Study of the accelerated stability of natural repellents against mosquito bites that transmit endemic diseases

Estudo da estabilidade acelerada de repelentes naturais contra picadas de mosquitos que transmitem doenças endémicas

Estudio de la estabilidad acelerada de repelentes naturales contra picaduras de mosquitos que transmiten enfermedades endémicas

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Abstract
The Aedesaegypti mosquito is the vector that transmits several diseases, such as dengue, yellow fever, zika virus, and chikungunya fever. The most used control of the spread of the Aedes aegypti mosquito today is chemical insecticides. However, over time, this mosquito created resistance in response to insecticides, requiring new alternatives capable of inactivating it without harming humans and the environment. In this sense, essential oils extracted from aromatic plants are widely used in the cosmetic and pharmaceutical industries due to their availability in the Brazilian flora. They have great potential to replace chemical insecticides. This project aims to produce a repellent cream by adding glycolic extract of monkey pepper (Piper aduncum) and clove (Syzygium aromaticum.) The emulsion will be prepared in two phases, the hydrophilic and the lipophilic phase; the quality control of the cream will be carried out based on accelerated stability in terms of the parameter’s centrifugation, freezing and thawing cycles, density, spreadability, organoleptic characterization, stability at extreme temperatures and pH.

Keywords: Mosquito. Repellent Cream. Essential Oils.

Resumo
O mosquito Aedesaegypti é o vetor que transmite várias doenças, como a dengue, a febre amarela, o vírus zika e a febre chikungunya. O controle mais utilizado da propagação do mosquito Aedes aegypti hoje é o dos inseticidas químicos. No entanto, com o tempo, esse mosquito criou resistência em resposta a inseticidas, exigindo novas alternativas capazes de inativá-lo sem prejudicar humanos e o meio ambiente. Nesse sentido, óleos essenciais extraídos de plantas aromáticas são amplamente utilizados nas indústrias cosmética e farmacêutica devido à sua disponibilidade na flora brasileira. Eles têm grande potencial para substituir inseticidas químicos. Este projeto tem como objetivo produzir um creme repelente adicionando extrato glicólico de pimenta de macaco (Piper aduncum) e cravo (Syzygium aromaticum.) A emulsão será preparada em duas fases, a fase hidrofílica e a lipofílica; o controle de qualidade do creme será realizado com base na estabilidade acelerada em termos de centrifugação do parâmetro, congelamento e ciclos de descongelamento, densidade, capacidade de espalhamento, caracterização organolética, estabilidade em temperaturas extremas e pH.


Resumen
El mosquito Aedesaegypti es el vector que transmite varias enfermedades, como el dengue, la
fiebre amarilla, el virus zika y la fiebre chikungunya. El control más utilizado de la propagación del mosquito Aedes aegypti en la actualidad es el de los insecticidas químicos. Sin embargo, con el tiempo, este mosquito creó resistencia en respuesta a los insecticidas, requiriendo nuevas alternativas capaces de inactivarlo sin dañar a los seres humanos y al medio ambiente. En este sentido, los aceites esenciales extraídos de plantas aromáticas son ampliamente utilizados en las industrias cosmética y farmacéutica debido a su disponibilidad en la flora brasileña. Tienen un gran potencial para reemplazar los insecticidas químicos. Este proyecto tiene como objetivo producir una crema repelente mediante la adición de extracto glicólico de pimiento de mono (Piper aduncum) y clavo (Syzygium aromaticum.) La emulsión se preparará en dos fases, la hidrofílica y la lipofílica; el control de calidad de la crema se llevará a cabo sobre la base de la estabilidad acelerada en términos de centrifugación del parámetro, ciclos de congelación y descongelación, densidad, untabilidad, caracterización organoléptica, estabilidad a temperaturas extremas y pH.

**Palabras clave:** Mosquito. Crema Repelente. Aceites Esenciales.

**Introduction**

Every year, across planet Earth, thousands of people are affected by diseases transmitted by insects. Mosquitoes are mainly responsible for transmitting specific pathogens. The Aedes aegypti mosquito is the most significant vector for dengue, chikungunya, zika virus, and yellow fever (Pimentel Stefani et al., 2009; POSSEL, 2019). The incidence of diseases caused by mosquitoes is more aggravating in tropical countries such as Brazil. In them, vectors find better conditions to develop. In the state of Maranhão, increasing cases of dengue, chikungunya, and zika virus have led to many people dying. 2019 more than 1,000,000 dengue cases were recorded, with 782 deaths, representing a percentage increase of 488 compared to 2018 (PORTAL DA SAÚDE, 2019).

The number of diseases transmitted by mosquitoes is relatively high, and according to the World Health Organization (WHO), it is estimated that 100 million dengue infections have occurred in recent years (Zeng et al., 2021). In Brazil in 2016, 1,438,624 cases of dengue were registered compared to 2018, an increase of 599.5% (MS, 2019). The mosquito vectors of these diseases, Aedes aegypti and Aedes albopictus, are easily adapted to the social dynamics and environment of cities, characterizing dengue as a disease typical of urban areas with a specific profile (Egid et al., 2022; LaDeau et al., 2015).
The discomfort and irritation caused by the bite of different species of mosquitoes can generate clinical symptoms of transmitted diseases and even lead to the individual’s death (Pimentel Stefani et al., 2009). These facts cause the incessant search for alternatives to combat and prevent these pathogens (Egid et al., 2022).

The most commonly used form of combat to contain the destructive action of mosquitoes is the application of synthetic insecticides consisting of organophosphate compounds and pyrethroids or even other compositions that are highly harmful to human health and the environment. The intensive consumption of these products aimed at exterminating insects alters the environmental balance, and consequently, adverse effects are inevitable (Demirak & Canpolat, 2022).

Brazil is a tropical country with a vast territorial area and a flora comprising many plants with a high potential for insecticidal activity. Such plants can be used as a viable alternative for integrated pest control. The insecticidal activity of plants found in the Brazilian flora results from bioactive substances, which have different active chemical structures against insects (POSSEL, 2019).

Certain essential oils can act in insect detoxification processes, in which binary mixtures of these oils increase their toxicity. Its synergistic effect can lead to a quantitative reduction of oil to control a particular pest, potentially reducing environmental impacts (ARAÚJO, 2014). Essential oils extracted from plants originate from complex mixtures of volatile organic compounds formed by monoterpenes, sesquiterpenes, and phenylpropanoids. Plant species of essential oils are called aromatic plants (Mohamed & Alotaibi, 2023).

Therefore, this project aims to develop repellent cream formulations whose composition contains essential oils extracted from monkey pepper and cloves, aiming to reduce production costs as well as hinder the development of arthropod larvae in the oviposition phase and redeem and/or eliminate the incidence of infectious diseases caused by mosquitoes. Therefore, it can be stated that this research has the possibility of bringing contributions to the academic community and society in general, as it is committed to the production of an insect repellent to alleviate the incidence of endemic diseases, the raw material of which can be easily found in the Zé Docá region and adjacent areas, where the research will be carried out. It could also serve as a basis for future research in the area of pharmaceutical production, with the use of other local raw materials, which could contribute to attracting investment, generating jobs and, therefore, growth for the local economy, resulting in improved quality of life of the residents of that municipality.
Methodology

2.1 Reagents Materials

The primary raw materials used in this work were ceto-stearic alcohol, almond oil, glycolic extract of monkey pepper and cloves, polawax, and tween 80.

2.2 Manufacture of Repellent Cream - Emulsion Preparation

The preparation of the lipophilic phase consisted of heating its components to 70°C. The mixture remained under constant stirring. The hydrophilic phase consisted of heating distilled water to 70°C and controlling its pH from 4.0 to 7.0, which is ideal for cream. Then, the hydrophilic phase was added to the lipophilic phase; the system was kept stirring for 5 minutes.

The heating system was deactivated, and the cream formed was stirred for another 45 minutes or until it reached room temperature. Finally, it was packaged in appropriate vials and made available for accelerated stability assessment.

2.3 Quality Control of The Cream Obtained

Centrifugation stands out among the quality control parameters of creams. It is a parameter called guiding in the production of this input. The non-occurrence of phase separation does not indicate the cream's stability; it only ensures that the product can be subjected to accelerated stability tests (Isaac et al., 2008). Therefore, an accelerated stability study was carried out for 28 years. This study consisted of centrifugation tests, stability tests at extreme temperatures, density tests, pH tests, and freezing and thawing cycles. The assays were performed in triplicate.

2.3.1 Centrifugation

This parameter was carried out for 28 days with an interval of every 7 days. In a typical test, 5g of each sample was added to a Falcon tube with a capacity of 15 mL. The samples were rotated at 3,000 rpm for 30 minutes, as recommended, (Chorilli, 2009).
2.3.2 Freeze and Thaw Cycles

Initially, 2 g of each sample was weighed into a porcelain crucible beaker. The aliquots were subjected to extreme temperature conditions, which were called cycles. The stability cycles were carried out alternating every 24 hours at high temperatures (40, 45, and 50°C) and low temperatures (-5°C) with a variation of ±2°C.

2.3.3 Organoleptic Characterization

The organoleptic characteristics, in terms of visual appearance, color, and aroma, were checked for 28 days at intervals of every 7 days. Samples consisting of 10 g of cream were subjected to three different controlled temperature conditions, namely: at room temperature (20-25 °C), at low temperature in a refrigerator (5 ± 2 °C), and in a drying oven at 45 ±2°C.

2.3.4 Hydrogenionic Product

The determination of the hydrogenionic product, pH, was determined every 28 days at an interval of 7 days.

Typically, a 10% (w/w) aqueous solution of the cream was prepared with distilled water. The tests were conducted under ambient conditions (20-25°C) in a digital pH meter (BRASIL., 2004)

2.3.5 Spreadability

The spreadability tests will be carried out over 28 days. Initially, 0.1 g of the sample was added between two glass slides with a width of 20.0 mm, a length of 50 mm, and a thickness of 5 mm, one of which will be impregnated with graph paper. Every 7 days, 1.0 g, 1.5 g, 2.0 g, and 2.5 g of the cream will be added every three minutes to the lower plate, which will promote the spread of the cream on the upper plate, which will be measured as extensibility in mm² (Isaac et al., 2008)
Results and Discussions

Three different formulations of repellents against mosquito bites were developed during this work. The formulations were coded as aqua green, Mint green, and turquoise green. Figure x shows the formulations above obtained throughout the development of the formulations.

![Images of repellent formulations](Figure 1 - Emulsions developed against mosquito bites)
*Source: Cesário (2022)*

Following development, the formulations obtained were stored in bottles (50 mL tube) and refrigerated at 10°C for 28 days. Accelerated stability was performed in quintuplicates with an interval of every 7 days (0 days, 7 days, 14 days, 21 days, and 28 days after emulsion development). For each physical-chemical quality control parameter, 5 tests were carried out, totaling 105 analyses. The aliquots destined for accelerated stability tests were chosen at random.

3.1 Guidance Parameter

The centrifugation test is a quality control parameter that ensures the possibility of continuing to carry out accelerated stability tests. During centrifugation tests, it is expected that phase variations will not occur. Two phases were formed for this parameter: a pasty and aqueous phase. Although the normative instructions of the National Health Surveillance Agency (ANVISA) specify that the formation of phases indicates the instability of cosmetic emulsions, it is worth highlighting that a specific dye for cosmetics and an essence were added during the production process. In this sense, the phase revealed during the centrifugation tests was directly related to the dye diluent and not precisely because the emulsions showed traces of instability. The remaining stability parameters were carried out, and the results did not indicate signs of physical-chemical instability of the mosquito bite repellent samples.
3.2 Evaluation of Organoleptic Properties

A repellent cream's physical and chemical instability can be assessed through its organoleptic characteristics: vision, smell, and touch. Such meanings allow for an analysis of the sample's chemical composition, thus preventing the instability of the formulations from spreading to consumers (Pereira De Souza & Sousa Da Nóbrega, 2018).

The results revealed during the organoleptic characterization during the 28 days at intervals of every 7 days did not show significant changes in aroma, color, and physical appearance. The aqua green and mint green samples had a characteristic aroma of dioecious pepper. At the same time, the turquoise blue sample revealed a clove aroma. The three samples showed no sudden color change and maintained a viscous, pasty appearance.

3.3 Measurement of the Hydrogenionic Product of Cosmetic Emulsions

pH measurements were taken using a portable digital pH meter. For this parameter, the analyzed samples presented a pH variation of 4.1-5.2 for aqua green, 4.0 to 4.81 for the Mint green sample, and 4.0 to 4.6 for the turquoise green sample. The pH variation occurred from the seventh day after the development of the emulsions. Although there was an increase in the hydrogenionic product in the three samples, this change does not prohibit the use of the repellent, which has a slightly acidic pH and its optimal range for consumption is 4.0 -10 (Borges, 2019).

3.4 Assessment of Spreadability

The spreading capacity of cosmetic emulsions is a parameter that evaluates how the cosmetic emulsion is distributed over human skin when subjected to force.

The rectangular plates exerted pressure on the surface of the emulsion, allowing it to spread in a shape like a rectangle. The results of the spreadability parameter evaluations are shown in the following Figure 2:
The Figure representation of the distribution of repellent samples showed different behavior between the samples analyzed 14 and 21 days after developing products related to the aqua and mint green samples. The turquoise green sample spread increasingly from the first day to the 28th day after production.

The investigation of the spreadability indicates a change in the consistency of the formulations when their viscosity is reduced. The visual appearance of the emulsions showed small changes, which explains the increasing spreadability behavior.

3.5 Variability of Repellent Emulsion Density

Considering that density is the ratio between the mass of the emulsion and the volume occupied by said cosmetic, it is noted that this parameter is directly proportional to its mass; this means that when losing mass, the density of the samples decreases. This mass variation is related to the phenomenon of evaporation or the vulnerability of emulsions to the oxidation reaction (BRASIL., 2004; Krzeczek et al., 2023). In this sense, storage and packaging conditions are of fundamental importance to avoid mass variation and maintain product stability. During the accelerated stability study for 28 days, the samples analyzed did not show a significant change in mass and density variation, indicating that the products are...
physicochemically stable. The Figure illustrates the results revealed for the density of the repellents.

**Conclusion**

The development of mosquito repellent formulations with the addition of essential oils of dioica pepper and clove, whose active ingredient, eugenol, which has repellent properties, allowed the production of three different formulations, which were called green water, Mint green, and turquoise green.

The samples were characterized using accelerated stability. In evaluating the physical, chemical, and organoleptic characteristics of the developed emulsions, it was noticed that the parameter results align with what is desirable for products applicable to the skin. Statistically, the results were not different, being a positive point for emulsions. More significant research time and other tests, such as viscosity and dermatological tests, are necessary for its effectiveness and commercialization.

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