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Development of Repellent Products Based on Plant Extracts as a Preventive Measure to Diseases Transmitted by the *Aedes aegypti* Mosquito

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Abstract: The aim of this research is to develop a variety of repellent products for personal use, made from extracts of natural plants from the southern region of the State of Mexico as a strategy to reduce the figures of diseases transmitted by the *Aedes aegypti* mosquito (dengue, chikungunia and zica). The research is the result of the collaborative work of the Academic Group UTSEM-CA-1. The plants used were *Ricinnus comunis*, *Artemisia ludoviciana*, *Cymbopogum citratus*, *Cucurbita maxima*, *Coffea arabica* and *Syzygium aromaticum*. After conditioning these plants, extracts were obtained using organic solvents, different mixtures with extracts were formulated and their efficacy was tested by *in vitro* laboratory tests and skin tolerance tests, measuring the repellency in a period of time from one hour to five hours. Five repellent products for personal use were obtained: a patch, two products for spray skin application, liquid hand soap and an environmental incense. These five products, proved to repel mosquitoes in a similar way to commercial repellants classified as synthetic. In this way, the exploitation and use of the physicochemical properties of plants through the elaboration of products represents a strategy to reduce the infectious incidence of diseases transmitted by *Aedes aegypti*, as a solution to current health problems in the region.

Keywords: Project, Repellent Plants, *Aedes aegypti*

1. Introduction

The Technological University of South of the State of Mexico is located in Tejupilco's municipality, State of Mexico, also, Amatepec, Tlatlaya and Luvianos, all of them are part the southern region of the state. This region is bordered by the states of Michoacan and Guerrero [1]. The region has a subtropical climate with high temperature (up to 40° C), abundant rain in summer and mountainous conditions; all these characteristics make it an ideal environment for the breeding and propagation of the *Aedes aegypti* mosquito, commonly known as mosquito or zancudo, as well as the transmission of diseases caused by this vector: dengue, chikungunia and zika; the risk of these deseases range from medium to high, according to epidemiological classification [2, 3].

The increase in the spread of dengue caused by the mosquito that transmits *Aedes aegypti* causes serious health problems among the habitants of the southern region of the state, few cases are registered and from experience it is known that many are not reported to the health sector and do not receive medical attention, despite of the prevention campaigns of the Institute of Health of the State of Mexico (ISEM) and the Panamerican Health Organization; it is also worth mentioning that the imbalance of the population diet is also one of the factors that affect the inmune system, which increases the probability of developing a desease transmitted by the mosquito bites, constituting a public health problema [4, 5].

1.1. Repellent Properties of Plants

There are references that many plants contain chemicals that are naturally effective in killing or repelling mosquitoes and other insects [6]. It is mentioned, for example, that cinnamon contains an effective oil to kill them, an effect similar to DEET (N, N-diethyl-meta-toluamide) or picaridin, which are chemical components that contained the most commercial repellents [7, 8]. Other plants contain substances such as flavonoids, phenolic acids and other chemicals that are attributed with the property of repelling insects [9]. Repellents do not kill the insect but are a good barrier alternative. DEET has passed many toxicity tests, although there is evidence showing its toxic effect from mild to severe levels [10] the effect is more if it is applied simultaneously with other personal products on the skin or the use in children which is not recommended at high concentrations and because of the resistance that can be generated [11, 12].

A great variety of flora with multiple utilities, which provide many and varied benefits, besides the predominant medicinal and edible uses are found in the region. One of them is its insect repellent effect, which are: 1) Castor seeds (*Ricinus communis)* in which germacrene D, trans-caryophyllene, bicyclogermacrene and germacrene B have been identified as the majority chemical components and have >50% mortality for *Aedes aegypti* [13]; 2) Stafiate plant (*Artemisia ludoviciana)* it was reported that extracts tested in macerate and infusion at 5% cause 78. 6% mortality in larvae [14, 15]; 3) Lemon tea (*Cymbopogon citratus*) contains a high content of geraniol, linalool, methylheptone, citronellol, limonenes and diterpenes and according to studies it is an insect repellent [16-18]; 4) pumpkin seed (*Cucurbita maxima*), containing triterpenes such as cucurbitacins, is employed as an insecticide; 5) the coffee bean (*Coffea arabica*) contains diteperenes (mainly cafestol and kahweol), waxes, free fatty acids, sterols and tocopherols, caffeine and other components, in toasting process some other components are generated, many of these have been proven toxic to different animal species [19-21]; 6) clove (*Syzygium aromaticum*) contains eugenol, carvacrol, thymol and cinnamaldehyde, proven safe for the skin and with good efficacy as a mosquito repellent for up to 225 minutes [6].

1.2. The Project

The need to find alternatives to control the bite of the *Aedes aegypti* mosquito, using natural extracts of fruits and plants from the region, formulated in appropriate proportions, is the focus of the development of repellent products for skin application, cleaning products, as well as food products that strengthen nutrition and the immune system, which reduce the impact caused by this insect as a measure to prevent contagion.

The above are also the “Development of personal use and food products using fruits and plants of the region as a measure of prevention of diseases transmitted by the *Aedes aegypti* mosquito" Project purposes " which in 2017 was proposed and approved in the Strengthening Academic group, with an amount of $296,000.00 for the UTSEM-CA-1 Food Technology Academic group.

As of April 2019, the progress that has been made consists of the development of a repellent patch prototype, two skin spray products, hand soap and environmental incense, food has also been developed but its effectiveness has not yet been tested. The following sections describe the development process for each of these products.

2. Materials and Methods

2.1. Methodological Process of the Project

Conducting a documentary and field research on the use of plants and fruits used to prevent mosquito bites or barriers in homes, gardens, and for personal products and food use.

Establishment of processes and formulations of products for personal use (spray lotions (repellent), soap, shampoo, or others) as well as foods rich in nutrients that help the immune system.

Testing the effectiveness of the use of personal products by means of pilot tests in the laboratory.

Determination of the composition of active chemical substances (which have a positive effect on the immune system) in food products.

Formulation of strategies for the dissemination of research findings and technology transfer.

2.2. Development Process of Repellents for Skin Application

Plant selection. Plants were identified and their availability in the region, based on the present active compounds and their repellency to *Aedes Aegypti,* dried castor seed (*Ricinus communis*), leaves of lemon grass (*Cymbopogon citratus*), leaves and stems of stafiate (*Artemisia ludoviciana*), pumpkin seeds (*Cucurbita máxima*), ripe coffee beans were selected, and cloves were also acquired from convenience stores. Plants and seeds were obtained dry and conditioned by removing excess dust and foreign matter.

Pretreatment. Each of the plant samples was immersed in a 0.35% colloidal silver disinfectant solution and left to stand for 10 min. The aerial parts of the plants were chopped, the seeds were slightly crushed with the help of a mortar and pestle. They were placed in aluminum trays with holes and were introduced into a Polinox electric dehydrator for four hours at a temperature of 60 to 70°C. At the end, they were left to cool for 15 min and were packed in metal vacuum bags until their use.

Extraction of oils. The extraction was carried out using Soxhlet equipment [22], the crushed castor seeds, lemon tea leaves, leaves and stems of stafiate were weighed separately, placed inside a cellulose cartridge and introduced into the extraction chamber, where the solvent was previously poured (petroleum ether or ethyl alcohol and methanol, this last component for coffee and clove). The extraction was carried out for 3 h, with a total of four discharges and before the last discharge the extractor was removed and the excess solvent

was emptied to a rotary evaporator for cleaning.

Then the flask with the sample was placed in the oven until constant weight and to volatilize the residual solvent.

Formulation of the repellent spray component. Trial and error formulations were carried out until a pleasant and long-lasting scent was obtained. Combinations of extracts were made for two types of repellents, the first with lemon tea, stafiate and castor seeds; and the second with lemon tea, castor seeds and pumpkin seed.

2.3. Effectiveness and Repellency Tests

a) Mosquito collection

Aedes aegypti samples were collected from castor seeds surroundings of UTSEM and the municipality of Tejupilco, in different places such as houses, stores, among others, near water ponds. Mesh traps were used. Once collected, they were taken to the laboratory to be stored without food (in closed containers with clean water) until the tests were carried out.

b) Bioassay

An equal number of mosquitoes were placed in closed containers and the test was performed: all experiments were carried out from 5 to 6 pm. In 3 jars in total with equal number of mosquitoes, 2 cotton balls were introduced, one as a host and the other as a sample of the repellent with different quantity in each jar, the first cotton was impregnated with chicken blood to attract the mosquito. The total number of those attracted by the blood was counted and compared with the mock test or reference test [23].

c) Tolerance test

A repeated application of the substance to be tested was made on an area of the skin (forearm anterior); this test was done to demonstrate the tolerance to a substance for which there is clinical suspicion [24]. The repellent was applied on different people (members of the work team), who were observed during each hour in a total period of 5 hours, emphasizing on the physical changes in the skin, such as odor´s duration of each spray and possible stains on clothing.

2.4. Liquid Soap and Environmental Incense Formulation

As a complement to the formulation of repellents for skin application, a liquid hand soap was formulated. An extraction was carried out as in the previous products, using ethyl alcohol as solvent for the unroasted and roasted coffee beans and cloves); a soap base formed by 5% texapon, carboxymethylcellulose (CMC), sodium lauryl sulfate and glycerin was used; a mixture of extracts of castor seeds, roasted coffee beans, unroasted coffee beans and clove extract was prepared, testing different formulations and ways of incorporating ingredients.

An environmental repellent, as a incense form, was also developed. Using a support material (ground wood) and incorporating a mixture of alcoholic extracts of green and roasted coffee and castor, using arabic gum as a stabilizing agent.

2.5. Process of the Repellent Patch Elaboration

The following diagram is the result of a bibliographic and patent search on the repellency of the plants studied, in order to propose a repellent patch (Figure 1).

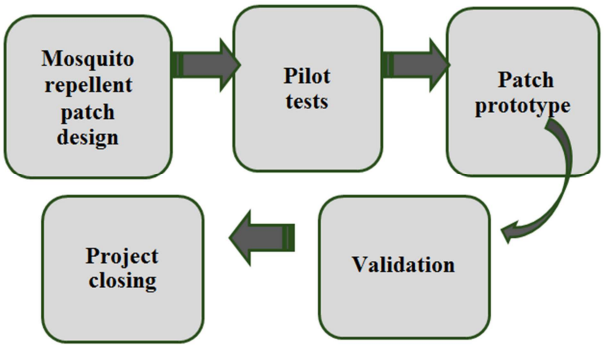

Figura 1. *Methodology for the development of a repellent patch.*

3. Results

As a result of the review of the art state on the subject, plants were chosen to work the products due to their repellent effect and their accessibility in the region that are: 1) castor seeds (*Ricinus communis*), 2) stafiate (*Artemisia ludoviciana),* 3) lemon tea (*Cymbopogon citratus),* 4) pumpkin seed (*Cucurbita máxima*), coffee (*Coffea arabica*) and clove (*Syzygium aromaticum),* the latter is not grown in the region but is commercially available.

Repellent with castor plant, stafiate and lemon tea. With the extracted oils, the necessary combinations were made to obtain an *Aedes aegypti* mosquito repellent formulation. The table 1 shows the formulation with the most durable aroma (according to the criteria of the work team) and pleasant to smell according to its bioactive compounds. This formulation was tested in a trial with a control (without repellent), a portion of 0.3 ml and another of 0.5 ml of the mixture, using a cotton impregnated with blood, where the formulated repellent was sprayed.

In the bioassay was found that, in bottle 1 marked as control (without repellent), the mosquitoes approached the cotton with blood without obstacles; in bottle 2 with 0.3 ml and in bottle 3 with 0.5 ml of the same formulation (Table 1) the mosquitoes moved away from the cotton with blood, these were anchored in the farthest parts of the cotton with repellent mixture. The sample with 0.5 ml of repellent managed to drive away 95% of the mosquitoes in the bottle. In the tolerance tests, no skin irritation occurred.

Tabla 1. *Bioactive repellent compound Formulation.*

Component	Used percentage
Ethyl alcohol	9.10%
Fixer	15.15%
Castor oil	30.30%
Stafiate oil	30.30%
Lemon tea oil	15.15%

Figura 2. *Bioassay and repellency.*

Repellent with castor seeds, lemon tea and pumpkin seed. Three formulations of the repellent spray (identified as #1 with 9% of each oil, #2 with 4.5% of each oil and #3 with 4.5% lemon tea extract, 13.6% of pumpkin seed and 9% of castor seeds) were tested for duration and effectiveness to choose the best formulation (Figure 2). Separation of the oils was observed due to the composition of the mixtures in formulations #2 and #3, formulation #1 remained stable. It was observed that the time or duration of the effect of the samples was variable, the formulation that prevailed most was #3, lasting 5 hours; formulation #2, 2 hours; and the #1, 4 hours.

With these formulations, a test was carried out using glass vials (with 15 mosquitoes in each vial), an absorbent cotton impregnated with sample was introduced, as shown in Table 2.

Figure 3. *Tolerance test of the skin application repellent.*

Table 2 shows the number of mosquitoes approached in the test.

Table 2. *Distribution of the bioassay on the repellent spray formulated with castor seeds, lemon tea and pumpkin seed extracts.*

Samples	Number of mosquitoes aproched in 5 minutes
Witness (sprayed with water)	5
With blood	4
With repellent #1	0
With repellent #2	3
With repellent # 3	1

Number of mosquitoes=quantity of mosquitoes that approached to the sample in a certain period of time.

Repellent one (#1) was the one that fulfilled the characteristics of a commercial repellent (duration of effect, did not cause alterations in the skin and did not leave stains on clothing), in addition to its level of repellency by ensuring that no mosquitoes of the *Aedes Aegypti* species came close to the sample. The use of dimethicone copolyol (fixer) was necessary for the union of the oils.

Liquid hand soap. After making several formulations, a mixture of 4% castor seeds extract, 3% roasted coffee, 2% green coffee and 1% clove was selected. Citral essence was used to reinforce the aroma of the soap, and yellow 5 and blue 1 dyes were used to provide color. In the repellency tests, 50% efficacy was obtained, this was to be expected due to the rinsing after washing. An observation on this product is that the people who tested it (with informed consent) commented that "their hands were very soft".

Incense-type environmental repellent. A cone-shaped, coffee-scented incense, weighing 5g, was made. With a combustion of approximately 45 minutes and aroma for at least two hours. Showing a high mosquito repellency (this project is in the process of registration of utility model).

Mosquito repellent patch. The repellent patch will be a small product (with a length of 3 cm and a maximum width of 2 cm), it will be flexible, thin (1 mm thick), with a soft texture, similar to an adhesive band; its design is leaf-shaped, to make it eye-catching and distinctive to people's eyes, evoking the natural origin of its components. The active substances (extracts of lemon tea, castor seeds and stafiate) it will be found between a double layer of polymeric material, it will have an adhesive that will keep it firm, and in addition, it will have a coating against water. When the patch is rubbed, the release of the active compounds from the plant extracts will begin. As the release process is slow, the patch can last for 48 hours, but if the body temperature is increased, its effect is reduced to 24 hours. This is the description of the prototype that is currently in the process of registration in the Mexican Institute of Industrial Property, for which the detailed information of the product is reserved.

It is worth mentioning that with the "Repellent Patch" project participated in the Program "Young Inventors and Innovators Award of the State of Mexico 2017" in the category of Pharmaceuticals, after a rigorous evaluation by leading scientists of the State of Mexico, the project resulted in a first place, receiving recognition and a cash prize of $50 thousand pesos during the Science and Engineering Fair edition of the State of Mexico (FECIEM) on September 25th, 2017, in charge of the Mexican Council of Science and Technology (COMECYT) at the Exhibition and Convention Center of Toluca facilities, from September 24th to 28th.

4. Discussion

In the region, the use of topical and environmental repellent products is common, and the population has adapted to their use to avoid being bitten or to reduce the

bites of *Aedes* species mosquitoes [24]; these products can be very effective and safe if they are used correctly, presenting no health risk.

Regarding the plants used, initially a pre-selection was made, based on comments from the population and later, a documentary research was made, finding references to the use of plants and their chemical components, which in general are phenolic and terpenic compounds.

Although the evidence is not conclusive, the data obtained will provide the background that the plants indicated in this study do indeed have repellent properties and can be safely used by families in the region.

This research also seeks to be a reference to give an alternative use to the diversity of flora in the region and to make better use of the available resources, being able to favor the cultivation of plants that grow wild.

5. Conclusion

Some plants used for both the repellent and the patch are taken as weeds, with the exception of lemon tea and pumpkin seed; coffee is grown in the municipality of Amatepec, and cloves are commercially available, plants easily found in the southern region of the State of Mexico. With this project we are giving an added value and rescuing Mexican herbal knowledge; taking advantage of their repellent properties for the benefit of the population and providing a solution to current health problems.

It is concluded that the mixture of essential oils of plants in an appropriate combination or formulation, are suitable to repel the females of the *Aedes aegypti* species, as well as commercial repellents that are generally classified as synthetic repellents because they have as active ingredient the chemical component DEET, by substituting this component with the essential oils of plants, a natural repellent is being created.

It is important to have knowledge about the toxicological effects of the natural resources used as repellents in the long term, so we will continue working on tests to demonstrate their non-toxicity. Likewise, the products proposed in this project of the UTSEM-CA-1 Food Technology Academic Group will continue to be developed.

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