Inhibitory Effects of Polyphenols from *Equisetum ramosissimum* and *Moringa peregrina* Extracts on *Staphylococcus aureus,* Collagenase, and Tyrosinase Enzymes: *In vitro* Studies

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ABSTRACT

Background: Skin problems caused by oxidative stress lead to the activation of collagenase and tyrosinase enzymes, contributing to skin aging, discoloration, and infections. *Equisetum ramosissimum* and *Moringa peregrina* were assessed for their potential uses in treating various skin conditions.

Objective: The present research aimed to investigate the positive effects of polyphenols in *Equisetum ramosissimum* and *Moringa peregrina* extracts as potential cosmetic products for the treatment of different skin conditions.

Methods: Total phenolic and flavonoid contents, antioxidants, and anti-collagenase and anti-tyrosinase activities of plant extract mixtures (PEM) at different ratios of (*M. peregrina*: *E. ramosissimum*) were determined using standard procedures. Inhibitory effects of PEM against acne-causing *Staphylococcus aureus* (ATCC 29213) were evaluated using the diameter (cm) of the inhibition zone method. A cream formulation containing PEM was developed and characterized for stability and potential skin irritation in rats using standard procedures.

Results: The PEM at a ratio of (2:1) showed the highest total phenolic and flavonoid content (150.15 ± 2.8 mg/g, equivalent to gallic acid, and 41.5 ± 1.2 mg/g, equivalent to quercetin, respectively). Antioxidant activities for PEM (2:1) were also optimal, as determined by the DPPH and ABTS methods (IC50 = 7.06 ± 0.12 µg/mL and 53.29 ± 3.3 µg/mL, respectively). Furthermore, PEM (2:1) exhibited superior inhibitory activities against collagenase and tyrosinase enzymes (IC50 = 32.4 ± 1.19 µg/mL and 8.4 ± 1.19 µg/mL, respectively). Antimicrobial activity of PEM (2:1) tested on *S. aureus* showed the largest zone of growth inhibition (2.8 cm) at a concentration of 60 mg/mL. Studies on the PEM (2:1) cream formulation revealed that it remained stable under room conditions. Skin irritation tests on rats showed no signs of oedema or erythema after treatment.

Conclusion: The PEM with a ratio of (2:1) demonstrated optimal activity as an oxidative stress-neutralizing agent, inhibitor of enzymes responsible for skin aging and hyperpigmentation, and antibacterial agent. The cream formulation containing PEM exhibited physical stability and no detectable risk of skin irritation throughout the research procedures.

Keywords: *Equisetum ramosissimum*, *Moringa peregrina*, *Staphylococcus aureus*, collagenase, tyrosinase.

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1. INTRODUCTION

Skin aging is considered a naturally occurring process influenced by several environmental factors, such as ultraviolet radiation (UVR) and oxidative stress [1,2]. Collagen, a major component of the skin, provides structural stability, firmness, elasticity, and flexibility, all of which are essential for maintaining skin health. Collagenases are enzymes that break down collagen in the skin. As humans age, the body produces more of these enzymes, leading to the appearance of wrinkles. Therefore, it is crucial to find substances that can inhibit collagenases to slow this process and delay skin aging [3].

Skin pigmentation is also one of the most distinctive and visible personal traits. Increased melanocyte activity and melanin production, driven by tyrosinase enzymes, result in hyperpigmentation disorders, such as post-inflammatory pigmentary alteration, senile lentigo, melasma, and ephelides [4]. Tyrosinase plays a crucial role in melanogenesis; therefore, inhibiting it is considered an effective approach, alongside other therapeutic techniques, to prevent the accumulation of melanin in the skin [5,6]. Tyrosinase inhibitors function through four distinct mechanisms: competitive, noncompetitive, uncompetitive, and mixed-type inhibition [6]. Many natural substances, such as hydroquinones and deoxyarbutins, act as competitive tyrosinase inhibitors [7]. Luteolin, a key component of *Moringa oleifera* and ginseng extracts, has been shown to employ an uncompetitive inhibitory mechanism against tyrosinase [8].

Acne vulgaris is a chronic inflammatory skin disorder that arises from infections in the pilosebaceous unit [9]. Hormonal imbalances can also contribute to acne [10]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common causes of skin and soft tissue infections [11,12]. The urgent need to discover novel treatments from non-traditional sources to combat MRSA infections has been highlighted as a current challenge [13].

Cosmetics containing herbal remedies have recently gained popularity as a solution for skin problems [14]. Thus, the inhibition of collagenase and tyrosinase activities by active components derived from herbs may have beneficial effects, such as delaying the degradation of collagen and other components of the extracellular matrix [15].

The *Moringa* genus belongs to the Moringaceae family and contains thirteen species found in tropical and subtropical regions [16]. The tree *Moringa peregrina* (M. peregrina), a member of the Moringaceae family, grows naturally in Jordan [17,18]. Previous studies investigating the phytocomponents of *M. peregrina* extracts have revealed numerous bioactive compounds, including phenolic acids, volatile isothiocyanates, flavonoids, alkaloids, and glucosinolates. These compounds contribute to the plant's diverse therapeutic activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective effects [19]. *Moringa peregrina* extracts have demonstrated potential benefits for skin health, such as moisturizing, anti-aging, and wound-healing properties. Traditionally, *M. peregrina* leaf extract is rubbed on the skin to manage rashes and paralysis [20]. The oil of *M. peregrina* is used to treat skin conditions such as freckles, scabies, and itching [21].

Several *Moringa*-based products are available in the pharmaceutical market. Cold-pressed *Moringa* oil is found in various products intended for application to the face, skin, and hair [22]. Additionally, anti-aging creams containing virgin seed oil from *Moringa* are marketed for use in skincare, hair care, aromatherapy oils, soaps, liquid body washes, face creams, massage oils, perfumes, and deodorants [23].

The plant *Equisetum ramosissimum* (E. ramosissimum), a member of the Equisetaceae family [24], is widely distributed in Europe, North America, and Asia. Several studies have shown that *E. ramosissimum* contains various compounds, such as flavonoids, alkaloids, phenolics, saponins, tannins, triterpenoids, and phytoesters, which possess a range of biological activities [25]. Traditionally, it has been used to treat various

ailments, including urinary tract disorders, skin conditions, and for wound healing [26].

Vanithamani et al. [27] highlighted several herbal mixtures used in skincare formulations, concluding that these combinations exhibited a potential skin-protectant effect with no noticeable side effects. Consequently, the combination of *M. peregrina* and *E. ramosissimum* extracts has been suggested as a potential agent with superior effects in inhibiting several skin-related disorders, such as bacterial infections and the activities of tyrosinase and collagenase enzymes. Cosmetic formulations containing the proposed plant extract mixtures as active ingredients can be investigated for their potential use in treating or reducing various skin conditions, including aging, infections, and hyperpigmentation, using standard in-vitro methods. The present research aims to investigate the positive effects of polyphenols in *Equisetum ramosissimum* and *Moringa peregrina* extracts as potential cosmetic products for treating different skin conditions.

2. MATERIAL AND METHODS

2.1. Materials

2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Sigma Aldrich, USA. Folin-Ciocalteu reagent and potassium persulfate were also purchased from Sigma Aldrich, USA. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) was obtained from Sisco Research Laboratories Pvt. Ltd., India. Dimethyl sulfoxide and gallic acid were purchased from GCC, UK, and quercetin was purchased from Santa Cruz Biotechnology, USA.

Emulsifying wax, Germall Plus, glycerin, isopropyl myristate, lanolin, mineral oil, lavender oil, and allantoin were obtained from LabChem Laboratory Chemicals, USA. Collagenase activity kits (ab196999) and tyrosinase activity kits (ab252899) were sourced from Abcam®, UK. All other organic solvents and materials were of HPLC, analytical, or pharmaceutical grade

2.2. Animals

For the skin irritation test, three healthy male Wistar rats weighing 250 ± 15 g were housed and acclimated at the Laboratory Animal Research Unit, Applied Science University, Amman, Jordan. The Applied Science University Ethics Committee granted clearance for this investigation (Clearance Number: 2023-PHA-32).

The rats were kept in separate cages with controlled temperature (20 ± 3°C), humidity (50 ± 15%), and a photoperiod cycle of 12 hours of light and 12 hours of darkness. They were fed a standard laboratory diet and had unlimited access to water.

2.3. Methods

2.3.1. Collection and processing of crude plant material

Moringa peregrina dried leaves and *E. ramosissimum* dried aerial parts were purchased from a local shop in Amman, Jordan. *M. peregrina* dried leaves were milled using an electric mill, while *E. ramosissimum* dried aerial parts were milled using a commercial hammer milling machine. The resulting powders of *M. peregrina* and *E. ramosissimum* were dried and stored separately in airtight jars at room temperature for further experiments.

2.3.2. Plant extraction methods

A total of 200 g of *M. peregrina* dried leaves or *E. ramosissimum* dried aerial parts was soaked in 1000 mL of pure ethanol for 48 hours. The resulting extract was then filtered using a Büchner funnel and dried using a rotary evaporator. The extract was left to dry completely for 48 hours in a fume hood and then stored at 4°C for subsequent tests. Different ratios of *M. peregrina* and *E. ramosissimum* plant extract mixtures (PEM) (2:1, 1:2, 1:1 w/w) were prepared from the dried extracts.

2.3.3. Phytochemical analysis of plant extract

2.3.3.1. Total phenolic content (TPC)

The Folin-Ciocalteu method, as reported by Lohvina et al. [28], was used to determine the TPC of each extract and mixture ratio. Ethanol was used to create a stock solution of each extract combination at a concentration of 2 mg/mL.

A 0.1 mL aliquot of Folin-Ciocalteu reagent, 1.6 mL of distilled water, and 0.3 mL of a 20% Na_2CO_3 aqueous

solution were mixed with 0.2 mL of the stock solution. The mixture was allowed to sit at room temperature for 1 hour in the dark, after which the UV absorption was measured at 750 nm.

Gallic acid (GA) was used as a reference standard at concentrations ranging from 100 to 6.25 µg/mL to construct a calibration curve. The TPC of the PEMs was calculated as mg/g dry extract equivalent of GA.

2.3.3.2. Total flavonoid content (TFC)

The aluminium chloride assay, as described by Chang et al. [29], was used to assess the TFC. Ethanol was used to create a stock solution of each extract combination at a concentration of 2 mg/mL. Then, 0.5 mL of the stock solution was combined with 0.1 mL of 10% aluminium chloride, 2.8 mL of distilled water, and 0.1 mL of 1M sodium acetate. The mixtures were allowed to sit at room temperature for 30 minutes. UV absorption was then measured at 415 nm.

Quercetin (QE) was used as a reference standard at concentrations of 25, 50, and 100 µg/mL to construct a calibration curve. The TFC of the PEMs was calculated as mg/g dry extract equivalent of QE.

2.3.4. The antioxidant activity of plant mixture extract

2.3.4.1. DPPH free radical scavenging activity assay

The 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH•) scavenging activity was determined as described by Nurzaman et al. [30]. Briefly, 2 mL of each extract mixture, at concentrations ranging from 100 to 6.25 µg/mL, was mixed with 2 mL of DPPH• (0.1 mM DPPH in pure ethanol, 1:1 v/v), stirred vigorously, and incubated for 30 minutes in a dark place at room temperature. The UV absorbance was measured at 517 nm.

The percentage of DPPH scavenging activity was calculated using Equation 1. The IC50 values of the PEMs were compared with the IC50 of ascorbic acid (Vitamin C) as a reference antioxidant compound.

$$\%\text{DPPH scavenging activity} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100\% \quad \text{-----(1)}$$

2.3.4.2. ABTS scavenging activity assay

The 2.2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation (ABTS•+) scavenging activity was determined as described by Re et al. [31]. Briefly, a solution of ABTS•+ was prepared by mixing 2.45 mM potassium persulfate solution (6.6 mg of potassium persulfate in 10 mL of water) with a 7 mM stock solution of ABTS (36 mg of ABTS in 10 mL of water). Ethanol was added as a diluent until the prepared ABTS•+ solution reached an absorbance value of 0.7 at 734 nm.

A stock solution of each extract mixture at different concentrations, ranging from 200 to 1.56 µg/mL, was prepared in ethanol. A 1 mL aliquot of each sample was added to 2 mL of the ABTS•+ solution. The absorbance was measured at 734 nm after 1 hour of incubation in a dark room. Ascorbic acid (Vitamin C) at a concentration range of 100 to 1.56 µg/mL was used as a reference compound [31].

The percentage of ABTS scavenging activity was calculated using Equation 2. The IC50 values of the PEMs were compared with the IC50 of ascorbic acid (Vitamin C) as a reference antioxidant agent.

$$\%\text{ ABTS scavenging activity} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100\% \quad \text{----------(2)}$$

2.3.5. Collagenase inhibition activity

The inhibition of plant extracts on collagenase enzyme (EC 3.4.24.3) was tested using the reference kit and the protocol provided by the supplier. The enzyme's activity was noted as 0.35 U/mL. In the experiment, collagenase enzyme solution vials were briefly centrifuged before use, and Collagenase Assay Buffer (pH = 7.6) was brought to room temperature. The collagenase substrate, synthetic peptide (FALGPA), was diluted with buffer. The inhibitor used was 1,10-Phenanthroline (1 M). A 96-well plate was employed for measurement.

Briefly, PEM (2 µL) at different concentrations were combined with buffer (88 µL) and collagenase enzyme (10 µL). A positive control contained enzyme (10 µL) and buffer

(90 µL). A substrate mixture (collagenase substrate: buffer, 4:6) was freshly prepared (100 µL) and added to each well. After 15 minutes of dark incubation at 37°C, UV absorbance was measured at 345 nm using a microplate reader. The percentage inhibition of collagenase enzyme activity by the PEMs was calculated using Equation 3.

$$\% \text{ Collagenase inhibition activity} = \frac{Abs\ control - Abs\ sample}{Abs\ control} x100\% \quad \text{------(3)}$$

2.3.6. Tyrosinase inhibition activity

The inhibition of plant extracts on tyrosinase enzyme activity was tested using the reference kit and protocol provided by the supplier. In this experiment, tyrosinase enzyme solution vials were briefly centrifuged. Sample wells containing PEM (2 µL) at different concentrations were combined with 3 µL of tyrosinase, 5 µL of enhancer, 10 µL of substrate, and 80 µL of assay buffer (pH = 7.6). The percentage inhibition of tyrosinase enzyme activity by the PEMs was calculated using Equation 4.

$$\% \text{ Tyrosinase inhibition activity} = \frac{Abs\ control - Abs\ sample}{Abs\ control} x100\% \quad \text{---(4)}$$

2.3.7. Anti-bacterial activity

Different ratios of PEMs were evaluated for growth inhibition activity on *Staphylococcus aureus* (ATCC 29213) using the method developed by Bauer-Kirby et al. (1966). For this experiment, Mueller-Hinton broth (Oxoid Ltd., UK) was used to cultivate *S. aureus*, which was then incubated overnight at 37ºC.

To test the antibacterial activity, an aliquot of 100 µL of PEM (2:1) at concentrations of 60, 30, and 10 mg/mL in water was transferred into each well. Plates were then incubated at 37ºC for 24 hours. The diameter (cm) of the inhibition zone was used for comparison. Tetracycline (30 µg) was used as a positive control.

2.3.8. Cream formulation and evaluation

PEM (2:1) was formulated as an oil-in-water emulsion using the formula in Table 1, as described by Ubaydee et al. [32]. Briefly, emulsifying wax, mineral oil, isopropyl myristate, phenoxyethanol, and lanolin were melted at 50°C and mixed to create the oil phase. PEM (2:1), allantoin, glycerin, and Germall plus were dissolved in deionized water to create the aqueous phase. The water phase was warmed to 50°C to dissolve all the components. The aqueous phase was gradually added to the oil phase with gentle agitation once both phases reached the same temperature. The mixture was continuously agitated until the temperature decreased to 35°C. Finally, lavender oil was added to the cream formulation. After gradually stirring the emulsion until it became homogenous, the cream was allowed to cool to room temperature.

Table 1: List of ingredients in the cream formulation with PMS (2:1)

Ingredient	Master formula (%w/w)	Uses
Isopropyl myristate	4 %	Lubricant and emollient
Mineral oil	12 %	Lubricant
Emulsifying wax	6 %	Non anionic emulsifier
Lanolin	1%	Emollient
Glycerin	3 %	Humectants
Allantoin	0.2 %	Healing agent
PEM (2:1)	1.5 %	Active ingredient
Water	71.8 %	Solvent
Lavender oil	q s	Flavoring agent
Germall plus	0.5	Preservative

2.3.8.1. Stability tests

The stability study of the prepared cream formulation was conducted as described by Bora et al. (2019) [33] over a one-month period under different conditions, including room temperature (25±2°C), refrigeration (4±2°C), and accelerated temperature (40±2°C). The physicochemical parameters (homogeneity, odor, color, and pH) were observed periodically. About 0.5 g of the cream was weighed and dissolved in 50 mL of distilled water, and its pH was measured [34]. The homogeneity of the freshly prepared cream formulation was determined using a centrifugation test [35]. This test was performed by placing a 5 g sample in a centrifuge tube and spinning it at 4000 rpm for 20 minutes, after which the appearance and phase separation were observed. Both color and odor properties were determined organoleptically by the research group.

2.3.8.2. Rheological study

The rheology of the prepared cream was tested using a rheometer (Physica MCR 302, Anton Paar) according to the method by Helgesen [36]. All measurements were carried out at a temperature of (25±1°C), using spindle Cp 50. About 0.5 g of the cream was loaded between concentric cylinders, and the flow curve was constructed by plotting controlled shear rate versus controlled shear stress. In addition, the viscosity–temperature curve was plotted [37].

2.3.8.3. Skin irritation/corrosive potential test

The evaluation of the PMS-cream formulation, intended for topical use, for its potential to cause skin irritation or corrosion, as well as the reversibility of dermal effects, was performed in accordance with the OECD Guideline for Acute Dermal Irritation/Corrosion [38]. Briefly, 3 healthy male Wistar rats were examined as follows: Rat #1 (negative control with no treatment), and Rats #2 and #3 were topically treated with the PMS-cream formulation. Prior to the experiment, the rats' fur was carefully trimmed from the dorsal part of their trunks with an electric clipper while they were held in a humane manner. The animals were ready for the cream formulation to be applied once it was confirmed that their skin was healthy and undamaged. A dosage of about 0.5 g of the prepared cream was applied to an area of about 6 cm^2, and the region was covered with a gauze patch. In accordance with the guidelines, one patch was applied for the first test and removed after 3 minutes. An hour later, a second patch was applied and removed after 3 minutes if no significant skin response was observed. A third patch was then applied and left in place for 4 hours. Upon removing the patch, the animals were checked visually for signs of erythema and edema. The appearance of cutaneous responses was assessed. A 4-hour exposure session on a different animal confirmed the negative reaction. Following the removal of the patch, the cutaneous reaction was assessed immediately, after 1 hour, and again 24 hours later, as recommended by Draize's dermal irritation scoring model [38] (Table 2).

Table 2. Draize dermal irritation scoring system [38].

Erythema and Eschar Formation	Value	Edema Formation	Value
No erythema	0	No edema	0
Very slight erythema (barely perceptible)	1	Very slight edema (barely perceptible)	1
Well-defined erythema	2	Slight edema (edges of area well defined by definite raising)	2
Moderate to severe erythema	3	Moderate edema (raised approximately 1 mm)	3

2.3.8.4. Antibacterial activity of the PEM-cream formula

The procedure used for the evaluation of the antimicrobial activity of PEM against *S. aureus* was also utilized to evaluate the antimicrobial activity of the prepared PEM-cream formulation on *E. coli* (ATCC 25922), *S. aureus*, and *P. aeruginosa* (ATCC 27853). The prepared PEM-cream formula containing Germall Plus preservative (T1), the PEM-cream formula containing Germall Plus and phenoxyethanol preservatives (T2), Germall Plus preservative (Pre1), and phenoxyethanol preservative (Pre2) were all tested for their antimicrobial preservative activities. The findings were compared to the reference positive control (Tetracycline) and the negative controls (1% DMSO / 1% Germall Plus) [39].

2.3.9. Statistical analysis

All statistical analyses were performed using Microsoft Excel. The results were presented as mean ± SD, and all experiments were conducted in triplicate.

3. RESULTS

3.1. Extraction yield

The resulting dry extracts were sticky with a dark green color. The extraction yield percentage was calculated for each plant, showing 31.3% for *M. peregrina* and 28.8% for *E. ramosissimum*.

3.2. Total phenolic content (TPC)

The total phenolic content for PEM (2:1) showed the highest value (150.15 ± 2.8 mg GAE/g), followed by PEM (1:1) and PEM (1:2) (133.25 ± 5.5 and 105.55 ± 1.9 mg GAE/g), respectively.

3.3. Total flavonoid content (TFC)

The total flavonoid content for PEM (2:1) also showed the highest value (41.5 ± 1.2 mg QE/g), followed by PEM (1:1) and PEM (1:2) (32.11 ± 0.8 and 26.3 ± 3 mg QE/g), respectively.

3.4. DPPH free radical scavenging activity

Using the DPPH antioxidant assay, the calculated IC50 for PEM (2:1) showed the highest antioxidant activity (7.06 ± 0.12 µg/mL), followed by PEM (1:1) and PEM (1:2) (10.18 ± 0.5 and 18.03 ± 0.28 µg/mL), respectively.

3.5. ABTS free radical scavenging activity

Using the ABTS free radical scavenging method, the calculated IC50 for PEM (2:1) showed the highest radical scavenging activity (53.29 ± 3.3 µg/mL), followed by PEM (1:1) and PEM (1:2) (67.4 ± 5.4 and 192.43 ± 13 µg/mL), respectively.

3.6. Anti-collagenase activity

The percentage inhibition and IC50 for collagenase enzyme activity using PEMs were calculated as shown in Figure 1. The findings revealed that the IC50 for PEM (2:1) was 32.4 ± 1.19 µg/mL, which was similar to the other samples: PEM (1:1) and PEM (1:2) were 33 ± 2.2 µg/mL and 33.3 ± 1.46 µg/mL, respectively. However, this activity increased significantly at higher concentrations, with comparable activities among the tested samples.

3.7. Anti-tyrosinase activity

The percentage inhibition and IC50 of tyrosinase enzyme activity using the PEMs were calculated as shown in Figure 2. The findings revealed that the IC50 for PEM (1:2) was 10.1 ± 0.4 µg/mL, which is lower than the other samples: PEM (2:1) and PEM (1:1) were 40.3 ± 2.3 µg/mL and 46.5 ± 0.9 µg/mL, respectively. However, this activity increased significantly, showing comparable differences among the tested samples at higher concentrations. Additionally, the results indicated that PEM (2:1) exhibited higher enzyme inhibition activity at concentrations greater than 60 µg/mL compared to the other samples tested.

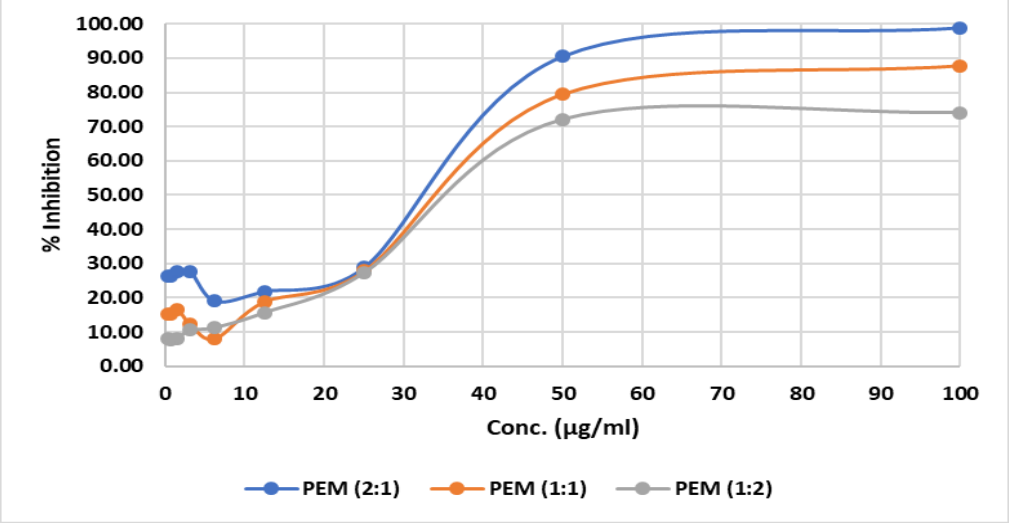

Fig. 1: The % inhibition on collagenase enzyme activity by PEMs

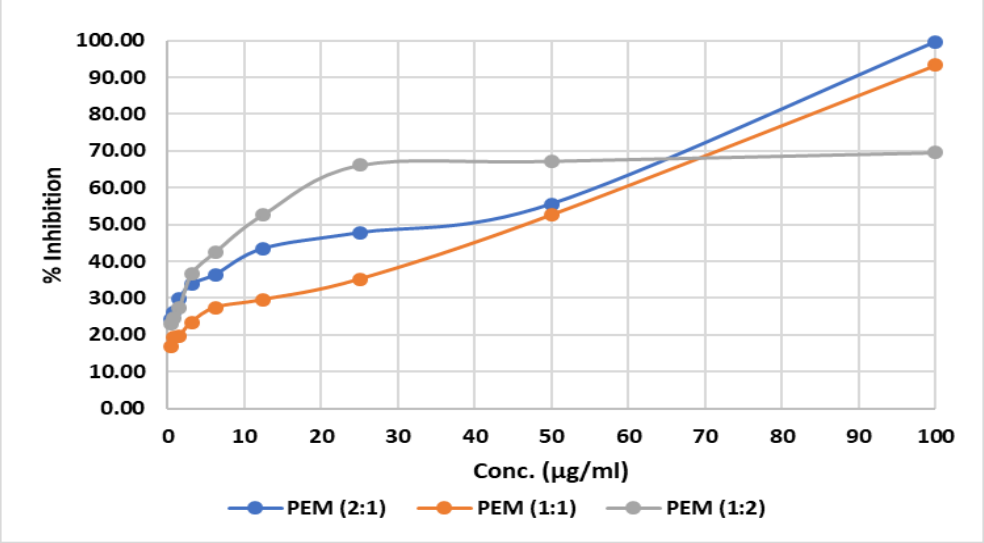

Fig. 2: The % inhibition on tyrosinase enzyme activity by PEMs

3.8. Antibacterial activity

Figure 3 shows the measured diameters of the inhibition zones (cm) for PEM (2:1) at three different concentrations against S. aureus. The findings revealed inhibition zone diameters of 2.3 cm, 2.55 cm, and 2.8 cm for PEM (2:1) at concentrations of 10 mg/mL, 30 mg/mL, and 60 mg/mL, respectively. In comparison, the positive control, Tetracycline, yielded an inhibition zone diameter of 1.45 cm. The tested PEM samples demonstrated superior antibacterial activity against S. aureus.

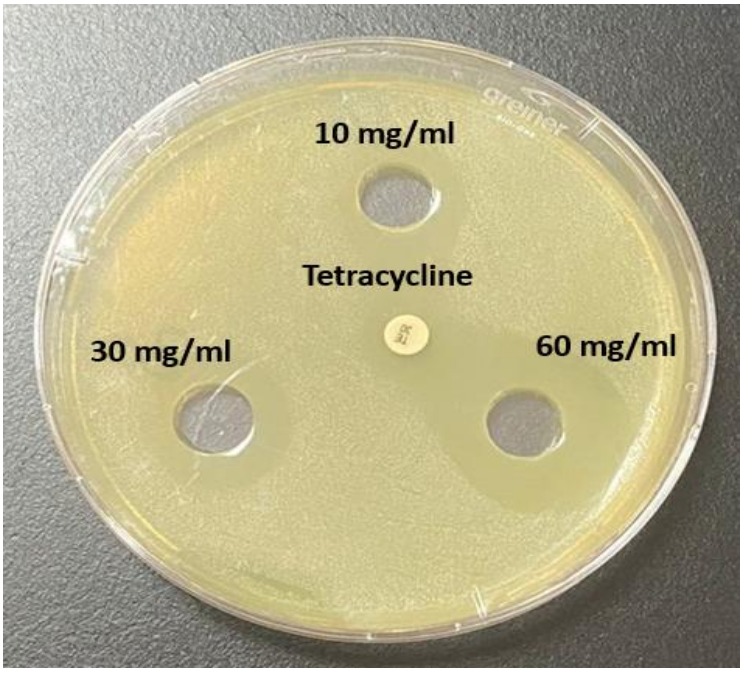

Fig. 3. Zone of inhibition (cm) of PEM (2:1) at different concentrations and tetracycline against *S. aureus*.

3.9. Evaluation of Cream formula

3.9.1. Stability studies

Over the study period, the PEM-cream formulations stored at room temperature (25°C) or in the refrigerator (4°C) exhibited no changes in physical characteristics. The results indicated that the PEM-cream formulations remained homogeneous with no signs of separation. They retained a pleasant lavender odor, a light green color, and a pH of 5.33, which is within the compatible skin pH range (4.5-6). In contrast, the cream formulation stored in the oven at 40°C developed a crust on the surface, indicating instability at this temperature.

3.9.2. Rheological study

Figure 4 shows the rheological pattern of the cream formulations, with a plot of shear stress versus shear rate. Figure 4.A illustrates a pseudoplastic rheological flow, characterized by a non-linear relationship between shear rate and shear stress. A shear-thinning or pseudoplastic system is the most common type of time-independent non-Newtonian fluid behavior.

The effect of shear rate on the viscosity of the PEM-cream formulations is presented in Figure 4.B. The findings revealed that as the shear rate increases, the viscosity of the formulations decreases. Thus, the rheological study results indicate that the PEM-cream formulation has good spreadability and homogeneity.

3.9.3. Skin irritation/corrosive potential

The interpretation of skin irritation and corrosive potential was based on Draize's Dermal Irritation Scoring Model [38], as described in Tables 2 and 3. Results indicated that the application of the formula on the rats' skin, serving as a model for human skin, showed no signs of irritation, erythema, or redness over a 24-hour period. This suggests that the prepared PEM-containing formulations can be safely applied to the skin (Figure 5).

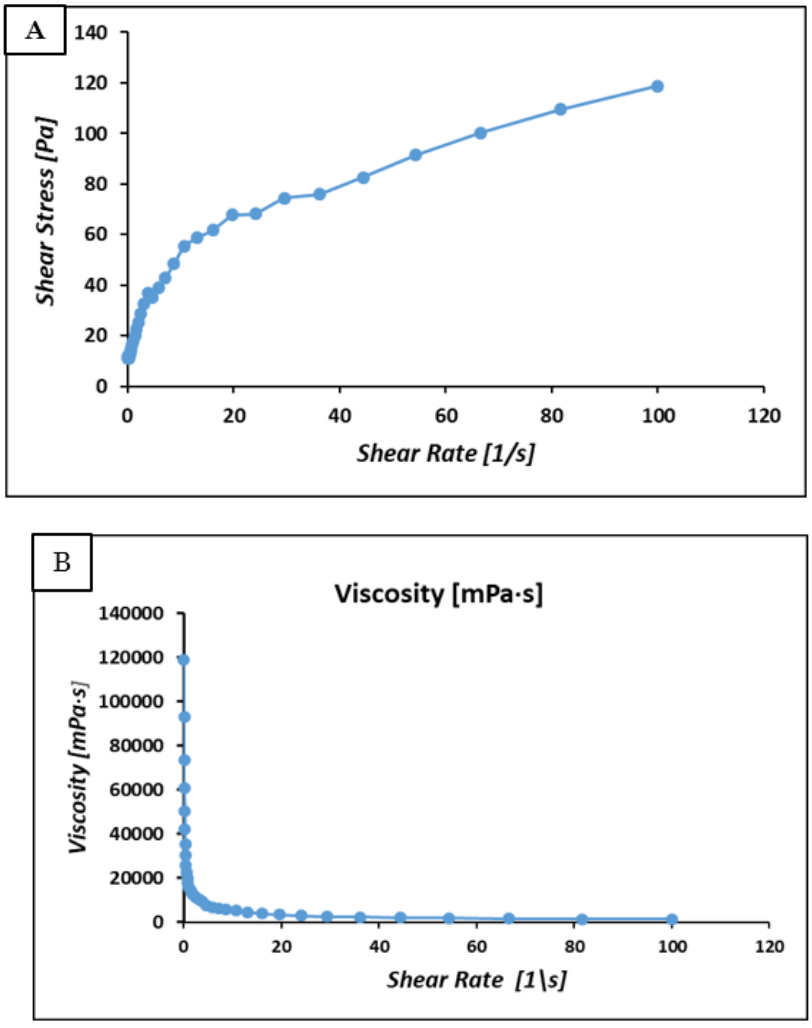

Fig. 4.1 Shear stress versus shear rate plot of cream showed non- Newtonian flow. (B) The effect of shear rate on the viscosity of the cream plant extract.

Table 3. Dermal Responses Observed in Individual Rats

Erythema			
Wistar Rat (1) control, Rat (2) test	**Evaluation after removal of test substance**		
	0 minutes	**60 minutes**	**24 hours**
(1) Control	0	0	0
(2) Test	0	0	0
Edema			
Wistar Rat (1) control. Rat (2) test	**Evaluation after removal of test substance**		
	0 minutes	**60 minutes**	**24 hours**
(1) Control	0	0	0
(2) Test	0	0	0

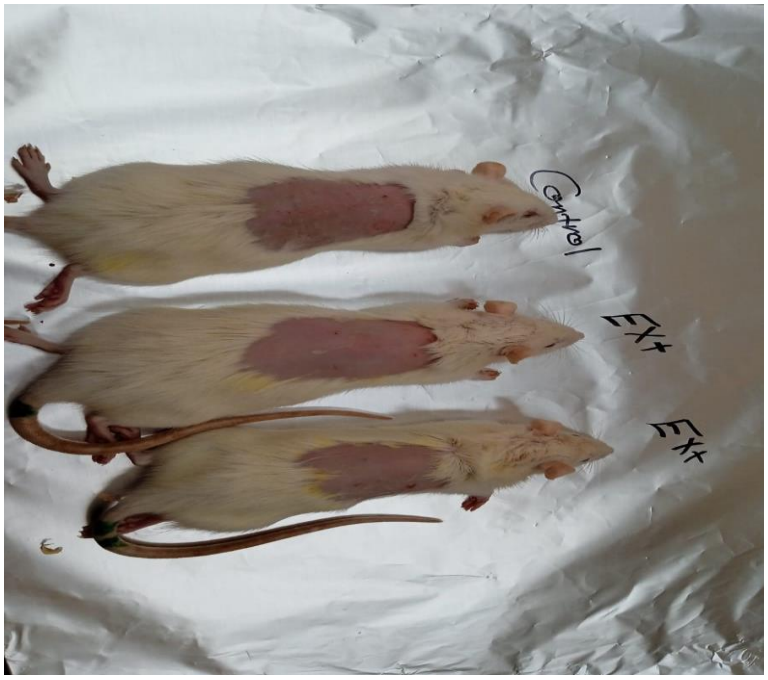

Fig. 5. Results of irritation test (Negative control with no treatment and duplicates with PEM-cream formulations after 24 hours).

3.9.4. Antibacterial activity for the PEM-cream formula

The antimicrobial activities of the prepared T1 and T2 formulas, as well as the Pre 1 and Pre 2 samples, are shown in Table 4. The findings suggest that incorporating a second preservative into the PEM-cream formula is unnecessary, as both T1 and T2 formulas (Figure 6D, E, F) demonstrated comparable antimicrobial effects to the positive control (Figure 6A, B, C). In contrast, Pre 2 (Figure 6D, E, F) showed no inhibition against the three bacterial strains and was therefore excluded from the final formula.

Table 4. Zone of inhibition (cm) for bacterial growth against different bacterial strains by DMSO (negative control), Tetracycline (positive control), T1, T2, Pre 1, and Pre 2.

Samples	*P. aeroginosa*	*E. coli*	*S. aureus*
DMSO	0 cm	0 cm	0 cm
Tetractcline	1 cm	2.9 cm	1.1 cm
T1	2.9 cm	3.2 cm	3.2 cm
T2	2.7 cm	3.2 cm	3.4 cm
Pre 1	0.6 cm	1.8 cm	1.2 cm
Pre 2	0 cm	0 cm	0 cm

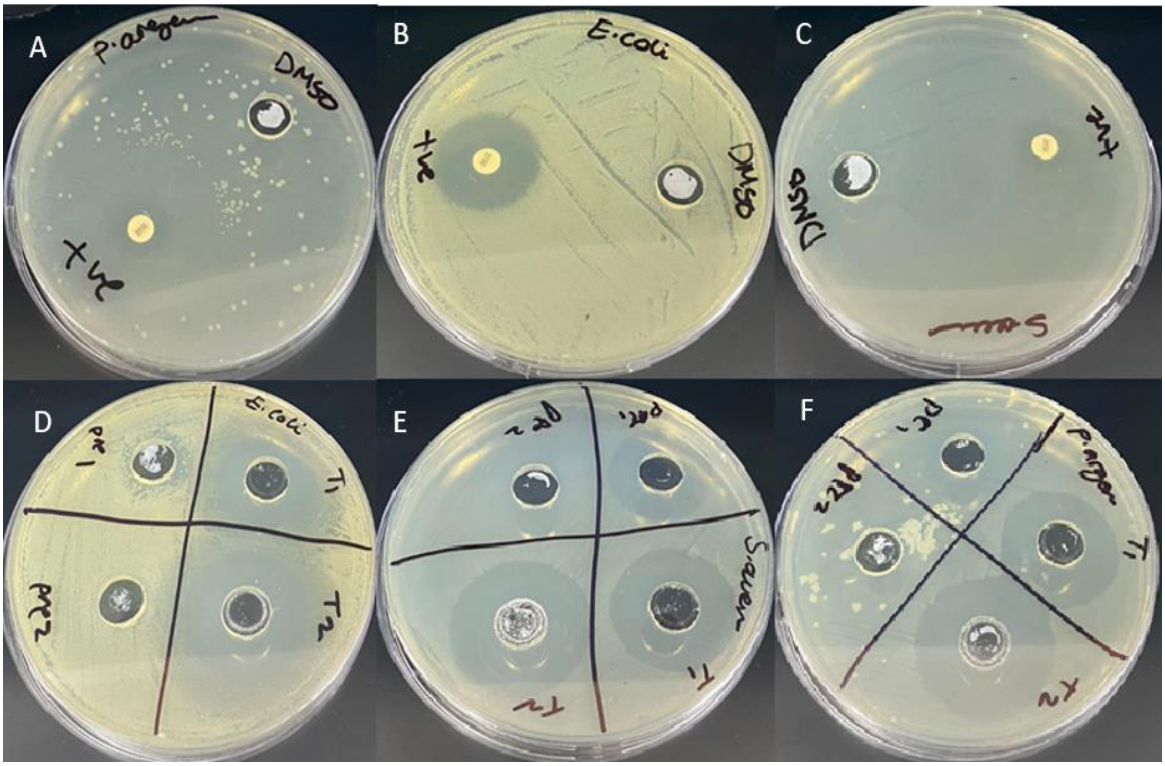

Fig. 6. Inhibition of bacterial growth against different bacterial strains by DMSO (negative control), andTetracycline (positive control) (A-C), T1, T2, Pre 1, and Pre 2 (D-F).

A: Tetracycline vs. DMSO against *P. aeruginosa,* B: Tetracycline vs. DMSO against *E. coli*, C: Tetracycline vs. DMSO against *S. aureus*, D: T1, T2, Pre 1, and Pre 2 against *E. coli,* E: T1, T2, Pre 1, and Pre 2 against *S. aureus,* F: T1, T2, Pre 1, and Pre 2 against *P. aeruginosa*.

4. DISCUSSION

Herbal medicine in skincare products is currently one of the most significant areas in the pharmaceutical industry [40,41]. Several modern herbs, such as Green Tea (Camellia sinensis), Liquorice (Glycyrrhiza glabra), and Centella Asiatica, have recently been introduced to the field of skincare [42]. Traditionally, M. peregrina and E. ramosissimum have been used to treat various skin conditions, including moisturizing, anti-aging, and wound-healing [21]. Furthermore, these plant extracts have been previously studied for their antimicrobial, anti-collagenase, and anti-tyrosinase activities [25,43]. To our knowledge, no previous work has been performed on the combination of M. peregrina and E. ramosissimum extracts.

The findings of the current study revealed that the PEM (2:1) mixture ratio exhibited the highest total phenolic content, flavonoid content, and antioxidant activities. This data is consistent with previous studies [16, 25]. M. peregrina's bioactive compounds and therapeutic effects were studied by Dehshahri et al. [19], who highlighted its bioactive compounds, including phenolic acids, isothiocyanates, flavonoids, alkaloids, and glucosinolates, which contribute to its diverse therapeutic effects such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective activities. The phytochemical analysis

of E. ramosissimum conducted by Savaya et al. [25] revealed the presence of flavonoids, phenolic acids, alkaloids, and saponins. A study by Parham [44] identified diverse compounds in E. ramosissimum, including flavonoids, alkaloids, phenolics, saponins, tannins, triterpenoids, and phyto-esters. Specific compounds like Myricetin, Quercetin, Kaempferol, and Kaempferol-3-O-glycoside were found to be the most abundant [45,46].

The antioxidant activities of the PEM were investigated using the DPPH and ABTS assays as generators of free radicals, which mimic reactive oxygen species (ROS) and reactive nitrogen species (RNS) under controlled laboratory conditions. These radicals impact biological systems and play a role in counteracting damage caused by oxidative stress and lipid peroxidation [47]. In this study, lower IC50 values against DPPH• and ABTS•+ radicals were obtained for PEM (2:1), which confirms our previous findings. The antioxidant activities of M. peregrina extract were previously tested by Hasan et al. [48], who found the extract to display significant scavenging activity against DPPH free radicals. Consistent with our research, Al-Bayati et al. [49] demonstrated notable free radical scavenging ability using the DPPH method in methanolic extracts of E. ramosissimum.

As expected, PEM (2:1) showed antimicrobial effects on S. aureus, as indicated by the measured inhibition zone using the well diffusion method. The antibacterial activity of M. peregrina ethanol extract was previously tested by Majali et al. [50], who found a correlation between extract concentration and inhibition zone against bacterial growth for E. coli, S. aureus, and K. pneumoniae. The antimicrobial potential of E. ramosissimum was illustrated by Karak [51] against various bacterial strains, revealing potential antimicrobial benefits for the extract. Similarly, Savaya et al. [25] examined E. ramosissimum extracts for phenolic content, antioxidants, and antibacterial effects against Propionibacterium acnes. The aqueous-methanol extract displayed potent antioxidant and antimicrobial effects, while other extracts exhibited varying levels of activity.

Studies on the tyrosinase enzyme inhibition effect revealed that PEM (2:1) was effective in a concentration-dependent manner. These data were expected, as the inhibitory effects of E. ramosissimum extracts on mushroom tyrosinase were previously examined [25]. Previous studies have shown that phytochemical compounds found in M. peregrina, including β-sitosterol, can potentially act as anti-tyrosinase agents [52]. Other plant extracts containing β-sitosterol, such as Arbutus andrachne L., have also shown potent inhibitory effects against tyrosinase [52]. Additionally, Prommaban et al. [53] demonstrated that β-sitosterol displays inhibitory effects on mushroom tyrosinase enzymes by suppressing the oxidation process of L-3,4-dihydroxyphenylalanine (L-DOPA), a reaction catalyzed by tyrosinase.

Studies on the collagenase enzyme inhibition effect revealed that PEM (2:1) was effective in a concentration-dependent manner. These data are consistent with a previous study by Da Costa et al. [54], who found that E. ramosissimum promotes collagen synthesis and inhibits enzymes related to skin aging, suggesting its potential use as a cosmeceutical ingredient. Additionally, several anti-collagenase compounds were identified in M. peregrina, such as lupeol acetate [55], which was tested for its ability to bind to matrix metalloproteinase-1 (MMP-1), a collagenase enzyme [56], and showed considerable results.

A cream formula was prepared using the emulsification method, incorporating the plant extract PEM (2:1) as an active ingredient at 1.5%. The prepared formula exhibited good stability at room temperature, as evidenced by its physicochemical appearance. Furthermore, the cream displayed pseudoplastic rheological behavior, meaning that it becomes easier to spread when subjected to rubbing force, contributing to its good spreadability and homogeneity. The antimicrobial test performed on the prepared cream formula revealed that the preservatives added to the formula were effective.

A skin irritation test was performed using a rat model. The results showed that this formula did not induce edema

or erythema, indicating that the cream's components are safe for application on skin models, pending further research.

5. CONCLUSION

Findings from the current study indicate that this plant extract mixture holds potential as an active ingredient for incorporation into pharmaceutical formulations for skin care. Its superior antioxidant activity, along with its inhibitory effects on tyrosinase and collagenase enzymes, and its antibacterial effect on the tested bacterial strains, underscore its promise. Furthermore, the PEM-cream formula demonstrated acceptable stability at room temperature and showed no risk of skin irritation, as confirmed by both in vitro and in vivo tests.

ABBREVIATIONS

PEM: Plant extract mixture; TPC: Total phenolic content; TFC: Total flavonoid content; ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; DPPH: 2,2-Diphenyl-1-picrylhydrazyl Radical; QE: Quercetin; GA: Gallic acid; IC_{50}: Half inhibitory concentration; MRSA: Methicillin resistant *Staphylococcus aureus*; UVR: Ultraviolet radiation; MMP-1: Matrix metalloproteinase-1; SD: Standard deviation.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design of the study. They were also involved in material preparation, data collection, and manuscript writing. All authors have read and approved the final manuscript.

CONFLICTS OF INTERESTS

The authors report no conflicts of interest in this work.

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التأثيرات المثبطة للبوليفينول من مستخلصات Equisetum ramosissimum و Moringa peregrina على المكورات العنقودية الذهبية والكولاجيناز وإنزيمات التيروزيناز: دراسات مخبرية تجريدية

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ملخص

خلفية: تؤدي مشاكل الجلد الناجمة عن الإجهاد التأكسدي إلى تنشيط إنزيمات الكولاجيناز والتيروزيناز ، والتي يمكن أن تسهم في شيخوخة الجلد وتغير لونه والالتهابات. سابقا تم تقييم النباتات Equisetum ramosissimum و Moringa peregrina لاستخداماتهما في حالات جلدية مختلفة. الهدف: يهدف البحث الحالي إلى التحقيق في التأثير الإيجابي للبوليفينول في مستخلصات Equisetum ramosissimum و Moringa peregrina كمنتج تجميلي محتمل يستخدم لعلاج مشاكل الجلد المختلفة. الطرق: تم تحديد المحتويات الكلية للفينول والفلافونويد ومضادات الأكسدة ومضادات الكولاجيناز ومضادات التيروزيناز لمخاليط المستخلصات النباتية (PEM) بنسب مختلفة من (M. peregrina: E. ramosissimum) باستخدام الإجراءات المعيارية. تم تقييم التأثيرات المثبطة ل ((PEMضد البكتيريا المسببة لحب الشباب المكورات العنقودية الذهبية ((ATCC 29213باستخدام طريقة قياس قطر منطقة التثبيط (سم). تمت صياغة تركيبة كريمية تحتوي على PEM والتحقق من ثباتها وتاثيها على تهيج جلد الفئران في المختبر. النتائج: أظهرمزيج PEM بنسبة (2: 1) على محتوى إجمالي من الفينول والفلافونويد (150.15 ± 2.8 مجم / جم مكافئ لحمض الغال) ، و (41.5 ± 1.2 مجم / جم مكافئ للكيرسيتين) ، على التوالي. كانت الأنشطة المضادة للأكسدة (2: 1) PEMكما تم الحصول عليها باستخدام طرق DPPH و IC50= 0.12 ± 7.06) ABTS ميكروغرام / مل و 3.3±53.29 = IC50 ميكروغرام / مل) ، على التوالي. علاوة على ذلك ، أظهر (2: 1) PEMأنشطة تثبيط متفوقة ضد إنزيمات الكولاجيناز والتيروزينات (= IC50 1.19 ± 32.4 ميكروغرام / مل و IC50=8.4±1.19 ميكروغرام / مل) ، على التوالي. أظهر النشاط المضاد للميكروبات PEM (2: 1)الذي تم اختباره على S. aureus أكبرقطر منطقة تثبيط النمو (2.8 سم) ، بتركيز 60 مجم / مل. كشفت الدراسات التي أجريت على تركيبة كريم (2: 1) PEM أنها ثابتة فيزيائيا في ظروف الغرفة. أظهر اختبار تهيج الجلد على الفئران عدم ظهور الوذمة أو الحمامي بعد العلاج. الخلاصة: أظهر PEM بنسبة (2: 1) الأنشطة المثلى كعامل تحييد الإجهاد التأكسدي ، ومثبط للإنزيمات المسببة لاكتساب الجلد وفرط التصبغ ، وكذلك مع تأثير مضاد للبكتيريا. كما أظهرت تركيبة كريمية تحتوي على PEM ثباتا فيزيائيا مع عدم وجود أي خطرا محتمل لتأثير تهيج الجلد خلال فترة إجراءات البحث.

الكلمات الدالة: نبات ذيل الفرس ، نبات المورينغا، المكورات العنقودية الذهبية، كولاجيناز ، تيروزيناز .

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