



# White pitahaya as a natural additive: potential usage in cosmetic industry

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Received 08.04.2022; Revised 03.06.2022; Accepted 05.07.2022; Published online 11.11.2022

## Abstract:

The awareness of some harmful side effects of the chemicals contained in synthetic cosmetics has increased the demand for herbal-based cosmetic products today.

White pitahaya fruit and peel methanol extracts were prepared to determine their usage potential in the cosmetic industry. Firstly, we investigated their antimicrobial activity against some test microorganisms using the disc diffusion assay. We also determined their minimal inhibition and minimal bactericidal or fungicidal concentrations. Then, we assayed the antimicrobial activity of a commercial cream containing white pitahaya extracts and the probiotic *Lactobacillus fermentum* MA-7 strain against the test microorganisms. Finally, we measured the sun protection factors of the white pitahaya fruit and peel extracts and the cream with the extracts.

The white pitahaya fruit and peel extracts exhibited antimicrobial activity against the test microorganisms. The cream formulation containing a pitahaya fruit extract had the highest inhibition zone diameter of 11.25 mm against *Escherichia coli* O157:H7. The highest sun protection value among the extracts and cream with extracts was determined for peel extract as 6.66 and 23.34, respectively.

The results indicate that pitahaya fruit and peel extracts have effective antibacterial and antifungal properties, as well as high sun protection factors, and therefore they could be used as natural preservatives in the cosmetic industry.

**Keywords:** Extract, antimicrobials, probiotics, cream formulation, solar protection

**Funding:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

**Please cite this article in press as:** Asan-Ozusaglam M, Celik I. White pitahaya as a natural additive: potential usage in cosmetic industry. *Foods and Raw Materials*. 2023;11(1):57–63. <https://doi.org/10.21603/2308-4057-2023-1-522>

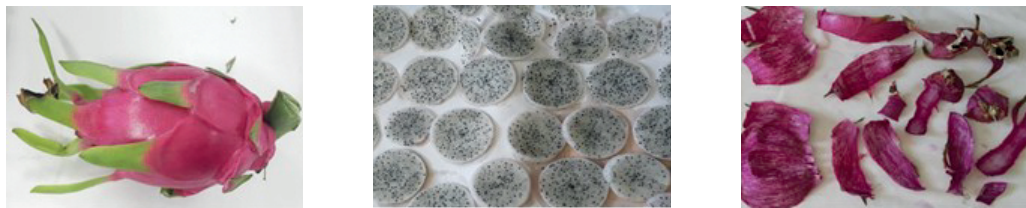
## INTRODUCTION

Pitahaya is a tropical fruit belonging to the *Cactaceae* family which attracts the attention of researchers and producers due to its appearance and color [1]. It was first used as an ornamental plant, and then its fresh fruit began to be consumed [2]. Pitahaya fruit is rich in vitamins and fiber, which helps the digestive system, blocks diabetes, removes toxic substances such as heavy metals, as well as controls cholesterol and blood pressure levels [2]. Pitahaya is known to have a high content of antioxidants which help fight many diseases such as arthritis, atherosclerosis, cancer, heart disease, inflammation, and brain dysfunction [3].

Food-borne and clinical pathogens pose a significant threat to human health. In developed countries, these

pathogens cause millions of infectious gastrointestinal diseases each year [4]. The influence of fungal microorganisms on human health has been increasing rapidly in recent years, with the global mortality rate from fungal diseases exceeding that from malaria or breast cancer [5]. Chemical-containing substances can be used against these pathogens, but they have harmful effects on human health, such as resistance to antimicrobials or toxicity.

Therefore, there is a growing interest in finding safer and more effective natural preservatives. Plants are natural sources of antimicrobials that can be used against various infectious pathogens, such as bacteria and fungi. More than 1340 plants with antimicrobial activity have been identified and more than 30 000 natural antimicrobial compounds have been isolated [6].



**Figure 1** White pitahaya fruit and peel

Formulations containing probiotics are increasingly in demand for skin care, prevention and treatment of skin aging and skin diseases. Thus, the use of probiotics in the cosmetic industry represents an emerging area for skin health [7]. Roudsari *et al.* reported that probiotics in cosmetic formulations have protective effects against atopic dermatitis, acne, eczema, allergic inflammation, or hypersensitivity of the skin to ultraviolet-induced damage [8]. The skin, one of the important organs of the human body, acts as a protective wall that prevents disease-causing pathogenic microorganisms from entering the internal system of the body.

It has been reported in many studies, e.g. by Nichols and Katiyar, that prolonged exposure to sunlight causes various skin conditions [9]. Solar UV rays are divided into three regions: UV-A (320–400 nm), UV-B (290–320 nm), and UV-C (200–290 nm). UV-A rays cause serious problems such as suppression of the immune system, premature skin aging, and endothelial cell necrosis that damages the dermal blood vessels [10]. UV-B rays cause sunburn, suppression of the immune system, photo carcinogenesis, and pigmentation [11]. UV-C rays react with oxygen atoms in the atmosphere and convert oxygen into ozone, which is absorbed by the atmosphere before reaching the Earth [12]. Sunscreen creams are used to protect the skin against the damage of these rays. However, chemical sunscreen creams can have some harmful effects on people with sensitive skin. Recent years have seen a trend towards using natural products to reduce or eliminate these harmful effects. In particular, researchers have focused on the development of herbal sunscreens that have no side effects by including naturally produced bioactive substances into sunscreens [10].

Fruit extracts are known to have ultraviolet (UV) protection, as well as antimicrobial and antioxidant activities due to the compounds they contain [13, 14]. Therefore, plant extracts have the potential to be used as bioactive natural products in food preservation, pharmaceutical industry, healthcare, and cosmetics [15].

We aimed to 1) evaluate the antimicrobial activity of pitahaya extracts; 2) develop a cream formulation containing pitahaya as a natural ingredient; 3) measure the sun protection factors of the pitahaya extracts to determine their potential use in the cosmetic industry as an additive in natural sunscreens; and 4) investigate the solar protection activity of the commercial cream with pitahaya extracts.

## STUDY OBJECTS AND METHODS

**Plant material.** The white pitahaya fruit was purchased in Antalya (Turkey) in September 2021.

**Preparation of extracts.** The pitahaya fruit samples were washed with distilled water. Then, the fruits and peels were air-dried separately at room temperature (Fig. 1). The dried fruit samples were ground with a Waring blender. For extraction, 10 g of the plant material was mixed with 30 mL of methanol (96%). The fruit samples were extracted every day for 6 h (3 days) in a hot water bath. The extracts were concentrated by using a rotary evaporator. The white pitahaya fruit and peel extracts were dissolved in dimethyl sulfoxide (DMSO) and then sterilized with a 0.45- $\mu$ m filter. The extracts were stored at 4°C until used.

**Determination of antimicrobial activity. Disc diffusion assay.** The disc diffusion method was used to determine the antimicrobial activity of the methanol extracts from white pitahaya fruit and peel against test microorganisms, including food-borne and clinical bacteria (*Salmonella enteritidis* RSKK 171, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 7644, *Enterococcus faecalis* ATCC 29212, and *Escherichia coli* O157:H7) and two yeasts (*Candida albicans* ATCC 10231 and *Candida glabrata* RSKK 04019).

The food-borne and clinical pathogens were cultured at 37°C in Tryptic Soy Broth and Nutrient Broth media, while the yeasts were cultured at 30°C in Yeast Peptone Dextrose media for 24 h. The test microorganisms were washed twice with a saline solution and their concentration was adjusted to 0.5 McFarland. 100  $\mu$ L of the prepared microbial suspension (0.5 McFarland) was spread onto agar media. Sterile discs (6 mm in diameter) were placed on the solid agar and 20  $\mu$ L (4000  $\mu$ g/disc) of the white pitahaya fruit and peel extracts were then dropped onto the discs in triplicates. The petri dishes were incubated at appropriate temperatures for 24 h. After incubation, the inhibition zones around the discs were measured with a caliper and recorded.

**Micro-dilution assay.** The minimum inhibition concentration and minimum bactericidal concentration or minimum fungicidal concentration of the extracts were determined using the micro-dilution assay. Test microorganisms adjusted to 0.5 McFarland were added to each tube containing the extract and the media. The mixtures in the tubes were incubated under the same conditions as in the disc diffusion assay for each

microorganism. After incubation, the concentration of the extract in the non-growth tube was recorded as minimum inhibition concentration values. Then, the samples from the tubes were inoculated onto solid media using the spot-dropping method. The petri dishes were incubated for 24 h at the same temperature for each microorganism which is indicated in the minimum inhibition concentration determination assay. At the end of the incubation, the extract concentrations that prevented bacterial growth on the solid media were evaluated as minimum bactericidal concentration or minimum fungicidal concentration values.

**Antimicrobial activity of the cream with pitahaya extract and probiotic.** A new method was designed by modifying the protocols of Handali *et al.* and Chen *et al.* [16, 17]. To formulate an antimicrobial cream, we used a commercial cream, white pitahaya fruit extracts, and the *Lactobacillus fermentum* MA-7 probiotic strain isolated from human milk [18].

In this study, three different experimental samples were assayed. The control was the cream (10% w/v) adjusted with distilled water to the final volume. The experimental samples contained: a) the cream (10% w/v) with the *L. fermentum* MA-7 probiotic strain; b) the cream (10% w/v) with the white pitahaya extract (20% w/v); and c) the cream (10% w/v) with the white pitahaya extract (20% w/v) and the *L. fermentum* MA-7 strain.

After vortexing, all the groups were sonicated for 15 min and then sterilized with a 0.45- $\mu$ m filter. The antimicrobial activity of the prepared mixtures was determined by the well diffusion method. The test microorganisms used in this study included *S. enteritidis* RSKK 171, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 27853, *E. coli* O157:H7, *L. monocytogenes* ATCC 7644, *C. albicans* ATCC 10231, and *C. glabrata* RSKK 04019. They were washed twice with a saline solution and their concentrations were adjusted to 0.5 McFarland. The microorganism suspensions (100  $\mu$ L) were spread onto solid media. 100  $\mu$ L of the mixture was added to each well (6 mm in diameter). The volume of the wells corresponded to 0.1. The experiment was carried out in three repetitions. The petri dishes were incubated for 24 h at 37°C for the food-borne and clinical pathogens and at 30°C for the yeasts. The inhibition zones around the wells were then measured with a caliper and recorded.

**Sun protection factor of pitahaya extract.** The sun protection factor (SPF) of the white pitahaya fruit and peel extracts was determined spectrophotometrically. The extract (0.006 g) was mixed with 3 mL of ethanol (96%) and homogenized. The homogeneous mixture was measured using a spectrophotometer (Beckman Coulter, USA) at 5-nm intervals in the wavelength range of 290–320 nm. The recorded values were calculated using the Mansur equation [19]:

$$\text{SPF} = \text{CF} \times \sum_{290}^{320} \text{EE}(\lambda) \times I(\lambda) \times \text{Abs}(\lambda)$$

where CF is the correction factor (= 10); EE( $\lambda$ ) is the erythemogenic effect radiation wavelength ( $\lambda$ ); I( $\lambda$ ) is the intensity of sunlight at wavelength ( $\lambda$ ); Abs( $\lambda$ ) is the absorbance of extracts at wavelength ( $\lambda$ ).

**Sun protection factor of the pitahaya extract and cream samples.** The sun protection factor of the pitahaya extract and commercial cream samples was determined using a modified method of Bambal *et al.* and Imam *et al.* [20, 21]. For this, 1 g of cream and 0.5 g of white pitahaya (fruit and peel) extracts were mixed, and 8.5 g of distilled water was added to the mixture. The mixture was stirred until it became homogeneous. 0.1 g of the homogeneous mixture was taken into another tube and completed to 10 mL volume with ethanol (40%). After sonication (30 kHz, 100% amplitude) for 5 min, the mixture was filtered through Whatman No.1 filter paper. The mixture (0.5 mL) was adjusted to 5.0 mL with ethanol (40%) in another tube. Then, 0.5 mL of this mixture was taken, and the volume was completed with ethanol (40%) to 2.5 mL. The mixtures adjusted to 2.5, 5.0, and 10.0 mL were measured in three repetitions using the spectrophotometer (Beckman Coulter, USA) at 5-nm intervals in the wavelength range of 290–320 nm. The sun protection factor values of the control and experimental samples were calculated using the Mansur equation presented above [19].

## RESULTS AND DISCUSSION

We investigated the antimicrobial and antifungal activities of the white pitahaya fruit and peel extracts on food-borne and clinical test microorganisms. Disc diffusion assay results are given in Table 1. As we can see, the highest inhibition zone diameter (11.57 mm) was found against *Pseudomonas aeruginosa* ATCC 2785 in the peel extract, while the lowest inhibition zone diameter (6.17 mm) was found against *Escherichia coli* O157:H7 in the fruit extract. As for the antifungal activity, the fruit and peel extracts had inhibition zone diameters of 11.66 and 13.15 mm, respectively, against *Candida albicans* ATCC 10231 and 12.21 and 12.93 mm, respectively, against *Candida glabrata* RSKK 04019.

Nurmahani *et al.* stated that ethanol, hexane, and chloroform extracts of white pitahaya peel showed an inhibition zone diameter of 7–9 mm against *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Enterococcus faecalis* ATCC 14506, *Salmonella typhimurium* ATCC 13311, *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Yersinia enterocolitica* ATCC 23715, and *Campylobacter jejuni* ATCC 29428 [22]. In our study, the inhibition zone diameters were larger compared to their results. In another study by Mahdi *et al.*, who used the well diffusion method, the inhibition zone diameters of 7–11 mm were obtained for white pitahaya aqueous extracts against *E. coli*, *Klebsiella* spp., *Staphylococcus epidermidis*, *S. aureus*, and *C. albicans* [23]. The

**Table 1** Antimicrobial activity of white pitahaya methanol extracts

Test microorganisms	Inhibition zone diameter, mm				
	Extracts		Antibiotics		
	Fruit extract	Peel extract	Ampicillin	Kanamycin	Fluconazole
<i>Salmonella enteritidis</i> RSKK 171	7.98 ± 0.21	9.28 ± 0.12	14.02 ± 0.30	15.48 ± 1.40	–
<i>Pseudomonas aeruginosa</i> ATCC 27853	9.87 ± 0.49	11.57 ± 0.79	23.53 ± 0.60	20.37 ± 0.20	–
<i>Listeria monocytogenes</i> ATCC 7644	6.30 ± 0.14	6.35 ± 0.38	29.57 ± 0.10	25.40 ± 1.30	–
<i>Escherichia coli</i> O157:H7	6.33 ± 0.09	7.06 ± 0.02	17.76 ± 0.00	19.33 ± 0.40	–
<i>Enterococcus faecalis</i> ATCC 29212	6.17 ± 0.22	6.82 ± 0.28	24.02 ± 0.30	13.48 ± 1.40	–
<i>Candida albicans</i> ATCC 10231	11.66 ± 0.07	13.15 ± 0.83	–	–	21.85 ± 1.76
<i>Candida glabrata</i> RSKK 04019	12.21 ± 1.44	12.93 ± 0.64	–	–	20.35 ± 0.10

**Table 2** Minimum inhibition and bactericidal or fungicidal concentrations of white pitahaya methanol extracts

Test microorganisms	Minimum inhibition concentration, mg/mL		Minimum bactericidal concentration or minimum fungicidal concentration, mg/mL	
	Fruit extract	Peel extract	Fruit extract	Peel extract
	<i>Salmonella enteritidis</i> RSKK 171	40	80	40
<i>Pseudomonas aeruginosa</i> ATCC 27853	20	40	20	80
<i>Listeria monocytogenes</i> ATCC 7644	40	80	> 80	80
<i>Escherichia coli</i> O157:H7	80	80	> 80	80
<i>Enterococcus faecalis</i> ATCC 29212	80	40	> 80	> 80
<i>Candida albicans</i> ATCC 10231	80	40	80	40
<i>Candida glabrata</i> RSKK 04019	40	20	40	20

**Table 3** Minimum bactericidal concentration/minimum inhibition concentration or minimum fungicidal concentration/ minimum inhibition concentration ratios of white pitahaya methanol extracts

Test microorganisms	Minimum bactericidal/inhibition concentration or minimum fungicidal/inhibition concentration	
	Fruit extract	Peel extract
	<i>Salmonella enteritidis</i> RSKK 171	1
<i>Pseudomonas aeruginosa</i> ATCC 27853	1	2
<i>Listeria monocytogenes</i> ATCC 7644	n.d.	1
<i>Escherichia coli</i> O157:H7	n.d.	1
<i>Enterococcus faecalis</i> ATCC 29212	n.d.	n.d.
<i>Candida albicans</i> ATCC 10231	1	1
<i>Candida glabrata</i> RSKK 04019	1	1

n.d. – not determined

differences in the antimicrobial activity assay results may be due to the differences in the origin of the pitahaya fruit samples, the solvents, as well as extraction and test methods used in the studies.

The disc diffusion assay results indicated the white pitahaya extracts' antimicrobial or antifungal activity against all the tested microorganisms. The minimum inhibition concentration, minimum bactericidal concentration or minimum fungicidal concentration values are shown in Table 2.

Among the tested white pitahaya extracts, the lowest minimum bactericidal concentration value was found to be 20 mg/mL for the fruit extract against *P. aeruginosa* ATCC 27853. The minimum bactericidal concentration values of the fruit

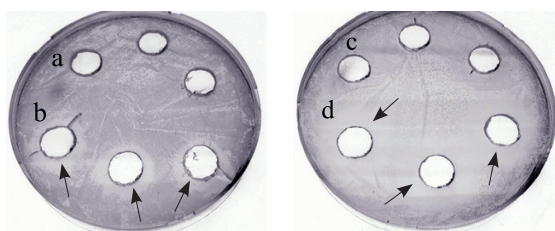
extracts varied from 20 to  $\geq 80$  mg/mL. The minimum bactericidal concentration values for the peel extracts were found to be 80 or  $\geq 80$  mg/mL against the tested bacterial strains. The lowest minimum fungicidal concentration value was 20 mg/mL for the peel extract against *C. glabrata* RSKK 04019.

The tested extract was considered bactericidal if the minimum bactericidal/inhibition concentration ratio was  $\leq 4$  and bacteriostatic if the minimum bactericidal/inhibition concentration ratio was  $> 4$  [24, 25]. The minimum bactericidal/inhibition concentration or minimum fungicidal/inhibition concentration ratios of some white pitahaya fruit and peel extracts were found to be 1 and 2. We established that those extracts had

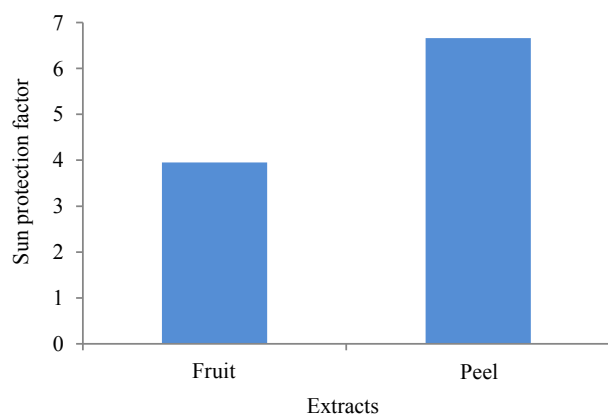
**Table 4** Antimicrobial activity of cream samples

Test Groups	Inhibition zone diameter, mm			
	Cream (control)	Cream with <i>Lactobacillus fermentum</i> MA-7	Cream with extract	Cream with extract and <i>Lactobacillus fermentum</i> MA-7
<i>Salmonella enteritidis</i> RSKK 171	–	1.62 ± 0.82	–	9.98 ± 5.57
<i>Enterococcus faecalis</i> ATCC 29212	–	–	–	2.72 ± 0.61
<i>Pseudomonas aeruginosa</i> ATCC 27853	6.44 ± 0.85	3.28 ± 0.30	3.76 ± 0.94	5.06 ± 0.58
<i>Escherichia coli</i> O157:H7	–	6.40 ± 0.24	–	11.25 ± 1.10
<i>Listeria monocytogenes</i> ATCC 7644	–	–	–	8.54 ± 0.38
<i>Candida albicans</i> ATCC 10231	–	6.76 ± 0.60	–	6.39 ± 0.11
<i>Candida glabrata</i> RSKK 04019	2.76 ± 0.53	8.73 ± 0.62	4.23 ± 1.53	3.54 ± 0.89

“–” is no inhibition zone



**Figure 2** Antimicrobial activity of cream samples against *Escherichia coli* O157:H7: a – cream (control); b – cream with *Lactobacillus fermentum* MA-7; c – cream with extract; d – cream with extract and *Lactobacillus fermentum* MA-7



**Figure 3** Sun protection factor of white pitahaya methanol extracts

antibacterial and antifungal activities against the tested microorganisms, except for the not determined group (Table 3).

The antimicrobial activity assay results of the cream samples are given in Table 4. As we can see, the cream with the extract and *Lactobacillus fermentum* formed an inhibition zone against all the tested microorganisms. The highest inhibition zone diameter (11.25 mm) among the tested microorganisms was obtained against *E. coli* O157:H7 in the cream with the extract and *L. fermentum*. The control cream and the cream with the extract did not

show an inhibition zone against *E. coli* O157:H7, while the cream with *L. fermentum* mixture had a diameter of 6.4 mm.

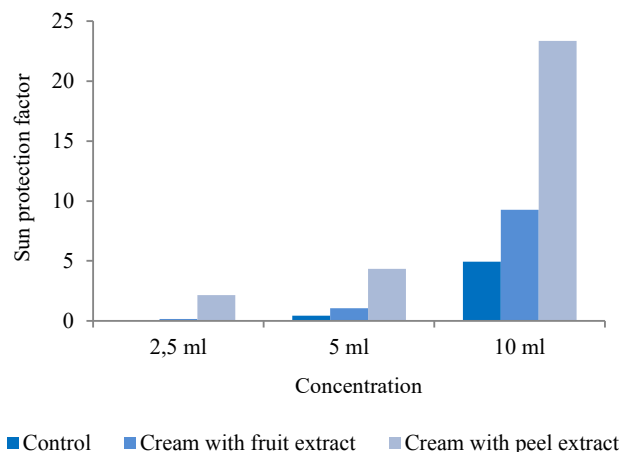
According to Fig. 2, the antimicrobial activity or increased inhibition zone diameters were observed in the cream with the extract and *L. fermentum*, compared to the control sample. The results for the control and all the test groups showed that the pitahaya extract and the probiotic candidate strain increased the antimicrobial activity of the commercial cream by creating a synergetic effect.

A recent study by Moysidis *et al.* indicated that the cream sample with *L. fermentum* MA-7 increased the rate of healing the wound [26]. Another recent study showed inhibition zone diameters of 2–10 mm in cream with *Vernonia ambigua* leave extracts against *C. albicans*, *S. aureus*, *E. coli*, and *P. aeruginosa* [27].

Sunscreens, which are widely used today to protect the skin from sun rays, constitute a large part of the cosmetic industry. In our study, the white pitahaya fruit and peel extracts had a sun protection factor of 3.95 and 6.66, respectively (Fig. 3). According to the UV blocking rates reported by Imam *et al.*, the white pitahaya fruit and peel extracts had a UV blocking capacity of 75 and 80%, respectively [21].

The sun protection factor values of the cream samples containing white pitahaya fruit and peel extracts are given in Fig. 4. The highest sun protection factor values of 9.26 and 23.34 were observed in the fruit and peel extracts at 10-mL concentrations, respectively. The highest sun protection factor value was observed at the 10-mL concentration for the samples with the extract as 9.26 and 23.34, while that for the commercial cream (control) was found to be 4.94 (Fig. 4). According to Imam *et al.*, the cream samples with pitahaya fruit and peel extracts had UV blocking rates of 90 and 95%, respectively [21]. Compared to the control, the fruit and peel extracts increased the sun protection factor values and the UV blocking rate of the cream.

Higher concentrations of the extract added to the commercial cream increased the sun protection factor value of the cream. The sun protective properties of plant extracts are due to the phenolic compounds and flavonoid substances they contain. Phenolic and



**Figure 4** Sun protection factor of commercial cream (control) and cream with white pitahaya methanol extracts

flavonoid compounds found in pitahaya have been reported to contribute to a high sun protection factor value and broad-spectrum UV-A and UV-B light protection [28]. The use of natural additives obtained from plants in various industries enables low-cost production in high quantities [29, 30].

## CONCLUSION

We studied the biological activities of pitahaya fruit and peel extracts to determine their usage potential in the cosmetic industry. The extracts exhibited good antibacterial and antifungal activities against the tested microorganisms. We also tested the cream samples containing the pitahaya extracts to determine their antimicrobial activity. The cream with the pitahaya fruit extract and *Lactobacillus fermentum* MA-7 with high antimicrobial activity could become an alternative to chemical preservatives. Additionally, the pitahaya fruit extracts and the cream with the pitahaya extracts exhibited high sun protection values. The results showed that pitahaya fruit and peel extracts could be suitable as natural preservatives for the cosmetic industry.

## CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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