

## The antimosquito properties of extracts from flowering plants in South Africa

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**Abstract.** Extracts of selected flowering plants, which are considered eco-friendly, are used for the treatment of numerous ailments and vector control worldwide. This has resulted in approximately 25 per cent of currently used drugs being derived from herbal sources. The aqueous and methanolic extracts of twelve plant species, *Psidium guajava* (pink fruit), *Psidium guajava* (white fruit), *Psidium cattleianum* var. *cattleianum*, *Psidium guineense* and *Psidium X durbanensis*, *Achyranthes aspera*, *Alternanthera sessilis*, *Guilleminea densa*, *Capparis tomentosa*, *Leonotis leonurus*, *Dichrostachys cinerea* and *Carpobrotus dimidiatus*, were tested for insecticidal activity, including larvicidal, adulticidal and repellent activities against the adult female mosquito, *Anopheles arabiensis*. The extracts of *P. guajava* (white fruit), *C. tomentosa*, *L. leonurus*, *D. cinerea*, and *C. dimidiatus* exerted a pronounced inhibitory effect on adult insects, while those of *P. guajava* (pink fruit), *P. X durbanensis*, *P. cattleianum* var. *cattleianum*, *P. guineense*, *A. aspera*, *A. sessilis*, and *G. densa* were ineffective and failed to satisfy the criteria set by the World Health Organization. In the tests for repellency against *An. arabiensis*, all the tested aqueous and methanolic plant extracts except those of *A. sessilis* repelled 80-100% of mosquitoes. The most effective mosquito repellents were the methanol and aqueous extracts of *P. guajava* (pink fruit), *P. X durbanensis*, *P. cattleianum* var. *cattleianum*, *P. guineense*, *G. densa*, *L. leonurus* and *D. cinerea*, which are potential sources of cost effective mosquito repellents to be utilized in malarial endemic areas.

### INTRODUCTION

Malaria is one of the world's most devastating causes of morbidity and mortality in the world. Despite the very significant regional decline in reported malaria cases in the 20<sup>th</sup> century, this disease still represents a significant public health problem in most developing countries in tropical and subtropical areas, where temperature and rainfall are suitable for the development of vectors and parasites (Greenwood *et al.*, 2008). It is estimated that the number of deaths due to malaria was 781 000 in 2009 (WHO, 2010). In Africa alone, malaria is the leading cause of death in children under five.

Malaria is transmitted by the bites of *Anopheles* mosquitoes. In Africa, the mosquito *Anopheles arabiensis* is one of the dominant vectors of malaria (Sinka *et al.*, 2010). Eliminating the source of infection is an essential component for the control of mosquito borne diseases (Mabaso *et al.*, 2004). During the past several decades, many synthetic organic insecticides have been developed and effectively used to eliminate mosquitoes. Unfortunately, the management of this disease vector by using synthetic insecticides has failed in part because the continuous and indiscriminate use of conventional chemical insecticides has resulted in the development of physiological

resistance (Severini *et al.*, 1993; Rao *et al.*, 1995). In addition, there are long-term harmful effects on non-target organisms and the environment (Wattal *et al.*, 1981). Most of the mosquito control programs target the larval stage in their breeding sites with larvicides, because adulticides may only reduce the adult population temporarily (El Hag *et al.*, 1999, 2001).

The chemicals derived from plants have been projected as weapons in future mosquito control programs as they are shown as ecologically sensitive pesticides (Isman, 2000). Generally they are safe to humans and other mammals (Templeton, 1969; Tripathi *et al.*, 2000, 2002). Several phytochemicals have been reported to exhibit detrimental effects on mosquitoes (Syamala & Vasudevan, 1995; Gleiser & Zygadlo, 2009). Protection against mosquito bites has been reported from plant sources *Cymbopogon* species (Ansari & Razdan, 1995), *Pelargonium citrosum* (Matsude, 1996), *Lantana camara* (Dua *et al.*, 1996), *Tagetes* species (Perich *et al.*, 1995; Gillij *et al.*, 2008), *Ocimum* species (Bhatnagar *et al.*, 1993), *Baccharis spartioides*, *Rosmarinus officinalis*, *Aloysia citriodora* (Gillij *et al.*, 2008), and *Lippia* species (Gleiser *et al.*, 2011) among others. Other essential oils from plants like *Myrtus communis*, *Origanum syriacum*, *Lavandula stoechos* and their pure compounds like thymol, carvacrol and  $\alpha$ -pinene have been documented for larvicidal activities towards *Culex pipiens molestus* (Traboulsi *et al.*, 2002). *Lippia turbinata* and *Lippia polystachya* showed larvicidal properties against *Culex quinquefasciatus* (Gleiser & Zygadlo, 2007) and sublethal concentrations of these essential oils can alter the locomotor activity of the larvae (Kembro *et al.*, 2009).

Personal protective measures, including repellents and larvicides are widely used to prevent the transmission of arthropod-borne diseases by minimizing the contact between humans and vectors. In contrast to vaccines and chemoprophylaxis as means of personal protection, repellents and larvicides are convenient, inexpensive, and offer advantages in protection against a wide range of vectors (WHO, 1995). They are also

the primary means of mosquito-borne disease prevention available in areas where vector control is not practical (Gupta & Rutledge, 1994). The majority of commercial repellent products contain the chemical DEET (diethyl-3-methylbenzamide, formerly known as diethyl-*m*-toluamide), which was first synthesized in 1954 (McCabe *et al.*, 1954). Although effective, DEET is not the ideal product, as allergic and toxic effects have been documented (TEACH, 2007) and its solvent characteristic can damage plastics and other synthetic materials. Because of the undesirable effects of DEET, research was actively carried out to find an alternative compound that is safer to use and equally or more effective (Robert *et al.*, 1990; Schreck & Leonhardt, 1991; Dua *et al.*, 1996; Walker *et al.*, 1997; Gleiser *et al.*, 2011). One promising new repellent is the piperidine compound, AI3-37220, which provides equal or better protection against certain mosquitoes than that obtained with DEET (Coleman *et al.*, 1994; Frances *et al.*, 1996; Walker *et al.*, 1997). Therefore, development of more efficient insect control materials which have no ill effects on the non-target population, and are easily degradable are necessary (Redwane *et al.*, 2002). The use of herbal products is one of the better alternatives for mosquito control.

The present work reports systematic investigations on the larvicidal, repellent and adulticidal effects of methanolic and aqueous extracts of twelve local medicinal plants against a malaria vector, the adult female mosquito *An. arabiensis*.

Currently there is only limited scientific data on the insecticidal effects of the following indigenous and exotic plants in South Africa, *Psidium guajava* (pink fruit), *Psidium guajava* (white fruit), *Psidium cattleianum* var. *cattleianum*, *Psidium guineense*, *Psidium X durbanensis*, *Achyranthes aspera*, *Alternanthera sessilis*, *Guilleminea densa*, *Capparis tomentosa*, *Leonotis leonurus*, *Dichrostachys cinerea*, and *Carpobrotus dimidiatus*. Information on these (African) medicinal plants is dominated by oral tradition and is not always scientifically well documented. This traditional information is further complicated

by loss of biodiversity and tradition and there is a clear need for accurate documentation of knowledge of traditional herbalists. The first step in understanding the insecticidal effect of these plants requires research to test for efficacy, safety and accuracy to validate traditional medicinal beliefs, and thus this investigation has been carried out to achieve this end.

## MATERIALS AND METHODS

### Sample Collection and Preparation

Twelve different plants used in this study were collected from different locations in KwaZulu-Natal, South Africa. These plants were identified using available taxonomic keys. Herbarium specimens were prepared and lodged at the Ward Herbarium, University of KwaZulu-Natal, South Africa. The plants used were *P. guajava* (pink fruit), *P. guajava* (white fruit), *P. cattleianum* var. *cattleianum*, *P. guineense*, *P. X durbanensis*, *A. aspera*, *A. sessilis*, *G. densa*, *C. tomentosa*, *L. leonurus*, *D. cinerea*, and *C. dimidiatus*. The selection of the plants was based on their traditional usage.

Plants were washed repeatedly with distilled water until no foreign material remained. Subsequently the plant was oven dried at 25°C for 12 h and samples were stored in Schott bottles until analysis. All analyses were conducted in duplicates and the reagents used were of analytical grade. Dried materials were used for all plants.

Methanolic and aqueous extracts of the dried plant material were prepared according to the procedure outlined in the literature (Jeremy & Whiteman, 2003) with minor modifications. For the methanolic extracts, 50 g of the powdered plant material were stirred in 200 mL of 80% methanol (v/v) and agitated for 24 h. The filtrate was collected using Whatman No. 1 filter paper. Solvents were removed by evaporation using a rotary evaporator (Buchi RE) connected to a water bath set at a temperature of 50°C. The remaining slurry was freeze dried in freeze Dryer (Virtis Benchtop) to form a powdery residue. For all experiments, the methanolic

plant extracts were diluted in acetone to give a final concentration of 1 mg/mL.

For the aqueous extract, 50 g of the powdered plant material was stirred in 200 mL of distilled water and agitated for 24 h, before centrifuging at 8000 rpm for 10 min. The supernatant was filtered using Whatman No. 1 filter paper and dried in an oven (Memmert, South Africa) set at 35°C. The powdery dried crude extract was dissolved in double distilled water to yield a final concentration of 1 mg/mL.

### Larvicidal activity

The larvicidal bioassay was performed according to the World Health Organization standard protocols (WHO, 1981a, b) with slight modifications. One millilitre of the extract solution was added to polypropylene containers (10 x 10cm) containing 0.25 litres of distilled water. Thirty third instar larvae of *An. arabiensis* were placed in the container. A negative control was set up in which the solvent was added instead of extract. A positive control was set up using temephos (*O,O,O'O'*-tetramethyl *O,O'*-thiodi-*p*-phenylene phosphorothioate; Mostop), an organo-phosphate used by the malaria control programme of South Africa as a larvicide. Each container was monitored for larval mortality (dead larvae were removed) at 24 h intervals for seven days and the larvae were fed regularly with specially made cat food pellets with reduced oil/fat content (50 mg/day) on a mesh type of floating container.

### Insecticidal activity

The adulticidal effect was assayed following a slightly modified version of the World Health Organization standard method (WHO, 1981a, b). One mL of plant extract solution was sprayed onto a clean dry non-porous ceramic tile using the pre-calibrated Potter's Tower. The Potter's Tower was cleaned with acetone between each different extract application. The sprayed tiles were air dried and assayed within 24 h of spraying. A standard bioassay cone was fixed in place over the area of the plant extract sprayed tile. Thirty blood-fed *An. arabiensis* females 3-5 days old were introduced into the cone. The mosquitoes

were then observed for knockdowns after 30 and 60 min of exposure. The test species were thereafter removed from the bioassay cone and transferred to a holding cage containing a nutrient solution. After 24 h, the number of dead mosquitoes was recorded and percentage mortality calculated. The positive control used in this experiment was deltamethrin (15 g/L; K-Othrine®). The negative controls in this experiment were acetone and distilled water.

### Repellent activity

The rodent *Mastomys coucha* was the test animal used for the general screening of plant extracts for repellent activity. Repellent activity was assessed by topical application of the compound to skin and subsequent exposure of the treated areas of skin to unfed female mosquitoes. Ethical approval for the use of *M. coucha* in these trials was approved from the MRC's Ethics Committee for Research on Animals.

### Animal preparation

Adult *Mastomys* were weighed individually, and injected intraperitoneally with the correct concentration of sodium pentobarbital in comparison to the weight of the animal. The anesthetized rodents were then shaved on the ventral surface and a measured volume of 1 mL of plant extract compound was applied to each of two rodent's abdomens. The third served as a negative control and the fourth as a positive control using DEET.

### Repellent assay

Paper cups (500 mL) were modified by replacing the base of the cup with mosquito netting held in place with a rubber band and covering the mouth of the cup with transparent plastic film. Thirty unfed 4-day old *An. arabiensis* females were introduced into the cup and held in contact with the treated ventral surface of each rodent. Mosquito activity was observed through the transparent plastic film. After a period of 2 min, the numbers of mosquitoes probing were recorded. Mosquitoes were then observed for 24 h. The rodent was then returned to the animal facility and allowed to recover from

anesthetic. Each rodent was monitored for 7 days for adverse reactions to the applied plant extracts.

Repellence of the extracts was calculated using the following formula.

$$\text{Percentage mosquitoes repelled} = \frac{\text{Number repelled}}{\text{Number introduced}} \times 100$$

### Statistical analysis

One-way analysis of variance followed by least significant difference (LSD) Fisher test was used to compare the mean repellence time for the plant extracts and controls on adult *An. arabiensis* mosquitoes. Adult knockdown and mortality data were subjected to a repeated measures analysis of variance (ANOVA) that examined the main effects of treatment (plant extracts and controls), time after application of treatment (30 min, 60 min and 24 h; the repeated measure) and their interaction, and LSD Fisher test was used for post hoc analyses. Before ANOVA testing, data were transformed to ranks (Shirley, 1987) to fit better the assumptions of the test. In all cases, a value of  $p < 0.05$  was considered statistically significant.

## RESULTS

None of the 26 extracts from twelve different plant species showed significant larvicidal effects on the immature third instar stage of *An. arabiensis* mosquitoes during the seven day period. No inhibition or mutation (irregularities) of growth pattern were detected throughout the seven day trial, compared to the positive control, which in this case was temephos, a commercial organophosphate which caused 100% mortality. All larvae developed to pupae normally.

The results from the *An. arabiensis* adulticidal assay using the different extracts are shown in Table 1. There was a significant effect of treatment ( $F_{28,41} = 83.83$ ;  $p < 0.001$ ), time from exposure ( $F_{2,82} = 165.16$ ;  $p < 0.001$ ), and their interaction ( $F_{56,82} = 7.75$ ;  $p < 0.001$ ) on mosquito knockdown/mortality. K-othrine

Table 1. Insecticidal activity of plant extracts (1 mg/mL) on adult *Anopheles arabiensis* mosquitoes. Activity is indicated by percentage knockdowns and mortality of mosquitoes against the plant extract. (All extracts are of leaves unless noted otherwise)

Sample	% Knockdown (min)*		% Mortality (h)
	30	60	24
<i>A. aspera</i> (AE)	15.0 ± 1.7 <sup>bc</sup>	36.7 ± 6.7 <sup>de</sup>	5.0 ± 1.7 <sup>ab</sup>
<i>A. aspera</i> (ME)	30.0 ± 10.0 <sup>e</sup>	53.3 ± 20.0 <sup>d</sup>	21.7 ± 5.0 <sup>ce</sup>
<i>A. sessilis</i> (AE)	31.7 ± 1.7 <sup>e</sup>	30.0 ± 3.3 <sup>ce</sup>	16.7 ± 0.0 <sup>c</sup>
<i>A. sessilis</i> (ME)	26.7 ± 6.7 <sup>ce</sup>	38.3 ± 11.7 <sup>de</sup>	21.7 ± 5.0 <sup>c</sup>
<i>C. tomentosa</i> (AE)	25.0 ± 1.7 <sup>ce</sup>	53.3 ± 6.7 <sup>d</sup>	18.3 ± 1.7 <sup>c</sup>
<i>C. tomentosa</i> (ME)	31.7 ± 8.3 <sup>e</sup>	50.0 ± 0.0 <sup>d</sup>	51.7 ± 1.7 <sup>d</sup>
<i>C. dimidiatus</i> (AE)	13.3 ± 1.6 <sup>bc</sup>	46.7 ± 0.0 <sup>d</sup>	43.3 ± 0.0 <sup>d</sup>
<i>C. dimidiatus</i> (ME)	15.0 ± 1.6 <sup>c</sup>	16.7 ± 1.7 <sup>c</sup>	33.3 ± 3.3 <sup>e</sup>
<i>D. cinerea</i> (AE)	25.0 ± 1.7 <sup>ce</sup>	48.3 ± 1.7 <sup>d</sup>	46.7 ± 5.0 <sup>d</sup>
<i>D. cinerea</i> (ME)	0.0 ± 0.0 <sup>a</sup>	6.7 ± 0.0 <sup>a</sup>	26.7 ± 5.0 <sup>ce</sup>
<i>G. densa</i> (AE)	23.3 ± 10.0 <sup>ce</sup>	31.7 ± 5.0 <sup>e</sup>	11.7 ± 5.0 <sup>ac</sup>
<i>G. densa</i> (ME)	18.3 ± 1.7 <sup>c</sup>	18.4 ± 1.7 <sup>c</sup>	18.3 ± 8.3 <sup>c</sup>
<i>L. leonurus</i> (AE)	6.7 ± 1.6 <sup>ab</sup>	43.3 ± 0.0 <sup>d</sup>	25.0 ± 1.7 <sup>ce</sup>
<i>L. leonurus</i> (ME)	56.7 ± 1.7 <sup>dg</sup>	56.7 ± 4.9 <sup>dg</sup>	43.3 ± 8.3 <sup>d</sup>
<i>L. leonurus</i> flower (AE)	30.0 ± 3.3 <sup>e</sup>	56.7 ± 3.3 <sup>dg</sup>	60.0 ± 0.0 <sup>g</sup>
<i>L. leonurus</i> flower (ME)	23.3 ± 3.3 <sup>ce</sup>	86.7 ± 0.0 <sup>g</sup>	26.7 ± 5.0 <sup>ce</sup>
<i>P. cattleanum</i> var. <i>cattleanum</i> (AE)	0 ± 0 <sup>a</sup>	24.4 ± 0.0 <sup>ce</sup>	23.3 ± 3.3 <sup>ce</sup>
<i>P. cattleanum</i> var. <i>cattleanum</i> (ME)	0 ± 0 <sup>a</sup>	13.3 ± 3.3 <sup>bc</sup>	3.3 ± 0.0 <sup>a</sup>
<i>P. durbanensis</i> (AE)	0 ± 0 <sup>a</sup>	23.3 ± 6.7 <sup>ce</sup>	28.3 ± 1.7 <sup>ce</sup>
<i>P. durbanensis</i> (ME)	0 ± 0 <sup>a</sup>	18.3 ± 8.3 <sup>c</sup>	5.0 ± 5.0 <sup>a</sup>
<i>P. guajava</i> pink fruit (AE)	0 ± 0 <sup>a</sup>	20.0 ± 6.7 <sup>c</sup>	18.3 ± 5.0 <sup>c</sup>
<i>P. guajava</i> pink fruit (ME)	0 ± 0 <sup>a</sup>	25.0 ± 8.3 <sup>ce</sup>	11.7 ± 5.0 <sup>bc</sup>
<i>P. guajava</i> white fruit (AE)	0 ± 0 <sup>a</sup>	15.0 ± 1.7 <sup>c</sup>	20.0 ± 6.7 <sup>c</sup>
<i>P. guajava</i> white fruit (ME)	0 ± 0 <sup>a</sup>	26.7 ± 6.7 <sup>ce</sup>	11.7 ± 5.0 <sup>bc</sup>
<i>P. guineense</i> (AE)	0 ± 0 <sup>a</sup>	16.7 ± 6.7 <sup>c</sup>	20.0 ± 3.3 <sup>c</sup>
<i>P. guineense</i> (ME)	0 ± 0 <sup>a</sup>	16.7 ± 3.3 <sup>bc</sup>	0 ± 0 <sup>a</sup>
Distilled water (negative control)	1.7 ± 1.3 <sup>a</sup>	6.7 ± 2.1 <sup>ab</sup>	7.8 ± 2.5 <sup>ab</sup>
Acetone (negative control)	5.0 ± 2.9 <sup>ab</sup>	6.6 ± 2.5 <sup>ab</sup>	7.8 ± 2.7 <sup>ab</sup>
K - othrine (positive control)	97.1 ± 1.6 <sup>g</sup>	99.7 ± 0.3 <sup>f</sup>	100.0 ± 0.0 <sup>f</sup>

\* (Knockdown: a rapidly and normally reversible paralysis). Values are mean ± SEAE: aqueous extract; ME: methanolic extract; C: control\*<sup>g</sup> Means without a common letter differ significantly (p<0.05)

(deltamethrine), a commercial insecticide, exhibited almost 100% knockdown and complete mortality at 60 min exposure time. Most extracts at some point showed significantly higher knockdown or mortality than the negative controls, but all values were lower than the positive control K-othrine. Highest mortality (60%) was recorded for *An. arabiensis* exposed to *L. leonurus* flower aqueous extract, followed by *C. tomentosa* (52%) and *D. cinerea* (47%). Knockdown after 60 min due to the methanolic extract of *L. leonurus* flower was the highest (87%), but mosquitoes recovered and mortality was only 27% after 24h. Other plant extracts such as *A. aspera* (methanolic extract) and *C. tomentosa* (aqueous extract) exerted an increased knockdown after 60 min but mosquitoes also recovered in 24 h.

The insecticidal activity of methanolic extracts of *P. guineense* and *P. cattleanum* var. *cattleanum* did not differ from the negative controls. Knockdown of mosquitoes exposed to methanolic extracts of *P. X durbanensis*, *P. guajava* and aqueous extracts of *A. aspera*, *A. sessilis* and *G. densa* showed a significantly increased knockdown after 60 min, but recovered after 24 h and did not show significant differences compared to the negative controls.

The results from the *An. arabiensis* repellency assay using the different extracts are shown in Table 2. There were significant differences in repellence between treatments ( $F_{28,41} = 17.57$ ;  $p < 0.001$ ). Most of the plant extracts were (statistically) as repellent as the positive control DEET, ranging from 85 to 100% repellence. On the other hand,

Table 2. The repellency activity of plant extracts (1 mg/mL) on adult *Anopheles arabiensis* mosquitoes. Activity is indicated by percentage mosquitoes repelled against the plant extract. (All extracts are of leaves unless noted otherwise)

Sample	Extract	
	Aqueous	Methanolic
<i>A. aspera</i>	26.67 ± 3.33 <sup>a</sup>	23.33 ± 9.99 <sup>ab</sup>
<i>A. sessilis</i>	85.00 ± 1.67 <sup>d</sup>	33.33 ± 0.00 <sup>ac</sup>
<i>C. dimidiatus</i>	97.00 ± 4.70 <sup>d</sup>	85.00 ± 1.67 <sup>d</sup>
<i>C. tomentosa</i>	97.00 ± 2.90 <sup>d</sup>	86.66 ± 4.70 <sup>d</sup>
<i>D. cinerea</i>	98.33 ± 1.67 <sup>d</sup>	93.33 ± 0.00 <sup>d</sup>
<i>G. densa</i>	55.00 ± 1.67 <sup>c</sup>	100.00 ± 0.0 <sup>d</sup>
<i>L. leonurus</i>	97.00 ± 9.10 <sup>d</sup>	91.67 ± 1.67 <sup>d</sup>
<i>L. leonurus</i> flower	78.33 ± 1.67 <sup>c</sup>	100.0 ± 0.0 <sup>d</sup>
<i>P. cattleanum</i> var. <i>cattleanum</i>	85.00 ± 1.67 <sup>d</sup>	100.00 ± 0.0 <sup>d</sup>
<i>P. durbanensis</i>	88.33 ± 1.67 <sup>d</sup>	98.33 ± 1.67 <sup>d</sup>
<i>P. guajava</i> pink fruit	95.00 ± 1.67 <sup>d</sup>	100.00 ± 0.00 <sup>d</sup>
<i>P. guajava</i> white fruit	50 ± 0.00 <sup>ac</sup>	96.67 ± 3.33 <sup>d</sup>
<i>P. guineense</i>	95.00 ± 1.67 <sup>d</sup>	100.00 ± 0.0 <sup>d</sup>
Negative control <sup>1</sup>	4.44 ± 2.81 <sup>b</sup>	42.22 ± 13.38 <sup>ac</sup>
Positive control (DEET)	100.00 ± 0.0 <sup>d</sup>	100.00 ± 0.0 <sup>d</sup>

Values are mean ± SE

<sup>1</sup>Negative control for aqueous extract was distilled water, for methanolic extract was acetone

<sup>a-d</sup> Means without a common letter differ significantly (p<0.05)

repellence of methanolic extracts of *A. aspera* were not different from the negative controls (water and acetone), and aqueous extracts of *A. aspera*, *G. densa*, *L. leonurus* flower, *P. guajava* white fruit and methanolic extracts of *A. sessilis* were not significantly different compared to the acetone negative control. No adverse reactions to the applied plant extracts were observed on any of the *Mastomys* rodents during the 7 days they were monitored.

## DISCUSSION

The findings of the present investigation indicate that the larvicidal trials of *P. guajava* (pink fruit), *P. guajava* (white fruit), *P. cattleanum* var. *cattleanum*, *P. guineense* and *Psidium* X *durbanensis*, *A. aspera*, *A. sessilis*, *G. densa*, *C. tomentosa*, *L. leonurus* (leaves and flowers), *D. cinerea*, and *C. dimidiatus* leaf extracts exhibited no significant effect on the immature stages of the anopheline mosquito during the seven day exposure period, compared to the positive control which in this case was temephos, a commercial organophosphate.

No inhibition of growth was detected with the extracts since all larvae grew to become pupae and subsequently adults. Earlier report (Bagavan, 2008) reveals that the ethyl acetate extract of *A. aspera* showed larvicidal activity against the early fourth-instar larvae of *Aedes aegypti* and *Cx. quinquefasciatus*. Either differences in susceptibility between mosquito species (Sukumar *et al.*, 1991) or variations in the composition of the extracts due to extraction method may explain the observed differences.

Regarding adulticidal effects, even though mortalities of mosquitoes exposed to some extracts such as *C. dimidiatus*, *C. tomentosa* and *L. leonurus* were significantly higher than the negative controls, they were also significantly lower than mortalities of *A. arabiensis* exposed to a commercial insecticide used as positive control. Moreover, for most extracts, knockdown recorded after 60 min exposure reverted 24h after mosquitoes were removed from the extract source (Table 2). Thus, the results for the insecticidal screening of the plant extracts in general reflected in this study have not been very encouraging based on the

criteria set for determining if an extract is a potential adulticide according to World Health Organization (1981b).

On the other hand, the repellent assays showed promising results, as the aqueous and methanolic extracts of *P. guajava* (pink fruit), *P. X durbanensis*, *P. cattleanum* var. *cattleanum*, *P. guineense*, *C. tomentosa*, *D. cinerea*, and *L. leonurus* have strong repellency effects against *A. arabiensis* and have potential as products for personal protection against the mosquitoes.

The WHOPES (2009) recommend testing of repellents on human subjects, because it utilizes the repellent end-user in the testing process and yields results that are relevant to the actual conditions of use. Results obtained in laboratory animals or artificial membranes tests cannot be directly equated to results expected in comparable tests on humans due to differences in skin temperature, sweat, lipid content among other specific factors that influence repellence effectiveness and duration (Debboun *et al.*, 2006). However, for reasons of economy and human safety, repellents intended for human use are frequently first tested on a surrogate species, most often the guinea pig, mouse, or rabbit, with an increase in human safety resulting from deferral of tests on humans to the late stages of repellent development (Rutledge *et al.*, 1994; Rutledge & Gupta, 2006). Although there is currently no standardized animal test system for general use for repellents, the mouse is considered a suitable model for screening topical mosquito repellents. For example, repellence values obtained in tests on mice exposed to different products, converted to values expected in tests on human volunteers (based on empirically correction terms) did not differ significantly from values obtained in tests on volunteers (Rutledge *et al.*, 1994).

In the present study repellence persistence was not evaluated, and repellence potential was assessed for a short period of time (2 min exposure), and thus we consider that interspecies effects on repellence were reduced. This screening allowed the identification of extracts that merit further evaluation including safety for humans, field confirmation trials and

repellency duration would be worthy to confirm their usefulness as alternative botanical repellents, as well as the development of new formulations.

The extracts of *C. tomentosa* showed good repellence and were as effective as DEET against the malarial vector *An. arabiensis*. This could be attributed to the fact that it belongs to the family Capparaeae which has a high concentration of chemicals such as stachydrine and 3-hydroxy-4-methoxy-3-methyl-oxindole (Raven *et al.*, 1999). The methanolic extract displayed greater activity because polar solvents extract more volatiles. Volatiles have been reported to have a strong repellency and mortality activity (Choochote *et al.*, 2004). Because of its repellent and insecticidal potential, *C. tomentosa* can therefore be recommended as a probable source of biologically active compounds useful in the development of potential alternatives for vector control, in areas where mosquitoes are resistant to conventional insecticides.

*Anopheles arabiensis* repellence assay using the leaves and flowers of *L. leonurus* showed strong repellency, and moderate knockdown and mortality. The methanolic flower extract of *L. leonurus* showed a greater repellency in comparison to the aqueous extract, as well as a comparatively high knockdown after 60 min exposure. This could be attributed to the fact that it belongs to the family Lamiaceae which has a high concentration of chemicals such as rosmarinic acid and other derivatives of caffeic acid (Raven *et al.*, 1999) and diterpenoids (Van Wyk & Gericke, 2003) that have shown repellent activity against mosquitoes (Tunón *et al.*, 1994). Plants such as *Ocimum basilicum* (basil), *Mentha citrata* (bergamot), *Nepeta cataria* (catnip), *Salvia sclarea* (clary sage), *Mentha arvensis* (cornmint), *Lavandula angustifolia* (lavender) and common herbs from the family Lamiaceae such as thyme, marjoram, sweet marjoram, oregano, rosemary, sage and spearmint all demonstrate a strong anti-mosquito activity because of the presence of monoterpenes, sesquiterpenes and phenols (Pavela, 2005; Gillij *et al.*, 2008; Gleiser *et al.*, 2011).

The methanolic and aqueous extracts of *D. cinerea* demonstrated good repellence and moderate mortality against *An. arabiensis*. This could be attributed to the chemicals such as polyphenols (especially flavonoids and tannins) present mostly in the family Fabaceae (Raven *et al.*, 1999). Repellence of methanolic and aqueous extracts of *C. dimidiatus* against *An. arabiensis* may be attributed to it belonging to the family Mesembryanthemaceae, which has a high concentration of chemicals such as tannins, malic acid and citric acid (Van Wyk & Gericke, 2003), compounds that have repellency activity (Choochote *et al.*, 2004). The use of plant extracts in insect control and/or personal protection is an alternative pest control method for minimizing the noxious effects of some synthetic compounds on the environment (Fatope *et al.*, 1993). *Dichrostachys cinerea* offers a potential source for bioactive compounds against *An. arabiensis*, particularly in its mosquito repellency ability. Further studies are required to isolate the active compounds involved.

A growing number of plant based products are very promising against mosquitoes and can be used as insecticides and/or repellents. They offer a safer alternative to synthetic chemicals and can be obtained by individuals and communities easily at a very low cost. Most of the plant extracts used in this study have shown repellent effects against *An. arabiensis* and may be useful for personal protection against the mosquitoes by individuals, thus minimizing the dependency on synthetic chemicals. These plant derivatives are probable sources of some biologically active compounds for mosquito control in the future. Since most of the plant based products are not as effective as synthetic insecticides and do not produce fast results, their use for mosquito control in a large scale programme under epidemic conditions may not be acceptable. However, the use of indigenous plant based products by individuals and communities can provide a prophylactic measure for protection against various mosquito borne diseases. There is a

need for promoting the use of herbal products through community based vector control programmes.

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## REFERENCES

- Ansari, M.A. & Razdan, R.K. (1995). Relative efficacy of various oils in repelling mosquitoes. *Indian Journal of Malariology* **32**: 104-111.
- Bagavan, A., Rahuman, A.A., Kamaraj, C. & Geetha, K. (2008). Larvicidal activity of saponin from *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research* **103**: 223-229.
- Bhatnagar, M., Kapur, K.K., Alers, S. & Sharma, S.K. (1993). Laboratory evaluation of insecticidal properties of *Ocimum basilicum* L. and *O. sanctum* L. plants essential oils and their major constituents against vector mosquito species. *Entomological Research* **17**: 21-26.
- Choochote, W., Teutun, B., Kanjanapothi, D., Rattanachanpichai, E., Chaithong, U., Chaiwong, P., Jitpakdi, A., Tippawangkosol, P., Riyong, D. & Pitasawat, B. (2004). Potential of crude seed extract of celery *Apium graveolens* L. against the mosquito *Aedes aegypti* (L) (Diptera: Culicidae). *Journal of Vector Ecology* **29**: 340-346.
- Coleman, R.E., Kumpitak, C., Ponlawat, A., Maneechai, N., Phunkitchar, V., Rachapaew, N., Zollner, G. & Sattabongkot, J. (1994). Infectivity of asymptomatic *Plasmodium*-infected human populations to *Anopheles dirus* mosquitoes in Western Thailand. *Journal of Medical Entomology* **41**: 201-208.
- Debboun, M., Frances, S.P. & Strickman, D.A. (Ed) (2006). *Insect Repellents: Principles, Methods, and Uses*. CRC Press - 495 pp.



- Dua, V.K., Pant, C.S. & Sharma, V.P. (1996). Determination of levels of HCH and DDT in soil, water and whole blood from bioenvironmental and insecticide-sprayed areas of malaria control. *Indian Journal of Malariology* **33**: 7-15.
- El Hag, E.A., Nadi, A.H. & Zaitoon, A.A. (1999). Toxic and growth retarding effects of three plant extracts on *Culex pipiens* larvae (Diptera: Culicidae). *Phytotherapy Research* **13**: 388-392.
- El Hag, E.A., Abd-Ei, R., El-Nadi, H. & Zaitoon, A.A. (2001). Effects of methanolic extracts of neem seeds on egg hatchability and larval development of *Culex pipiens* mosquitoes. *Indian Veterinary Journal* **78**: 199-201.
- Fatope, M.O., Ibrahim, H. & Takeda, Y. (1993). Screening of higher plants reputed as pesticides using brine shrimp lethality assay. *International Journal of Pharmacognosy* **31**: 250-254.
- Frances, S.P., Eamsila, C., Pilakasiri, C. & Linthicum, K.J. (1996). Effectiveness of repellent formulations containing DEET against mosquitoes in northeastern Thailand. *Journal of American Mosquito Control Association* **12**: 331-333.
- Gillij, Y.G., Gleiser, R.M. & Zygadlo, J.A. (2008). Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. *Bioresource Technology* **99**: 2507-2515.
- Gleiser, R.M. & Zygadlo, J.A. (2007). Insecticidal properties of essential oils from *Lippia turbinata* and *Lippia polystachya* (Verbenaceae) against *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitology Research* **101**: 1349-1354.
- Gleiser, R.M. & Zygadlo, J.A. (2009). Essential oils as potential bioactive compounds against mosquitoes. In: *Recent Advances in Phytochemistry* Ed. F. Imperato. Research Signpost, Kerala. pp.53-76.
- Gleiser, R.M., Bonino, M.A. & Zygadlo, J.A. (2011). Repellence of essential oils of aromatic plants growing in Argentina against *Aedes aegypti* (Diptera: Culicidae). *Parasitology Research* **108**: 69-78.
- Greenwood, B.M., Fidock, D.A., Kyle, D.E., Kappe, S.H.I., Alonso, P.L. & Collins, F.H. (2008). Malaria: progress, perils, and prospects for eradication. *Journal of Clinical Investigation* **118**: 1266-1276.
- Gupta, R.K. & Rutledge, L.C. (1994). Role of repellents in vector control and disease prevention. *American Journal of Tropical Medicine and Hygiene* Suppl **50**: 82-86.
- Isman, M.B. (2000) Plant essential oils for pest and disease management. *Crop Protection* **19**: 603-608.
- Jeremy, C.K.L. & Whiteman, M. (2003). Antioxidant activities of some Chinese herbs. *Free Radical Biology and Medicine* **26**: 1231-1237.
- Kembro, J.M., Marin, R.H., Zygadlo, J.A. & Gleiser, R.M. (2009). Effects of the essential oils of *Lippia turbinata* and *Lippia polystachya* (Verbenaceae) on the temporal pattern of locomotion of the mosquito *Culex quinquefasciatus* (Diptera: Culicidae) larvae. *Parasitology Research* **104**: 1119-1127.
- Mabaso, M.L., Sharp, B. & Lengeler, C. (2004). Historical review of malaria control in Southern Africa with emphasis on the use of indoor residual house-spraying. *Tropical Medicine and International Health* **9**: 846-856.
- Matsude, B.M., Surgeoner, G.A., Heal, J.D., Tucker, A.O. & Maciarelo, M.J. (1996). Essential oil analysis and field evaluation on the citrus plant *Pelargonium citrosum* as a repellent against populations of *Aedes* mosquitoes. *Journal of the American Mosquito Control Association* **12**: 69-74.
- McCabe, E.T., Barthel, W.F., Gertler, S.I. & Hall, S.A. (1954). Insect repellents. III. *N,N*-diethylamides. *Journal of Organic Chemistry* **19**: 493-498.

- Pavela, R. (2005). Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. *Fitoterapia* **76**: 691-696.
- Perich, M., Wells, C., Bertsch, W. & Tredway, K.E. (1995). Isolation of the insecticidal components of *Tagetes minuta* (Compositae) against mosquito larvae and adults. *Journal of the American Mosquito Control Association* **11**: 307-310.
- Rao, D.R., Mani, T.R., Rajendran, R., Joseph, A.S., Gajanana, A. & Reuben, R. (1995). Development of high level of resistance to *Bacillus sphaericus* in a field population of *Culex quinquefasciatus* from Kochi, India. *Journal of the American Mosquito Control Association* **11**: 1-5.
- Raven, P.H., Evert, R.F. & Eichhorn, S.E. (1999). Families yielding important phytopharmaceuticals, 6<sup>th</sup> edition. WH Freeman, New York.
- Redwane, A., Lazrek, H.B., Bouallam, S., Markouk, M., Amarouch, H. & Jana, M. (2002). Larvicidal activity of extracts from *Quercus lusitaniavar infectoria* gals (Oliv). *Journal of Ethnopharmacology* **79**: 261-263.
- Robert, V., Verhave, J.P. & Carnevale, P. (1990). *Plasmodium falciparum* infection does not increase the precocious mortality rate of *Anopheles gambiae*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**: 346-347.
- Rutledge, L.C., Gupta, R.K., Wirtz, R.A. & Buescher, M.D. (1994). Evaluation of the laboratory mouse model for screening topical mosquito repellents. *Journal of the American Mosquito Control Association* **10**: 565-571.
- Rutledge, L.C. & Gupta, R.K. (2006). Animal models for research and development of insect repellents for human use. In *Insect Repellents: Principles, Methods, and Uses*, Debboun, M., Frances, S.P. & Strickman, D.A. (Ed). CRC Press, pp. 125-146.
- Schreck, C.E. & Leonhardt, B.A. (1991). Efficacy assessment of Quwenling, a mosquito repellent from China. *Journal of the American Mosquito Control Association* **7**: 433-436.
- Severini, C., Rom, R., Marinucci, M. & Rajmond, M. (1993). Mechanisms of insecticide resistance in field populations of *Culex pipiens* from Italy. *Journal of the American Mosquito Control Association* **9**: 164-168.
- Shirley, E.A. (1987). Application of ranking methods to multiple comparison procedures and factorial experiments. *Applied Statistics* **36**: 205-213.
- Sinka, M.E., Bangs, M.J., Manguin, S., Coetzee, M., Mbogo, C.M., Hemingway, J., Patil, A.P., Temperley, W.H., Gething, P.W., Kabaria, C.W., Okara, R.M., Van Boeckel, T., Godfray, H.C.J., Harbach, R.E. & Hay, S.I. (2010). The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. *Parasites and Vectors* **3**:117 <http://www.parasitesandvectors.com/content/3/1/117>. Accessed 15 May 2012.
- Sukumar, K., Perich, M.J. & Boobar, L.R. (1991). Botanical derivatives in mosquito control. *Journal of the American Mosquito Control Association* **7**: 210-237.
- Syamala Devi, G. & Vasudevan, P. (1995). Indigenous plants and their extracts for the control of pests. *Changing Villages* **14**: 15-20.
- TEACH (2007). Toxicity and Exposure Assessment for Children's Health. Diethyltoluamide (DEET) Chemical Summary U.S. EPA. <http://www.epa.gov/teach/> Accessed 15 May 2012.
- Templeton, W. (1969). *An introduction to the chemistry of terpenoids and steroids*. Butterworths, London.
- Traboulsi, A.F., Taoubi, K., El-Haj, S., Bessiere, J.M. & Ramal, S. (2002). Insecticidal properties of essential plant oils against the mosquito *Culex pipiens molestus* (Diptera: Culicidae). *Pest Management Science* **58**: 491-495.

- Tripathi, A.K., Prajapati, V., Aggarwal, K.K., Khanuja, S.P.S. & Kumar, S. (2000). Repellency and toxicity of oil from *Artemisia annua* to certain stored product beetles. *Journal of Economic Entomology* **93**: 43-47.
- Tripathi, A.K., Prajapati, V., Verma, N., Bahl, J.R., Bansal, R.P. and Khanuja, S.P.S. (2002). Bioactivities of the leaf essential oil of *Curcuma longa* (var. ch-66) on three species of stored-product beetles (Coleoptera). *Journal of Economic Entomology* **95**: 183-189.
- Tunón, H., Thorsell, W. & Bohlin, L. (1994). Mosquito repelling activity of compounds occurring in *Achillea millefolium* L. (Asteraceae). *Economic Botany* **48**: 111-120.
- Van Wyk, B. & Gericke, N. (2003). *Peoples Plants – a guide to useful plants of South Africa*. Briza Publications, Pretoria.
- Walker, T.W., Robert, L.L. & Copeland, R.A. (1997). Field evaluation of arthropod repellents, DEET and Piperidine compound A13-37220, against *Anopheles funestus* and *Anopheles arabiensis* in western Kenya. *Journal of the American Mosquito Control Association* **12**: 172-176.
- Wattal, B.L., Joshi, G.C. & Das, M. (1981). Role of agricultural insecticides in precipitating vector resistance. *Journal of Communicable Disease* **13**: 71-73.
- World Health Organization (1981a). Instructions for susceptibility or resistance of mosquito larvae to insecticides: WHO \VBC\81.807.
- World Health Organization (1981b). Instruction for determining susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate diagnostic tests: WHO \VBC\81.806.
- World Health Organization (1995). Traditional medicine. World Health Organization, Geneva.
- World Health Organization (2010). World malaria report 2010. Geneva: WHO; pp. 60-61.
- World Health Organization Pesticide Evaluation Scheme (WHOPES) (2009). Guidelines for efficacy testing of mosquito repellents for human skin. WHO/HTM/NTD/WHOPES/2009.4 World Health Organization, Geneva.