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ACTIVITY OF THE COMPOUNDS ISOLATED FROM *BLIGHIA SAPIDA* (SAPINDACEAE) STEM BARK AGAINST *AEDES AEGYPTI* LARVAE

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ABSTRACT

Blighia sapida (Sapindaceae) is commonly known as Ackee and in the South West Nigeria it is called Isin. The wood is resistant to termites and the pounded fruit and the stem bark is used as fish poison. The methanol extract of the stem bark reported to be active against the fourth instar larvae of *Aedes aegypti* was suspended in water and partitioned into *n*-hexane, dichloromethane and ethyl acetate. The most active *n*-hexane fraction with activity that was statistically insignificant to that of the methanolic extract gave LC_{50}, LC_{90} values of 1.81 ± 0.02, 3.17 ± 0.02 mg/mL at 24 h and 1.65 ± 0.05, 2.82 ± 0.35 mg/mL at 48 h. Endosulphan the positive control, had LC_{50}, LC_{90} values of 0.93 ± 0.06, 1.61 ± 0.12 mg/mL at 24 h and 0.90 ± 0.09, 1.44 ± 0.11 mg/mL at 48 h. The *n*-hexane fraction was subjected to chromatographic separations that led to the isolation of friedelin and α-amyrin. Friedelin and α-amyrin gave $LC_{50,}$ LC_{90} 0.07 ± 0.01, 0.13 ± 0.01 mg/mL and 0.05 ± 0.00, 0.08 ± 0.01 mg/mL respectively at 24 h and at 48 h there was 100% mortality. The activity of both compounds was statistically higher than that of Endosulphan.

Keywords: *Blighia sapida*, extract, chromatography, friedelin, α-amyrin, *Aedes aegypti*.

INTRODUCTION

Blighia sapida (Sapindaceae) is commonly known as Ackee and in the South West Nigeria it is called Isin. It is an evergreen tree growing to a height of 10 to12 meters at maturity. Its fruit is relished by humans (Ubulom *et al.,* 2012), the wood is known for its termite resistance and the pounded fruit and the stem bark is used as fish poison (Kayode, 2006). It is used to treat oedema, fever, intercostal pain, dysentery and diarrhea, migraine, yaws, ulcers, yellow fever and epilepsy (Asamoah *et al.,* 2010). The hypoglycemic, antidiarrheal and nutritional potential (Abolaji *et al*., 2007; Antwi *et al*., 2009) have been reported. It is well known for its poisonous unripe aril that contains hypoglycin A, a water-soluble liver toxin that inhibits gluconeogenesis thus leading to hypoglycemia. The activity of the aqueous, ethanol and ethylacetate extracts of the leaf against *Anopheles gambiae, Culex quinquefasciatus* and *Aedes aegypti* larvae had been reported (Ubulom *et al.,* 2012). The methanol extract of the stem bark was reported to be highly active against the fourth instar larvae of *Aedes aegypti* (Adebajo *et al.,* 2012). This work reports the activity-directed purification of the methanol extract of *B. sapida* stem bark which led to the isolation of two terpenoid compounds from the active fractions.

MATERIALS AND METHODS

Extraction and Isolation

The stem bark collected in Shaga via Ile-Ife, Osun State, Nigeria was authenticated and a voucher specimen was deposited at the Ife Herbarium, Department of Botany, Obafemi Awolowo University Ile-Ife, Osun State, Nigeria with reference number IFE 17248. The stem bark was cut into small pieces, oven-dried at 40 °C and milled in a commercial blender. The powder (5.00 kg) was macerated in methanol for 72 h with constant shaking. The extract was filtered and concentrated *in vacuo* at 35 °C. The procedure was repeated twice. The combined extract (170.00 g) was suspended in water, successively partitioned and concentrated *in vacuo* to give *n*-hexane (BSB_1), dichloromethane (BSB_2), ethyl acetate (BSB_3) and aqueous (BSB_4) fractions. Each fraction was tested for activity against *A. aegypti* larvae. The most active *n*-hexane fraction (37.58 g) was separated on a silica gel column (70-230 mesh, 350 g), eluted with gradient solvent systems from Hex 100%, Hex-DCM, DCM-EtOAc to EtOAc-MeOH (25:75) to give eight bulked fractions (A–H). The third bulked fraction C (9.90 g) that was eluted with gradient solvent systems from Hex-DCM 75:25 to DCM-EtOAc 75:25 was further subjected to column chromatographic separations

that yielded compounds **1** (140mg) and **2** (125mg).

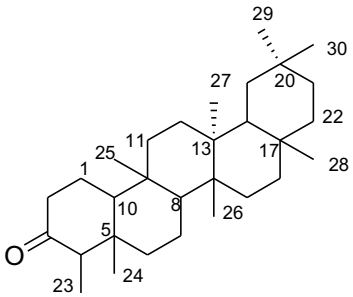
Larvicidal Assay

The larvicidal assay of the extract, partitioned and column fractions and the isolated compounds were carried out according to World Health Organisation, 2005 guidelines with slight modifications (Adebajo *et al.,* 2012). The partitioned fractions, column fractions and isolated compounds were initially dissolved in Tween 80 at a concentration of 0.2 % (v/v). Twenty five larvae were introduced into each assay cup. Each concentration was replicated six times. Endosulphan, a commercial insecticide and Tween 80 were used as the positive and negative controls respectively. The data was subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Student Newmann Keul's post hoc's test.

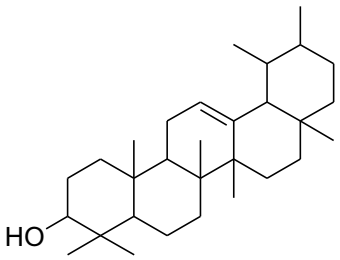
RESULTS

Compound **1** was isolated from the fraction eluted with n-hex- DCM 25:75. It is a white needle-like solid (m. p. 264 – 265°C). The ^{13}C-NMR (75 MHz, $CDCl_3$) spectrum gave signals for thirty carbon atoms and the signal at 213.1 ppm is characteristic of a carbonyl of ketone. The ^{13}C and DEPT analysis showed eight methyl, eleven methylene, four methine and seven quaternary carbon atoms which is consistent with the molecular formula, $C_{30}H_{50}O$. The ^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of the compound showed signals for eight methyl groups at 0.76 (3H, s), 0.91 (3H, s), 0.93 (3H, s), 0.99 (3H, s), 1.04 (6H, s), 1.09 (3H, s) and 1.22 (3H, s). The ^{13}C-NMR (75 MHz, $CDCl_3$) spectrum of the compound showed signals for thirty carbon atoms at 6.8, 14.7, 17.9, 18.3, 18.7, 20.3, 22.3, 28.2, 30.0, 30.5, 31.8, 32.1, 32.5, 32.8, 35.0, 35.4, 35.7, 36.1, 37.5, 38.3, 39.3, 39.7, 41.3, 41.5, 42.2, 42.8, 53.1, 58.3, 59.5 and 213.1. The spectroscopic data compares very well with those of friedelin in literature (Queiroga *et al.,* 2000). It has been previously isolated from *Maytenus ilicifolia* (Queiroga *et al.,* 2000), *Maytenus robusta* (Sousa *et al.,* 2012), *Terminalia avicennioides* (Mann et al., 2011), *Synadenium grantii* (Munhoz *et al.,* 2014), *Azima tetracantha* (Antonisamy *et al.,* 2015), *Blighia inijuigata* (Ongarora *et al.,* 2009), *Kielmeyera neglecta* (Souza *et al.,* 2012) and *Garcinia mangostana* (Parveen, 2009). It has however not been previously reported in *B. sapida*. Friedelin and its selenadiazole derivatives had been reported to be highly active against various pathogenic bacteria (Parveen *et al.,* 2009). Several other activities including antitumour (Lu *et al.,* 2010), antimycobacterial (Mann *et al.,* 2011), antiulcerogenic (Queiroga *et al.,* 2000), antidiarrhoeal (Antonisamy *et al.,* 2015), antifeedant and anti-inflammatory (Duke, 1992) had been reported for the compound. The result of larvicidal activity of LC_{50} 0.07 ± 0.01 mg/mL obtained in this study corroborated an earlier insecticidal activity against adult *Musca domestica* (LC_{50}129.27µg/g at 48 h) reported by Huang *et al.,* 2009.

Compound **2** is a white powder (m. p. 186–188 °C). The ^{13}C-NMR (75 MHz, $CDCl_3$) spectrum gave signals for thirty carbon atoms and the signals at 121.7 and 140.8 ppm are characteristic of vinyl carbon atoms. The ^{13}C and DEPT analysis showed eight methyl, nine methylene, four methine, one hydroxylated methine and seven quaternary carbon atoms which is consistent with the molecular formula, $C_{30}H_{50}O$. The ^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of the compound showed signals for six tertiary methyl groups at 0.89, 0.90, 0.91, 1.01, 1.02 and 1.08, two secondary methyl groups at 0.74 and 0.96, a carbinol proton at 3.60 and vinyl proton at 5.40. The ^{13}C-NMR (75 MHz, $CDCl_3$) spectrum of the compound showed signals for thirty carbon atoms at 19.0, 19.1, 19.4, 19.9, 21.1, 23.1, 24.4, 25.4, 26.1, 28.3, 29.0, 29.2, 31.7, 31.9, 31.9, 34.0, 36.2, 36.5, 37.3, 39.8, 40.5, 42.4, 45.9, 50.2, 51.3, 56.0, 56.9, 71.8, 121.7 and 140.8. The spectroscopic data compare very well with those of α-amyrin in literature (Hernández –Vázquez *et al.,* 2012; Ebajo Jr. *et al.,* 2015).



compound 1: friedelin



compound 2: α-amyrin

Fig. 1: Structures of the isolated compounds

Larvicidal Activity

Table 1: The activities of the extract, partitioned fractions and the isolated compounds against *A. aegypti* larvae

Code	Activity at 24 h		Activity at 48 h	
	LC_{50} (mg/mL)	LC_{90} (mg/mL)	LC_{50} (mg/mL)	LC_{90} (mg/mL)
BSB	2.00 ± 0.09 [d]	3.84 ± 0.14 [d]	1.71 ± 0.09 [c]	3.26 ± 0.11 [d]
BSB_1	1.81 ± 0.02 [d]	3.17 ± 0.35 [d]	1.65 ± 0.05 [c]	2.82 ± 0.02 [c]
BSB_2	2.52 ± 0.10 [e]	4.62 ± 0.20 [e]	2.52 ±0 .10 [d]	4.62 ± 0.02 [e]
BSB_3	3.43 ± 0.36 [f]	5.99 ± 0.56 [f]	3.44 ± 0.36 [e]	5.99 ± 0.56 [f]
BSB_4	6.15 ± 0.61 [g]	9.78 ± 1.18 [g]	6.15 ± 0.61 [f]	10.19 ± 1.35[g]
BSB_{1c}	0.71 ± 0.00 [c]	1.40 ± 0.00 [c]	0.58 ± 0.00 [a]	1.14 ± 0.00 [a]
1	0.07 ± 0.01 [b]	0.13 ± 0.01 [b]	IND	IND
2	0.05 ± 0.00[a]	0.08 ± 0.01 [a]	IND	IND
Endosulphan	0.93 ± 0.06 [c]	1.61 ± 0.12 [c]	0.90 ± 0.09 [b]	1.44 ± 0.11[b]

Key: BSB: methanol extract; **BSB_1:** *n*-hexane fraction; **BSB_2**: dichloromethane fraction; **BSB_3**: ethyl acetate fraction; **BSB_4**: aqueous fraction; **BSB_{1c}**: third bulked column fraction of *n*-hexane fraction; **1:** friedelin; **2**: α-amyrin; **Endosulphan**: positive control. **IND**: Indeterminable (all the larvae died at the concentrations tested); Values with different superscripts within the columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test). **LC_{50} and LC_{90}:** Values ± SEM.

DISCUSSION

The methanol extract of *B. sapida* stem bark was reported to have high larvicidal activity (LC_{50}, LC_{90} 2.00 ± 0.09, 3.84 ± 0.14 mg/mL) against the fourth instar larvae of *A. aegypti* (Adebajo *et al.,* 2012) at 24 h. The ethyl acetate extract of the leaf had also been found active against the fourth instar larvae of *Anopheles gambiae*, *Culex quinquefasciatus* and *A. aegypti* (Ubulom *et al.,* 2012). The high insecticidal and repellent activities of the powder and extract of the leaf against *Dinoderus porcellus,* an important pest of stored yam chips was reported (Loko *et al.,* 2017). In furtherance of the work, the methanol extract was partitioned into *n*-hexane, dichloromethane and ethyl acetate; the resulting fractions were tested against the fourth instar larvae of *A. aegypti*. The *n*-hexane fraction with LC_{50}, LC_{90} values of 1.81 ± 0.02, 3.17 ± 0.02 mg/mL at 24 h and 1.65 ± 0.05, 2.82 ± 0.35 mg/mL at 48 h, respectively was the most active and had activity that was statistically insignificant to that of the extract (Table 1). On further purification of the *n*-hexane fraction by column chromatography, the third bulked fraction had the highest activity (LC_{50} 0.71 ± 0.00 and LC_{90} 0.58 ± 0.00 mg/mL at 24 and 48 h respectively). Its activity was higher than the partitioned fraction and comparable to the positive control used (Table 1). Friedelin and α-amyrin isolated from this third bulked fraction (BSB_{1c}) had LC_{50} LC_{90} 0.07 ± 0.01, 0.13 ± 0.01 mg/mL and 0.05 ± 0.00, 0.08 ± 0.01 mg/mL respectively at 24 hours when tested. At 48 hours there was 100% mortality

therefore their lethality values could not be determined. Marked antifeedant, larvicidal and pupicidal activities had been reported for friedelin, a pentacyclic triterpenoid, against *Helicoverpa armigera* and *Spodoptera litura* (Baskar *et al*., 2014; Kekuda and Raghavendra, 2017). The effect of the α-amyrin acetate isolated from *Catharanthus roseus* Linn, on mortality and blood feeding behavior in females of *Anopheles stephensi* on *in vivo* exposure on treated guinea pig skin had been reported. 1.6% of the acetate caused a rapid mosquito knock down and 76.9% mortality after 24 hours of exposure (Kuppusamy and Murugan, 2012). The acetate is more active than the α-amyrin (0.05 mg/mL) at 24 hours recorded in this study. This could be due to the more lipophilic nature of the acetate. Lipophilic compounds are more active insecticides based on their easier transport through insect cell wall and cytoplasmic membrane (Mann and Kaufman, 2012). The difference in activity may also be due to the susceptibility of the vectors.

CONCLUSION

The larvicidal compounds of the methanol extract of *B. sapida* stem bark reside in the *n*-hexane fraction. The friedelin and α-amyrin isolated from the *n*-hexane fraction were more active than Endosulphan (the positive control) against the fourth instar larvae of *A. aegypti*. These compounds could be formulated and used against the larvae stage of *A. aegypti* thereby controlling the diseases transmitted by this mosquito.

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