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Determination of Biological Activity of Hylocereus polyrhizus Methanol Extracts: Antimicrobial Activity, Probiotic-Promoting Effect, Photoprotective Activity

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***Abstract*— In our study, the biological activity of Hylocereus polyrhizus obtained from Türkiye was determined. Firstly, antimicrobial activity of methanol extracts obtained from H. polyrhizus against test microorganisms was determined by disc diffusion susceptibility assay, minimum inhibition concentration (MIC) and minimum fungicidal or bactericidal concentration (MFC or MBC) tests. Then, viable cell counting against Limosilactobacillus fermentum MA-7 using the macro-dilution method was used to determine the probiotic-promoting effect of the fruit extracts. Finally, the photo-protective activity of the extracts and extract-cream mixtures was obtained spectrophotometrically. The inhibition zone diameter of the peel extract against the test microorganisms is in the range of 9.60-12.37 mm and the fruit extract is in the range of 7.85-12.00 mm. MBC values ranged from 40 µg/µl to 80 µg/µl in peel extract and between 10 µg/µl and >80 µg/µl in the fruit extract. The inhibition zone diameters of the extracts against probiotics were determined as 6.10 -10.25 mm. As a result of the viable cell count, the viability rate of L. fermentum MA-7 increased as the fruit methanol extract concentration and time increased. The photoprotective activities of the peel and fruit extracts were determined as 13.17 and 12.26. It was determined that the extracts increased sun protection factor (SPF) value of the cream. The results indicated that H. polyrhizus extracts may have potential for use in the nutraceutical products, cosmetic and pharmaceutical industries.**

Keywords*— *Antimicrobial activity, Extract, Pitahaya, Prebiotic, Solar protection factor

I. INTRODUCTION

H. polyrhizus (red pitahaya) is a tropical fruit consumed in many parts of the world, popular for its taste, aroma, nutrition, beneficial health effects and attractive appearance. About one-third of a pitaya fruit is the peel, contains an abundance of nutritive and functional compounds, such as the flesh, thus offering great recycling potential [1]. *H. polyrhizus* contains bioactive phytochemicals such as polysaccharides, terpenoids, betacyanin and phenolic compounds. *H. polyrhizus* offers pharmacological values (helpful in coping with cancer, other metabolic syndromes, type 2 diabetes and obesity) thanks to the components it contains [2]-[4].

The antimicrobial and antioxidant activities of plant and fruit extracts have become the basis of many uses such as pharmaceuticals, food preservatives, natural therapies, cosmetics and medicine [5], [6]. Antimicrobial properties of plant extracts are related to phenolics, alkaloids, terpenoids, pectin, essential oils, polypeptide, and others [7]. Plant extracts inhibit the growth of pathogens by other mechanisms than conventional antibiotics. Thus, extracts are important in the treatment of diseases caused by pathogens showing antibiotic resistance [8].

Probiotic bacteria cannot grow well in the digestive tract without prebiotics, and their combination has a synergetic effect on host health [9], [10]. The prebiotics are food ingredients that are not absorbed in the gastrointestinal tract but are broken down by the gut microbiota, stimulating the growth or activity of gut bacteria [11]. In recent years, consumers' increasing awareness of the relationship between food and health has led to an increased demand for functional foods. Prebiotics are one of the most promising functional foods as a component of food [12]. It has been reported that plant extracts have health-promoting properties due to their biological activities. There is a need for new prebiotics derived from plant extracts that stimulate the growth of lactic acid bacteria (LAB) [13].

The demand for plants and plant extracts is increasing by day by with the discovery of natural bioactive molecules with photoprotective activity [14], [15]. Plants may act synergistically with physical and chemical filters to increase sun protection activity and inhibit the acceleration of transcription factors occurring in skin cells [16], [17]. Antioxidants used in sunscreens provide high ray protection by preventing damage caused by sun rays [18]. In recent years, researchers have focused on the development of side-effect-free herbal sunscreens by incorporating natural bioactive substances from plants into sunscreens.

The purpose of this study is to determine the biological activities of H. polyrhizus methanol extracts. First of all, antimicrobial activities of H. polyrhizus methanol extracts against test microorganisms and lactic acid bacteria were

investigated. Afterwards, the potential of the fruit extract to exert a probiotic-promoting effect against probiotic candidate L. fermentum MA-7 was determined by the macro-dilution method. Finally, the photoprotective activity of H. polyrhizus methanol extracts was determined in vitro.

II. Materials And Methods

A. H. polyrhizus Sample and Methanol Extracts Preparation

H. polyrhizus samples taken from Antalya/Türkiye were washed with tap water and rinsed with distilled water (Fig. 1). The fruits and their peels were separated and dried at room temperature, then ground with a Waring blender. The peel and fruit of H. polyrhizus were separately (10 g) extracted with 99.7% methanol (30 ml) using a sonication device on ice for 10 min each day in 2 replicates (2 days). The extracts were dissolved with dimethyl-sulfoxide (DMSO) and sterilized with a syringe filter (0.45-µm).

Fig. 1. *H. Polyrhizus* fruit

B. Preparation of Test Microorganisms

Candida glabrata RSKK 04019 were cultured at 30ºC in Yeast-Peptone-Dextrose (YPD) media for 24 hours. *Enterococcus faecalis* ATCC 29212, *Escherichia coli* O157:H7, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* RSKK 171, *Staphylococcus epidermidis* ATCC 12228 and *Staphylococcus aureus* ATCC 25923 were grown in Nutrient-Broth (NB) at 37ºC for 24 hours.

C. Preparation of Probiotic Candidate Strains Originated from Breast Milk

Limosilactobacillus fermentum MA-7, *Lactobacillus gasseri* MA-1, *Lactobacillus vaginalis* MA-10 and *Lactobacillus delbrueckii* MA-9 were cultured at 37ºC in Man, Rogosa and Sharpe (MRS) media for 24 hours. *Streptococcus thermophilus* MAS-1 was cultured at 37ºC in M17 (broth acc. to Terzaghi) media for 24 hours.

D. Disc Diffusion Susceptibility Assay

Disc diffusion test was used to determine the inhibitory effect of the peel and fruit methanol extracts from *H. polyrhizus*. The prepared culture suspension (0.5 Mcfarland) was spread on agar medium and sterile discs (6 mm diameters) were placed on the agar. *H. polyrhizus* peel and fruit extracts (4 mg/disc for pathogenic microorganism strains and for probiotic candidate strains) were dropped onto the discs. Kanamycin (CN-30 µg/disc) antibiotic discs were used as controls for pathogenic microorganism strains, and Fluconazole (FCA-25 µg/disc) was used for yeast. The culture dishes were incubated for 24 hours at the appropriate temperatures indicated previously. Then, the inhibition zone diameter around the wells was measured using a caliper. All experiments were done in triplicate.

E. Micro-Dilution Assay

The micro-dilution assay was used to determine the MIC and MBC or MFC values of the *H. polyrhizus* extracts. The extracts were added to each growth medium to obtain a final concentration of 80 µg/µl and diluted to 40, 20, 10, 5 and 2.5 µg/µl in tubes. The culture suspension at 0.5 McFarland concentration was added to each tube containing the extract and medium. The tubes were then incubated under the conditions required for each microorganism as mentioned above. At the end of the incubation, the extract concentration in the tube without microbial growth was recorded as the MIC value. MBC or MFC concentrations were determined by inoculating samples from the tubes onto a solid medium using the spot-dropping method. The culture dishes were incubated for 24 hour at the appropriate temperature for the test microorganisms. The lowest concentration without growth at the end of incubation was defined as MBC or MFC values.

F. Macro-Dilution Method

The antimicrobial activity of *H. polyrhizus* fruit methanol extract against *L. fermentum* MA-7 was determined by modifying the viable cell count method of Sousa et al. [18]. The culture suspension of *L. fermentum* MA-7 adjusted to 0.5 Mcfarland (0.1% v/v) concentration was added to the medium containing fruit extract (50 mg/ml and 100 mg/ml) and medium. The microbial suspension without extract was used as the control group. Afterwards, the control group and microbial suspensions containing the extract were incubated at 37°C. The samples from the mixtures were then diluted and inoculated into MRSA medium. These procedures were performed at 0, 24, and 48-hour incubation times. At the end of the incubation periods, viable cells were counted and recorded as $\log_{10}$ CFU/ml. The viability of the cells was then calculated by the formula of % viability.

G. Determination of Photoprotective Activity of H. polyrhizus Extracts by UV-VIS Spectrophotometry

Sun protection factor (SPF) values of *H. polyrhizus* methanol extracts were determined by spectrophotometric measurements. Each extract (0.002 g/ml) was mixed with ethanol (96%) until homogeneous. The absorbance values of homogeneous mixtures were measured in 3 repetitions using a spectrophotometer device (Beckman Coulter, USA). Measurements were measured at 5 nm intervals in the UV-B (290-320 nm) wavelength range. The SPF of the recorded absorbance values was calculated using the Mansur equation (1) [20].

$$\textbf{SPF:}\ \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times \mathrm{I}(\lambda) \times \mathrm{Abs}(\lambda) \qquad (1)$$

CF: 10 (correction factor); **EE (λ)**: Erythemogenic effect of radiation with a wavelength of λ; **I (λ)**: The intensity at the wavelength of sunlight; **Abs (λ)**: The absorbance value of the

extracts in the UV-B wavelength range. $\mathbf{EE(\lambda) \times I(\lambda):}$ This value has constant values determined by Sayre et al. [21].

H. Determination of Photoprotective Activity of H. polyrhizus methanol extracts and cream mixture by UV-VIS Spectrophotometry

A modified method was used to determine the effects of homogeneous mixtures containing *H. polyrhizus* extract and cream on UV-B blocking capacity [22]. The extract (0.5 g) and cream (1 g) were mixed and made up to 10 g with distilled water. After the mixture was homogenized, 0.1 g of sample was taken and made up to 10 ml volume with ethanol (40%). After 5 minutes of sonication (100% amplitude, 30 kHz), the mixture was filtered through Whatman (No. 1) filter paper. The absorbance values of the prepared samples were measured in the UV-B wavelength range (290-320 nm) in a spectrophotometer device (Beckman Coulter, USA) with 3 repetitions. The recorded absorbance values were evaluated with the formula (1) given above.

I. Statistical Analysis

The data were analyzed using the SPSS (GNU) program (version 25.0) and statistical significance was confirmed by Tukey's post hoc test (one-way ANOVA) analysis of variance. The difference between test microorganisms was considered statistically significant at the $p<0.05$ level.

III. Results And Discussion

A. Disc Diffusion Susceptibility Assay

Plants have been used for their antimicrobial properties resulting from phytochemicals (such as flavonoids, phenolic compounds, alkaloids, and tannins) synthesized as secondary metabolites [23], [24]. In the present study, the antimicrobial activities of *H. polyrhizus* methanol extracts against test microorganisms (yeasts, food-borne and clinical origin) were determined. The disc diffusion susceptibility assay results of *H. polyrhizus* extracts against the tested microorganisms were statistically evaluated (Table 1). The highest inhibition zone diameter of *H. polyrhizus* peel extract against *C. glabrata* RSKK 04019 was determined as 12.37 mm. The difference in the inhibition zone diameters between the test microorganisms in the peel extract is not statistically significant except ($p>0.05$) *E. coli* ATCC O157:H7 ($p<0.05$). The highest inhibition zone diameter of *H. polyrhizus* fruit extract against *S. epidermidis* ATCC 12228 was determined as 12.00 mm.

TABLE I. ANTIMICROBIAL ACTIVITY OF *H. POLYRHIZUS* METHANOL EXTRACTS

Microorganism Strains	Inhibition zone diameters (mm±SD)			
	Extracts		Antibiotics	
	HPM (4mg/disc)	HFM (4mg/disc)	CN (30µg/disc)	FCA (30µg/disc)
C. glabrata RSKK 04019	12.37±0.42[a]	11.50±0.36[a]	-	20.35 ± 0.10
E. faecalis ATCC 29212	10.68±0.79[a]	10.58±0.16[a,c,d]	13.48 ±1.40	-
E. coli ATCC O157:H7	9.60±0.34[b]	7.85±1.11[b]	18.37 ±0.17	-
P. aeruginosa ATCC 27853	10.97±0.49[a]	8.32±0.18[b]	15.89 ±0.05	-
S. enteritidis RSKK 171	10.63±0.24[a]	9.37±0.52[b,d,e]	12.05±0.14	-
S. epidermidis ATCC 12228	10.50±1.61[a]	12.00±0.59[a,c]	-	-
S. aureus ATCC 25923	11.15±0.62[a]	10.43±0.94[a,c,e]	15.89±0.05	-

[a.] HPM: *H. polyrhizus* Peel Methanol, HFM: *H. polyrhizus* Fruit Methanol, CN: Kanamycin, FCA: Fluconazole. Different letters in the column indicate significant differences between samples at $p< 0.05$.

Rohin et al. [25] determined the antimicrobial activity of red pitahaya flesh methanol extract against *S. epidermidis* (15.00 mm), *S. aureus* (19.00 mm), *E. faecalis* (18.50 mm), *P. aeruginosa* (15.00 mm) and *E. coli* (14.50 mm). As a result of the study, it was indicated that fruit extracts can be used as a potential source for the production of a wide spectrum of medicine.

B. Micro-Dilution Assay

The disc diffusion assay alone is not sufficient to obtain whether the antimicrobial activity is a static or a cidal effect [26]. MIC values were found as 40 µg/µl or 80 µg/µl in the peel extract, and in the range of 10 µg/µl or 40 µg/µl in the fruit extract. The concentration at which 99.9% of the test microorganisms are killed at the lowest concentration is called MBC [27]. The lowest MBC value in peel extract was determined as 40 µg/µl against *S. epidermidis* ATCC 12228 and *S. aureus* ATCC 25923. The lowest MBC value in fruit extract was determined as 10 µg/µl against *S. aureus* ATCC 25923.

TABLE II. MIC AND MBC OR MFC VALUES OF *H. polyrhizus* METHANOL EXTRACTS

Microorganism Strains	MIC (µg/µl)		MBC or MFC (µg/µl)	
	HPM	HFM	HPM	HFM
C. glabrata RSKK 04019	40	40	40	40
E. faecalis ATCC 29212	40	20	40	20
E. coli ATCC O157:H7	40	20	40	20
P. aeruginosa ATCC 27853	40	40	40	40
S. enteritidis RSKK 171	80	20	80	20
S. epidermidis ATCC 12228	40	40	40	40
S. aureus ATCC 25923	40	10	40	10

[a.] HPM: *H. polyrhizus* Peel Methanol, HFM: *H.polyrhizus* Fruit Methanol

Yong et al. [28] determined the MIC values of red pitahaya methanol (70%) extract against *S. aureus* ATCC 29213, *S. aureus* ATCC 6538P, *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC BAA-47 and *P. aeruginosa* ATCC 10145. MIC values were obtained in the range of 6.25-25 mg/ml. In our previous study investigating the antimicrobial activity of fruit and peel methanol extracts obtained from white pitahaya, MIC values of the extract against *P. aeruginosa* ATCC 27853, *E. coli* ATCC O157:H7, *E. faecalis* ATCC 29212 were determined in the range of 20-40 mg/ml [21].

LAB or their metabolic products with antimicrobial properties are used as bio-preservatives to inactivate unwanted microorganisms that are contaminants [29]. The inhibition zone diameter against *L. fermentum* MA-7 was determined as 10.06 mm in the peel extract and 8.61 mm in the fruit extract. The lowest inhibition zone diameter was determined as 6.10 mm on *S. thermophilus* MAS-1. In the fruit extract, the difference between the mean inhibition zone diameters of other probiotic candidate strains except *L. gasseri* MA-1 and *L. vaginalis* MA-10 are significant ($p<0.05$) (Table 3).

TABLE III. DISC DIFFUSION SUSCEPTIBILITY VALUES OF *H. polyrhizus* METHANOL EXTRACTS ON LACTIC ACID BACTERIA

Microorganism Strains	Inhibition zone diameters (mm±SD)	
	Extracts	
	HPM (4 mg/disc)	HPM (4 mg/disc)
L. fermentum MA-7	10.06±0.20[a]	10.06±0.20[a]
L. gasseri MA-1	10.25±0.16[a]	10.25±0.16[a]
L. vaginalis MA-10	9.45±0.13[a,c]	9.45±0.13[a,c]
L. delbrueckii MA-9	10.05±0.55[a]	10.05±0.55[a]
S. thermophilus MAS-1	7.45±0.07[b]	7.45±0.07[b]

[a.] HPM: *H. polyrhizus* Peel Methanol, HFM: *H. polyrhizus* Fruit Methanol. Different letters in the column indicate significant differences between samples at $p<0.05$.

Morais et al. [30], were inoculated *Lactobacillus acidophilus* LA-05 and *Bifidobacterium lactis* BB12 into red pitahaya pulp and their biological effects were investigated. As a result, *L. acidophilus* LA-05 and *B.* lactis BB12 microorganisms could grow in red pitahaya pulp and survive in this fruit during exposure to simulated gastrointestinal conditions. In the literature, studies on the antimicrobial activity of *Hylocereus* extracts on lactic acid bacteria are limited, so more studies are needed.

C. Micro-Dilution Method

L. fermentum belongs to the LAB group, which is widely found in nature, isolated in various foods (including dairy products, fermented meat products, bread, human milk and plant materials) and used as ferment in the food industry [31]. In this study, additionally, the probiotic-promoting potential of the *H. polyrhizus* fruit extract was determined by the macro-dilution method on *L. fermentum* MA-7. The viability ratios of *L. fermentum* MA-7 with *H. polyrhizus* fruit extract are presented in Fig. 2. The increase in *L. fermentum* MA-7 viability was observed at 50 mg/ml and 100 mg/ml concentrations after 24 and 48 hours compared to the control group (Fig. 3). As a result of the macro-dilution test, it was determined that *H. polyrhizus* fruit extract stimulates *L. fermentum* MA-7 growth and may have a have potential probiotic-promoting effect.

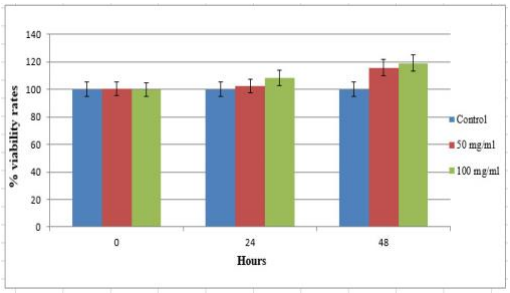

Fig. 2. Viability rates of *L. fermentum* MA-7 with *H. polyrhizus* fruit methanol extract

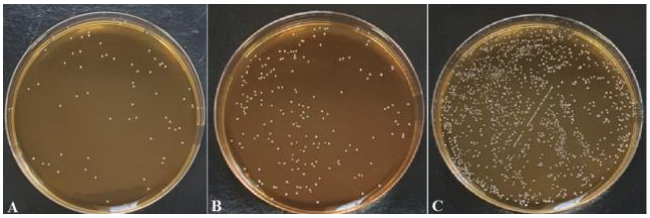

Fig. 3. 48.h petri display of probiotic promoting activity of *H. polyrhizus* methanol extract. **A:** Control group**, B:** 50 mg/ml**, C:** 100 mg/ml

In a study conducted by Wichienchot et al. [32], hemocytometry measurement at 0, 24 and 48 hours was performed to determine the growth of *L. delbrueckii* BCC 13296 and *B. bifidum* 702715 using oligosaccharide and reference prebiotic (inulin) from white pitaya and red pitahaya flesh. It has been reported that pitahaya exhibits prebiotic properties, including the ability to promote the growth of *L. delbrueckii* BCC 13296 and *B. bifidum* 702715. Therefore, pitahaya is a potential source of prebiotics that can be used as an ingredient in functional food and nutraceutical products.

D. Photoprotective Activity of Extracts

Prolonged exposure to the sun's rays can cause degenerative changes in skin cells that lead to premature aging, sunburns and skin cancers [33]. Although it has been known for years that chemical-containing sunscreens can protect people from the harmful effects of sunlight such as aging or skin cancer, they also have some negative side effects. Extracts from plants can reduce the cost of currently used preservatives and can be used in pharmaceutical products [34]. In the current study, the sun protection potential of *H. polyrhizus* extracts was investigated under in vitro conditions. The photo protective activity results of peel and fruit extracts are presented in Table 4 and Table 5. The SPF values of the peel and fruit extracts were determined as 13.17 and 12.26. The method used in the evaluation of SPF used in this study is simple, economical, fast and easy.

TABLE IV. PHOTOPROTECTIVE ACTIVITY OF *H. polyrhizus* PEEL METHANOL EXTRACT

UV-B wavelength	$\mathbf{EE(\lambda) \times I(\lambda)}$	Abs	$\mathbf{CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)}$
290	0.0150	1.6760	0.2514
295	0.0817	1.4770	1.2067
300	0.2874	1.3800	3.9661
305	0.3278	1.2950	4.2450
310	0.1864	1.1945	2.2970
315	0.0839	1.1890	0.9951
320	0.0180	1.1623	0.2092
Solar Protection Factor (SPF)			**13.17**

TABLE V. PHOTOPROTECTIVE ACTIVITY OF *H. polyrhizus* FRUIT METHANOL EXTRACT

UV-B wavelength	$\mathbf{EE(\lambda) \times I(\lambda)}$	Abs	$\mathbf{CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)}$
290	0.0150	1.5480	0.2322
295	0.0817	1.3126	1.0724
300	0.2874	1.2663	3.6394
305	0.3278	1.2093	3.9641
310	0.1864	1.1986	2.2343
315	0.0839	1.1186	0.9363
320	0.0180	1.0276	0.1849
Solar Protection Factor (SPF)			**12.26**

The prepared different concentrations of *H. polyrhizus* ethanol extracts presented broad spectrum UV protection and high SPF values. *H. polyrhizus* is highly proficient in replacing synthetic sunscreen agents to serve as natural ingredients in the cosmetic industry [35].

E. Photoprotective Activity of Extract-Cream Mixtures

The toxic effects and safety of sunscreen products containing commercial and synthetic additives are questioned by researchers [36]. The potential of using natural preservatives as an alternative to the possible and identified dangers of the synthetic substances used is being investigated [37]. SPF values of peel extract and fruit extract cream mixtures were determined as 10.70 and 9.40 (Table 6). Compared to the control (cream) group, it was determined that the extracts increased the SPF value of the cream group ($p<0.05$).

TABLE VI. PHOTOPROTECTIVE ACTIVITY OF CREAM WITH *H. polyrhizus* METHANOL EXTRACTS

Samples	SPF Value
Cream (control)	4.94±0.01[a]
HPMC	10.70±0.03[b]
HFMC	9.40±0.02[c]

[a.] HPMC: *H. polyrhizus* Peel Methanol-Cream, HFMC: *H. polyrhizus* Fruit Methanol-Cream. Different letters indicate significant differences between samples at $p< 0.05$.

In our previous study, the SPF values of cream mixtures of white pitahaya methanol extracts were evaluated in vitro. SPF values of white pitahaya peel and fruit extract cream mixtures were determined as 23.34 and 9.26 [22]. The potential to use pitahaya extracts with high SPF values as a natural preservative in the cosmetic industry and to replace synthetic sunscreen agents has been determined because of in vitro studies. This study may be a pioneer for future in vivo studies.

IV. CONCLUSIONS

The biological activity of extracts obtained from *H. polyrhizus* grown in Türkiye was investigated to determine the potential for use in different industries. *H. polyrhizus* extracts exhibited good antimicrobial activity against the test microorganisms. The extracts have the potential to be natural antimicrobial agents with synergistic effect. The fruit extract has been observed to promote the development of *L. fermentum* MA-7. Therefore, *H. polyrhizus* may be used as a probiotic-promoting source like prebiotic that as ingredients in functional foods and nutraceutical products. The extracts have the potential to be used as natural ingredients instead of synthetic sunscreens due to their high SPF values. The determined properties of *H. polyrhizus* extracts have shown that the peel and fruit extracts of *H. polyrhizus* have potential for use in the nutraceutical products, pharmaceutical and cosmetic industries.

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