptions at: δ 213.66 (for C=O, C3), δ 130.96 (for two C=C, C8), δ 132.35 (C9) δ 125.15 (C24) and δ 135.54 (C25). These data

observation conformed to Agauriasterone B, a tetranortriterpene with lanostane skeletone which has been isolated from this plant [8].
EI Mass spectrum confirmed the molecular mass, it showed molecular ion at 410.6 in agreement with the formula $C_{29}H_{46}O$ (calculated 410.686)

Insecticidal test

The crude extract from which Acetylaldehyde (**1**) and Agauriasterone (**2**) was isolated had a mortality of 90% at 1 % concentration. The compound **1** and **2** had LD_{50} of 0.04 and 0.15 µg/insect. Their insecticidal activity is closer to the standard Rotenone with LD_{50} 0.015 µg/insect.
Compound **1** and **2** have great potential to be the base for exploiting natural insecticides since they contain steroidal triterpenes. It is supported by the fact that insects are unable to biosynthesize the steroidal skeleton. Steroids are required in their diet for their normal development and productivity [9]; therefore triterpenic compounds can acts as false steroids hence killing the insect.

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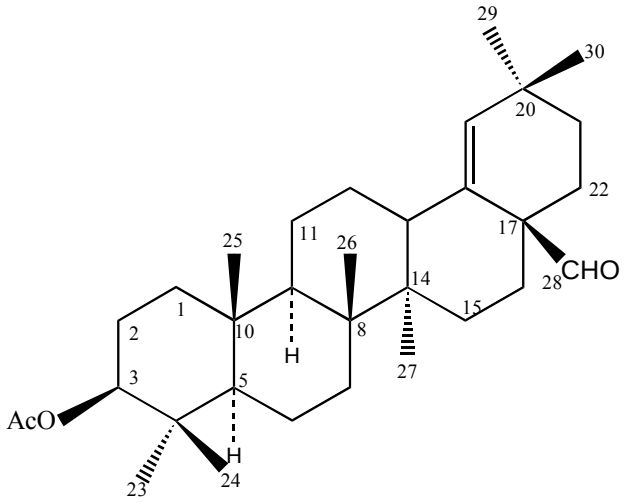
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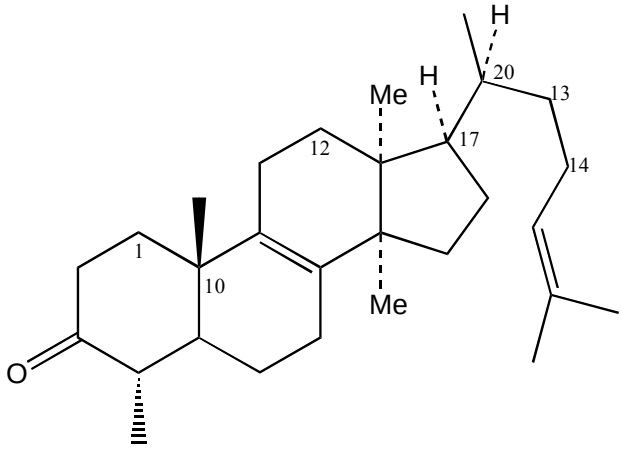
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