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Antipruritic Ointment *Randia Monantha*

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Abstract

Objective: Obtain *Randia Monantha Benth* extract, use it as the main component in the formulation of an ointment with antipruritic characteristics. Investigate and analyze its effect through microbiological, activity, validation, and sensory assays.

Methods: Soxhlet process with different solvents was performed to obtain the extract.

Results: Comparing the different solvents (petroleum ether, acetone and ethanol). It was determined that the best solvent for extraction is 20% ethanol. After the different experiments, the ointment made of *Randia Monantha Benth* presented no contamination through the microbiological assay. Using statistical technique ANOVA along with the activity tests it was proved that the application of the ointment can reduce the pruritic effect.

Conclusion: The use of *Randia Monantha Benth* extract as part of a formulation was possible, antipruritic effects were successful and the results obtained suggest a favorable potential for future tests and research.

Keywords: *Randia Monantha Benth*; Crucetillo; Antipruritic ointment; Soxhlet extraction; ANOVA analysis; Irritation reduction

Introduction

When the skin is exposed to different xenobiotics, these have the potential to induce a skin reaction where different immune and histological responses are raised. Therefore, it is defined as skin irritation to the reversible damage done to the derma. The skin is composed of three layers, which are predominantly populated by keratinocytes, the latter being the key factor in many functions, including the immune responses that follow a chemical exposure.

The mechanism by which chemicals can induce skin irritation can vary depending on the properties of the same. The most investigated is by initiating a disruption of the cell membranes resulting in an extracellular release of proinflammatory cytokines, which normally reside within the keratinocytes. This activates a cascade response of inflammatory signaling, which results in skin irritation [1].

Traditional treatments with medicinal plants from different regions of the country have been passed down from generation to generation and have even begun to be commercialized. these home remedies are still in force thanks to their notorious effectiveness in counteracting the effects of bee and snake bites among other poisonous animals. In states of México such as Veracruz, the use of the *Randia Monantha benth* plant denominated as "Crucetillo" in home remedies is very common due to their effectiveness in countering the effects of bee (*Apis mellifera*) stings, snake (*Bothrops asper*) bites among other poisonous animals. This plant belongs to the genus Randia and to the family Rubiaceae. In addition, is a plant with flower and fruit, the latter with the pharmacological properties mentioned above [2].

Other therapeutic applications that are also mentioned among the population are: antidiabetic, reduce cholesterol, inflammation among others. However, the study of these properties has not yet been carried out formally and its research can be said to be recent. Due to the empirical medicinal management that has been given to *Randia Monantha Benth* (Figures 1-3) studies have been done evaluating the toxicity of the drug consumed as an antidote, these tests showed that the consumption of the preparation using different parts of the plant, is innocuous and that it does not present any toxicity, however, the need for another type of tests to check the possible pharmaceutical activity of the plant is stated [2].

Figure 1: *Randia Monantha Benth.*

It is possible to say to this day, that there are no products with well-established protocols that make use of this plant in a formulation, so it is possible to say that this text is a pioneer in the field of watermelon research, it is expected that this search continues until the complete characterization of the plant and its components.

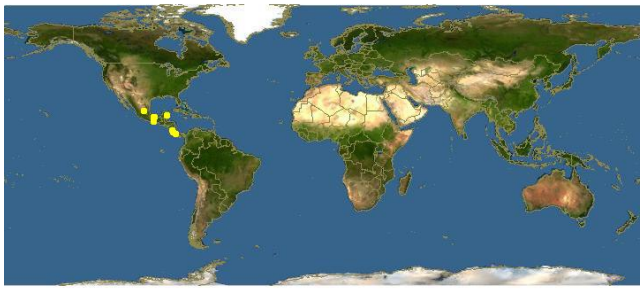

Figure 2: Geographical distrubution of *Randia Monantha Benth* across the globe.

Figure 3: *Randia Monantha* fruit.

Materials and Methods

Extraction of *Randia Monantha Benth* compounds by Soxhlet

Solid-liquid extraction was carried out with the use of Soxhlet equipment brand Kimax Kimble, 3 different solvents were used for the extraction to verify which solvent had a higher yield. 2.5 grams of the previously cut fruit were placed in the Soxhlet chamber with 70 ml of petroleum ether in a first Soxhlet at a temperature of 60°C, in another place the same amount of fruit was placed with 70 ml of acetone at 60°C registered in the muffle of the grill, and finally 5 grams of chopped and dried fruit with 80 ml of alcohol were placed in duplicate at 110°C marked on the muffle.

Ointment preparation

For this step different concentrations of *Randia Monantha Benth* extract were proposed: 5%, 10% and 15% of total volume (100 gr). Fruit of *Randia Monantha Benth* were collected from Córdoba, Veracruz, Mexico, in September 2017 and dried for 3 hours at 60°C.

The excipient was heated in a water bath until it melted at 80°C, once in a liquid state, in a separate container *Randia Monantha Benth* extract was placed and the excipient was added gradually with a constant agitation for the formation of the ointment, this procedure was carried out at room temperature. Once mixed, it was set at room temperature also and the consistency was measured.

pH test

Randia Monantha Benth ointment solution was diluted with Milli-Q grade water ointment samples were diluted to determine the acidity of the product with the use of pH strips.

Microbiological analysis

As a first reportable method to verify the potential microbiological growth of the solution, four petri dishes were prepared with nutritious Agar medium, which serves for the growth of non-demanding bacteria and fungi with respect to their nutritional requirements. This medium is used to check the health requirements of products [3]. A sample of the ointment was taken with a sterile handle and the technique of surface-spread with heat sterilized loop was performed in the petri dishes, finally were left in incubation for 9 days at 37°C.

Life-time analysis

The solution was placed in a plastic container, and in an incubator to similar conditions as indicated in the NORMA Oficial Mexicana NOM-073-SSA1-2005, which is a norm for the stability of medicines of different types, and for topical use [4]. This analysis was performed for 2 weeks for reasons of time at 37°C with 26% humidity.

Activity test

To measure the possible antipruritic activity of the ointment, the plant known in México as "Ruda" (*Ruta graveolens L*) was used, this plant has the property of causing irritation and itching in the areas where the skin comes into contact [5]. Skin of a test subject was exposed to the previously mentioned plant and at the start of irritation symptoms (itching, burning and redness of the skin) the ointment was applied, and its activity was checked in relation to time by observing the improvement in the coloration and sensitivity (itching and burning) of the skin. It was also done an experiment which the lesion was not treated. The MINITAB software was used to perform a hypothesis test and verify if the results were favorable.

Results

Extraction by Soxhlet

From the solid-liquid extraction with Soxhlet method and the different solvents the following yields were obtained, for the solvent with petroleum ether a yield of 0.3138 grams of oil/gram of sample was obtained, with acetone solvent a yield of 0.66 grams of oil/gram of sample. When performing these first extractions it was possible to analyze that the extraction with ether has a very low yield, for which we did not consider this solvent for the preparation of the final ointment, the extraction with acetone solvent offered a better performance in comparison to the ether, however, when the extraction

by Soxhlet was performed, it had a complicated behavior to control, since it had projections and completed 6 cycles in 7 hours.

Finally, 20% ethanol was used, with the best yield being 0.7086 grams of oil/grams of sample and process took approximately 5 hours. As an attempt to volatilize the solvent, extract-solvent solutions were placed in the same Soxhlet equipment with the empty chamber to recover the solvent and then it was placed in an extraction hood, during this process the extract of *Randia Monantha Benth* got stuck in the walls of the flask, which complicates obtaining the compound of interest to add it to the ointment formulation. A percentage of alcohol was considered for the final formulation

Ointment elaboration and pH

The solution obtained through the preparation process was a semi solid solution with texture of an ointment. For the pH test done by duplicate the obtained results were that the ointment has a pH of 7.

Microbiological and life-time analysis

After monitoring the plates every 24 hours, the analysis was completed 9 days after planting, there was no contamination. For the lifetime method, after the 2 weeks established and at 37°C, the ointment presented phase separation, the excipient became liquid, however 3 hours later at room temperature the ointment returned to its natural state.

Activity test with ANOVA

With the mentioned procedure it was measured the time of action of the ointment 4 times, the results of these tests indicate an average time of 1:20 minutes to reduce the effects of burning and itching in the affected area and 4 minutes to control and eliminate the reddish hue of the skin. As an additional note the experiment that did not receive treatment with the ointment stopped at 20 minutes because the irritation grew, and the skin became very sensitive.

An One-Way ANOVA test was done using the software MINITAB® to verify if there was a significant difference between the mean times of the treatments.

Ho: μ1(time with ointment)=μ2(time without ointment)

Ha: μ1(time with ointment)≠μ2(time without ointment)

We accepted Ha because the results show with a significance level of 5% (α=5%) that there is a difference between the mean times of the treatments, in other words, that there are favorable effects using the ointment.

To corroborate these results, we decided to perform a hypothesis test comparing means of the times, our hypothesis is mentioned below:

Ho: μ1 (time with ointment)-μ2 (time without ointment)

Ha:=μ1 (time with ointment)<μ2 (time without ointment)

We accepted Ha with a significance level of 5% (α=5%), and can be concluded that the irritation time with *Randia Monantha Beth* ointment treatment is less than the irritation time without it. With both assays we can determine an effective activity of the ointment reducing the effects of burning and itching, also eliminating the reddish hue of the skin.

Discussion

Although a good yield was obtained by Soxhlet method with ethanol, we consider that it is a better option to perform microwave assisted extraction, which could offer better yields with a shorter production time. The range of pH that can be found in the skin varies between 4 and 6 [6], given the results obtained it would be important to add some acid to lower the pH, citric acid is recommended to obtain the desired pH. For a complete successful result of Lifetime analysis and to obtain the required results to accomplish NORMA Oficial Mexicana NOM-073-SSA1-2005, it is necessary to repeat this experiment for a longer time and at the temperature indicated by the standard (30°C) to assume that the useful life of the ointment is 2 years.

The performance of the activity tests as well as their analysis using the MINITAB software was very important since it was proved that the use of the ointment in effect, stops and helps to eliminate the reddish hue characteristic of an irritation as well as the itching and burning. However, a more extensive and specific study is necessary to determine at what intensity range of irritation the treatment through the application of the ointment is possible.

Due to the lack of information available regarding the characteristics of the species known as "Crucetillo" (*Randia Monantha Benth*), the realization of each experiment was a new discovery, from the way of obtaining the extract of its fruit, its behavior for the elaboration of a semi-solid substance in conjunction with other components, life-time, microbiological, pH and antipruritic activity tests. It is possible to indicate that the experiments present a favorable result that allows in the future to continue discovering the true potential of the plant. It was proven that the formulation of the ointment does not present potential for microbial growth as well as reducing the effects of mild irritation- reducing levels of oxidative stress but other important assays are needed to confirm this, such as *Staphylococcus aureus* test.

Finally, the formal search of the active site that *Randia Monantha Benth* offers is planned, since there is no information on this, being pioneers in the discovery of the essential components that make this plant unique and traditionally used, represents an authentic opportunity of knowledge.

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