Original article

Development of an Antibacterial Impregnated Support using *Citrus Sinensis* Essential Oil as Active Ingredient

Desarrollo de un soporte impregnado antibacteriano usando como activo aceite esencial de *Citrus sinensis*

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ABSTRACT

Introduction: Nowadays one of the products with the highest consumption in the cosmetic industry are impregnated supports due to their different functions, which has made them one of the most common items in the daily hygiene of many individuals and families.

Objective: To develop antibacterial impregnated carrier using *Citrus sinensis* essential oil as active ingredient.

Methods: The essential oil was obtained by microwave radiation-assisted hydro distillation from the pericarp of the fruits; the chemical composition was evaluated by gas chromatography/mass spectrometer. Impregnated supports were prepared using essential oil as active ingredient at concentrations of 1.5 % and 2 %, then microbiological parameters were evaluated on bacterial strains of Echericha coli ATCC 25922 and Staphylococcus aureus ATCC 25923.

Results: The essential oil extracted from *Citrus sinensis* presented limonene as the major component and it was found that the suitable formulations were those in which orange essential oil was found at concentrations of 2 %, as they presented activity against the bacterial strains in question.

Conclusions: The antibacterial impregnated carriers formulated from 2 % of Citrus sinensis essential oil showed promising chemical and microbiological indicators; and provide an indication of correct formulation from the galenic point of view.

Keywords: *Citrus sinensis*; cosmetic; orange; Escherichia coli, Staphylococcus aureus.

RESUMEN

Introducción: En la actualidad uno de los productos con mayor consumo en la industria cosmética son los soportes impregnados debido a sus distintas funciones, lo que los ha convertido en uno de los artículos más habituales en la higiene diaria de muchas personas y familias.

Objetivo: Desarrollar soporte impregnado antibacteriano usando como activo aceite esencial de *Citrus sinensis*.

Métodos: El aceite esencial se obtuvo por hidrodestilación asistida por radiación con microondas, a partir del pericarpio de los frutos; la composición química se evaluó mediante cromatografía de gases/espectrómetro de masa. Se elaboraron soportes impregnados usando como activo aceite esencial a concentraciones de 1,5 % y 2 %, posteriormente se evaluaron parámetros microbiológicos sobre cepas bacterianas de *Echericha coli* ATCC 25922 y *Staphylococcus aureus* ATCC 25923.

Resultados: El aceite esencial extraído del *Citrus sinensis* presentó como componente mayoritario el limoneno y se encontró que las formulaciones idóneas fueron aquellas en las que el aceite esencial de naranja se encuentra a concentraciones del 2 %, al presentar actividad contra las cepas bacterianas en cuestión.

Conclusiones: Los soportes impregnados antibacterianos formulados a partir de 2 % de aceite esencial de *Citrus sinensis* presentaron indicadores químicos y microbiológicos promisorios; y brindan indicio de correcta formulación desde el punto de vista galénico.

Palabras clave: *Citrus sinensis*; cosmético; naranja; *Escherichia coli, Staphylococcus aureus.*

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Introduction

Colombia is a country that has a great diversity of ecosystems and microclimates, which makes its vegetation very varied, enriched with endemic species and 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 perspectives to carry out research and development of new products.[1,2,3,4]

The term "essential oil" (EO) is used to refer to liquid, volatile, lipophilic substances with aromatic properties. These substances are synthesized by plants as secondary metabolites and can be extracted by physical methods such as steam distillation or hydro distillation.[5,6,7]

EOs play an important role in the protection of plants, as they act as antibacterial, antiviral, antifungal and insecticidal agents.[8,9,10,11,12,13,14,15,16,17] They have a complex chemical composition consisting of a mixture of organic substances such as hydrocarbons, alcohols, aldehydes, ketones, esters, etc.[12,18]. It should be noted that EOs

have become an integral part of our lives because they have numerous pharmacological actions, so they constitute the basis of aromatherapy, but they are also widely used in perfumery and cosmetics, in the pharmaceutical industry and in the food, liquor and confectionery industry.[19]

It has been shown that currently one of the products with the highest consumption in the cosmetic industry are impregnated supports, which are impregnated paper wipes that have different functions among which we highlight refreshing, eliminating impurities, absorbing excess fat or favouring the penetration of active ingredients, therefore, it has become one of the most common items in the daily hygiene of many people and families.[20] Therefore, the objective of this research was to develop an antibacterial impregnated support using *Citrus sinensis* as an active ingredient.

Methods

Collection of plant material

The pericarp of orange fruits (Citrus sinensis) was collected in the city of Cartagena, located in the north of the department of Bolívar (10°25′25″N 75°31′31″O), Colombia. 1000 g of pericarp per week were taken.

Processing of plant material

The pericarp of the collected fruits was washed with deionized water, and those that were fresh, whole, without signs of deterioration were selected. They were immediately chopped, weighed and processed.[6]

Extraction of Essential Oil

The essential oil (EO) was obtained by the method: microwave assisted hydro distillation. For that, it was used a hydro distillation equipment with 4 L of capacity. 500 g of plant material were taken, then they were introduced into the extraction flask, which contained 500 mL of distilled water, the extraction time was 3-4 h. As a source of

microwave addition, a conventional modified oven brand Samsung was used, with a 1-cycle irradiation of 60 m and a power of 70%.[4,5] The EO was collected in a Dean Steark type vessel.

Determination of the major components of EO by gas chromatography-mass spectrometry (GC / MS)

An Agilent 7890A / 5975C chromatograph was used. Each EO sample (50 µL) was dissolved in 450 µL dichloromethane, the injector temperature was 250 °C, a HP-5MS 5% Phenyl Methyl Silox capillary column was used; Helium was used as carrier gas at constant flow rate of 1 mL / min, pressure of 7.6354 psi and linear velocity of 36 cm /sec. Initial temperature 45 °C and transfer line temperature 280 °C. Mass spectra were obtained by electron ionization (70 eV), with automatic scanning at a range of m / z 30-400 u.m., at 3.85 scan / s. The components identities were assigned by comparison of each spectrum with the database standards reported in the literature.[4,5,6]

Development of antibacterial impregnated support type formulation

Several impregnated support units were acquired to which concentrations of 1.5 and 2% of essential oil (EO) of *Citrus sinensis* were incorporated, which in previous studies demonstrated potent antibacterial activity.[14]

Antibacterial activity in vitro

The bacterial inoculate of *Staphylococcus. Aureus* and *Escherichia coli* were prepared according to the indications established by the Institute of Clinical and Laboratory Standards (CLSI),[21] between 3 and 4 well-differentiated and morphologically similar colonies of bacteria previously planted in petri dishes with MH agar were taken, then those were suspended in test tubes with sterile MH broth, were incubated at 35 ± 2 °C and the optical density (OD) at 620 nm was constantly checked in the microplate reader (Multiscan EX Thermo, USA), until the bacterial suspension reached a DO 620 between 0.08 - 0.1 units, which is equivalent to 0.5 on the McFarland scale (1 × 108 CFU/mL),

which was diluted in order to obtain a working suspension of 5 × 105 CFU/mL in the biological assays.[11].Once this value was reached, 5 mL of broth was mixed with 5 mg of the impregnated support using a Vortex agitator, in microbiological test tubes with sterile lid, leaving in incubation for 24 h at 35 ± 2 °C in constant stirring. After this time, and to evaluate the bactericidal capacity of the impregnated support, roasts taken from the tubes were sown in the specific agar for the two strains and again incubated at the same conditions for 24 h.

Statistical Analysis

All trials were performed by sextuplets. The results were expressed as the mean ± SD (standard deviation). Significant differences were determined by ANOVA analysis followed by Dunnett's or Tukey's test or as deemed appropriate.

Results

The efficiency of extracting *Citrus sinensis.* essential oil is presented (fig.).

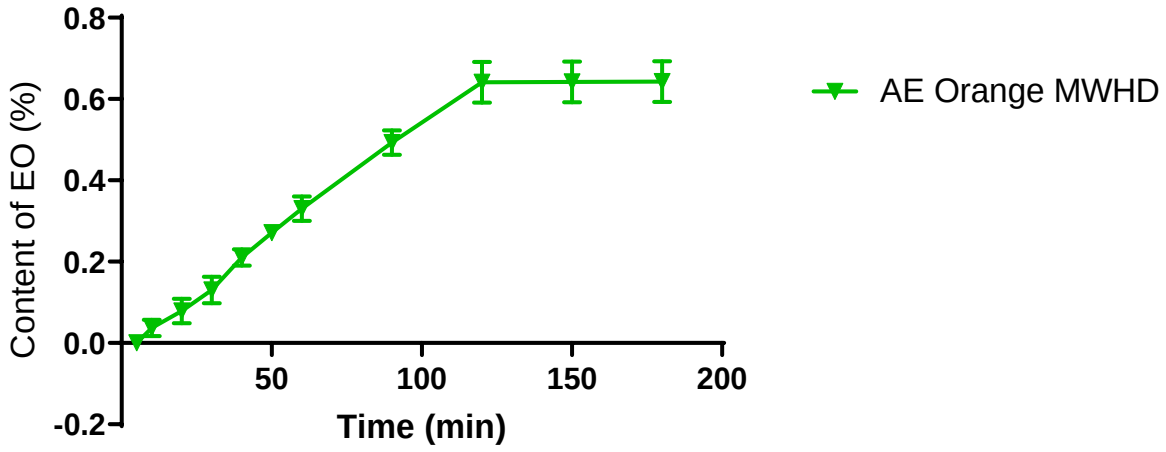

Fig. 1 - Efficiency of the extraction of *Citrus sinensis* essential oil obtained through the microwave assisted hydro distillation method (MWHD).

Table 1 presents the chemical composition of the essential oil of *C. sinensis* which was carried out by gas chromatography/mass spectrometry (GC/MS).

Table 1 - Major components detected in the OE of *C. sinensis* obtained through the microwave-assisted hydro distillation method

Component	% Relative abundance (Rt, min) *
	C. sinensis
α-pinene	0,48 (9,40)
β-Myrcene	1,22 (11,25)
Limonene	89,11 (11,68)
Linalool	0,85 (12,33)

*Retention time (Rt) and relative abundance (%) of essential oils, identified by comparison with reference mass spectrum of the NIST database - 2008.

To determine whether the EO maintains its antibacterial activity when incorporated into the impregnated support, the *in vitro* antibacterial activity of the finished product was determined, the results of which are presented (table 2).

Table 2. *In vitro* antibacterial activity of the finished product

Impregnated support		CFU of *E. coli**	CFU of *S. aureus**
Citrus sinensis	1.5%	Countless colonies	Countless colonies
	2.0%	0 CFU/mg of impregnated support	CFU / mg of impregnated suppo
Control		Countless colonies	Countless colonies

*CFU: colony-forming unit

Discussion

The determination of the antiseptic activity of plant extracts is an inexhaustible issue, because although this is well known since primitive times, using them specifically by

selecting a species, adjusting a dose, stabilizing a formulation based on its composition, is a task that cannot be done with generic information.[14] It should be noted that the MWHD technique is an effective method in the extraction of essential oils.[5,6] This is due to the action of microwaves on the glandular walls contained in the essential oil, which causes the plant material to break down faster and more efficiently.[14] Microwave-assisted hydro distillation uses three forms of heat transfer within the sample irradiation, conduction, and convection. As a result, it produces heat more quickly inside and outside the glands.

The essential oil of *Citrus sinensis* has a high content of monoterpenes, highlighting the presence of limonene, β-myrcene and linalool. These major compounds define the biological activity of the essential oil, where not a single one can be framed as responsible for it, which explains the antibacterial activity found in the impregnated support. The components of the essential oil could exert antibacterial activity by interfering with the phospholipid bilayer of the cell membrane causing the increase of its permeability and loss of cellular constituents, since it destroys the system of enzymes including those that involve the production of cellular energy (proton motive force) and bacterial respiration, when it comes to low concentrations of essential oils; while at high concentrations they would cause severe damage to the structural components of the bacterial cell, such as the loss of homeostasis or inactivating or destroying the genetic material, leading to cell death. In the mechanism of action of essential oils as antibacterial agents, the large number of chemical compounds that are present in them must be considered, whose antibacterial activities do not present a specific mechanism.[16,22,23].

The antibacterial impregnated supports formulated from 2% of *Citrus sinensis* essential oil, presented promising chemical and microbiological indicators; which gives us an indication of the correct formulation from the galenic point of view.

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Conflict of interests

The authors report no conflict of interest.

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