Evaluation of antibacterial activity in essential oils and by-products used in the artisan cosmetic industry

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Abstract. The experimental study allowed us to learn about antibacterial activity in essential oils in Eucalyptus, Rosemary, Myrtle, Orange plants and their by-products used in the industry. Phase 1. Extraction of essential oils through steam distillation. Phase 2. Determination of antibacterial activity through diffusion of the agar disc. Phase 3. Determination of the minimum inhibitory concentration through macrodilution in broth. Phase 4. Product effectiveness tests. The results showed antibacterial activity in eucalyptus and rosemary oils against \textit{Escherichia coli} at concentrations of 100\%, 75\% and 50\%, whereas for \textit{S. aureus} only orange and eucalyptus oils were effective at concentrations of 75\% and 100\%, however, eucalyptus oil was the only one with inhibition against \textit{Pseudomonas aeruginosa} at 100\% concentration. It is concluded that the antibacterial activity found in the EO of orange, eucalyptus, myrtle and their by-products such as myrtle gel opens the door for the development of subsequent studies in the search for its application in the cosmetic industry as a viable alternative to the treatment of skin and gastric system related conditions, given the ability to inhibit the growth of \textit{S. aureus} and \textit{E. coli}.

1. Introduction

Colombia is a country with great diversity in its ecosystems and microclimates, which means that it has a very varied vegetation, enriched with endemic species and a very high genetic diversity. Some of the plants that can be found have essential oils with active ingredients that have demonstrated biological or industrial activity, with broad prospects for research and development of new products [1-3]. Thus, in recent years, essential oils and their constituents from different plant species have shown an extraordinary increase in the chemistry of natural products worldwide, placing them among the three groups of products of botanical origin that are most likely to have the greatest impact on plant protection in the next decade [4].

Essential vegetable oils or "essences" are the liquid and volatile fractions, which can be obtained from various parts of the plant (root, stem, leaf, flower or fruit), usually by steam trawling, which contain the substances responsible for the aroma and biological activity of the plants, their use depends largely on their major components and the absence or presence of these depends on geobotanical conditions, cultivation methods, harvest season, plant material storage method, production method and the age of the plant [5].
Studies have shown that the bacteria resistance to antibiotics is a natural phenomenon and the indiscriminate use of synthetic antibiotics in both humans and animals is accelerating the process to dangerous levels and is now one of the greatest threats to global health [6]. Given the above, researchers around the world are constantly searching for active ingredients that can act against the main disease-causing organisms in humans and animals, which is causing complementary and traditional medicine to be increasingly considered by health care teams and patients alike [7].

Given this reason, for the artisan cosmetic industry, the study of essential oils as a basic raw material for the production of finished products in the cosmetic industry is of vital importance. This is an area with a high potential in the field of research and development in several countries that seeks biological solutions for the different pathologies mainly related to skin care [8].

2. Materials and methods

2.1. Extraction of essential oils through steam distillation

Plant material from orange peels, moringa (cold pressed), rosemary, eucalyptus and myrtle tree were used. Each of these samples was subject to a stream of water vapor (100-105°C) for 2 hours, so the essence was washed away, condensed and separated from the aqueous fraction. The separated essential oil was measured in a burette and stored in an amber bottle at 4°C [9]. The performance of the EC obtained was calculated as follows (Equation 1):

\[
\% \text{yield} = \frac{\text{Essential oil ml} \times 100\%}{\text{Vegetal material weight g}}
\]  

(1)

2.2. Determination of antibacterial activity through diffusion of the agar disc

A bacterial suspension of approximately \(10^8\) microorganisms per milliliter equivalent to Mc Farland's 0.5 standard was prepared for each of the microorganisms to be tested (E. coli ATCC 25922, S. aureus ATCC 25923, Pseudomonas aeruginosa). Once this was achieved, a sterile, dry swab was immersed in the bacterial suspension and the surface of a Mueller-Hinton agar plate was inoculated with it at room temperature. Using sterile forceps, a paper filter disc embedded in 90% alcohol (negative control) was placed on each plate (μl), reference antibiotics for each strain (positive control) and a disc of filter paper embedded in the essential oil at 100%, 75% and 50% concentrations, pressing them firmly on the surface of the agar. It was left to dry for 2 to 3 minutes, and then incubated for 24 hours at 37°C.

2.3. Determination of the minimum inhibitory concentration by macrodilution in broth

Decimal dilutions of \(1 \times 10^0\) to \(1 \times 10^9\) of the 0.2% 0.4% 0.4% 1.5% and 3% Myrtle gel were performed, each tube containing 9ml of peptone water and 1 ml of the bacterial suspension corresponding to the 0.5 tube in the Mac Farland scale. Each of the strains to be evaluated was incubated at 37°C for 24 hours, and hence the minimum inhibitory concentration, defined as the lowest concentration of the product that can inhibit the visible growth of a microorganism after incubation for 24 hours, was determined.

2.4. Product effectiveness tests

The effectiveness of the myrtle gel product at three exposure times (1 minute, 5 minutes and 10 minutes) was determined using the modified ecometric method, which can be used in the evaluation of disinfectants as a semi-quantitative technique. The product is put against two pure micro-organisms in suspension such as Staphylococcus aureus and Escherichia coli by placing 1 ml of them in tubes with 10 ml of the product (myrtle gel). After exposure in the first quadrant the sowing of the pure strain is carried out, in the second quadrant the strain is sown at the first minute, in the third quadrant it is sown at the fifth minute, and in the fourth quadrant at the tenth minute.
3. Results and discussion

Table 1 shows the estimated yield of essential oils obtained from dried leaves at room temperature for 72 hours using steam distillation.

| Material under study | Extraction time (hours) | Performance (%) |
|----------------------|-------------------------|-----------------|
| Eucalyptus tree      | 2                       | 0.10            |
| Rosemary             | 2                       | 1.20            |
| Myrtle               | 2                       | 0.10            |
| Orange               | 2                       | 0.08            |

The results show that Rosemary is the plant with the highest yield in terms of oil per gram obtained, this can be directly related to the reported studies for well-known aromatic species, such as oregano [10], thyme, ginger and pine in which case it is stated that the percentage yield of essential oil presents an appreciable variability according to the state of the leaf and the place of harvest. Where inhibition halos <13mm are resistant and inhibition halos ≥13mm are sensitive to hydrolate or essential oil.

Table 2 shows how the largest antibacterial inhibition halos are present in eucalyptus and rosemary oil at the three concentrations evaluated against *Escherichia coli*, however, it can be observed that their greatest activity is had when using pure oil, with inhibition halos of 38mm and 35mm respectively.

| Concentration essential oil/hydrolate | Inhibition halos (mm) |
|---------------------------------------|------------------------|
|                                       | *Escherichia coli*     | *Staphylococcus aureus* | *Pseudomonas aeruginosa* |
| Orange Oil (50%)                      | <13                    | 16                     | <13                     |
| Orange Oil (75%)                      | <13                    | 17                     | <13                     |
| Orange Oil (100%)                     | <13                    | 18                     | <13                     |
| Eucalyptus oil (50%)                  | 23                     | 10                     | <13                     |
| Eucalyptus oil (75%)                  | 25                     | 25                     | <13                     |
| Eucalyptus oil (100%)                 | 38                     | 25                     | 25                      |
| Rosemary hydrolylate (50%)            | 25                     | <13                    | <13                     |
| Rosemary hydrolylate (75%)            | 30                     | <13                    | <13                     |
| Rosemary hydrolylate (100%)           | 35                     | <13                    | <13                     |
| Diluted rosemary                      | <13                    | <13                    | <13                     |
| 0.2% Myrtle Gel                       | <13                    | <13                    | <13                     |
| 0.4% Myrtle Gel                       | <13                    | <13                    | <13                     |
| Myrtle 1.5% Gel                       | 16                     | <13                    | <13                     |
| Myrtle Gel 3%                         | 18                     | 14                     | <13                     |
| Diluted Myrtle                        | <13                    | <13                    | <13                     |
| Myrtle oil (100%)                     | 35                     | 34                     | <13                     |
| Myrtle oil (75%)                      | 28                     | 22                     | <13                     |
| Myrtle oil (50%)                      | 21                     | 18                     | <13                     |
| Myrtle oil (35%)                      | 16                     | 18                     | <13                     |

Similarly, the largest antibacterial inhibition halos were present in orange and eucalyptus oil at the three concentrations evaluated against *Staphylococcus aureus*. Given the above, it can be observed that the activity of orange oil does not depend on the concentration of EC as it does not present significant differences between each of them, handling inhibition halos of 17mm on average. Similarly, the greatest activity of eucalyptus oil is obtained when used in concentrations of 75% and
100%, with 25mm inhibition halos. However, the myrtle gel product also has high activity against this microorganism when used at concentrations of 3% with an MIC of 100% (Table 3). A similar case occurs with the E. coli microorganism where this product (Myrtle gel) has a greater activity against the microorganism in question when used at concentrations of 1.5% and 3% with an MIC of 100% (Table 3).

| Table 3. Minimum inhibitory concentration of oils with antibacterial activity. |
|-----------------------------|-----------------|-----------------|-----------------|
|                            | Escherichia coli | Staphylococcus aureus | Pseudomonas aeruginosa |
| Ae/product                  | Mic             | Ae/product        | Mic             |
| Eucalyptus tree             | 100%            | Orange oil        | 100%            |
| Rosemary                    | 0.01%           | Eucalyptus oil    | 0.1%            |
| Myrtle tree gel 1.5%        | 100%            | Myrtle tree gel 3%| 100%            |
| Myrtle tree gel 3%          | 100%            | Myrtle oil       | 0.001%          |
| Myrtle oil                 | 0.1%            | Orange oil        | 100%            |

These results confirm what Gómez [11] has said, stating that arrayan species produce bioactive substances with antimicrobial effects, which contributes to the strengthening and credibility of the ancestral knowledge of myrtle oil, which is the one with the greatest antimicrobial activity [11-13].

On the other hand, none of the oils and products evaluated presented antibacterial inhibition halos against Pseudomonas aeruginosa, being this result similar to that obtained by Gualtieri et al. [14]. However, eucalyptus oil is the only oil with 100% action and a 25mm inhibition halo.

These results may be related to the external membrane of P. aeruginosa which plays a major role in antibiotic resistance by limiting the penetration of small hydrophilic molecules and excluding larger molecules. According to Luján [15], small hydrophilic antibiotics such as β-lactams and quinolones can only cross the external membrane through watery channels made up of proteins designated as porins, for this reason, cautious use of antibiotics should be taken, as well as adequate infection control practices that can limit the emergence and spread of antibiotic resistance in this bacterium.

4. Conclusion
The antibacterial activity found in the AE and by-products of Orange Oil, Eucalyptus Oil, Myrtle Oil and Eucalyptus Oil enables the development of subsequent studies that help the cosmetic industry determine the greatest antibacterial effectiveness and yield according to the crop's eco physiology.

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