SCREENING OF THE ANTIMICROBIAL ACTIVITY OF GRAPEFRUIT OIL

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RESEARCH ARTICLE

Abstract
Citrus fruits, notably grapefruit, are renowned for their health benefits. This tropical, pink-hued fruit boasts a distinctive sweet-bitter taste and a trove of nutrients—ascorbic acid, vitamin B6, phenolic compounds, flavonoids, and pectins—making it among the healthiest in its family. Beyond consumption, grapefruit's biologically active components like peptides, essential oils, vitamins, and polysaccharides are prized in cosmetics. Its essential oil, packed with flavonoids, carotenoids, and phenolic compounds, showcases antibacterial, antioxidant, and antimicrobial prowess against various bacteria, doubling as a remedy for chronic skin conditions like dermatitis and acne. Leveraged extensively in the cosmetic industry, particularly for acne treatment, its anti-inflammatory properties combat excessive sebum, reducing blemishes. The antimicrobial activity of grapefruit essential oil was proven through a standardized culture of Staphylococcus aureus (ATCC 25923), where we applied 4 micro-presses with 4 different antibiotics and 3 different amounts of essential oil.

Keywords: (max. 5) Grapefruit; acne; flavonoids; essential oil.
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INTRODUCTION

Acne remains a pervasive dermatological challenge, prompting exploration into novel treatments sourced from nature's bounty. Amidst the vast array of citrus fruits cultivated worldwide, the grapefruit emerges as a potent contender, celebrated not only for its tropical allure, pink color and bittersweet flavor but also for its multifaceted beneficial properties (Russo et al, 2021) (Figure 1). Rich in an array of nutrients, antioxidants, and bioactive compounds, ascorbic acid, vitamin B6, phenolic compounds, flavonoids, and pectins, grapefruit claims its place among the healthiest citrus fruits. (Santana de Oliveira et al, 2023).


Figure 1. **Grapefruit pulp**

Peptides, essential oils, vitamins and polysaccharides are the biologically active ingredients present in cosmetics (Hoang et al, 2021; Herman, 2019). Laden with flavonoids, carotenoids, and phenolic compounds, this oil demonstrates compelling antibacterial, antioxidant (Warale et al, 2023; Zayed et al, 2021), antimicrobial properties against bacteria such as Staphylococcus aureus, Enterococcus faecalis and Escherichia coli (Meryem et al, 2023; Visakh et al, 2022). Its efficacy spans beyond mere antibacterial action, exhibiting antiallergic, and photoprotective traits, positioning it as a viable candidate for managing chronic dermatological conditions like acne vulgaris (Mutha et al, 2021). Due to these properties, grapefruit essential oil is used in the cosmetic industry, especially for acne (Anitha & Sujatha, 2023). Acne is an inflammatory disorder, triggered by excessive sebum production, causing the appearance of comedones and pustules on the neck and face (Leung et al, 2021).

Numerous medical remedies from various plant extracts are used in the treatment of acne, being included in various topical preparations offering beneficial results (Decker & Graber, 2012, Asnaashari et al, 2023). Citrus essential oil can be used as a natural antibacterial agent that represents a useful alternative in the food industry to reduce the amount of synthetic additives used in an attempt to satisfy consumer demands, as long as they accept their effects on the organoleptic properties of products. Consuming grapefruit has been shown to be helpful in insulin resistance but also for weight loss (Fujioka et al, 2006).

As the cosmetic industry increasingly capitalizes on its potential, this article aims to dissect the intricate role of grapefruit essential oil in acne treatment, exploring its application and potential implications in skincare formulations.

MATERIAL AND METHOD

For determination of the antimicrobial activity of grapefruit oil, the reference strain of Staphylococcus aureus ATCC 25923, lot 983413, expiration date 16.11.2021 was used, and 4 antibiotics and 6 different volumes of grapefruit essential oil were tested in parallel.

For the formulation of grapefruit oil emulsions, we used 5 different emulsifiers in different amounts, 1mL of oil, and completed up to 10mL with distilled water. As a preparation technique, we introduced the oil in a mortar and emulsified it in the aqueous solution to which we previously added the emulsifier.

The determination of the pH of the emulsions was taken with the help of a pH meter to allow the establishment of acidity or basicity, because for example for people with sensitive skin, a pH that is not high is necessary to avoid the appearance of eczema.

Determining the refractive index is important because it can confirm the consistency of the product from batch to batch and determine the presence of the components in the correct concentrations to have the desired effects and quality.

The viscosity of a fluid is a measurement of its resistance to deformation. To determine the viscosity, we used the Fungilab viscometer.

To determine the density we used a pycnometer, by weighing the mass of micellar water and applying the formula $\rho=m/v$ (g/mL).

RESULTS AND DISCUSSIONS

The diameters obtained by grapefruit essential oil and antibiotics can be seen in Table 1.

Table 1

Diameters achieved by grapefruit essential oil and antibiotics

Grapefruit oil volume	Diameter	Antibiotic	Diameter
0,2µL	28mm	Azithromycin	26mm
0,4µL	30mm	Erythromycin	30mm
0,5µL	32mm	Tetracycline	32mm
0,6µL	32mm	Levofloxacin	34mm

The antimicrobial activity of grapefruit essential oil followed in a study on the standardized culture of Staphylococcus aureus (ATCC 25923), which can be followed in figures 3 and 4 where we have applied 4 micro presses with 4 different antibiotics and 3 different amounts of essential oil (Figure 2 and Figure 3).

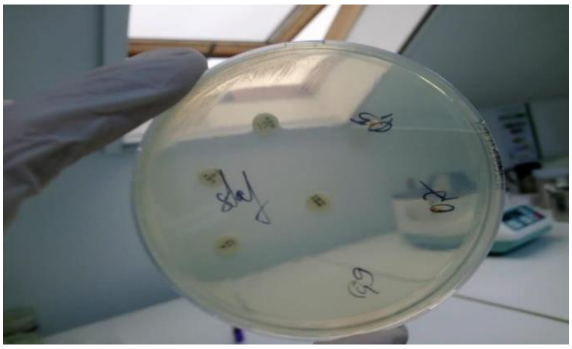

Figure 2. **Antibiogram on S. aureus culture with the 4 antibiotics and essential oil (0.5 mL, 0.7 mL and 0.9 mL)**

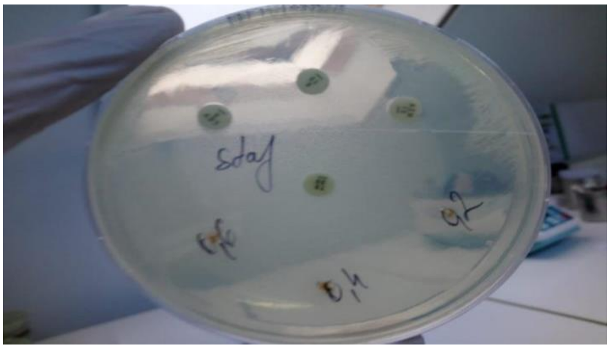

Figure 3. **Antibiogram on S. aureus culture with the 4 antibiotics and essential oil (0.2 mL, 0.4 mL and 0.6 mL)**

In this case of Staphylococcus aureus (ATCC 25923) cultures, the zone of inhibition of azithromycin is 26mm, of erythromycin is 30mm, of tetracycline is 32mm and of levofloxacin is 34mm. The essential oil was used in 6 different amounts and the minimum diameter of the inhibition zone was 28mm as shown in Table 1.

The formulation and preparation of emulsions with grapefruit oil is shown in table 2. Thus we obtained 5 different emulsions in which we incorporated different emulsifiers.

Table 2

Formulation of emulsions with grapefruit oil

Emulsion	Lipophilic phase	Emulsifiers	Hydrophilic phase
E1	Grapefruit oil 1mL	Tween 80 0,6g	8,4mL distilled water
E2	Grapefruit oil 1mL	Sodium lauryl sulfate 0,4g	8,6mL distilled water
E3	Grapefruit oil 1mL	Surfactant SCI 1g	8 mL distilled water
E4	Grapefruit oil 1mL	Coconut surfactant 0,5g	8,5 mL distilled water
E5	Grapefruit oil 1mL	Surfactant SCS 1,5g	7,5 mL distilled water

To evaluate the pharmaco-technical properties of cosmetic emulsions, we determined the pH, refractive index, viscosity, and density.

Determination of pH:

The values obtained vary between 5-10 due to the type and amount of surfactant used, so the amount of water used in the formulation of the emulsions also varies, affecting the pH, as we can see in Table 3.

Table 3

pH values of the 5 obtained emulsions

Emulsion	E1	E2	E3	E4	E5
pH	7,041	9,061	4,983	5,925	10,036

Emulsions with slightly acidic or neutral pH will be well tolerated on the skin, but those with alkaline pH can also be used for cleaning.

Determination of refractive index:

The results determined on the 5 emulsions that can be followed in Table 4, prove to us that they can be used with efficiency including on the face.

Table 4

Refractive index values of the 5 emulsions

Nr. Crt.	E1	E2	E3	E4	E5
1	1,3470	1,3470	1,3420	1,3480	1,3651
2	1,3471	1,3469	1,3421	1,3482	1,3652
3	1,3470	1,3471	1,3421	1,3481	1,3650
Average	1,3470	1,3469	1,3421	1,3481	1,3651

Determination of viscosity:

To determine the viscosity we used the following formula:

$\eta = K (\rho 1 - \rho 2) t$

K - Ball constant

ρ1 - density of the ball

ρ2 - density of the measured liquid

t - falling time of the ball (s)

The values obtained for the viscosity of the emulsions are shown in Table 5.

Table 5

Emulsion viscosity values

Emulsions	E1	E2	E3	E4	E5
Viscosity	1.1612	0.9939	0.9939	1.2174	1.2856

Determination of density:

Following the measurements and calculations, we found that the densities of the five emulsions have close values (table 6), Therefore the amount of emulsifier does not significantly influence the density of the analyzed preparation

Table 6

The values of the densities of the emulsions

Emulsions	Density (g/mL)
E1	0,992
E2	0.991
E3	0.991
E4	0.995
E5	1.007

CONCLUSIONS

The antimicrobial activity of grapefruit essential oil was proven through a standardized culture of Staphylococcus aureus (ATCC 25923), where we applied 4 micro-presses with 4 different antibiotics and 3 different amounts of essential oil. Thus, the zone of inhibition for azithromycin was 26 mm, for erythromycin 30 mm, for tetracycline 32 mm, and for levofloxacin 34 mm. The essential oil was used in 6 different amounts and the minimum diameter of the inhibition zone was 28 mm and the maximum 34 mm.

Grapefruit oil was incorporated into 5 emulsions using the same concentration of oil as the internal phase. For the formulation and preparation of the emulsions, we used 5 different emulsifiers, in different amounts.

The refractive index of the epidermis at the level of the skin is between 1.35-1.49, so the obtained emulsions can be used with efficiency on the face.

Emulsions with slightly acidic pH (E3-4,983; E4-5,925) or neutral (E1-7,041) are well tolerated on the skin, and those with alkaline pH (E2-9,061: E5 – 10,036 can be used for cleaning.

Following the measurements we found that the densities of the five emulsions (E1-0.992; E2-0.0.991; E3-0.991; E4-0.995; E5-1.007) have close values, therefore the amount of emulsifier does not significantly influence the density the analyzed preparation.

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