

Research Article



Formulation of A Plant Based Repulsive Candle from Oils of *Azadirachta indica*. JUSS (Meliaceae) Seeds and of *Cymbopogon citratus* (DC.) STAPF (Poaceae) Leaves

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ABSTRACT

Malaria is endemic to sub-Saharan Africa where it kills thousands of people every year mainly children under five years age. The burden of the disease has increased these recent years due to the rising costs as well as the development of resistances and toxicity associated with synthetic insecticides used to kill the malaria vector, the mosquitoes *Anopheles*. Therefore, it is important and urgent the search for other sources of insecticides. Plant-based products have emerged as a solution providing low-cost products with neglected side effects. In the present study, we prepared and evaluated repulsive candles containing oils of *Azadirachta indica* and *Cymbopogon citratus*, two plants well known for their repulsive effect against mosquitoes. Steam distillation of *C. citratus* dried leaves and mechanical pressing of dried seeds of *A. indica* afforded oils with a yield of 0.44 % and 16.98%, respectively. The presence of terpenoids was detected in both oils. The group of candles containing 2.5% of essential oils of *C. citratus* was found to be the most active exhibiting 80 - 95 % repellency. This study demonstrates the importance of using oils from medicinal plants for the formulation of repellent candles.

Keywords: Formulation, *Cymbopogon citratus*, *Azadirachta indica*, candle.

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INTRODUCTION

Malaria is the most widespread endemic disease worldwide with 229 million cases reported in 2019 causing 409,000 fatalities with 94 % of death occurring in Africa. There was an estimated of 33 million pregnant women, of which 35 % were exposed to malaria infection during pregnancy.¹ The management of the disease is compromised by the increase in resistances of the parasite *Plasmodium* and the vector *Anopheles* to available antimalarial drugs and insecticides, respectively.¹ In Cameroon, more than 2 million cases occur every year.¹ This deathful disease caused by the *Plasmodium spp.* is transmitted to humans through mosquito bites belonging to the genera of *Anopheles* and the mosquitoes species *Anopheles gambiae* Giles which is the main vector responsible for transmission, and are among the most disturbing blood sucking insects afflicting human beings. Deforestation, high temperatures, lack of hygiene, standing water, unused plastics pools, old tires or buckets,

dirty gutters, leaks and swampy areas are responsible for the alarming increase in the number of mosquitoes.¹⁻² The use of chemical mosquito's repellents, vaccination and sleeping under mosquitoes nets are represent the first major solutions for the fight against the spread of *Anopheles*.¹ Regarding mosquitoes repellents, they are very expensive, scarce and most of people dislike the smoke of the synthetic pyrethroid containing mosquito coils. In addition, most of the mosquito's repellents available in markets are based on toxic reagents.³ Their toxicity against the skin and nervous system can cause rashes, swelling, eye irritation and other serious health problems.³ Therefore, the development of bio-based mosquito's repellents is needed to replace chemical mosquito's repellents.

Bio-based repellent are insect management tools which active ingredients are derived from natural sources such as plants.⁴ Plant products are emerging as a potential source of mosquitoes control and among them essential oils belonging to various plant species and their mixtures have been shown to act as effective repellent against various mosquitoes and pests, *Azadirachta indica* oil has been used as an alternative for mosquitoes repellent.⁵⁻⁷ It has also been demonstrated that essential oils of *Hyptis suaveolens* and *Cymbopogon citratus* possess repellent properties against mosquitoes.⁸ The preparation of repellents from different essential oils represents an alternative to fight



against mosquitoes, hence, reducing malaria burden. The aim of the present study is to formulate candles from oils of *Cymbopogon Citrullus* and *Azadiracta indica* and evaluate their physicochemical properties and repellency against mosquitoes were also evaluated.

MATERIALS AND METHODS

Vegetable Materials

Cymbopogon citratus leaves were harvested in Soa, Center region of Cameroon and *A. indica* seeds were harvested in Garoua, North region of Cameroon. Both plants were identified and authenticated at the national herbarium in comparison with Voucher number N°18628/SRF Cam for *C. citratus* and N° 19223/SRFK for *A. indica*.

Extraction of Essential Oil

Essential oil *C. citratus* was obtained by steam distillation using a modified Clevenger Apparatus while oil of *A. indica* was obtained by mechanical pressing using a local mechanical press. The yield of the oil was calculated using the formula below.

$$\text{Yields} = \frac{\text{Mass of oil (g)}}{\text{Mass of vegetable (g)}} \times 100$$

Qualitative Phytochemical Screening of *C. Citratus* and *A. Indica* Oils

The identification of secondary metabolites was done following the method described by Harbone et al.⁹

Formulation of Repulsive Candles

Three groups of candles were formulated, a group containing *C. citratus* oil, another group with *A. indica* oil and the last group containing the combination of both oils. For each lot 975g of bee wax were put in a mixer and set on at a temperature of 80°C to melt the bee wax. The wicks for the candle were prepared simultaneously by cutting them at a precise length and attaching them to sticks. They were immersed into the melted wax to harden them. Once the wax was melted, 25g of the essential oils was added and well homogenized. The obtained mixture (100 mL) was poured in glasses containing the prepared wick stick and allow to cool. After 24 h, the glasses containing candles were covered with parafilm and labelled following the European Pharmacopoea. The formulated candles contained 2.5% of essential oils and in the case of the mixture of both oils 1.4% and 1.1 % of *A. indica* and *C. citratus* oils, respectively.

Performance Tests on the Prepared Candles

Uniformity of mass:

Ten candles of each group were selected at random and weighed individually. The weight of individual group was noted. Average weight was calculated, and the individual weights were compared with the average weight.

Organoleptic test of repellent candles:

This test was done visualizing the formulation to evaluate the texture, color and scent.

Melting point of repulsive candles:

The melting point was determined by the maximum temperature that allow the changing of state of candle. For this, small quantities of candles were put in a capillary tube and immersed in the disintegrator. The temperature was set at 50°C. Temperature was increased until a change in state was observed and the temperature noted.

Flame test of repulsive candles:

The wick was lighted to appreciate the behavior, color, residue and stability of the flame

Burning rate (BR) of candles:

For this test, initial mass of candle (M1) and the time of lighting (T1) were noted. Once the candle stops burning the time (T2) and mass (M2) were once again noted. The time of burning was T2 – T1 in hours and the mass burnt was M2 – M1 in grams. The burning rate was calculated according to the follow formula $\frac{M2-M1}{T2-T1}$ in grams per hour.

Field candle mosquito repellent test:

The repellent tests were conducted in October 2021 (rainy Month) from 6 to 9 pm during 3 days in the botanical garden of Institute of Medical research and medicinal Plants studies (IMPM) in Cameroon according to the method described by Muler et al.¹⁰ The site was selected based on the high density and large diversity of mosquitoes species previously prospected around the place. The average temperature of the site during the experiments ranged from 26 to 28°C with relative humidity fluctuating from 68 to 72 %. To start the test, three volunteers (2 males and 1 female) were selected and were therefore fully informed of the nature and purposes of the test. The legs and arms of each volunteer were used as the test areas. Before each trial, the exposed body parts on each volunteer were cleaned with distilled water and volunteers avoided to apply any perfume or fragrance products during the whole test period. For test design, 4 tests and control candles were lighted and placed all at distance of 1 m for the first trial, 2 m for the second trial and 3 m for the third trial around each volunteer. Between each test set and control, a distance of 10 m from each other was maintained and the volunteers rotated their positions during the repetitions. Each test and control were done for 20 minutes with 5 minutes break for the three replicates. Thus, mosquitoes biting, probing, and landing on volunteers were recorded. Mean totals were converted to percentage of repellency following the equation below:

$$\text{Repellency rate (\%)} = \left[1 - \frac{\text{number of mosquitoes recorded in the test}}{\text{number of mosquito recorded in the control}} \right] \times 100$$

Statistical Analysis

Data converted to the percentage of repellency were submitted to Analysis of Variance using SPSS version 16.0 software. Tukey test at P=0.05 was performed for mean



separation. Graphs were designed using Microsoft Excel software.

RESULTS AND DISCUSSION

Results

Extraction Yields of Essential Oils and Phytochemical Screening

Steam distillation of the dried leaves of *C. citratus* afforded a spicy pale yellow with a lemon-like odor essential oil with a yield of 0.44%. The mechanical pressing of *A. indica* dried seeds afforded a bitter dark green oil with strong garlic-like small with a yield of 16.98%. Results of the phytochemical screenings of the two oils are reported in Table 1 below.

Table 1: Phytochemical profile of oils of *C. citratus* dried leaves and *A. indica* dried seeds

Metabolites	<i>C. citratus</i>	<i>A. indica</i>
Alkaloids	+	-
Phenolic compounds	+	+
Flavonoids	+	-
Terpenoids	+	+
Steroids	-	+
Tannins	+	+
Glucosides	-	-
Anthraquinones	+	+
Coumarins	+	+
Anthocyanin	+	+
Saponins	-	-

+: Presence of class of secondary metabolites highlighted, -: Absence of class of secondary metabolites highlighted

Formulation of Repellent Candles

Candles were formulated using bee wax. The bee wax used was white in color, semi-solid, having no odor or taste, with a density of 0.9473g/cm³ and a melting point of 65 °C. Three groups of candles were prepared with 2.5% essential oils of *C. citratus* (group 1), 2.5% of oil of *A. indica* (group 2) and 1.1% of *C. citratus* and 1.4% of *A. indica* oils (group 3) and named cycital, azical and azacymal, respectively (Figure 1). The measure melting point of the prepared candle was also 65 °C.

Performance Test on Candles

- **Uniformity of mass:** The masses of *C. citratus* candles ranged from 87.5g to 93.7 g. Those of *A. indica* candles from 82.4g to 93.9g and the masses of candles containing both oils from 85.5 to 98.1g. The masses of the candles were in accordance with the recommendation of the Pharmacopeia which stipulates that no two candles should deviate by 5% and no single candle by 10% of the average masse¹¹.
- **Organoleptic parameters of repulsive candles:** The candles aspect including color, texture, odor, cracks at the surface level and cross-section level was examined to evaluate the degree of homogeneity and the conformity of the candles. The results are grouped in table 2.



Figure 1: Illustration of the prepared candles

Table 2: Organoleptic parameters of the prepared candles

Candle Area	Parameters	Cycital	Azical	Azacymal
Surface	Aspect	Bright	Bright	Bright
	Color	Ecrú white	Creamy white	Ivory white
	Texture	Semi solid	Semi solid	Semi solid
	Cracks	Absence	Absence	Absence
	Odor	Lemon like	Garlic odor	Lemon like
Cross section	Homogeneity	Homogeneous	Homogeneous	Homogeneous
	Cracks	Absence	Absence	Absence
Conformity		Yes	Yes	Yes
Burning rates (g/h)		6.97	5.46	7.94

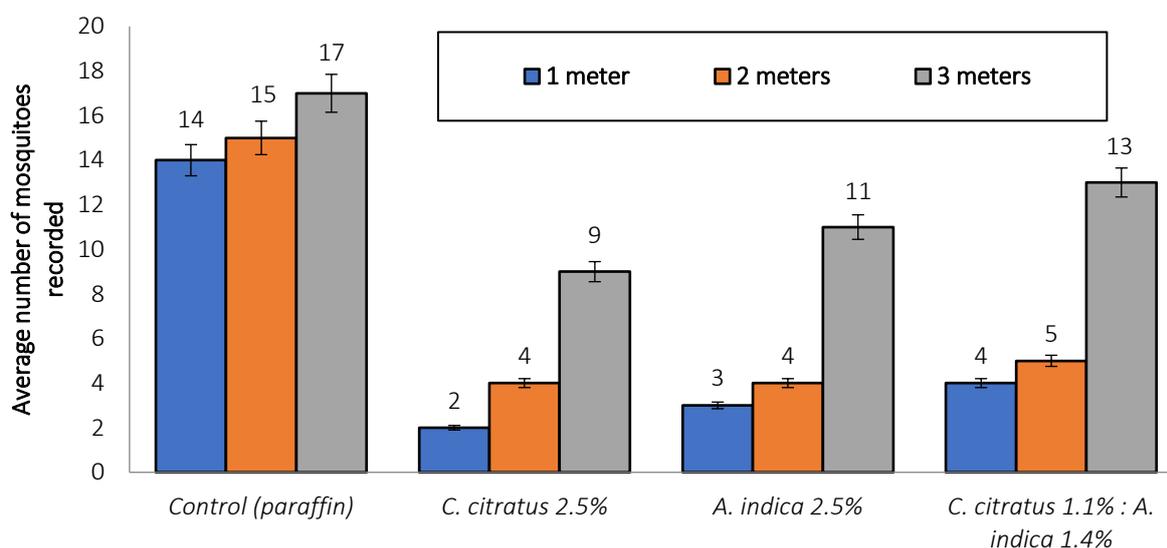


Figure 2: Average number of mosquitoes recorded in the volunteers submitted to tests of *C. citratus* 2.5 %, *A. indica* 2.5 % and *C. citratus* 1.1 % and *A. indica* 1.4 % candles formulations in the field conditions.

Table 3: Protection rate of *C. citratus* 2.5%, *A. indica* 2.5% and the mixture *C. citratus* 1.1% + *A. indica* 1.4% candles from the bites of mosquito species in field conditions.

Concentration of candle Formulation	Percentage of protection (%)			F (2, 6)
	1 meter	2 meters	3 meters	
Control (paraffin)	0.00±0.00 ^d	0.00±0.00 ^c	0.00±0.00 ^d	-
<i>C. citratus</i> 2.5%	86.03±0.54 ^{aA}	72.69±1.09 ^{aB}	46.06±3.42 ^{aC}	281.33***
<i>A. indica</i> 2.5%	79.04±0.82 ^{bA}	72.69±1.09 ^{aA}	31.71±6.95 ^{bB}	118.16***
<i>C. citratus</i> 1.1%: <i>A. indica</i> 1.4%	72.06±1.09 ^{cA}	65.87±1.35 ^{bA}	19.90±5.87 ^{cB}	158.23***
F (2, 6)	200.27***	32.43**	14.96**	

Each value represents mean±standard deviation of 3 replicates data. Means in columns followed by the same small letter and in rows followed by the same capital letter did not differ significantly according to Tukey test (P=0.05). **P<0.01 and ***P<0.001

- **Flame and burning rate:** The prepared candles were observed while burning. It was noticed they burned with a yellow, long and steady flame suggesting a complete combustion. The fragrance of the candle started diffusing after ten minutes of burning. The burning rate results are also consigned in Table 2.
- **Field candle mosquitoes repellent test**

Landing, probing, and biting pressure of mosquitoes: The average number of mosquitoes in the environment of the lighted prepared candles was recorded. A paraffin candle without oils was used as control. Figure 2 show the obtained results.

It appears from **Figure 2** that the average number of mosquitoes landing, probing, and biting varied significantly with plant candles formulation product and also the distance between candles and volunteers and ranged from 2 to 17 mosquitoes. The low average number (2 mosquitoes) of mosquitoes was obtained with the essential oils *C. citratus* candle placed at 1m of volunteer's participants and the high average number (17) of mosquitoes was obtained with the paraffin was placed at 3 m of volunteer's participants. The average landing, probing,

and biting pressure of mosquitoes toward the volunteers at 1.0 m with *A. indica* 2.5 % candle was 3 mosquitoes and 4 with the mixture *C. citratus* 1.1 % + *A. indica* 1.4 % candle compared to the high number obtained in the control (14 mosquitoes). At 2.0 m, the average number of mosquitoes landing, probing, and biting were 4, 4, 5 and 15 mosquitoes in the tests with *C. citratus* 2.5%, *A. indica* 2.5 %, *C. citratus* 1.1 % + *A. indica* 1.4% candles and control respectively. At the furthest distance (3.0 m), high number of mosquitoes landing, probing, and biting in the volunteers was recorded in *C. citratus* 2.5% (9 mosquitoes), *A. indica* 2.5 % (11 mosquitoes), *C. citratus* 1.1% + *A. indica* 1.4% (13 mosquitoes) candles and control (17 mosquitoes). These results suggest that the *C. citratus* essential oils candle has an important effect on the repellency protection.

Protection repellency rate: The protection repellency rate was done in order to evaluate the percentage of protection of each formulated candle supplemented with essential oils. Results are presented in Table 3.

From the **Table 3**, the protection percentage significantly (P<0.05) varies with distance of candles from the volunteers and also with the type of repellent candle formulated. In

comparison with the control, the repellency rate of *C. citratus* 2.5 % candle at the closest distance from the volunteers (1.0 m) toward female mosquitoes was 86.03 %, significantly high compared to *A. indica* 2.5 % candle (79.04%) and the mixture *C. citratus* 1.1% + *A. indica* 1.4 % candle (72.06 %). At 2.0 m, the repellency rate of *C. citratus* 2.5 % (72.69 %) and *A. indica* 2.5% (72.69 %) candles remained still higher compared to the mixtures of *C. citratus* 1.1% + *A. indica* 1.4% candle (65.87 %). At the furthest distance (3.0 m), the repellency rate of *C. citratus* 2.5 % candle was 46.06 %, of *A. indica* 2.5 % candle (31.71 %) and the mixture *C. citratus* 1.1 % + *A. indica* 1.4 % candle (19.90 %).

DISCUSSION

The present study was carried out to formulate and evaluate candles supplemented with *C. citratus* and *A. indica* oils for their repellency against mosquitoes. Oils were extracted from *C. citratus* dried leaves and from the dried seeds of oils *A. indica* with a yield of 0.44% and 16.98%, respectively. The essential oil yield obtained from steam distillation of dry leaves of *C. citratus* which was greater than that obtained by Hien Tran et al¹². A difference in yield was also observed with the study conducted by Cassel et al. who obtained a yield of 0.8 % after four hours extraction using steam distillation method.¹³ This difference in yield can be explained by the different equipment used, the method of extraction, site of harvest, time of harvest, plant age, farming practices and geographical origin. This yield extraction of *A. indica* obtained was lower than that obtained by Jijakli et al. (18.3 %) using mechanical pressing method while a yield of 44.29% was obtained by Liauw et al. when extracting neem seeds using ethanol and hexane solvents by Soxhlet extraction.¹⁴⁻¹⁵ Though a good yield is obtained while using solvent extraction, oil from this extraction is of low quality due to the fact that at high temperatures metabolites substances are denatured and the obtained oil is contaminated with solvents.

Three groups of candles were prepared including candles with 2.5% *C. citratus* essential oil, candles with 2.5% *A. indica* oil and candles containing both oils at 1.1% for *C. citratus* and 1.4% of *A. indica*. The prepared candles showed good conformity regarding the surface and cross section parameter including the physical aspect, the absence of cracks and homogeneity. A good combustion with a steady flame was also observed for all the three groups. The formulated candle supplement with combination of the two essential oils showed the highest burning rate of 7.94 g/h and those containing *C. citratus* oil and *A. indica* oil showed a burning rate of 6.97 g/h and 5.46 g/h, respectively. The observed burning rates was lowest than that observed for a molded wax candle in glasses without oil (9 g/h).¹⁶ This result also can be explained by the fact that, oils present in the wax reduces their melting point thus, candles melt faster. Also, oil feed the flame of the candle increasing its rate of burn.

In the field condition, prepared candles with *C. citratus* were the most repulsive against mosquitoes. The observed

repellent activity varies with the distance between candles and volunteers in the testing field. Human-landing catch experiments at the high biting pressure site (oasis) showed that under high pressure of biting insects, geraniol candles provided protection of above 50 %, which is the accepted USEPA target for claiming that a compound is repellent.¹⁷ The repellent activity observed for all the three groups of candles was above 50 %. This activity could be due to the presence of compounds such as azadirachtin in *A. indica* oil and geraniol, linalool, and citronella from *C. citratus*. Azadirachtin is a complex of tetranorterprenoid limonoid compounds which are responsible for the toxic effects in insects.¹⁸ *C. citratus* is rich in geraniol which have been showed to have good repellence against mosquitoes.^{10,19}

Far from chemicals harmful to health, and whose effectiveness has not been irreproachable, mosquito repellent candles has established itself as one of the best natural alternatives to fight against this pernicious insect.

CONCLUSION

In our study, we pre formulated repulsive candles from oils of *A. indica* seeds and *C. citratus* leaves. Extraction of oils from these plants was done using mechanical press for *A. indica* seeds and hydrodistillator for *C. citratus* leaves. The extraction yields obtained were 16.98 % and 0.44 % for *A. indica* and *C. citratus*, respectively. Phytochemical screening revealed the presence of secondary metabolites including terpenoids which might be responsible for the repulsive effect of mosquitoes. Three types of candles including, *A. indica* *C. citratus* and candles containing both oils were formulated and their repellent activity evaluated. The results identified *C. citratus* EO candles as the most active with 86.03 % following by *A. indica* oil candle with 79.04 %. Candles containing mixture of both oils were the least active (72.06 %). Our study shows that repellent candles from oils can be used to fight against the burden rate of malaria. In addition, they provide light, are not toxic, cheap and accessible to all individual.

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