Biol. Stud., 2024, 18(4), 79–92 • doi: https://doi.org/10.30970/sbi.1804.804
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 57.02.084:[582.746.21:615:661.12]

BIOACTIVE RICH FINGERED CITRON LEAVES: INVESTIGATION OF USAGE POTENTIAL IN COSMETIC AND PHARMACEUTICAL PRODUCTS

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Tasbasi, H. & Asan-Ozusaglam, M. (2024). Bioactive rich fingered citron leaves: investigation of usage potential in cosmetic and pharmaceutical products. *Studia Biologica*, 18(4), 79–92. doi:10.30970/sbi.1804.804

Background. Fingered citron is one of the important plants attracting attention with its important bioactive components. The aim of the study was to evaluate the potential for use of fingered citron leaves in the cosmetic and pharmaceutical industries.

Materials and Methods. The antimicrobial activity of fingered citron leaf ethanol extract was determined by disc diffusion and micro-dilution methods against clinical pathogens. Furthermore, fingered citron leaf ethanol extract sun protection factor (SPF) was spectrophotometrically evaluated.

Results and Discussion. The inhibition zone diameters obtained as a result of the disc diffusion method were 9.16 mm against *Candida albicans* ATCC 10231 and 9.63 mm against *C. glabrata* RSKK 04019. *Staphylococcus aureus* ATCC 25923 was inhibited by fingered citron leaf ethanol extract with 7.76 mm of inhibition zone. Minimal inhibition (MIC) and bactericidal or fungicidal (MBC or MFC) concentrations values varied between 2.5 and 40 mg/mL. Additionally, the biological activity of the cream formulation obtained with cream, probiotic strain *Limosilactobacillus fermentum* MA-7 and fingered citron leaf extract was evaluated using the well diffusion method. The inhibition zone diameters of cream, *L. fermentum* MA-7, and fingered citron leaf extract cream group against *C. albicans* ATCC 10231, *C. glabrata* RSKK 04019 and *S. aureus* ATCC 25923 were determined as 2.73 mm, 4.37 mm, and 5.21 mm, respectively. Furthermore, the SPF value of fingered citron leaf ethanol extract was determined as 25.82. Then, fingered citron leaf ethanol extract-cream mixtures were prepared at various concentrations. It was determined that the SPF values of the extract and cream mixtures were higher at all concentrations compared to the commercial cream (control). The highest SPF value was determined as 6.7 at 10 mL concentration.

Conclusion. The results indicated that fingered citron leaf ethanol extract can be a valuable resource for the cosmetic and pharmaceutical industries in the development of natural origin and effective products.

Keywords: biological activity, *Citrus medica* L. var. *sarcodactylis*, extract, cream, pathogens

INTRODUCTION

One of the biggest problems people face is the development of antibiotic resistance. Microorganisms resistant to multiple drugs are rapidly proliferating and spreading worldwide (Church & McKillip, 2021). Therefore, the need for new and natural antimicrobial agents is increasing. Plants are one of the sources that offer potential use as therapeutic agents due to their antimicrobial properties (Najmi *et al.*, 2022). In recent years, the positive effects of natural compounds on health have become scientifically prominent (Antonić *et al.*, 2020). In addition, consumers are increasingly choosing herbal products that may have fewer side effects than synthetic components (Hoang *et al.*, 2021). Since the past, people have used herbal products as an effective method of treating diseases (Chaughule & Barve, 2024). Fingered citron (FC) belongs to the *Rutaceae* family and generally grows in subtropical regions. It is a morphologically different fruit and with this feature it differs from other *Citrus* species (Karp & Hu, 2018). In traditional Chinese medicine, FC stands out as an important raw material used in the treatment of various chronic diseases. It is a very rich source of terpenoids. FC has high antioxidant content and can be consumed safely as a food additive (Chen *et al.*, 2021; Xu *et al.*, 2022). In addition, FC fruit and its byproduct, the leaves, have high antifungal effect. A study reports that essential oils of flowers, fruits and leaves obtained from FC can be considered as natural preservatives alternative to chemical additives to meet safety needs in the food industry and to meet consumer demand for natural ingredients (Wu *et al.*, 2021).

The organ with the largest surface area in the human body is the skin. It plays a critical role as an immunological and physical barrier against pathogens (Vale de Macedo *et al.*, 2021). A number of antimicrobial lipids, peptides, toll-like receptors and chemokines produced by the epidermis constitute cutaneous innate immunity (Elias, 2005). This protective mechanism occurs through a complex immune response and a community of beneficial microorganisms known as the microbiota (Smythe & Wilkinson, 2023). Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill *et al.*, 2014). These microorganisms are commonly lactic acid bacteria (LAB) (Mishra and Acharya, 2021). Probiotics are also used in cosmetic products to support skin health, prevent skin aging and whitening, and reduce inflammation and UV-induced damage (Dou *et al.*, 2023). In the cosmetic industry, fractions or lysates are generally preferred (Gueniche *et al.*, 2022). Lysates of lactic acid bacteria contribute to improving skin epidermis (Voloshyna & Shkotova, 2022). At the same time, topical use of probiotic products strengthens the skin’s natural defense barrier, creating direct effects from the application area (Habeebuddin *et al.*, 2022). Therefore, probiotics and plant extracts have the potential to create beneficial effects by improving and protecting the skin structure (Flores-Balderas *et al.*, 2023). One of the effective methods of protecting the skin from the harmful effects of the sun is the use of natural topical sunscreens (Li *et al.*, 2023).

The skin is constantly exposed to different environmental factors, including ultraviolet (UV) rays (Chinnasamy *et al.*, 2022). Ultraviolet radiation, invisible to the human eye, is filtered by the ozone layer. The ozone layer absorbs UVC completely, largely UVB, and partially UVA, preserving life (Kwon *et al.*, 2022). These rays can cause DNA damage in the skin, causing health problems such as signs of premature aging, sunburn and even skin cancer (Anbualakan *et al.*, 2022). Compounds with sunscreen effects play an effective role in reducing the effects of UV radiation. For this reason, sunscreens containing various synthetic filters are used today to protect the skin from negative UV radiation (Ghazi, 2022). In addition to chemical UV filters, bioactive components such as flavonoids or polyphenols found in plant extracts act as UV filters, reducing the risk of sunburn and absorbing UV rays. These compounds also have anti-inflammatory and antioxidant activities (Li *et al.*, 2023). UV exposure leads to oxidative reactions by causing the production of reactive oxygen species (ROS); such damage can cause significant damage to cell metabolism (Nakai and Tsuruta, 2021). Many studies have found that terpenoids are powerful natural antioxidants that protect against oxidative reactions in various organs, including the skin, liver, kidney, cardiovascular system, brain and during the aging process (González-Burgos & Gomez-Serranillos, 2012). Limonene, a monoterpene found in high amounts in FC leaves, protects keratinocytes from UVB-induced photodamage and photoaging by inhibiting intracellular ROS formation and cell death (Kumar *et al.*, 2022). In this context, FC leaves may have potential in skin protection as a natural UV filter due to their richness in terpenes and natural sunscreen properties.

FC fruit is widely used as a traditional herbal medicine in the treatment of many diseases (Prc, 2008). However, its flowers and leaves are by-products of fruit processing. The leaves are often discarded, which pollutes the environment and also wastes functional ingredients and resources (Wu *et al.*, 2021). FC leaves may have potential as a natural preservative alternative to chemical additives to meet consumer demand for natural additives.

To date, there have been few studies on FC leaves in the literature, therefore, the primary aim of this study was to determine the biological activity of FC leaf ethanol extract (FCLEE) against yeasts and clinical pathogen. Then, the antimicrobial activity of cream groups developed with FCLEE and *L. fermentum* MA-7 on some pathogens was evaluated. Additionally, it was aimed to determine the sun protection capacity of FCLEE and FCLEE cream mixtures. Thus, we aim to contribute to both the environment and various areas of the industry by investigating the use of leaves qualified as waste in various industries.

MATERIALS AND METHODS

Plant samples and preparation of leaf extract. FC leaves were obtained from Muğla Köyceğiz Plant World (October 2022, Turkey) and dried under room temperature conditions (**Fig. 1**). Extraction was performed using 10 g of powdered leaf material with 30 mL of ethanol solvent in a water bath at 70 °C for 24 h (2 days). After this process, the remaining solvent was removed by evaporation. The extract was prepared with dimethyl sulfoxide at a concentration of 100 mg/mL. The stability of its content was ensured by storing the extract in a refrigerator at +4 °C until use.

Fig. 1. Fingered citron (FC)

Growth conditions of yeasts, clinical pathogen and lactic acid bacteria. The yeast strains *C. albicans* ATCC 10231 and *C. glabrata* RSKK 04019 at 30 °C (Yeast-Peptone-Dextrose), clinical bacterial pathogen *S. aureus* ATCC 25923 at 37 °C (Nutrient-Broth) and probiotic candidate LAB strain *L. fermentu*m MA-7 at 37 °C (Man-Rogosa-Sharpe) were grown for 24 h.

Antimicrobial assay of leaf extract. The biological activity of FCLEE was evaluated using the disc diffusion method (Tasbasi & Asan-Ozusaglam, 2024). The microbial cultures were washed with saline solution. The suspension adjusted to the standard of 0.5 McFarland (100 µL) was inoculated onto solid media. Sterile discs of 6 mm diameter (in triplicate) were placed onto solid culture medium. 2 mg/mL FCLEE was then added to the discs. Petri dishes were incubated for 24 hours at the appropriate temperature as stated above.

Micro-dilution. The micro-dilution method was used to determine the MIC and MBC or MFC values of FCLEE (Tasbasi & Asan-Ozusaglam, 2024). Appropriate medium was added to the tubes and diluted twofold from the initial concentration of 40 mg/mL (20, 10, 5, 2.5, 1.25 mg/mL) and 0.5 McFarland was added to each tube separately. The mixture was incubated at the temperature specified for each pathogen for 24 hours. The

extract concentration at which there was no growth was determined as the MIC value. After incubation, they were inoculated onto solid media. The concentration at which microbial growth stopped on the solid media was recorded as the MBC or MFC value.

Antimicrobial assay of cream formulation developed with extract and probiotic strain. The biological activity of the extract and cream formulation developed in the study was determined using the modifying method as described by H. Tasbasi and M. Asan-Ozusaglam (2024). The cream formulation containing FCLEE and/or *L. fermentum* MA-7 was prepared. The experimental groups were cream (C, control), cream-*L. fermentum* MA-7 (CLF), cream-FCLEE (CF), cream, FCLEE, *L. fermentum* MA-7 (CLFF). In the preparation of four different cream groups, firstly, 10 % w/v commercial cream was added for each group. 20 % w/v extract was added to the groups containing FCLEE. While the final volume of the groups containing probiotic bacteria was completed with *L. fermentum* MA-7, the final volume of the other groups was completed with distilled water to prepare cream formulations. The cream formulations were sonicated (15 min). They were then sterilized with a filter (45 µm). The biological activity of the groups was determined using the well diffusion method. The microbial suspension prepared at a density of 0.5 McFarland was spread onto solid medium. Then, 100 µL of cream formulations were added to the 6 mm diameter wells in the solid medium. The experiments were performed in triplicate. The Petri dishes were incubated at the appropriate temperature as mentioned above. After the incubation period, the inhibition zone diameters were recorded.

Determination of photoprotective activity of finger citron leaf ethanol extract. The SPF of FCLEE and cream-FCLEE mixtures was determined spectrophotometrically in 3 replicates (Tasbasi & Asan-Ozusaglam, 2024). FCLEE extract (0.006 g) was dissolved in 3 mL of ethanol (96%). Then, for the cream-FCLEE mixtures, 1 g cream and 0.5 g FCLEE were made up to 10 g with water. The prepared mixture (0.1 g) was taken into another tube and made up to 10 mL with ethanol (40%). It was then sonicated for 5 minutes. After the mixture was filtered through No:1 Whatman filter paper, 0.5 mL was taken into another tube and made up to 5 mL with ethanol. Then, 0.5 mL of the 5 mL mixture was taken to make up to 2.5 mL. The spectrophotometric measurements were made at 5 nm intervals between $OD_{290–320}$ of FCLEE extract and diluted cream-FCLEE mixtures (2.5, 5 and 10 mL). The SPF values were calculated using the Mansur equation (Mansur *et al.*, 1986). The equation is given below.

$$SPF = CF \cdot \sum_{290}^{320} EE(\lambda) \cdot I(\lambda) \cdot Abc(\lambda)$$

CF = correction factor (= 10); EE (λ) = erythemetogenic effect radiation wavelength (λ); I (λ) = intensity of sunlight at wavelength (λ); Abs (λ) = optical density value at wavelength (λ) of FC ethanol extract.

Statistical analysis. The antimicrobial activities were analyzed using GNU SPSS software (version 25.0). One-way analysis of variance (ANOVA) was performed. The significance of differences between mean values was evaluated using the Tukey test and considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Pathogenic microorganisms have been a serious threat to human health since their emergence in human history and stand out as the cause of many diseases (Górniak *et al.*, 2019). Therefore, antimicrobial activity is a feature of great importance for the

prevention and treatment of diseases. In our study, the biological activity of FCLEE was tested against the pathogens *C. albicans* ATCC 10231, *C. glabrata* RSKK 04019, and *S. aureus* ATCC 25923, as shown in **Table 1**. The higher inhibition zone diameter against *C. glabrata* RSKK 04019 (9.63 mm) was determined than *C. albicans* ATCC 10231 (9.16 mm). Recently, *Candida* spp. has been seen as the main cause of hospital-acquired infections (Hameed *et al.*, 2021). The potent antifungal effect of FCLEE may offer an approach for the effective control of these infections. The inhibition zone diameter against *S. aureus* ATCC 25923 was 7.76 mm. MIC and MBC or MFC values of FCLEE against pathogens varied between 2.5–40 mg/mL. The lowest MIC value among yeasts was 2.5 mg/mL for *C. glabrata* RSKK 04019, while the MFC values for both yeast strains have been determined as 20 mg/mL. The MIC and MBC values against *S. aureus* ATCC 25923 were 10 mg/mL and 40 mg/mL, respectively. In our study, Fluconazole (FCA) at 25 µg concentration showed higher antimicrobial activity compared to FLEE, but appropriate concentrations of FCLEE can be used for antifungal activity.

It was previously reported that Limonene (329.75 mg/mL) was the highest among the monoterpene hydrocarbons in the FC leaf (Wu *et al.*, 2021). Most studies have reported cell membrane damage and changes in membrane permeability as the mechanism of antibacterial action of limonene (Gupta *et al.*, 2021). Limonene also has antifungal effects against various yeasts and molds. The antifungal activity of limonene against *C. albicans* was explained by the change in membrane permeability of up to 82–88% of the cells exposed to limonene (Pekmezovic *et al.*, 2016). Limonene is also reported to be active against many pathogenic bacteria that cause skin diseases (Gupta *et al.*, 2021). In the present study, both antibacterial and antifungal activity may be due to Limonene, a monoterpene hydrocarbon found in high amounts in the leaves of FC.

Table 1. **Disc diffusion and micro-dilution assay results of FCLEE against pathogens**

Yeasts and clinical pathogen	Inhibition zone diameters (mm ± SD)			MIC (mg/mL)	MBC or MFC (mg/mL)
	FCLEE	AM	FCA		
C. albicans ATCC 10231	9.16±0.82[a]	-	20.35±0.10	20	20
C. glabrata RSKK 04019	9.63±0.57[a,b]	-	21.85±1.76	2.5	20
S. aureus ATCC 25923	7.76±0.64[a]	21.04±0.80	-	10	40

Note: FCLEE – fingered citron leaf ethanol extract; AM – ampicillin; FCA – fluconazole (25 µg); "-" – not detected. Different superscripts represent significant differences between values in the column. These differences were determined by one-way ANOVA and Tukey's post-hoc test ($P < 0.05$). F – 6.016; Sig – 0.037

Although there is a great deal of information on the antimicrobial activity of common Citrus, little is known about the antimicrobial activity of FC. In a previous study, the antimicrobial activity of essential oil obtained from FC leaf against *S. aureus* and *C. albicans* was determined by disc diffusion method. The inhibition zone diameter was recorded as 9.15 mm against *S. aureus* and 8.02 mm against *C. albicans* (Himawan *et al*., 2017). In our study, lower inhibitory activity was observed against *S. aureus* ATCC 25923, while higher inhibitory activity was detected against *C. albicans* ATCC 10231. In a study conducted by A. Abirami *et al.* (2013), the antimicrobial activity of *C. hystrix* leaf methanol extract was determined. The inhibition zone diameter against *S. aureus* 3160 was

10 mm and the MIC value was 50 mg/mL. Compared to these results, although FCLEE created a lower inhibition zone on *S. aureus* in our study, higher antimicrobial activity was observed with lower MIC value. In addition, in another study conducted with the *C. hystrix* species, the antimicrobial activity of three leaf extracts (*n*-hexane, ethyl acetate and ethanol) on *C. albicans* ATCC 90028 was investigated. According to the results, the MIC value for *n*-hexane and ethyl acetate extracts was determined as 50 mg/mL, while this value was >100 mg/mL for the ethanol extract. MBC values were recorded as >100 mg/mL for all three extracts (Buakaew *et al.*, 2022). In another study, the antimicrobial activities of the methanol extract obtained from the leaf of the *C. medica* were evaluated. The inhibition zone diameter against *C. albicans* NCIM 3471 and *S. aureus* MTCC 3103 was 15.46 mm and 18.46 mm, respectively. The MIC value was determined against *C. albicans* NCIM 3471 (48.75 mg/mL) and *S. aureus* MTCC 3103 (6.09 mg/mL) (Indira *et al.*, 2023). Compared to these studies, in the current study, FCLEE showed higher antimicrobial activity with lower MIC and MFC values. In the studies of A. N. Sah *et al.* (2011), it was found that the extract obtained from *C. medica* Linn. leaves with 70% ethanol created a 10 mm inhibition zone against *S. aureus* 737 strain in tests performed with the disc diffusion method, but did not show any inhibitory effect against *C. albicans* 227. MIC and MBC values were recorded as 10 and 25 mg/mL against *S. aureus* 737. Our results were closely similar for *S. aureus*.

The development of resistance to traditional antifungal drugs constitutes a significant health problem. The emergence of resistance has become a significant clinical problem that limits the successful treatment of *Candida* infections. Therefore, there is a need to discover and develop new and effective antifungal agents (Perlin *et al.*, 2017). FC may have the potential to be used as a new alternative antifungal agent.

The biological activities of the developed cream formulation against pathogens were determined and the results are given in **Table 2**. The inhibition zone diameter of C (control) group was not observed except *C. glabrata* RSKK 04019 (2.08 mm). However, FCLEE together with *L. fermentum* MA-7 and cream increased antifungal activity against *C. glabrata* RSKK 04019 (4.37 mm) (P <0.05). The cream, which had no antifungal activity, showed an inhibitory effect against *C. albicans* ATCC 10231 in combination with *L. fermentum* MA-7 and FCLEE (2.73 mm). Interestingly, against *S. aureus* ATCC 25923 (no inhibitory effect of the cream), CLF and CF showed low but high antibacterial effect in the CLFF group due to the synergistic effect of *L. fermentum* MA-7 and FCLEE (P <0.05). *L. fermentum*, one of the probiotic LAB, is one of the most commercialized heterofermentative *limosilactobacillus*. The bacterium produces bacteriocins, various antimicrobial peptides and H_2O_2. The higher antimicrobial activity of CLFF than CLF and CF on pathogens may be due to the combined effect of the antimicrobial substances of *L. fermentum* MA-7 and the FCLEE. This cream formulation containing natural ingredients can be an important guide for the cosmetic and pharmaceutical industries in order to provide effective protection against *Candida* and *Staphylococcus* species. Additionally, this natural resource can increase the reliability of products by reducing the risk of potential contamination by pathogens.

In a study conducted by S. Indriaty *et al.* (2024), cream formulations prepared with tea tree and lemon oil (1:1, 1:2 and 2:1) showed significant inhibition against *Propionibacterium acnes*, demonstrating their potential antimicrobial effects. As a result of the disc diffusion method, the inhibition zone diameters were determined as 12.3, 11.2 and 11.2 mm. In a different study, *C. aurantium* fruit peels extracted with ethyl

acetate were used for ointment formulations at various concentrations (0.25%, 0.5% and 1%). The wound healing activities of the prepared natural-based ointment was determined on albino rats. The ointment has been shown to heal skin wounds and significantly reduce surface area (Cherukuri *et al.*, 2023). Additionally, herbal ointments formulated with *Curcuma longa*, *C. aurantium dulcis* and *Cymbopogon citratus* extracts have shown that the product structure can be preserved intact by utilizing the medicinal properties of these ingredients. It has been reported that such ointments can be developed by utilizing the medicinal properties of the extracts (Borah *et al.*, 2022). Herbal lotion developed with lemon grass extract also supports the safe and effective use of natural cosmetic products by offering high moisturizing properties without side effects such as redness, edema and irritation (Ghortale & Somani, 2023). Similarly, it has been reported that the medicinal properties of essential oils obtained from the leaves and different parts of citrus fruits provide valuable contributions to the development of natural ingredient formulations in the cosmetic and pharmaceutical industries (Borah *et al*., 2022; Osman, 2019). In our study, it was clearly shown that the cream formulation containing FCLEE and probiotics provided effective protection against pathogens. In this way, it emphasizes the importance of the pharmaceutical and cosmetic industries being able to successfully develop products such as creams and ointments by enriching formulations with natural ingredients of this kind.

Table 2. **Disc diffusion assay results of creams developed with FCLEE and probiotics against pathogens**

Yeasts and clinical pathogen	Inhibition zone diameters (mm ± SD)				
	C	CLF	CF	CLFF	F(Sig)
C. albicans ATCC 10231	-^{a}	2.21±0.44^{a}	1.42±0.14^{a}	2.73±0.48^{b,a}	7.416(0.011)
C. glabrata RSKK 04019	2.08±0.19^{a}	4.01±1.16^{b}	2.21±0.18^{a}	4.37±0.24^{b}	11.540(0.003)
S. aureus ATCC 25923	-^{a}	1.25±0.10^{b}	1.81±0.38^{b}	5.21±0.74^{c}	84.758(0.000)

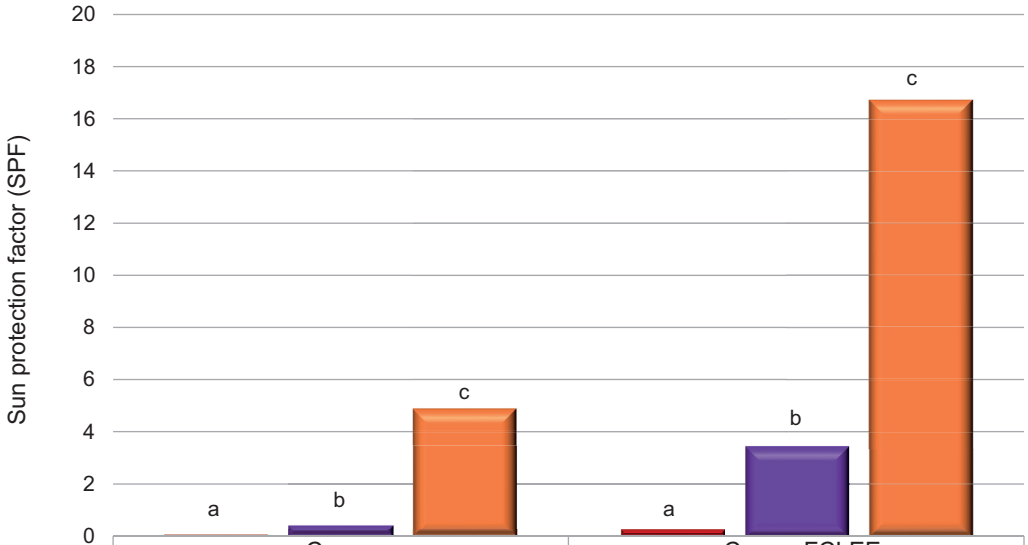
Note: FCLEE – fingered citron leaf ethanol extract; C – cream; CLF – cream-*L. fermentum* MA-7; CF – cream-FCLEE; CLFF – cream-*L. fermentum* MA-7-FCLEE; "-" – not detected. Different superscripts represent significant differences between values in the line. These differences were determined by one-way ANOVA and Tukey's post-hoc test (P <0.05)

The SPF values of FCLEE and FCLEE-cream mixtures are shown in **Figure 2**. The SPF value of the FCLEE was 25.82. Considering the high SPF value of FCLEE, SPF values were determined for various concentrations (2.5, 5, and 10 mL) of the FCLEE-cream mixture. As shown in **Figure 2**, different concentrations of FCLEE (2.5, 5, and 10 mL) increased the SPF value of the cream compared to the control group (cream) and a statistically significant difference was determined between them (P <0.05). The highest SPF value for FCLEE-cream was determined as 16.7 at a concentration of 10 mL. According to S. Imam *et al*. (2015), FCLEE and FCLEE-cream mixture (10 mL concentration) showed high UV blocking activity over 96% and 93%. These results demonstrate the usability of FCLEE as an effective ingredient in sun protection products.

It is stated in literature that bioactive components such as carotenoids, coumarins, terpenoids and polysaccharides found in plant extracts may help reduce photoaging and UV-induced harmful effects (Boo, 2020). In a study it was reported that limonene

protects keratinocytes against UVB-induced photodamage and photoaging by preventing intracellular reactive oxygen species (ROS) formation and cell death (Kumar *et al*., 2022). Therefore, it is thought that the sun protection capacity observed in our study may be due to the terpenes, especially limonene, found in the leaves.

Different letters represent significant differences between values on the column; These differences were determined by one-way ANOVA and Tukey's post-hoc test (P <0.05). F(Sig): 57199.225(0.000) for cream, 397248.800(0.000) for cream-FCLEE.



	Cream	Cream-FCLEE
2.5 mL	0.07	0.24
5 mL	0.44	3.44
10 mL	4.94	16.7

Fig. 2. Photoprotective activities of FCLEE and FCLEE-cream mixtures

In the study, SPF values of kumquat (*C. fortunella*) fruit and leaves extracted with methanol solvent were determined as 7.35 and 26.06. The sunscreen properties of kumquat fruit and leaf may enable the development of new and effective sun protection formulas in the cosmetic industry (Asan-Ozusaglam *et al*., 2021). In our study, the SPF value of FCLEE was obtained to be close to kumquat leaf extract. F. R. Putri and I. Sailah (2022) developed sunscreens with different concentrations using green tea, lemon and chitosan as natural ingredients in their study. When the SPF values of sunscreen formulations were examined using the Mansur equation, it was determined between 10.98–14.80. In another study, the SPF value of the cream developed with lemon grass extract was determined as 22 (Yaseen *et al*., 2018). In our study, the SPF value of the 10 mL FCLEE-cream mixture was found to be lower than the creams developed with lemon grass and higher than the one developed with lemon. In addition, the safety of 33 components obtained from citrus flowers and leaves, which are most frequently used in the cosmetic industry, was evaluated. It has been reported that these components are safe in current usage and concentration applications when formulated in a way that is not irritating and sensitizing (Burnett *et al*., 2021).

CONCLUSION

In the study, the biological activities of FCLEE against yeasts and clinical pathogens was examined to determine its use in various industries. FCLEE exhibited good antimicrobial activities against *Candida* strains and *S. aureus*. FCLEE may be a natural antimicrobial alternative, especially against antimicrobial resistance of pathogenic strains. Additionally, the extract with *L. fermentum* MA-7 and cream formulation showed inhibitory activity against these pathogen strains. The high SPF values of FCLEE and FCLEE cream concentrations demonstrated effective sun protection potential. In CLFF, various antimicrobial substances produced by *L. fermentum* MA-7 and the antimicrobial activity of FCLEE increased the antimicrobial activity with a synergetic effect. The statistical significance of the antimicrobial and SPF activities of FCLEE strengthens the claim that FCLEE is a promising ingredient for cosmetic or pharmaceutical applications. Limonene, a terpene found in high amounts in FC leaves, also protects against UVB-induced photodamage and photoaging, indicating that FCLEE may have a potential for skin protection as a natural UV filter. Therefore, the bioactive properties of FCLEE can be evaluated as an alternative to develop natural and effective cosmetic or pharmaceutical products.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that they have no conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: this article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceptualization, [H.T.; M.A-O.]; methodology, [H.T.; M.A-O.]; validation, [H.T.; M.A-O.]; formal analysis, [H.T.; M.A-O.].; investigation, [H.T.; M.A-O.]; resources, [H.T.; M.A-O.]; data curation, [H.T.; M.A-O.]; writing – original draft preparation, [H.T.; M.A-O.]; writing – review and editing, [H.T.; M.A-O.]; visualization, [H.T.; M.A-O.] supervision, [H.T.; M.A-O.]; project administration, [M.A-O.]; funding acquisition, [M.A-O.].

All authors have read and agreed to the published version of the manuscript.

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Received / Одержано 06 October, 2024 | Revision / Доопрацьовано 06 November, 2024 | Accepted / Прийнято 18 December, 2024 | Published / Опубліковано 23 December, 2024