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Article

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Chemical composition and repellent activity against Anopheles gambiae of essential oils from three plant taxa collected in the Democratic Republic of the Congo.

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Abstract

Essential oils (EOs) derived from aromatic plants are currently being investigated as potential mosquito repellents that could be used to complement existing preventive measures. *Anopheles gambiae* is one of the major malaria vectors in sub-Saharan Africa. The aim of this study was to evaluate the repellent activity against *Anopheles gambiae* and the chemical composition of essential oils extracted from three plant taxa collected in the Democratic Republic of the Congo : *Aframomum stipulatum*, *Lippia multiflora*, and *Leptonychiopsis* sp. The essential oils were obtained by hydrodistillation and subsequently analyzed using gas chromatography-mass spectrometry (GC-MS). Repellent activity was assessed against adult female *A. gambiae* using a human-skin bioassay. Ethanol was used as the negative control, while a 13% DEET formulation served as the positive control. The protection rate and duration of protection were measured. The essential oils investigated exhibited different chemical profiles. 1,8-Cineole was the major compound identified in the essential oils of *L. multiflora* (59.01%) and *Leptonychiopsis* sp. (61.53%), whereas it accounted for only 3.10% of the essential oil of *A. stipulatum*. The reported protection rates were 68% for *A. stipulatum*, 77% for *L. multiflora*, 93% for *Leptonychiopsis* sp., and 86% for the DEET-based positive control. The longest protection duration was recorded for the essential oil of *Leptonychiopsis* sp., reaching 82.3 minutes. Under the experimental conditions described, the essential oil of *Leptonychiopsis* sp. exhibited the highest protection rate and the longest protection duration among the essential oils tested. These preliminary findings warrant further investigation, particularly through standardized efficacy and safety studies.

Keywords:
Anopheles gambiae; essential oils; GC-MS; plant-based repellent; Democratic Republic of the Congo.

Résumé

Les huiles essentielles (HE) issues de plantes aromatiques font actuellement l'objet de recherches en tant que répulsifs potentiels contre les moustiques, pouvant être utilisés en complément des moyens de prévention existants. *Anopheles gambiae* constitue l'un des principaux vecteurs du paludisme en Afrique subsaharienne. La présente étude avait pour objectif d'évaluer l'activité répulsive vis-à-vis de *Anopheles gambiae*, ainsi que la composition chimique des huiles essentielles extraites de trois taxons végétaux récoltés en République démocratique du Congo : *Aframomum stipulatum*, *Lippia multiflora* et *Leptonychiopsis* sp. Les huiles essentielles ont été obtenues par hydrodistillation, puis analysées par chromatographie en phase gazeuse couplée à la spectrométrie de masse (CPG-SM ou GC-MS). L'activité répulsive a été évaluée sur des femelles adultes d'*A. gambiae* au moyen d'un bioessai réalisé sur la peau humaine. L'éthanol a été utilisé comme témoin négatif, tandis qu'une formulation de DEET à 13 % a servi de témoin positif. Le taux de protection ainsi que la durée de protection ont été mesurés. Les huiles essentielles étudiées ont présenté des profils chimiques différents. Le 1,8-cinéole constituait le principal composé identifié dans les huiles essentielles de *L. multiflora* (59,01 %) et de *Leptonychiopsis* sp. (61,53 %), contrairement à celle de *A. stipulatum*, dans laquelle il ne représentait que 3,10 %. Les taux de protection rapportés étaient de 68 % pour *A. stipulatum*, de 77 % pour *L. multiflora*, de 93 % pour *Leptonychiopsis* sp. et de 86 % pour le témoin positif à base de DEET. La durée de protection la plus longue a été enregistrée avec l'huile essentielle de *Leptonychiopsis* sp., soit 82,3 minutes. Dans les conditions expérimentales décrites, l'huile essentielle de *Leptonychiopsis* sp. a présenté le taux de protection le plus élevé ainsi que la durée de protection la plus longue parmi les huiles essentielles testées. Ces résultats préliminaires justifient la poursuite des recherches, notamment à travers des études d'efficacité et d'innocuité reposant sur des protocoles standardisés.

Mots clés :
Anopheles gambiae; huiles essentielles ; GC-MS; répulsif à base de plantes; République démocratique du Congo.

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1. Introduction

Malaria remains a major public health problem, particularly in sub-Saharan Africa, where it contributes substantially to morbidity and mortality, especially among children under five years of age and pregnant women (WHO, 2023). In the Democratic Republic of the Congo (DRC), *Plasmodium falciparum* is the predominant malaria parasite, and members of the *Anopheles gambiae* complex are important vectors (PNLP, 2022). Synthetic repellents such as DEET (N,N-diethyl-m-toluamide) are widely used for personal protection against mosquito bites (Nerio et al., 2010). Growing insecticide resistance in malaria vectors has also reinforced interest in complementary plant-based vector-control products (Ranson et al., 2011). Essential oils contain volatile compounds that may exhibit repellent or insecticidal activity (Isman, 2020), and several plant-derived repellents have shown activity against mosquitoes (Maia & Moore, 2011; Nerio et al., 2010). However, the repellent activity and chemical profiles of many plant taxa occurring in the DRC remain insufficiently characterized. The present study therefore evaluated the chemical composition and repellent activity against *A. gambiae* of essential oils obtained from *A. stipulatum, L. multiflora*, and *Leptonychiopsis sp*. collected in the DRC.

2. Materials and Methods

2.1. Harvesting and packaging of biological material

2.1.1. Plants used

Three plant taxa were used in this study: *Aframomum stipulatum, Lippia multiflora*, and *Leptonychiopsis sp*. After collection, the plant material was identified at the herbarium of the National Institute for Agricultural Research located at the Faculty of Science of the University of Kinshasa. Voucher specimen numbers were not reported in the manuscript and should be provided, if available, to ensure taxonomic traceability.

2.2. Female mosquitoes

A total of 1 000 female Anopheles gambiae mosquitoes were reported as being used for the repellency experiments. The mosquitoes were reared at the insectarium of the Entomology Laboratory of the National Institute for Biomedical Research (INRB), Kinshasa. The manuscript reports maintenance with 10 % glucose solution and rabbit blood meals. The strain or origin of the mosquito colony and the allocation of the 1 000 mosquitoes across experimental replicates were not specified and should be documented for reproducibility.

2.3. Methods

2.3.1. Extraction of essential oils

A 350-g quantity of fresh leaves, described as having been dried away from light, was subjected to hydrodistillation for two hours. This description is internally inconsistent (fresh versus dried material) and the actual condition of the plant material should be verified from the laboratory record. The recovered oils were separated by decantation and stored at 4 °C until analysis. Major constituents were identified by gas chromatography-mass spectrometry (GC-MS) following the method cited as Loham et al. (2015). Details of the GC-MS instrument, column, temperature program, carrier gas, identification library/criteria, and quantification procedure were not reported and should be added from the original analytical record.

2.3.2. Repellency test

Repellency bioassays were reported as being conducted according to WHO guidance (2009). Adult female mosquitoes, 72 hours post-emergence, were maintained on glucose solution and deprived of a blood meal before testing. For each treatment, 0.5 mL of essential oil preparation was applied to a human hand. Ethanol (0.5 mL) served as the negative control, whereas a commercial formulation containing 13% DEET (Pendaffaran-Medisoft) served as the positive control. The manuscript states that treated hands were introduced into a 30 × 30 cm cage containing 100 female mosquitoes and that mosquito contacts/bites were recorded at 15-minute intervals. The number of human participants, essential-oil concentrations/formulation, exposure time per interval, randomization or treatment order, washout procedure, environmental conditions, and number of biological/technical replicates were not reported and should be verified from the original protocol.

2.3.3. Protection rate

The number of mosquito bites was recorded at 15-minute intervals. The protection rate was calculated relative to the negative control using the formula below:

Protection (%) = [(C − T) / C] × 100, where T is the number of bites recorded on skin treated with the test product and C is the number of bites recorded for the negative control. This definition is consistent with the protection percentages reported in Table 2; using DEET as C, as stated in the original

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text, would not reproduce the reported values.

Statistical analysis: The manuscript states that bite-count and protection-duration data were analyzed using a Scheffé test at $\alpha = 0.05$ with SAS software (1995). However, the underlying omnibus model (e.g., ANOVA), the experimental unit, the number of replicates, measures of variability, test statistics, degrees of freedom, and exact p-values are not reported. Consequently, the inferential analysis cannot be independently verified from the information available in the manuscript; these elements should be checked against the original dataset and analysis output.

2.3.4. Ethical considerations

The repellency test involved direct application of test products to human skin and exposure of human hands to mosquitoes. Ethical approval, informed-consent procedures, participant eligibility, and safety monitoring are not reported in the manuscript. These details must be verified from the original study records and reported before publication ; no approval number should be added unless documented.

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3. Résultats

3.1. Chemical composition of the essential oils

Table 1 summarizes the major constituents reported for the essential oils analyzed by GC-MS.

Table 1: Major components of the essential oils of the plants studied

Plants	Major constituents	Retention time (minutes)	Content (%)
A. stipulatum	α-pinene	6.375	0.80
	β-pinene	6.825	1.20
	β-caryophyllene	11.262	2.53
	α-humulene	11.567	5.40
	Caryophyllene [verify identity]	12.567	0.65
	α-terpinene	13.413	0.10
	1,8-cineole	14.200	3.10
L. multiflora	α-thujene	9.23	0.27
	α-pinene	9.31	1.00
	β-pinene	9.71	0.70
	α-phellandene	10.04	0.27
	α-terpinene	10.21	4.08
	1,8-cineole	10.31	59.01
	Eugenol	13.61	0.70
	β-caryophyllene	14.45	3.04
	α-humulene	14.55	0.35
	Caryophyllene oxide	14.61	2.10
Leptonychiopsis sp.	β-caryophyllene	6.13	0.40
	α-pinene	6.52	0.80
	Isoamyl acetate	6.72	0.12
	Limonene	8.30	5.05
	1,8-cineole	9.40	61.53
	β-pinene	11.02	0.42
	p-cymene	12.13	8.11
	β-linalool	15.82	2.00
	O-cinenol [verify compound name]	20.95	0.86
	α-terpinol (α-terpinene) [verify compound identity]	22.63	10.52

GC-MS analysis identified multiple reported constituents in the essential oils of the three plant taxa (Table 1). *L. multiflora* and *Leptonychiopsis sp*. were reported to contain 1,8-cineole at 59.01% and 61.53%, respectively, whereas *A. stipulatum* contained 3.10%. Therefore, the statement that all three oils were dominated by 1,8-cineole is not supported by Table 1. Several compound names in the *Leptonychiopsis sp*. profile are ambiguous or internally inconsistent and should be verified against the original GC-MS chromatograms and library-identification output before publication. Because no inferential comparison of chemical composition is reported, differences among oils should be described as observed rather than statistically significant.

3.2. Repellent activity against Anopheles gambiae

Table 2. Observed mosquito bites, protection rate, and duration of protection for the tested essential oils and controls.

Plants	Number of bites	Protection rate (%)	Duration of protection (minutes)
Aframomum stipulatum	5.3	68	41.0
Lippia multiflora	3.8	77	69.44
Leptonychiopsis sp.	1.2	93	82.3
Positive control (13% DEET)	2.3	86	72.1
Negative control (ethanol)	16.7	-	-

Table 2 shows the observed repellency outcomes. Leptonychiopsis sp. had the highest reported protection rate (93%) and the longest reported protection duration (82.3 min), followed by L. multiflora (77%; 69.44 min) and A. stipulatum (68%; 41.0 min). The 13% DEET positive control showed 86% protection and a reported duration of 72.1 min. Although the observed value for Leptonychiopsis sp. was numerically higher than that for the DEET control, no valid statistical comparison can be confirmed because replicate-level data, variability estimates, and inferential test results are not provided. The findings should therefore be interpreted as preliminary observations. The chemical profile may help generate hypotheses about constituents contributing to repellency, but the present data do not establish which compound or combination of compounds caused the observed effect. In addition, efficacy and safety in field use, including use in children or pregnant women, cannot be inferred from this laboratory bioassay.

4. Conclusion

This study characterized the reported chemical composition and evaluated the repellent activity against A. gambiae of essential oils from *A. stipulatum*, *L. multiflora*, and *Leptonychiopsis sp*. Under the reported experimental conditions, protection rates ranged from 68% to 93%, and *Leptonychiopsis sp.* showed the highest observed protection rate (93%) and longest reported protection duration (82.3 min). L. multiflora and *Leptonychiopsis sp*. oils were reported to be rich in 1,8-cineole, whereas *A. stipulatum* was not. Because key information on replication, participant ethics, formulation/concentration, and inferential statistics is missing, these results should be considered preliminary. Further standardized studies with complete methodological reporting, appropriate replication, statistical analysis, and safety assessment are required before practical recommendations can be made.

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