# LARVICIDAL POTENTIAL AND MOSQUITO REPELLENT ACTIVITY OF *CASSIA MIMOSOIDES* EXTRACTS

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**Abstract.** This study aims to investigate larvicidal activities of extracts of *Cassia mimosoides* leaves and pods as a potential agent in vector control of malaria and to evaluate repellent effect against *Anopheles gambiae* mosquito of the extract formulated in an aqueous cream base. Petroleum spirit, ethanol, water and dichloromethane extracts were tested against third and fourth instar *Anopheles gambiae* larvae. The petroleum extract was formulated in an aqueous cream base and repellency determined using N-N-diethyl-m-toluamide (DEET) as control. Phytochemical analysis showed the presence of saponins, tannins, anthraquinones, steroids, and flavonoids but absence of cardiac glycosides and alkaloids in powdered *C. mimosoides.* A dose related response was observed in the mortality rate of the extracts, with 2 mg/ml petroleum ether and dichloromethane extracts achieving 100 % mortality. Larvicidal activity of extracts based on $LC_{50}$ values was petroleum ether > dichloromethane > ethanol > water. The formulated petroleum ether extract cream had a characteristic odor, hard and smooth texture, skin feeling of smoothness, ease of application by rubbing, easy removal using soap and water, non-irritating effect on skin and an acceptable pH value. The cream containing 2%-6% (w/w) extract and control achieved 100% repellency against mosquitoes after an exposure time of 5 minutes. There was a linear relationship between percent concentration of plant extract in the cream samples and repellent activity. These results suggest that crude extracts of *C. mimosoides* can be developed as eco-friendly larvicide and mosquito repellent and encourage further effort to investigate the bioactive compounds in the extracts.

**Keywords:** *Anopheles gambiae, Cassia mimosoides,* aqueous cream, larvicide, mosquito repellent

## INTRODUCTION

Insect-transmitted disease remains a major source of illness and death worldwide. Although insect-borne disease currently represents a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk. Mosquitoes alone transmit diseases to more than 700 million persons annually (WHO, 2010). Mosquitoes are considered as being nuisance pests; they are major vectors for the transmission of several life threatening diseases, such as malaria, dengue fever, yellow fever, filariasis, schistosomiasis, and Japanese encephali-

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tis, causing millions of deaths every year. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions, such as angioedema. Malaria kills just under one million persons each year, mainly children in sub-Saharan Africa, with 200-450 million infections annually worldwide (WHO, 2010).

Insect repellent is known to play an important role in preventing vector-borne diseases by reducing man-vector contact. Currently a variety of repellents are marketed to control mosquito-human contact in the form of mats, coils, lotions and vaporizers. There also are mosquito repellent products available based on sound production, particularly inaudible high frequency ultrasounds (Sylla *et al,* 2000).

Mosquito control has become increasingly difficult because of the indiscriminate use of synthetic chemical insecticides, which have adverse impacts on the environment and disturb ecological balance as some are non-biodegradable. A number of repellents of synthetic origin may cause skin irritation and affect the dermis. The majority of commercial repellents are prepared by using chemicals such as allethrin, N-N-diethyl-m-toluamide (DEET), dimethylphthalate (DMP) and N, N-diethyl mendelic acid amide (DEM) (Lipscomb *et al*, 1992). It has been reported that these chemical repellents are not safe for public use because of their unpleasant smell, oily feeling and potential toxicity (Lipscomb *et al*, 1992).

Plants may provide an alternative source of compounds for control of mosquitoes because they are rich in bioactive chemicals, which are active against a limited number of species including specific target insects, and are biodegradable (Elumalai *et al*, 2010). Considerable efforts have been made to develop and encourage the use of environmental friendly insecticide materials that are not persistent, have low mammalian toxicity and are relatively safe. Extracts or essential oils from plants may be alternative sources for mosquito larval control, as they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products. Many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Amer and Mehlhorn, 2006) and there are documented evidence of repellant activities (Muthukrishnan *et al*, 1997; Das *et al*, 2000; Pushpanathan *et al,* 2008; Aremu *et al,* 2009).

*Cassia mimosoides* is a medicinal herb native to China that now grows wild in many parts of the world. Traditional uses include the use of leaves or whole plant parts to facilitate urine voiding and for anti-inflammatory purposes, and roots as cure for diarrhea (Sugimoto, 2001). However, there appears to be little or no information on the larvicidal properties of crude extracts of *C. mimosoides.* This study reports the larvicidal activity of *C. mimosoides* extracts and formulation as a dermatological cream as a mosquito repellent.

## MATERIALS AND METHODS

**Materials**

Dichloromethane, ethanol and purified water were obtained from MBS Scientific (Wickford, UK) and hard paraffin, petroleum spirit and white soft paraffin from BDH Chemical (Poole, UK). Fresh leaves and pods of *C. mimosoides* were identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Nigeria. Larvae of *Anopheles gambiae* were collected from stagnant water in district

Table 1
Larvicidal effect of *C. mimosoides* extracts against third instar larvae of *Anopheles gambiae* ($n$ = 20).

| Type of solvent | Conc (mg/ml) | Period of exposure | | | |
|---|---|---|---|---|---|
| | | 24 hours | | 48 hours | |
| | | Mortality (%) | Survival (%) | Mortality (%) | Survival (%) |
| Petroleum ether | 0.25 | 40 | 60 | 50 | 50 |
| | 0.50 | 77 | 23 | 85 | 15 |
| | 1.00 | 95 | 5 | 97 | 3 |
| | 2.00 | 100 | 0 | 100 | 0 |
| Dichloromethane | 0.25 | 32.5 | 67.5 | 37.5 | 63.5 |
| | 0.50 | 65 | 35 | 77 | 13 |
| | 1.00 | 72 | 28 | 83 | 17 |
| | 2.00 | 100 | 0 | 100 | 0 |
| Ethanol | 0.25 | 25 | 75 | 27 | 73 |
| | 0.50 | 30 | 60 | 42 | 58 |
| | 1.00 | 32 | 68 | 40 | 60 |
| | 2.00 | 35 | 65 | 67 | 33 |
| Water | 0.25 | 10 | 90 | 12 | 88 |
| | 0.50 | 12 | 88 | 17 | 83 |
| | 1.00 | 22 | 78 | 22 | 78 |
| | 2.00 | 22 | 78 | 27 | 73 |

of Sagamu, Nigeria and mosquitoes were disease-free laboratory-reared female mosquitoes (8-11 days of age).

**Phytochemical analysis**

Assays for alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponin, steroids, and tannins were carried out according to established procedures (BP, 1998).

**Extraction of plant materials**

Powdered leaves and pods of *C. mimosoides* were extracted successively with ethanol (100%), petroleum spirit, water and dichloromethane in a Soxhlet apparatus for 18 hours and the extracts were filtered through Whatman filter paper No. 4, and then were concentrated at 40ºC under vacuum and stored at 4ºC.

**Determination of larvicidal activity**

Larvicidal activity of the crude plant extracts were assessed using standard method (WHO, 2009). In brief, extracts (0.25, 0.5, 1, and 2 mg/ml) were prepared and tested against freshly molted (0-6 hours) third instar larvae of *An. gambiae.* Larvae ($n$ = 20) were introduced into 20 ml of aqueous medium of each plant extract. Larval mortality was recorded after 24 and 48 hours of exposure. Larvae were considered dead if they did not move after touching with a needle or showed discoloration and unnatural position. Experiments were conducted in duplicate.

**Formulation of *C. mimosoides* extract cream**

Plant extract with the highest larvicidal activity was used in the formula-

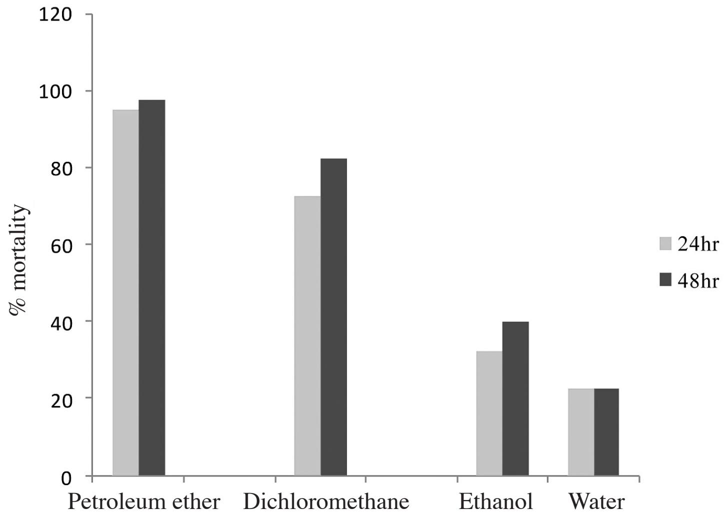


Fig 1–Larvicidal activity of *C. mimosoides* crude extracts against *Anopheles gambiae* third instar larvae. Larvae (*n* = 20) were exposed to 1 mg/ml plant extract.

tion of cream to produce 25 g of aqueous cream containing 0.5%, 1%, 2%, 4%, and 6% (w/w) extract and stored in sterile containers.

**Characterization of the creams**

Physical evaluation of the creams was carried out by observing the appearance, color, texture, odor, ease of application, ease of removal, and skin feeling and irritation. The pH value was determined. Stability of the creams was evaluated by leaving the preparations at 0, 4, 25, and 37°C and were observed for separation of the drug from the base after 24 hours, 72 hours, 7 days, 2 weeks and 3 weeks. Rheology of the creams was measured using a Brookfield viscometer.

**Mosquito repellent activity**

Repellent efficiency of the cream formulation was performed on human volunteers as previously described (Tawatsin *et al*, 2006). In brief, host seeking behavior of the mosquitoes was tested prior to the experiment by placing a precleaned (with alcohol) hand into a 75 × 60 × 60 cm net cage containing approximately 200 non-blood fed female (5-7days old) *An. gambiae* mosquitoes and the numbers of mosquitoes that alighted within 5 minutes were counted. The repellency test was performed using 0%, 0.5%, 1%, 2%, 4%, and 6% (w/w) repellent formulations, and with 12% (w/w) DEET cream as control. The cream was applied on each volunteer's forearm up to the wrist and the arm was introduced into the cage and the numbers of mosquitoes that alighted or bit the treated area of each volunteers hand within 5 minutes were recorded. Three volunteers were used for each cream sample. Repellent efficiency was calculated using the formula: percent repellency = 100 - [(number of bites on treated arm) / (number of bites on untreated arm)] × 100. The volunteers were informed about the test and consents were taken.

## RESULTS

The ethanol extract gave the highest yield of 12%, followed by water extract (10.4%), dichloromethane (3.6%) and petroleum spirit (1.5%). Phytochemical screening of powdered *C. mimosoides* revealed the presence of anthraquinones, flavonoids, saponins, steroids, and tannins, but absence of alkaloids and cardiac glycosides (data not shown).

Highest larvicidal activity of *C. mimosoides* extracts (1 mg/ml) against *An. gambiae* third instar larvae was found after 48 hour exposure in petroleum ether extract,

Table 2
$LC_{50}$, $LC_{90}$ and statistical analysis of *C. mimosoides* extracts against third instar larvae ($n$ = 20) of Anopheles gambiae at 24 hours of exposure.

| Plant extract | $LC_{50}$ (mg/ml) | $LC_{90}$ (mg/ml) | $R^2$ |
|---|---|---|---|
| Petroleum ether | 0.28 | 1.36 | 0.6526 |
| Dichloromethane | 0.41 | 1.61 | 0.8664 |
| Ethanol | 4.85 | 12.92 | 0.8072 |
| Water | 5.53 | 11.07 | 0.7217 |

$LC_{50}$, concentration that kills 50 % of exposed larvae; $LC_{90}$, concentration that kills 90 % of exposed larvae.

Table 3
Percent repellency of formulated petroleum ether extract of *Cassia mimosoides* after exposure of 5 minutes.

| Cream (% w/w) | % repellency |
|---|---|
| 0 | 3.7 |
| 0.5 | 48.1 |
| 1 | 88.9 |
| 2 | 100 |
| 4 | 100 |
| 6 | 100 |
| Control[a] | 100 |

[a]12% (w/w) DEET.

followed by that of dichloromethane, ethanol and water (equal activity at 24 and 48 hours) (Fig 1). This indicates that the more non-polar the extraction solvent the higher the larvicidal activity of *C. mimosoides* extract. A dose related response was observed in the mortality rates of the extracts, with 2 mg/ml petroleum ether and dichloromethane extracts achieving 100 % mortality (Table 1). Petroleum ether extract also was the most active against *An. gambiae* larvae with a significantly lower $LC_{50}$ than the other extracts ($p$< 0.05) (Table 2). The $LC_{90}$ of the petroleum ether extract was also the lowest. Larvicidal activity based on the $LC_{50}$ was highest with petroleum ether, followed by that of dichloromethane, ethanol and water.

Based on the larvicidal results, petroleum ether extract of *C. mimosoides* was chosen to be formulated into a mosquito repellent cream. The cream samples showed good physical properties and were stable, with no layer separation over a period of 3 weeks at 37°C indicating drug-vehicle compatibility. All *C. mimosoides* formulated cream samples had acceptable pH values, falling within the range of 4 to 6.5, pH values suitable for the skin (Yosipovitch *et al*, 2003). The formulation had a characteristic odor, and was hard and smooth in texture, smooth on skin, easy to apply by rubbing, easy to remove with soap and water and nonirritant to skin. Mosquito repellency of the creams showed a linear dose-response effect over the concentration range 0.5%-2% (w/w) petroleum ether extract, with 100% repellency at ≥ 2% (w/w), equal to that of 12% (w/w) DEET (Table 3).

## DISCUSSION

Although higher yields of *C. mimosoides* extracts were obtained using polar solvents (water and ethanol), mosquito larvicidal properties resided in the non-polar extracts (petroleum ether and dichloromethane). Several reports have attributed the various biological activities in plants to the presence of alkaloids, cardiac glycosides, saponins and sterols (Akinpelu and Soetan, 2006), but *C. mimosoides* leaves and pods lacked alkaloids and cardiac glycosides. Creams formulated from *C. mimosoides* petroleum ether extract were at least 6-fold more effective than DEET-containing cream in repelling mosquitoes. These data indicate that future efforts should be directed in identifying the bioactive compounds in *C. mimosoides* petroleum ether extract that can be developed as eco-friendly larvicides and safe mosquito repellent products.

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