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Adulticidal Activity of *Combrentum micratum* leaf extract against Female *An. gambiae (Culucidae: Diptera*)

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Abstract
Adulticidal activity of Combrentum micratum was carried out against Anopheles gambiae. Combretum micratum leaves were obtained from Goron-maje town, Dambatta Local Government Area in Kano Nigeria State. The prepared test concentration of plant extract was volumetrically diluted in methanol solvent using standard method. One (1) mL Combretum micratum was diluted in methanol and prepared using 2, 4 and 6% concentrations respectively . Each test concentration was repeated four (4) times and each replicate was subjected to different volunteers to nullify any effect of repellent on their kin. Volunteers were asked to follow the testing protocol. The World Health Organization (WHO) recommended percentage protection in relation to concentration and method was used. Combretum micratum extracts was evaluated for their repellent activities against adult female Anopheles gambiae using the human bait technique. The results showed that C. micratum had adulticidal activity against Anopheles mosquitoes tested with strong repellency actions on volunteers A, B, and C with complete protection time (CPT); A (30.73%), B (33.33%) and C (16.66%) when compared to the control employed. For all the treated groups, methanol extract showed low biting activity compare to control with highest biting deterrence recorded for each concentration 0.2mL, 0.3mL and 0.4mL respectively. The findings in this study has revealed that the extracts from the leaves of C. micratum possessed potent effect on repellency against adult female Anopheles mosquitoes and may therefore serve as a potential source for the control of adult mosquitoes

Keywords:
Adulticidal, Biting deterrence, *Combretum micratum, Anopheles gambiae*

INTRODUCTION
Mosquito control methods mainly rely on the chemical insecticides, but has led to environmental pollution (Barnard, 1999). Mosquito control has become more difficult due to the unsystematic use of synthetic chemical insecticides which have inauspicious effect on the environment (Das *et al*., 1997), These synthetic chemical insecticides equally affect man and animals because they are not properly degradable and spread their toxic effect (Aktar *et al*., 2009). The larval stages of mosquitoes can be easily eradicated for control operations because they are less movable in larval forms than the adults (Benelli, 2015). To control or eliminate mosquito population highly effective pesticides have been employed (Downe, 1975). These pesticides are threatened due to the developing resistance of mosquitoes against them (Cheng *et al*., 2004). Therefore, alternative biological mosquitocides are urgently needed. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents (Arsenaul*et al*., 2008). Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programmes due to their excellent larvicidal and adulticidal properties (De Souza *et al*., 2011).

Plants could be an alternative source for mosquito repellents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects (El-Badry, 2010). The chemicals derived from plants have been projected as weapons in future mosquito control programmes as they have proven function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent (Ferdouse, 2001; Islam, 2005). Utilizing endogenous knowledge concerning plants with traditional medicinal value has proven fruitful in identifying potential sources of phyto-extracts with insecticidal activity (FMoH, 2016). Plant extracts are best options for the eradication of mosquitoes as they are less harmful to environment and non-targeted species (Conti *et al*., 2010). The top priority for finding a new insecticide is that they may be originate from plants and they must be eco-safe with different plant extracts such as saponin, steroids, flavonoids (Govindarajan, 2010).

There have been many attempts to assay the activity of plant extracts against vectors of human disease, in particular through the utilization of plants for which such knowledge exists (Hafeez *et al*., 2011). Plant extracts contain botanical insecticides or phytochemicals that could be used to limit reproduction and survival of various pest species including mosquitoes (Haque *et al.,* 2009). Mosquitoes are of much concern to public health and well-beings of the global human population (James, 2016). Since these mosquitoes transmit a number of dreadful diseases like filarial, malaria, elephantiasis and dengue, control measures using non-conventional insecticides like botanicals and phytochemical derivatives are gaining much attention in recent days due to a number of favorable reasons (Gericke, 2002). The These phytochemicals have been tested have been tested to be specific in action against target insects and are non-toxic on the ecosystem and man as compared to the chemical compounds (Jeyabalan *et al*., 2003).

MATERIALS AND METHODS

Collection and rearing of mosquitoes

Reared adult *Anopheles* mosquitoes were obtained from the insectary unit, Department of Biochemistry, Bayero University Kano, Nigeria.

Collection and Identification of Plant Materials

Combretum micratum leaves were obtained from Dambatta Local Government Area Kano State. The leaves were identified with the help of experts in plant taxonomy, in the Department of Plant Biology, Bayero University Kano.

Preparation of Plant Extracts

Healthy leaves of *Combretum micratum* were washed in tap water, cut into small pieces and air dried. After the leaves were completely dried they were ground into powder and then macerated in methanol solvents at room temperature for 3 days and then filtered. The combined filter was concentrated to dryness by rotary evaporation at 50 °C and kept in a freezer. The prepared test concentration of the plant extract was volumetrically diluted in methanol solvent.

Repellency tests procedure

Combretum micratum extracts were evaluated for their repellent activities against *Anopheles* using the human – bait technique (Fradin and Day, 2002). First, 1 mL *Combretum micratum* was diluted in methanol and 2, 4 and 6% concentrations. For the test, 20 diseases free, laboratory-reared female mosquitoes were placed into separate laboratory cages. Before the test, the volunteer's skin was washed with unscented soap and the extract was applied from the elbow to the finger tips. In each cage, one arm was inserted for one test concentration and the other arm applied without methanol which served as control. The treated and control arms were interchanged regularly to eliminate bias. Each test concentration was repeated four (4) times and in each replicate subject different volunteers to nullify any effect of repellent on skin. Volunteers were asked to follow the testing protocol. Volunteers conducted their test of each concentration by inserting the treated and control arms alternatively into the same cage for one full minute for every five minutes. If they were not bitten within 20 minutes, then the arms were reinserted for 1 full minute for every 15 minutes, until the first bite occurred. The percentage (%) repellency was calculated using
The following formula below:

$$\% \text{ Repellency} = \left(\frac{Ta - Tb}{Ta}\right) \times 100\%$$

Where Ta is the number of mosquitoes recorded on the control group and Tb is the number of mosquito recorded in the treatment group.

Biting deterrence

The percentage protection in relation to concentration and dose method recommended by WHO (1996) was adopted to derive the biting deterrence. Blood starved female *Anopheles* mosquitoes were kept for about 3-4 days old, in an insect net cage. The arms of the test persons or volunteers were cleansed with sterile water. After air drying the arms, a 25m area of the dorsal side of the skin was exposed, the remaining portion was covered by rubber gloves. The plant extracts were dissolved in methanol, with other hand served as control without extract. Different concentrations of the leaf extracts were applied. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over five (5) minutes at every protected period. The experiment was conducted five times.

The percentage protection was calculated by using formula:

$$\% \text{ protection time (CPT)} = \frac{\text{No.of bited recieve by control arm} - \text{No.of bite recieved by treated arm}}{\text{No.of bites recieved by control arm}}$$

Statistical analysis

All data was subjected to analysis of variance (ANOVA), Statistical analysis was carried out using the excel statistical package (version 16). Significant different at $p>0.05$ would be used to determine efficacy of plant extract at different concentration.

RESULTS AND DISCUSSION

Repellency effect of *Combretum micratum* leaf extract against *Anopheles gambiae*.

Concentrations of plant extract used exhibited repellency activity against female *Anopheles* mosquitoes which indicates that the methanol extraction of *Combretum micratum* was effective in exhibiting a repellency action against the mosquitoes tested compared to the control employed (Table 1). With strong repellency actions on volunteers A, B, and C couple with complete protection time (CPT) (30.73, 33.3 3and 16.66 sec) when compared to the control respectively (Table 2). Table 1 shows high level of counted mosquitoes on control arm compared to treated arm for all volunteers.

Effect of *C. micratum* extract on the biting deterrence of *Anopheles gambiae*

Results of laboratory testing of *C. micratum* leaf extract against the biting of deterrence of *Anopheles* mosquitoes are shown in Table 3. For all treated groups with methanol extract recorded low biting activity compared to the control that record highest biting across all the concentration levels employed.

Table 1: Repellency effects of *C. micratum* extract on treated and control arms tested against *Anopheles* mosquitoes

Plant Material		Concentration (mL)	CPT (Sec)	Treatment Arms	Control
C. micratun	A	0.2	15	1	16
	B	0.2	30	2	8
	C	0.2	25	1	21
	A	0.3	3	2	8
	B	0.3	20	4	13
	C	0.3	30	6	23
	A	0.4	25	3	10
	B	0.4	20	2	14
	C	0.4	15	1	13

CPT= Complete protection time.

Table 2: Repellency effect of *C. micratum* extract against *Anopheles* mosquitoes (CPT)

Plant Material		Concentration Methanol (mL)	Complete protection time (CPT) min Treatment	Control
Conbretum micratun	A	0.2	15.13±1.2	6.0±0.1
	B	0.2	25.00±1.0	6.0±0.1
	C	0.2	8.33±1.3	5.0±0.1
	A	0.3	0.07±1.4	4.0±0.1
	B	0.3	33.33±0.7	3.0±0.1
	C	0.3	33.33±1.3	4.0±0.1
	A	0.4	30.73±1.3	5.0±0.1
	B	0.4	33.33±1.3	6.0±0.1
	C	0.4	16.66±0.4	3.0±0.1

Mean ±SE= Standard error, which represent that there is significant difference between the treatment and control groups at 5% where F Calculated value is 15.16, compared to critical table value using ANOVA.

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Table 3: Effects of *Combretum micratum* on biting deterrence against *Anopheles* mosquitoes

Treatment	Concentration (mL)	Biting Deterrence (Prevention)
Control		21
Combretum micartum	0.2	1
	0.3	0
	0.4	4

In the present study, concentrations of plant extract used exhibited repellency activity against female *Anopheles gambiae* mosquitoes which indicates that the methanol extraction of *Combretum micratum* was effective in exhibiting a repellency action against the mosquito tested compared to control (Table 1). The result shows that *C. micratum* had repellent activity against the female *Anopheles* mosquitoes tested. With strong repellency actions on volunteers A, B, and C with complete protection time (CPT) (341.0 sec, 307.0 sec and 210.0 sec) when compared to control with different concentrations 0.2, 0.3 and 0.4mL respectively (Table 1).

These findings have reemphasized the need to explore the possibility of using herbal-based repellents (Osmani *et al.,* 1972) as supplementary and complimentary measures for malaria control. This will reduce the chemical burden on the environment. The group treated with the extract showed low biting activity compared to the control with highest biting deterrence. This finding is in agreement with the report of Murugan *et al.* (1999) on *C. micratum* having more potential effect against mosquito larvae.

The early instars larvae were more susceptible than the later ones and the pupae, which was not much affected by all the solvents. This may be due to the non-feeding behavior of pupae, whereas the bio-pesticide enters the insect system through oral feeding and affects the gut and other organs. Previous study of phytochemical screening results indicated that the leaves extracts of these plants were rich in alkaloids, flavonoids and tannins, saponins which may be responsible for the insecticidal properties observed in these plants (Kabaru and Gichia, 2001). These phyto-chemicals have earlier been reported to have larvicidal and insecticidal abilities (Sofowora, 1993). Crude extract of *C. micartum* has specifically been reported to inhibit metamorphosis thereby disallowing pupation or adult emergent of the mosquito (Kabaru and Gichia, 2001). The results of this study agree with the finding of Okumu *et al.* (2007) where it was reported that *C. micratum* is highly toxic to mosquito and delay pupation on *Aedes* and *Anopheles*. Exposure of *A. gambiae* larvae to sub lethal doses of *C. micartum* and catnip leaves extract in the laboratory prolonged larvae development and pupation (Su and Mulla, 1999b).

CONCLUSION

Due to the results obtained in this study, it can be concluded that based on the repellent effect that the extract of *C. micratum* exhibited mosquitocidal properties. *C. micratum* extract can be employed as effective mosquitocidal agent against female *Anopheles* mosquitoes having recorded complete protection time (CPT) (341.0, 307.0 and 210.0 sec) among three volunteers in this study. It is recommended that further research studies should be conducted with a view to assessing the ovicidal activity of *C. micratum* against the larvae of mosquitoes.

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