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## LARVICIDAL EFFECT OF THE PET. ETHER, CHLOROFORM FRACTIONS AND METHANOL EXTRACT OF *BUCCHOLZIA CORIACEA* ENGLE SEED

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### ABSTRACT

#### Keywords:

*Buchholzia coriacea*,  
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*Buchholzia coriacea* Engle (Capparidaceae 'wonderful cola') a tropical plant is used in traditional medicine in the management of diabetes, malaria and hypertension. This study aims to investigate the larvicidal activities of the Petroleum ether, chloroform fractions and methanol extract of *B. coriacea* seed as a potential agent in vector control for malaria. Third and fourth instar larvae of *Anopheles gambiae* were used for the study. Five doses (62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml and 1000 µg/ml) of the methanol extract petroleum ether and chloroform fractions tested against the larva with 1% ethanol used as control. The mortality rates were determined 24 and 48 hr after exposure to extract following the WHO procedure. The chloroform fraction exhibited the highest mortality rate of 100% at 250, 500, 1000 µg/ml after 24 hr exposure, the pet ether exhibited mortality rate of 77.5% (1000 µg/ml) after 48 hr exposure while the methanol extract had the lowest effect of 22.5 % after 48 hr. This study reports for the first time the larvicidal activity of *B. coriacea* which is used in the folklore medicine as treatment for malaria.

**INTRODUCTION:** Mosquito-borne diseases are great health problems in tropical and subtropical climates<sup>1</sup>. *Anopheles gambiae* is one of the main vector responsible for transmitting malaria parasites and in Africa alone, the malaria disease prevalence is estimated to constitute about 300 - 500 million clinical cases every year<sup>2</sup> including death of one child in every thirty seconds<sup>3</sup>.

There is a recent increase in the search for natural products of plant origin with insecticidal properties for the control of a variety of pest insects and vectors<sup>4</sup> because they are safe, effective, inexpensive, environment friendly, biodegradable and target specific against mosquitoes<sup>4,5</sup>.

*Buchholzia coriacea* Engle (Capparidaceae) known as musk tree<sup>6</sup> is found mostly in Guinea, West Cameroon, East Cameroon and Gabon<sup>7</sup>. It is a tree characterized with a dense brown, large glossy leathery leaves arranged spirally and with conspicuous cream-white flowers. The seed has pungent taste with hot spicy flavor like *Capsicum frutescens*<sup>8</sup>.

In the African folklore medicine, *Buchholzia coriacea* is used in the treatment of various diseases. The decoction of the seed in lime or local gin is used for the treatment of diabetes mellitus, hypertension, rheumatism, cold cough and catarrh by traditional healers<sup>8</sup>, the ground seeds mixed in palm oil is taken orally as treatment for malaria<sup>9,10</sup>.

Also, the inhaling of the bark pulp helps to relieve headache, sinusitis, bronchitis and nasal congestion<sup>6,7</sup> and as an anti-helminthic in Northern Nigeria.

Biological studies have reported the antidiabetic effect of the seed in streptozotocin induced diabetic mice and normoglycemic rats<sup>8</sup>, the anti-trypanosomal and anti-helminthic effects of the extract of the seed<sup>10,11,12</sup> and the antimicrobial activity of the stem bark and seed<sup>13,14</sup>.

This study therefore, aims at investigating the larvicidal activities of the petroleum ether, chloroform fractions and methanol extract of the seed of *B. coriacea* against the third and fourth instar larva *Anopheles gambiae*.

**Plant Material:** *Buchholzia coriacea* Engl. seeds were purchased from Oje market in Ibadan, Oyo State and authenticated at the Department of Pharmacognosy, Olabisi Onabanjo University, Sagamu. Ogun State.

**Preparation of Plant Extracts:** Finely powdered, dried seeds of *Buchholzia coriacea* (250g) were macerated with methanol for five days. The filtrate was reduced under pressure in the rotary evaporator; the residue was reconstituted in distilled water and partitioned successively with pet. ether and chloroform to yield the pet. ether and chloroform fractions.

**Larvae Collection:** Larvae of *A. gambiae* were collected from stagnant water in Ojoo district of Ibadan, Oyo

state, Nigeria. Collected larvae were washed in clean well water, stored in several plastic bowls and were fed with biscuits.

**Larvicidal Activity:** The effects of five doses (62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml) of the extract and fractions were tested for their larvicidal activities against *A. gambiae*.

The larvicidal bioassay used in this study followed the WHO standard protocols<sup>15</sup> with little modification. Five doses of the crude methanol extract, pet ether and chloroform fractions were prepared in distilled water and transferred into sterile glass petri dishes. Twenty third and fourth instars larva of *Anopheles gambiae* were introduced into the petri dishes containing appropriate concentrations.

Mortality rates were recorded after 24 and 48 hr exposure. The larvae were considered dead if the larva did not move after touching with needle<sup>16,17</sup> or showed discolouration and unnatural position. The experiment was carried out in triplicates at 25°C and 80% relative humidity. 1% ethanol was used as reference.

**Statistical Analysis:** All values are expressed as mean ± S.E.M and Percentage mortality.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *BUCHHOLZIA CORIACEA* SEEDS

Saponin	Tannins	Anthraquinone	Alkaloids	Cyanogenetic glycosides	Cardiac Glycosides
+	-	+	++	+	-

++ = Present in large amount; + = Present; - = absent

TABLE 2: EFFECT OF THE METHANOL CRUDE EXTRACT, PET. ETHER FRACTION AND CHLOROFORM FRACTIONS OF *BUCCHOLZIA CORIACEA* ON THE THIRD AND FOURTH INSTAR LARVAE *ANOPHELES GAMBIAE* AFTER 24H EXPOSURE

Dose (µg/ml)	Methanol extract	% Mortality ± S.D Pet. Ether fraction	Chloroform fraction
1000	7.5 ± 0.71	55 ± 1.41	100 ± 0.00
500	0.0 ± 0.00	30 ± 1.41	100 ± 0.00
250	0.0 ± 0.00	17.5 ± 0.76	100 ± 0.00
125	0.0 ± 0.00	0.0 ± 0.00	90 ± 2.83
62.5	0.0 ± 0.00	0.0 ± 0.00	15 ± 0.00
Control	0.0 ± 0.00	0.0 ± 0.00	2.5 ± 0.55

TABLE 3: EFFECT OF THE METHANOL CRUDE EXTRACT, PET. ETHER FRACTION AND CHLOROFORM FRACTIONS OF *BUCCHOLZIA CORIACEA* ON THE THIRD AND FOURTH INSTAR LARVAE *ANOPHELES GAMBIAE* AFTER 48 H EXPOSURE

Dose ( $\mu\text{g/ml}$ )	Methanol extract	% Mortality $\pm$ S.D	Pet. Ether fraction	Chloroform fraction
1000	22.5 $\pm$ 0.71	77.5 $\pm$ 0.71	-	-
500	17.5 $\pm$ 0.00	52.5 $\pm$ 3.54	-	-
250	0.0 $\pm$ 0.00	7.5 $\pm$ 0.6	-	-
125	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	-	-
62.5	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	45 $\pm$ 0.00	-
Control	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	2.5 $\pm$ 0.55	-

**RESULTS AND DISCUSSION:** There are several reports attributing the various biological activities in plants to the presence of alkaloids, cardiac glycosides, saponins and sterols<sup>18, 19, 20</sup>. The phytochemical analysis of the seed of *B. coriacea* in this study revealed the presence of alkaloids, anthraquinone, sterols and saponins which could be responsible for the larvicidal activities determined in the pet ether, chloroform fractions and methanol extract.

The extract and fractions of *B. coriacea* tested showed that the chloroform fraction was more effective against the third and fourth instar larva of *A. gambiae* than the pet ether fraction and methanol extract. The chloroform fraction achieved maximum mortality of 100% at a dose of 250, 500 and 1000  $\mu\text{g/ml}$ ; 90% at 125  $\mu\text{g/ml}$ ; 15% at 62.5  $\mu\text{g/ml}$  24 hr after exposure to the larvae.

Also, after 48 hr exposure, the pet ether fraction exhibited a dose-dependent larvicidal activity of 17.5%, 25.5% and 77.5% mortality at 250  $\mu\text{g/ml}$ , 500  $\mu\text{g/ml}$ , and 1000  $\mu\text{g/ml}$  dose respectively while the least activity was exhibited by the methanol extract.

Though, the exact mechanism of action is unknown, it is suggested that the larvicidal activity exhibited by *B. coriacea* in this study might be acting independently or synergistically as reported in other studies that plants that contain larvicidal agents may act in combination or independently<sup>21, 22, 23</sup>.

In addition, the study suggests that the active compound(s) responsible for these larvicidal effects in the chloroform and pet. ether fractions are diverse in nature.

This study however, reports for the first time the larvicidal activity of *B. coriacea*.

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