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Usnea barbata Extract in Karanja Oil—An Innovative Combination with Antioxidant, Antimicrobial, and Sunscreen Properties, with Potential Applications in Skincare

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Abstract

Plant extracts in vegetable oils are foundational and eco-responsible for skin care, acting as potent antimicrobials, antioxidants, photoprotectants, and emollients. The present research aims to conduct a comprehensive investigation of Usnea barbata extract in Karanja oil (KO). The lichen extract (UBKO) was obtained through cold maceration. Phytochemical screening was performed using the Folin-Ciocalteu method and Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS). Physicochemical properties were evaluated by Atomic Force Microscopy (AFM) and Fourier Transform Infrared Spectroscopy (FTIR). The rheological characteristics and oxidative resistance of both oil samples, with and without *U. barbata* (UBKO and KO), were investigated. The bioactive potential of UBKO was assessed by evaluating antioxidant properties, sunscreen efficacy, and antibacterial and antifungal activity. UBKO had a slightly lower density (0.827 vs. 0.955) and pH (4.22 vs. 4.86) than KO, and a slightly higher oxidative resistance, quantified as the induction period (IP) value (6.45 vs. 6.00). The total phenolic content was significantly greater in UBKO than in KO (567.16 ± 14.96 vs. 433.26 ± 22.96 mg GAE/mL, $p < 0.05$). The antibacterial effect against *S. aureus* was higher for UBKO than KO (25 ± 0.00 vs. 31.25 ± 18.75 mg/mL, $p < 0.05$), while the inhibitory activity of UBKO against C. albicans was considerably higher than that of KO (6.75 ± 0.00 vs. 37.50 ± 12.50 mg/mL, $p < 0.05$). Finally, our results showed that UBKO had an appreciable sun-protective factor (SPF) slightly higher than KO (30.9 vs. 29.8). Therefore, our study suggests that *U. barbata* extract in Karanja oil could be used to develop pharmaceutical formulations with antimicrobial and photoprotective effects, with potential applications for skincare.

Keywords: *Usnea barbata*; Karanja oil; total phenolic content; heavy metals; rheological properties; oxidative resistance; antioxidant activity; antimicrobial potential; sunscreen efficacy

1. Introduction

Modern skincare science is increasingly oriented toward multi-active botanical formulations that deliver broad-spectrum efficacy while reducing reliance on synthetic chemical agents [1]. Natural products often enhance their action when combined rather than when isolated and concentrated;

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therefore, plant extracts and vegetable oils, which are rich sources of physiologically active chemicals, are widely utilized in cosmetics for their capacity to shield the skin from damaging external or internal factors [2,3]. Moreover, plant extracts in vegetable oils constitute a versatile, scientifically substantiated category of skincare ingredients that have successfully bridged traditional medicine and evidence-based cosmetic science. For example, flowers of *Arnica montana* L. (Asteraceae), a perennial mountain herb native to Europe, are macerated in sunflower or olive oil to produce arnica-infused oil. The active constituents transferred to the oil phase include sesquiterpene lactones (helenalin, dihydrohelenalin), flavonoids, and carotenoids, and the oil extract has anti-inflammatory properties [4]. Araújo et al. revealed that leaves or roots of *Symphytum officinale* L. (Boraginaceae) macerated in sunflower (Helianthus annuus) seed oil produce comfrey macerated oil, with significant wound healing and pain-reduction activities [5].

Other ingredients listed in the International Nomenclature of Cosmetic Ingredients (INCI) database are also known for their use in traditional medicine from ancient times, but they were still not used in combination: *Pongamia Glabra* Seed Oil (available online at https://cosmileeurope.eu/inci/detail/12565/pongamia-glabra-seed-oil/), known as Karanja oil (KO), and *Usnea barbata* (available online at https://cosmileeurope.eu/inci/detail/16750/usnea-barbata-extract/). Karanja oil — also known as pongamia oil — is a non-edible vegetable oil cold-pressed from the seeds of *Pongamia pinnata* (L.) Pierre (a medium-sized leguminous tree belonging to the Fabaceae family), while *Usnea barbata* (L.) F.H. Wigg. is a lichen belonging to the Parmeliaceae family [6,7].

Karanja oil's main unsaturated fatty acids are oleic acid (44–71%), linoleic acid (10–18%), palmitic acid, and stearic acid [8]; it also contains three phenolic constituents (ongamol, karanjin, and cycloart-23-ene-3β,25-diol) with potential UVA and UVB sunscreen activities [9]. Modern chemical analysis has identified numerous secondary metabolites from *Usnea* species [10]: usnic acid is the predominant bioactive constituent, depsides and depsidones (barbatic acid, diffractaic acid, evernic acid), and phenolic acids (caffeic acid, ellagic acid, chlorogenic acid, *p*-coumaric acid, gallic acid, cinnamic acid) [11]. Both have accrued substantial evidence for antimicrobial, anti-inflammatory, antioxidant, and photoprotective activities — yet they act through distinct molecular mechanisms, occupy different chemical polarities (lipophilic oil versus lichen-acid complex), and target complementary portions of the UV spectrum [12–18]. Moreover, both are already used in skincare in various formulations. Karanja oil is described as a high-polarity active oil and is traditionally used for various chronic skin conditions [19]. Patents claim the use of Karanja oil in sunscreen formulations [20]. Dhule et al. incorporated Karanja oil in a herbal antifungal spray [21]. Karanjin, isolated from seed oil, can be used in cosmetic products such as soaps, body oils, and shampoos [22]. Recent studies report that its incorporation into modern formulations may benefit psoriasis and acne [23,24]. On the other hand, Engel et al. demonstrated that *U. barbata* standardized extract can be a promising ingredient for the development of UV-protective skin care products or anti-inflammatory topical preparations [18]. Romanian researchers incorporated *U. barbata* extract into bioadhesive oral films with antimicrobial and anticancer properties for potential use in complementary therapy for oral cancer [25]. Usnic acid was loaded into electrospun fibers and hydrogel membranes to optimize wound-healing efficiency [26,27].

INCI decoder, the new INCI application (available online at https://incidecoder.com/), indicates that Karanja oil (used at 3-5% concentration) and *U. barbata* can serve as natural preservatives in cosmetic products, due to their antimicrobial activity. In this context, our study aims to investigate the physicochemical, functional, and pharmacological properties of *U. barbata* extract in Karanja oil, for possible applications in skincare. To our knowledge, a combination of these 2 natural ingredients has not yet been analyzed in scientific literature.

2. Materials and Methods

2.1. Materials and Equipment

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Karanja oil (KO) was obtained by cold-pressing the seeds of *Pongamia pinnata* (L.). Pierre (Fabaceae) and supplied by Fagron, Greece. The oil is highly pure and suitable for cosmetic applications. Its liquid wax composition, primarily long-chain esters, provides oxidative stability, a non-greasy texture, and compatibility with human skin.

U. Barbata thalli were collected in March 2024 from the Călimani Mountains, Romania (47°28′ N, 25°13′ E, at an altitude of 900 m). The freshly gathered lichen thalli were cleaned of impurities, then dried at 18–25 °C in an herbal room, protected from sunlight. Preservation of dried lichen for an extended period was carried out under similar conditions. It was identified by the Department of Pharmaceutical Botany at the Faculty of Pharmacy, Carol Davila University of Medicine and Pharmacy, using standard methods. A voucher specimen is kept in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Carol Davila University of Medicine and Pharmacy (UBL 3/2024, Ph-UMFCD).

All chemicals, solvents, and reagents used were of analytical grade. Dimethyl sulfoxide (DMSO), sodium carbonate (Na_2CO_3), and Folin–Ciocalteu reagent were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). High-purity 69% nitric acid (trace grade), standard stock solutions containing 1000 mg/L H_3AsO_4 in 0.5 M nitric acid, and 1000 mg/L $Pb(NO_3)_2$ in 0.5 M nitric acid, along with 30% H_2O_2, 96% ethanol, methanol, crystal violet, acetic acid, and ultrapure deionized water, were obtained from Merck Millipore (Burlington, MA, USA).

Microbial strains were obtained from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. Culture media were sourced from two suppliers. Tryptic Soy Agar for bacterial strains was purchased from Sigma-Aldrich Merck (Dartmouth, Germany), and RPMI 1640 for fungi was supplied by American Biorganics (Buffalo, NY, USA).

2.2. U. barbata Extract in Karanja Oil

The oil extract (UBKO) was prepared by cold maceration, as previously described [40]. Almost 20 g of this mass was accurately weighed using a Kern analytical balance, placed in a 1000 mL brown glass container, and 500 mL of KO was added. The sample was macerated for 3 months in a light-protected location at a constant temperature (21-22 °C). The brown container was manually shaken daily; after 3 months, UBKO was filtered through cotton-mesh gauze into a brown vessel with a sealed plug and preserved in a plant room, sheltered from sunlight.

The UBKO density (g/mL) was slightly lower than that of KO (0.827 < 0.955).

The pH values for KO and UBKO were determined with a CONSORT P601 pH meter (CONSORT nv, Turnhout, Belgium) using a pH electrode. UBKO has a lower pH-value than KO (4.22 < 4.86).

2.3. Total Phenolic Content

The determination of total phenolic content (TPC) was performed using the Folin–Ciocalteu colorimetric method, as previously described [39]. The samples (KO and UBKO) were diluted 1:1 (v/v) with DMSO to ensure uniform dispersion and to maintain a measurement within the calibration curve's linear range. To achieve a homogeneous mixture, polyethylene glycol 400 (PEG 400) was used as a cosolvent to facilitate the uniform dispersion of the oil and the solubilization of phenolic compounds in the analysis medium. Two milliliters of each sample (KO and UBKO) were mixed with eight milliliters of PEG 400, then diluted 1:1 (v/v) with DMSO to ensure uniform dispersion and keep measurements within the linear range of the calibration curve. Absorbance was measured at 765 nm using a FlexStation 3 UV–Vis spectrophotometer (Molecular Devices, GA, USA). The results were reported as µg of gallic acid equivalents (GAE) per 1 mL of oil sample (µg GAE/mL) or µg GAE/g of oil sample, depending on the analyzed matrix. The assay was performed in comparison with PEG 400 alone to provide clear evidence of the oils' composition.

2.4. FTIR Analysis

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Fourier Infrared (FTIR) spectra were acquired using a Fourier-transform infrared (FTIR) spectrometer (model FT/IR-4200, JASCO, Tokyo, Japan) equipped with an attenuated total reflectance (ATR) accessory (ATR PRO450-S), as previously described [53]. Spectral measurements were performed over the wave range of 4000 to 400 cm^{-1}, at a spectral resolution of 4 cm^{-1}. All spectra were recorded at ambient temperature and are presented in transmittance values.

2.5. AFM Analysis

Atomic Force Microscopy (AFM) analysis was conducted as previously described [54]. Oil samples (20 µL each) were diluted in 2 mL of 96% ethanol and deposited onto clean glass substrates. Then, the samples were heated at 200 °C for 30 min to ensure proper adhesion and solvent evaporation. AFM imaging was performed in enhanced contrast mode, and the resulting line scans, displayed below the images, clearly illustrate the surface profiles of both oil samples.

2.6. Heavy Metals Content

Heavy metals, arsenic (As) and lead (Pb), were identified using Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) as previously described. A SOLAAR 6M (Thermo Electron Inc., Waltham, MA, USA) atomic absorption spectrometer equipped with a deuterium lamp for background correction served as the platform, and the wavelengths for As and Pb were 193.7 nm and 283.3 nm, respectively. Both heavy metal concentrations in each oil sample were calculated from the corresponding regression lines, namely, absorbance as a function of concentration. All measurements were performed in duplicate [53].

2.7. Rheological Properties of the KO and UBKO

The rheological determinations were made using a B One Plus rotary viscometer (Lamy Rheology, Champagne-au-Mont-d'Or, France), with an accuracy of ± 1% of full scale and a repeatability of 2%. It is equipped with seven probes (spindles), identified as RV1-RV7, each measuring a specific viscosity range. The RV3 probe was used to determine oil viscosity by immersing it in 50 mL of each sample. The measurements were performed at rotation speeds of 50, 100, 150, 200, and 250 rpm for 10 seconds each; the temperature remained constant (22 °C) [53].

The spreading behavior of all samples was assessed using an extensometer (Epsilon Technology Corp., Jackson, WY, USA). Two glass plates were used; 0.5 g of oil was placed at the center of the lower plate. The upper plate, weighing 50 g, was placed on top, and the diameter of the oil spread was measured. Additional weights of 50, 100, 200, and 500 g were gradually added. After 1 minute of rest, the diameter occupied by the oil sample was measured, and the area was calculated as πr^2 [53].

2.8. Oxidative Stability

The Velp OXITEST reactor (Velp Scientifica Srl, Usmate Velate, MB, Italy), which accelerates oxidation under controlled, repeatable conditions, was used to assess the oxidative stability of UBKO and KO by measuring the induction period [34], as previously described [53].

2.9. Antioxidant Activity

2.9.1. DPPH Method

We tested the radical-scavenging capacity of the UBKO using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, as previously described [55–57]. The DPPH method is based on the discoloration of a solution containing the DPPH free radical (Sigma-Aldrich, USA) upon interaction with antioxidants present in the tested sample. For each sample, several dilutions in methanol were prepared (1:1, 1:5, 1:10, and 1:25) to assess the dose dependency of the antioxidant effect.

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Each sample was used at a 1:2 ratio with the DPPH working solution, which had an initial OD of approximately 0.8.

We performed a kinetic assessment, recording the decrease in optical density (OD) at 517 nm over 1 hour. Also, we conducted endpoint measurements after 5, 30, and 60 min respectively, analyzing the decrease in OD:

$\Delta OD\ (\%) = 100 \times (OD_{DPPH} - OD_{DPPH + sample})/OD_{DPPH}$.

This was considered to express the antioxidant effect of the tested oil samples.

2.9.2. ABTS Method

The 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) is based on measuring a sample's ability to scavenge the ABTS+· free radical. The stock reagent, containing ABTS at 7 mM and $K_2S_2O_8$ at 2.45 mM, was kept in the dark for 16 hours at 4 °C to generate the green ABTS+· free radical. The working solution was prepared by diluting the stock reagent with distilled water until an appropriate optical density (0.8) was reached at 714 nm.

The oil samples (undiluted and diluted 1:1, 1:5, 1:10, and 1:25) were incubated with the ABTS working solution at a 1:3 ratio, and the OD was measured at 714 nm after 10 min. A blank was prepared using ethanol instead of the sample. Results are presented as the % decrease of optical density (ΔOD):

$\Delta OD\ (\%) = 100 \times (OD_{blank} - OD_{sample})/OD_{blank}$.

These are directly related to the antioxidant capacity of KO and UBKO [58].

2.10. Antimicrobial Activity

To evaluate the antimicrobial activity of KO and UBKO against the most common pathogens (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *C. albicans* ATCC 10231), we first determined the minimum inhibitory concentration (MIC). Then, using sub-MICs (MIC/2 and MIC/4), we evaluated the oil samples' capacity to inhibit microbial adhesion to an inert substrate (ACI, expressed as a percentage).

2.10.1. Determination of Minimum Inhibitory Concentrations

Suspensions with an optical density of 0.5 McFarland for bacteria and 1 McFarland for fungi were prepared, and the following were distributed in the 96-well plates: 100 µL of TSA or RPMI 1640, 100 µL of a 100 mg/ml solution of the substances investigated in the first well, after which a binary serial dilution scheme was performed up to well 10, and 20 µL of suspension from the strains to be analyzed, from well 1 to well 11 (11 was the positive growth control, and 12 was the negative control). The absorbance readings were performed at 620 nm using a Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) [59].

2.10.2. Evaluation of the Oil Samples' Influence on Microbial Adherence Capacity to the Inert Substratum

The influence of UBKO and KO on biofilm formation is evaluated using the same method previously described at 4.9.1. The degree of adhesion to the inert substrate of the tested strains was assessed at sub-MICs after fixation with methanol, staining with a 1% crystal violet solution resuspended in 33% acetic acid.

The adherence capacity - AC (%) – of the microbial strains was determined using the following formula:

$AC\ (\%) = 100 \times (A_{sample} - A_{blank})/(A_{control} - A_{blank})$

where A_{sample} is the absorbance at 490 nm of the tested oils, and $A_{control}$ is the absorbance at 490 nm of the negative control represented by untreated microbial strains [59].

2.11. Sunscreen Properties

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Volumes of 1 mL of each oil sample were transferred to a 100 mL volumetric flask and diluted to volume with ethanol. The samples were then ultrasonicated for 5 minutes. The SPF values of the oil samples were measured using a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer (Perkin-Elmer Inc., Waltham, MA, USA) in transmission mode. Absorption values were recorded for samples in microcuvettes with a 10 mm light path over the range of 290 to 320 nm, at 5 nm intervals. Each measurement was performed three times, and the average value was used. SPF values were calculated using the Mansur equation [58] (Eq. 3).

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (3)$$

where CF = correction factor (10), EE(λ) = erythemogenic effect of radiation at wavelength λ, I(λ) = intensity of solar light at wavelength λ, and abs (λ) = absorbance of wavelength λ by the preparation solution. The value of EE × λ is constant.

2.12. Data Analysis

Almost all measurements were performed in triplicate to ensure reproducibility, and the results are expressed as mean ± standard deviation. Data analysis was performed using XLSTAT Premium v.2025.2.0.1232 (Lumivero, Denver, CO, USA) and Microsoft Excel v. 16.0 19328 (Microsoft Corporation, Redmond, WA, USA) [60]. A single-factor ANOVA was used to detect significant differences between variables ($p < 0.05$) [61].

3. Results

3.1. Total Phenolic Constituents

The results obtained by the Folin–Ciocalteu method (Table 1) show significant differences in the total phenolic content (µg GAE/mL) between the KO and UBKO. Karanja oil contained appreciable levels of phenolic metabolites (Table 1). The *U. barbata* extraction in KO resulted in a significant increase in TPC concentration (567.16 vs. 433.26 µg GAE/mL in oil sample, $p < 0.05$, Table 1). The oil samples emulsified with PEG 400 had higher TPC levels, with significant differences between KO and UBKO (Table 1).

Table 1. TPC concentrations in both oil samples, expressed as µg GAE/mL oil or µg GAE/g.

Sample	TPC			
	µg GAE/mL oil + PEG 400	µg GAE/mL oil sample	µg GAE/g oil + PEG 400	µg GAE/g oil sample
KO	693.75 ± 22.96 [a]	433.26 ± 22.96 [b]	652.61 ± 20.28 [c]	407.57 ± 20.28 [d]
UBKO	827.66 ± 14.96 [a]	567.16 ± 14.96 [b]	773.55 ± 13.98 [c]	530.08 ± 13.98 [d]
PEG 400	260.49 ± 0.27 [a]	-	229.23 ± 0.24 [c]	-

TPC – Total phenolic constituents; KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil; PEG 400 – polyethylene glycol 400. In the same column, the values marked with the same superscripts are significantly different.

3.2. FTIR Analysis

The IR spectrum of KO reveals molecular structures rich in saturated aliphatic chains, evidenced by C–H stretching vibrations at 2922.6 cm^{-1} and 2853.2 cm^{-1}, which are typical of methylene (−CH2) groups in alkane derivatives (Figure 1, blue line) [28]. The C–H bending vibrations are observed at 1463.7 cm^{-1} and 1376.0 cm^{-1}, confirming the presence of alkyl chains. The absorption at 722.2 cm^{-1} corresponds to the rocking vibration of methylene groups, supporting the existence of long-chain aliphatic backbones. The weak band at 3006.5 cm^{-1} indicates a =C–H stretching vibration, but the high transmittance suggests a low proportion of double carbon–carbon bonds compared to the saturated (single-bonded) carbon atoms in the oil components (Figure 1, blue line). The presence of ester groups is confirmed by the strong band at 1743.3 cm^{-1}, produced by the carbonyl (–C=O) stretch vibration,

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while the C–O stretching vibrations are observed at 1230.4 cm^{-1}, 1162.9 cm^{-1}, and 1116.6 cm^{-1}. The spectra also show that free acids are below the detection limit, as indicated by the absence of the characteristic broad O–H band in the range of 3500 to 2500 cm^{-1}, caused by hydroxyl stretching. The IR spectrum suggests that triglycerides may be the most abundant components (Figure 1, blue line). The IR spectrum of UBKO shows some differences in the 1600-400 cm^{-1} region, but the spectral shapes are similar (Figure 1, red line). These differences in the fingerprint region suggest slight structural alterations, such as changes in the chemical environment, cation substitution, or various lattice vibrations/degrees of ordering between the two oil samples, thereby maintaining a pattern similar to the KO. This similarity, together with the absence of significant band shifts or new absorption bands, demonstrates that UB and KO do not undergo chemical interactions upon incorporation.

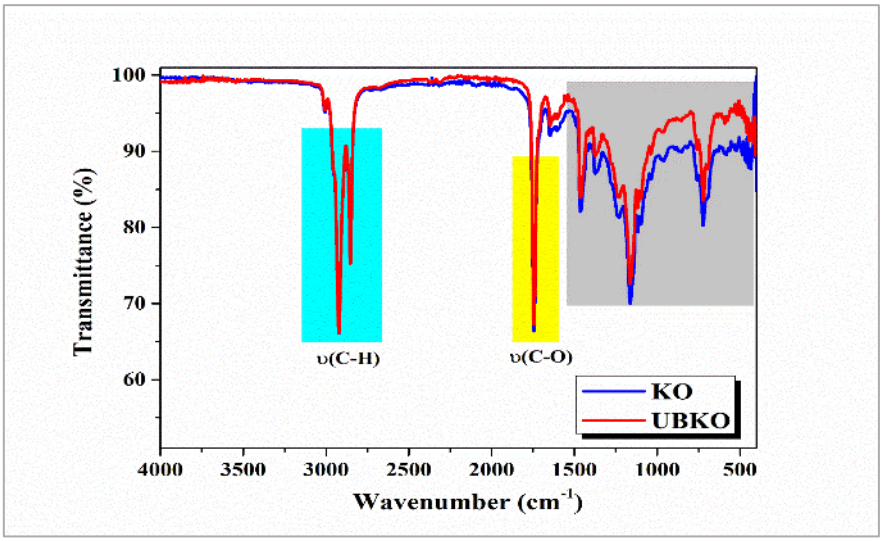
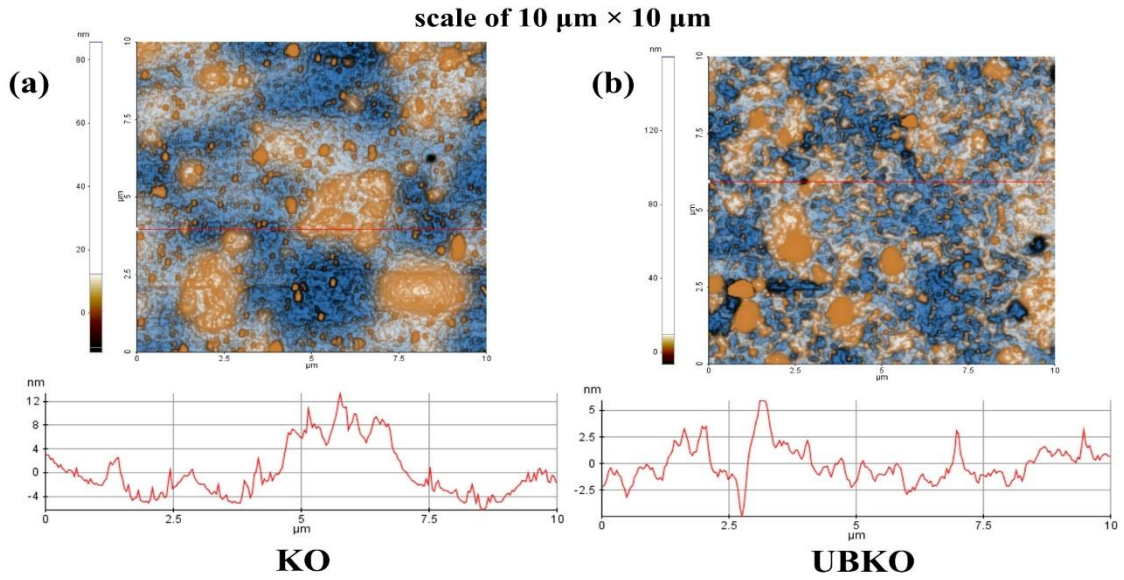


Figure 1. Overlapped IR spectra of KO (blue line) and UBKO (red line).

3.3. AFM Analysis

The AFM images of KO and UBKO are shown in Figure 2. Figure 2a displays the scan over a (10 × 10) μm^2 area for KO, while Figure 2b shows a similar scan for UBKO. Figures 3c and 3d present scans over a (4 × 4) μm^2 area for KO and UBKO, respectively. The characteristic line-scan profiles are indicated at the position marked by the red line. Additionally, Figures 2e-h show histograms of the roughness (Rq) and peak-to-valley (Rpv) parameters.



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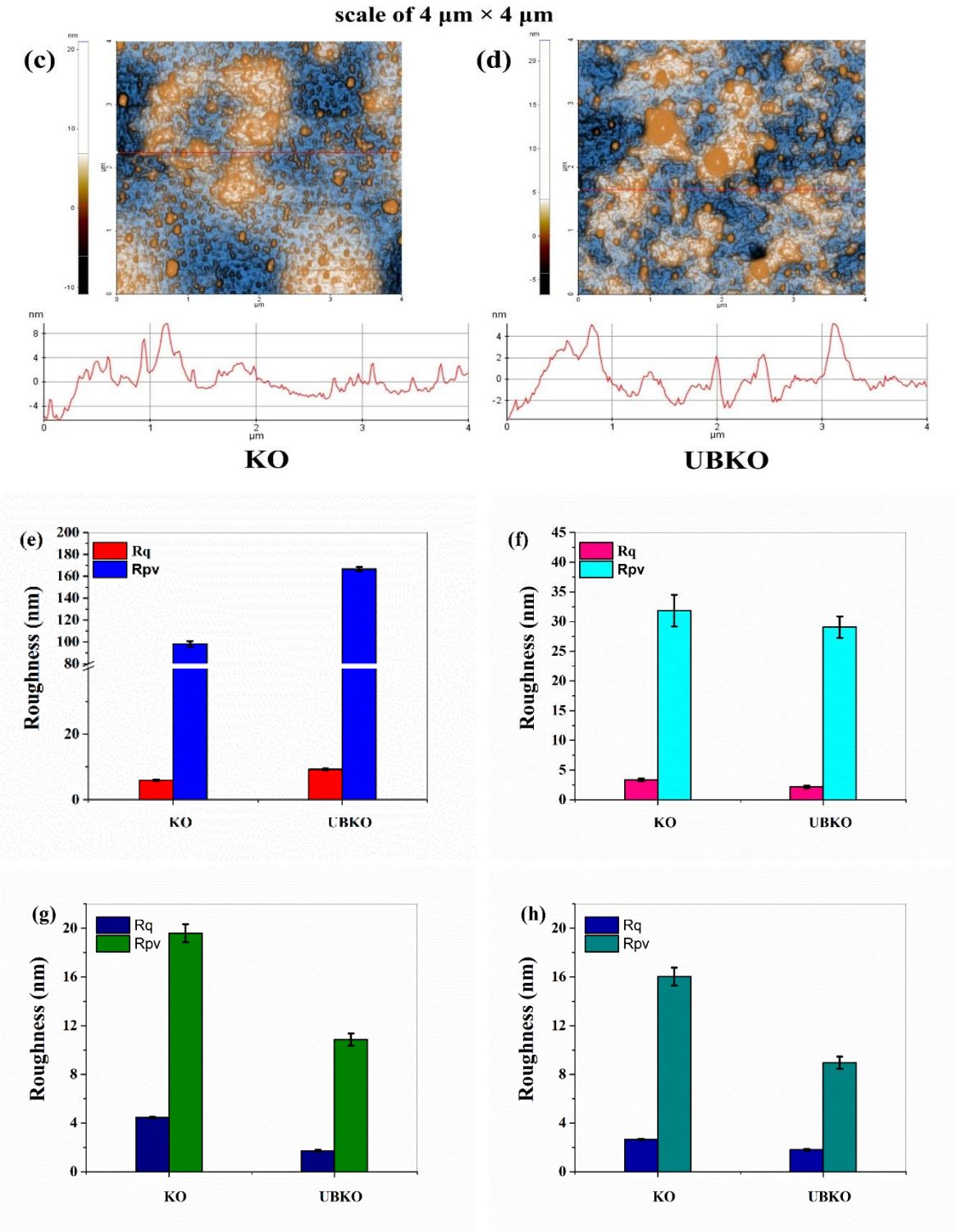

Figure 2. AFM images together with the characteristic red scan line profiles for KO (**a,c**) and UBKO (**b,d**). The AFM images are scanned over areas of (10 ×10) µm² (**a** and **b**) and (4 × 4) µm² (**c** and **d**); Histogram of roughness (Rq) and peak-to-valley (Rpv) parameters over the entire scanned areas (**e**) (10 × 10) µm² and (**f**) (4 ×4) µm² and along the line scan for (**g**) 10 µm and for (**h**) 4 µm.

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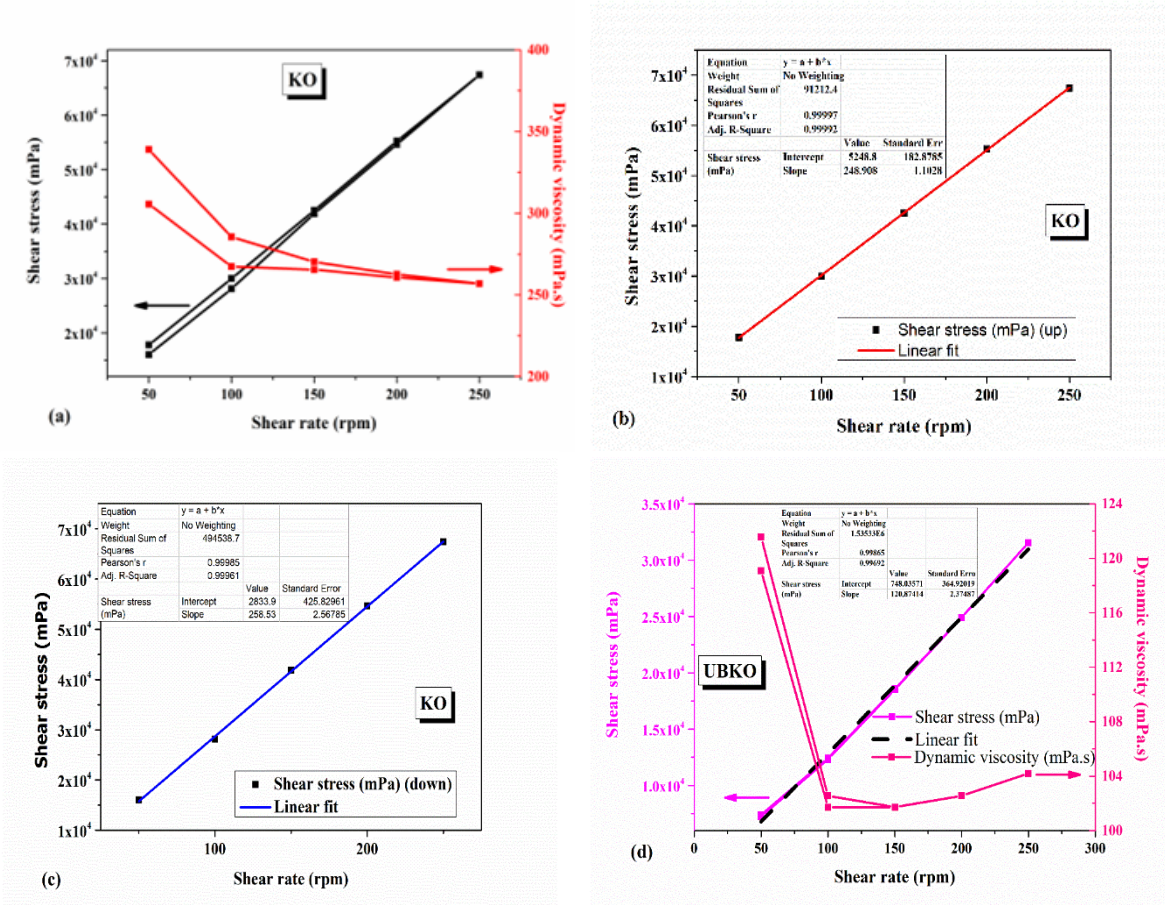

Figure 3. Rheological analysis of KO (a); Linear fit of Shear stress for KO (up mode) (b); Linear fit of Shear stress for KO (down mode) (c); Rheological analysis of UBKO (d). KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil.

AFM analysis shown in Figure 2 highlights several morphological differences between KO and UBKO at both examined scales of (10×10) μm² and (4×4) μm². KO exhibits a mostly uniform surface, characterized by larger structures or aggregates that are sporadically dispersed. This suggests the presence of microdomains on the surface, leading to a vertical red line scale variation of approximately 17 nm (from -5 to 12 nm), specific to its lipid composition (Figure 2a). In contrast, UBKO displays a much more heterogeneous surface, characterized by smaller structures that are more numerous and evenly distributed (Figure 2b). This results in lower variations of around 9 nm (from −3 to 6), as indicated by the red line scan in Figure 2b. These formations suggest possible interactions between the lichen's bioactive compounds and the KO matrix. The AFM topography of both compounds is also confirmed at a smaller scale (4×4) μm², where the morphological details become more evident (Figure 2c, d).

The analyzed roughness parameters, Rpv (peak-to-valley parameter) and RMS (root mean square) roughness (Rq), respectively, show lower values in the case of KO despite the presence of larger aggregates, confirming its smooth and homogeneous character (Figure 2e, f). The KO sample is characterized by an Rq of 5.9 nm and an Rpv of about 98.1 nm across the entire scanned area for (10×10) μm² (Figure 2e). The relatively low density of KO (0.9548 g/cm³) suggests a less dense molecular association, typical for lipid systems. This KO structure allows molecular mobility, supporting the formation of larger, but sporadically distributed, aggregates that lead to a relatively smooth surface.

In contrast, for the lichen extract, the *U. barbata* phytochemicals in Karanja oil resulted in much higher roughness parameter values across the entire scanned area and along the corresponding red line profile (Figure 2e-h). This indicates a noticeable increase in the roughness and local height

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variations. UBKO is characterized by an RMS roughness (Rq) of about 9.3 nm and a peak-to-valley parameter (Rpv) of about 166.6 nm. Lichen constituents generate supplementary interactions that induce reorganization of the surface, possibly through the formation of smaller nanometric-scale structures, thereby increasing surface roughness.

3.4. Heavy Metals Content

Arsenic content (μg/g) is higher in UBKO than in KO (0.203 versus 0.131), while lead content is lower (0.056 versus 0.065). The results are shown in both measurement units (μg/L and μg/g) in Table 2. The As levels in both oil samples remain within a range considered safe for cosmetic or unrefined vegetable oils [29]. The results obtained are consistent with the values reported in recent literature, whereas As concentrations in vegetable oils generally range from 0.05 to 0.20 mg/kg [30]. Both oil samples have similar Pb levels (0.065 and 0.056 μg/g), which are significantly lower than the permissible limit of 0.100 μg/g set by Regulation (EC) No. 1881/2006 for lead in vegetable oils and fats [31].

Table 2. The As and Pb content in KO and UBKO.

Metal	Oil sample	Sample weight	Heavy metal concentration	
		g	μg/L	μg/g
As	KO	0.315	4.122	0.131
	UBKO	0.318	6.450	0.203
Pb	KO	0.315	2.049	0.065
	UBKO	0.318	1.788	0.056

KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil.

3.5. Rheological Properties

The rheological curves of KO and UBKO, together with the linear fit equations for shear stress, are represented in Figure 3. The rheological behavior of the KO was evaluated by analyzing the relationship between shear stress (mPa) and shear rate (rpm) (Figure 3 a,b and c). A linear correlation was observed over the investigated range, indicating Newtonian behavior, shear stress being directly proportional to shear rate.

The experimental data were fitted using a linear regression model of the form (Eq. 1)

$$y = a + bx \quad (1)$$

where y is the shear stress (τ), x is the shear rate ($\dot{\gamma}$), and slope (b) represents the dynamic viscosity (η), according to Eq. 2 [32].

$$\eta = \frac{\tau}{\dot{\gamma}} \quad (2)$$

The high correlation coefficient ($r^2 \approx 0.999$) confirms a strong linear dependence between shear stress and shear rate. Although a rapid decrease in dynamic viscosity was observed with increasing or decreasing shear rate, in the initial range of 50 to 100 rpm (at low shear rates), the KO exhibits shear-thinning behavior. Subsequently, the dynamic viscosity ranges from approximately 250 to 260 mPa·s at higher shear rates, indicating a transition to Newtonian behavior. This variation can be attributed to the low torque values (0.115-0.485 mN·m) recorded during measurements. Additionally, the composition of higher fatty acids influences KO's shear-thinning behavior, leading to non-Newtonian behavior at lower shear rates due to molecular interactions involving monounsaturated fatty acids, which result in higher dynamic viscosity at low shear rates. Therefore, the linear shear stress–shear rate relationship is considered a more reliable parameter for determining KO's fluid behavior. Overall, these results demonstrate that the analyzed KO sample behaves as a Newtonian fluid within the tested shear rate range.

The rheological behavior of UBKO was assessed by analyzing the relationship between shear stress and shear rate (Figure 3d). A linear correlation was observed throughout the entire tested shear rate range, and the dynamic viscosity remained mostly constant between 100 and 250 shear rates,

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confirming that the UBKO also displays Newtonian behavior, similar to KO. Linear regression was conducted using the same model as before (Eq. 1). Additionally, the high correlation coefficient ($r^2 \approx 0.997$) supports the strong linear relationship between shear stress and shear rate and indicates no yield stress. The nearly constant dynamic viscosity suggests that UBKO's flow resistance is independent of shear rate, a hallmark of Newtonian fluids.

Regarding the spreadability of the samples, initially, after applying the upper plate, the stretching surface of UBKO was smaller than that of KO (Figure 4).

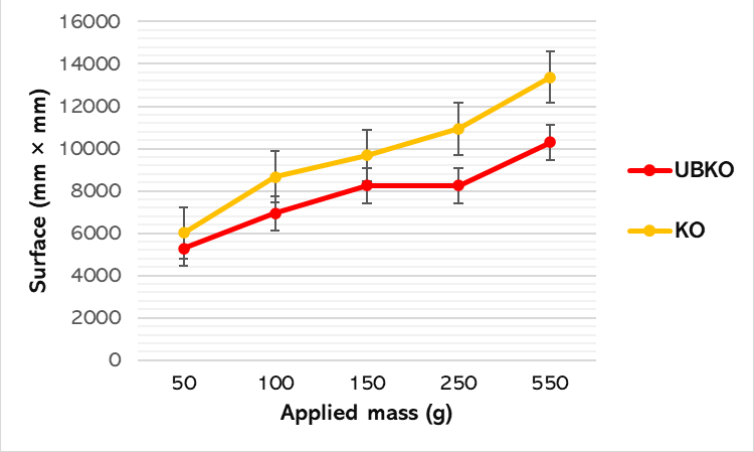

Figure 4. Spreadability analysis of UBKO and KO; KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil.

Later, when additional weights are added, the extensibility of UBKO remains lower than that of KO (7800.88 ± 1851,54 vs. 9727.16 ± 2723,88, $p > 0.05$; Figure 4). The spreadability of the samples aligns with their viscosity behavior, indicating that KO will likely spread quickly and evenly over the skin surface without requiring high pressure. Additionally, extensibility reflects the KO behavior when incorporated into other fluid or semisolid systems. The results demonstrate that KO can uniformly spread across different carriers without requiring high energy. Formulation cohesiveness determines extensibility performance, and KO was found to preserve the structural properties and flexibility. Gobi et al. have shown that viscoelastic properties strongly influence adhesion [33]. A significant correlation between the spreading and viscoelastic properties of UBKO is observed, similar to that for KO. Nevertheless, based on the recorded slight differences, it can be concluded that lichen constituents form new interparticle bonds in the oily system, as evidenced by changes in rheological properties.

3.6. Oxidative Stability

The quantitative measurement of oxidative stability was the induction period (IP), measured in hours. It is defined as the time when the tangent lines drawn before and after the inflection point intersect. This period indicates how long it takes for oxidation to begin, whether it leads to noticeable rancidity, or to a sudden increase in the oxidation rate.

Generally, a longer IP correlates with higher oxidation resistance and shelf life [34]. Our results show that UBKO had slightly greater oxidative stability (IP = 6.45) than KO (IP = 6).

3.7. Antioxidant Activity

To accurately evaluate the antioxidant activity of PJO and UBPJO, the samples were diluted with methanol (1:1, 1:5, 1:10, and 1:25) and compared with a solvent-based standard to evaluate dose-response behavior.

The in vitro radical-scavenging capacity of KO and UBKO, assessed using the DPPH method, was measured using both kinetic and endpoint tools (Figure 5a).

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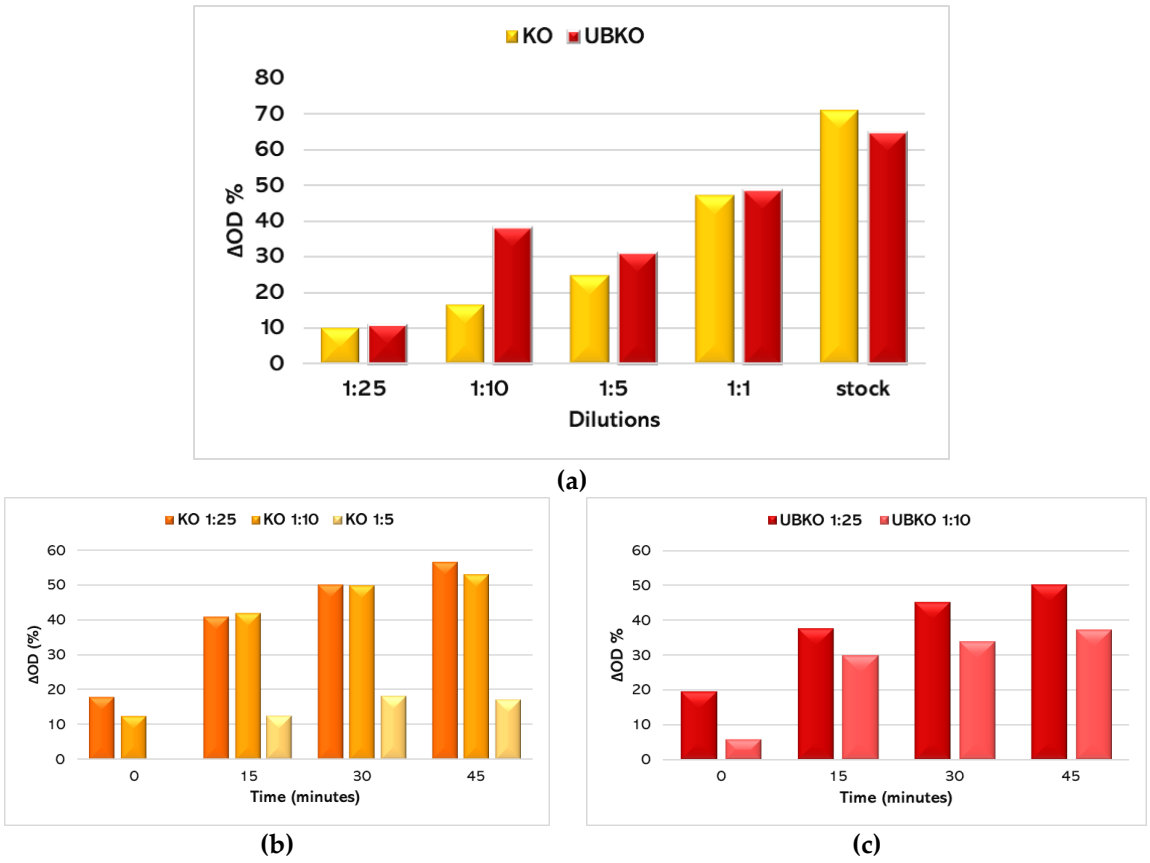

Figure 5. Antioxidant capacity (%) expressed as decrease of optical density determined by the DPPH method at different dilutions of both oil samples (a) and ABTS method, for KO (b), and UBKO (c); - ΔOD (%) – decrease of optical density; KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil.

The KO stock solution reported the strongest DPPH radical-scavenging activity (ΔOD = 70.92%), higher than UBKO (ΔOD = 64.64%). UBKO dilutions exhibited greater antioxidant capacity than KO, which generally decreased with dilution grade from 1:1 to 1:25 (Figure 5a).

Results from the ABTS method are shown in Figures 5b and 5c. We were unable to assess the UBKO undiluted samples or the lowest dilutions because an emulsification process rendered them unsuitable for reading at the specified OD. The KO dilutions ranged from 1:5 to 1:25. The antiradical capacity of both oil samples increased over time. The results showed that KO had antioxidant capacity, with insignificant differences at 1:25 and 1:10. The 1:25 and 1:10 dilutions exhibited the strongest antioxidant effects for both oil samples, but the differences between them were greater at UBKO (Figure 5c).

3.8. Antimicrobial Activity

The results of the antimicrobial activity assessment focused on two areas: determining the minimum inhibitory concentration (MIC) and evaluating the ability of KO and UBKO to inhibit microbial biofilm formation.

To evaluate the inhibitory activity of the oil samples, a 20% Tween 80 (T80) solution in ethanol was used for solubilization, allowing the bioactive compounds to disperse in the aqueous culture medium. Tween 80 was included as a positive control in the microbiological analyses.

All determined MIC values (mg/mL) are illustrated in Table 3, compared with those of conventional antibiotics (μg/mL) reported by Rankovic et al. for the same ATCC bacterial and fungal lines [35]. UBKO had the highest inhibitory activity against *S. aureus* and *C. albicans*, and the lowest antibacterial efficacy against *E. coli* (Table 3).

Table 3. Minimum inhibitory concentration (MIC) values.

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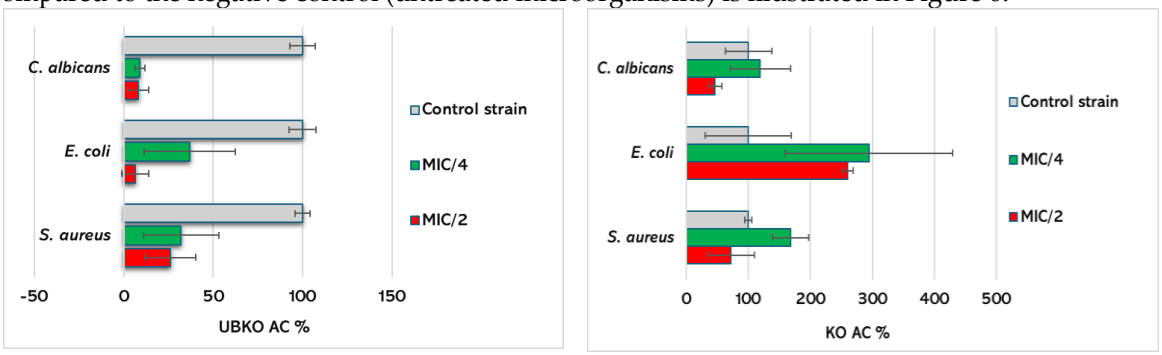
Microbial cell line	UBKO	KO	T80	*Standard Antibiotic [35]
		MIC (mg/mL)		MIC (µg/mL)
S. aureus ATCC 25923	9.62±2.87 ^{a,b}	31.25±18.75 ^{a}	50±0.00 ^{b}	31.35 [35]
E.coli ATCC 25922	50±0.00	37.50±12.50	37.5±12.50	21.25 [35]
C. albicans ATCC 10231	5.06±1.68 ^{a,b}	37.50±12.50 ^{a}	50±0.00 ^{b}	1.95 [35]

KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil, T80 – Tween 80. In the same row, the values marked with the same superscripts are significantly different. *Standard antibiotics were Streptomycin for bacterial strains and Ketoconazole for *C. albicans* [35].

The influence of KO and UBKO at sub-MICs (MIC/2 and MIC/4) on microbial adherence compared to the negative control (untreated microorganisms) is illustrated in Figure 6.



(a) **(b)**

Figure 6. The influence of KO (a) and UBKO (b) on the adherence capacity of microbial strains; the negative controls were untreated microbial strains, and their adherence capacity was considered 100%. KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil. AC % = adherence capacity expressed as percentages.

The results show that UBKO inhibited the adherence of all bacterial and fungal strains at both sub-MICs tested, and the effect was directly proportional to the concentration tested. The AC values for all pathogens were significantly lower than those of negative controls ($p < 0.05$, Figure 6a). The highest antiadherence capacity was observed against *C. albicans*, with very low differences in AC (%) values at both sub-MICs (MIC/2 vs. MIC/4, 7.89 vs. 8.49, Figure 6a). Higher AC values were recorded on *S. aureus* (31.99 vs. 26.62, Figure 4a). UBKO at MIC/2 showed an *E. coli* adherence inhibition almost 6 times higher than MIC/4 (6.19 vs. 36.77, Figure 6a).

Contrariwise, KO at MIC/4 stimulated biofilm formation for all pathogens; the highest adherence capacity was observed at *E. coli*, followed by *S. aureus* and *C. albicans* (294.52 vs. 167.91 and 119.33, Figure 6b). At MIC/2, KO considerably inhibited the adherence of *C. albicans* and *S. aureus* (46.55% and 72.24%, respectively), while its stimulatory effect on *E. coli* remained very strong and recorded a very low diminution (AC = 260.30 at MIC/2 vs. 294.52 at MIC/4, Figure 6b).

3.9. Photoprotective Activity

SPF values serve as a standard for evaluating the efficacy of sunscreen formulations against UV-B radiation. SPF value represents the ratio of UV energy required to produce a minimum erythematous dose (MED) in protected skin versus unprotected skin.

The absorbance values of the KO and UBKO samples obtained using the UV spectrophotometric method are shown in Table 4.

Table 4. The results for KO and UBPO absorption using the spectrophotometric method.

Wavelength (cm^{-1})	EE(λ) × I(λ)	Absorbance	
		KO	UBKO
290	0.0150	2.80294	2.96479

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295	0.0817	2.92969	3.06945
300	0.2874	2.98885	3.11607
305	0.3278	3.00255	3.10471
310	0.1864	2.97773	3.09127
315	0.0837	2.96323	3.03929
320	0.0180	2.91667	3.01506

KO – Karanja oil; UBKO – *U. Barbata* extract in Karanja oil. EE(λ) - the erythemogenic effect of radiation at wavelength λ; I(λ) - is the intensity of solar light at wavelength λ; Abs (λ) - the absorbance of wavelength λ by the preparation solution.

The SPF value of KO is 29.8, and for UBKO is 30.9. It was observed that extracting *U. barbata* bioactive constituents into Karanja oil increased the SPF by approximately 1 unit. The results demonstrate that the KO and UBKO can be successfully used in the development of sunscreen formulations, as evidenced by their higher SPF values.

4. Discussions

The current study is based on the results of our previous research. Our team analyzed the composition of *U. barbata* collected from the same unpolluted area in the Calimani Mountains at 900 m altitude, as reported in previously published studies. *U. barbata* extracts were prepared using various solvents (ethanol, methanol, ethyl acetate, acetone, and Canola oil) through different methods (maceration and Soxhlet extraction). A detailed elemental analysis was performed on dried lichen harvested in 2020 [30,38]. Twenty-three metals were investigated by inductively coupled plasma mass spectrometry (ICP-MS), and the results reveal that autochthonous *U. barbata* contains all metals at levels below the permissible limits for medicinal plants [36]. Usnic acid and other phenolic secondary metabolites were identified and quantified using UHPLC (including caffeic acid, p-coumaric acid, ellagic acid, chlorogenic acid, cinnamic acid, and gallic acid) [17,36–40]. We also isolated usnic acid from the dry *U. barbata* extract in ethyl acetate via semi-preparative chromatography [41], and validated a UHPLC method to determine usnic acid content in *U. barbata* extract in Canola oil [40]. Moreover, the antimicrobial, antioxidant, and cytotoxic potential of different *U. barbata* extracts was previously investigated [25].

Total phenolic constituents account for almost all the bioactive constituents of *U. barbata* and Karanja oil. Watti et al. quantified total phenolic compounds in KOs extracted from *Pongamia glabra* seeds harvested from two different zones in India and reported 13.5–15.2 mg GAE/g [42]. Our results indicate lower TPC levels in both KO (407.57 µg GAE/g) and UBKO (530.08 µg GAE/g), probably due to differences in the KO origin and preparation.

The potential synergistic effect arises from the interaction between the phenolic compounds in the extract and the bioactive constituents of KO, which together could boost UBKO's pharmacological potential.

The present research was designed as a focused screening that emphasizes the most critical toxic elements instead of a comprehensive multi-element analysis. Choosing arsenic (As) and lead (Pb) as representatives for heavy metal measurement was based on their toxicological importance, regulatory significance, and well-known affinity for plant- and lichen-based matrices. In our previous heavy metal determination in dry lichen, the Pb concentration was 1.296 µg/g, and As was undetected [36]. The heavy metal levels in UBKO and KO are below the maximum permitted threshold, indicating compliance with European safety standards. The results also align with data reported in recent literature, which shows that typical Pb concentrations in vegetable oils range from 10 to 80 µg/kg. This correlation verifies the method's accuracy and confirms the absence of external contamination in both oil samples. Therefore, the HNO_3/H_2O_2 mineralization method in the Ethos Easy microwave system, combined with GFAAS detection, proved to be efficient, reproducible, and sensitive for measuring trace levels of heavy metals in vegetable oils [43]. The World Health Organization (WHO) classifies arsenic and lead as priority contaminants because of their cumulative

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toxicity, carcinogenic and neurotoxic effects, and the lack of a safe exposure threshold, especially for lead. Additionally, lichens like *U. barbata* are well-known bioaccumulators of atmospheric pollutants, with arsenic and lead among the most frequently reported metals in the literature [44]. From an analytical perspective, both elements are known to migrate into lipid-based matrices and can be accurately measured at trace levels using GF-AAS [45]. In contrast, other metals such as Fe, Zn, and Cu are either essential, naturally abundant, or less relevant from a safety standpoint in the present study.

Excessive UV radiation can promote the development of stress-resistant microorganisms. Thus, the antimicrobial activity of KO and UBKO was evaluated against the most common pathogens (*S. aureus, E. coli,* and *C. albicans*); our results revealed a significant antimicrobial and antibiofilm activity of UBKO on *S. aureus* and *C. albicans.* They are similar to literature data that confirm broad-spectrum antibacterial efficacy, particularly against Gram-positive organisms [36,46]. In UBKO, usnic acid is a potent antibacterial agent against Gram-positive bacteria; its dibenzofuran structure inhibits RNA and DNA synthesis and blocks DNA replication and elongation [46]. Karanja oil-specific phenolic constituents (karanjin, pongamol, and cycloart-23-ene-3β,25-diol) act through a different route — their bactericidal activity is primarily due to inhibition of bacterial cell membrane synthesis [13]. The antibacterial and antifungal activities of Pongame Oil Tree seeds are well documented in the literature. Additional studies confirmed inhibitory effects on *S. aureus, E. coli,* and *C. albicans* [47–50]. Devidas et al. reported that unsaturated fatty acids (linoleic, oleic, linolenic) in the oil also independently contribute to antimicrobial activity through membrane-disruptive mechanisms [22]. Thus, the UBKO anti-staphylococcal and anti-candida efficacies are significantly higher than those of KO. Moreover, the KO biofilm-formation stimulation across all pathogens at MIC/4 can be explained. All our results suggest a potential synergistic effect of triterpenoids and flavonoids, which could represent a new approach for treating bacterial infections [51].

Phenolic metabolites and unsaturated fatty acids also contribute to the antioxidant and sunscreen properties of UBKO and KO. UBKO's higher phenolic content, derived from lichen secondary metabolites, gives it stronger DPPH-radical scavenging activity, better oxidative stability, and a greater photoprotective effect than KO.

Beyond bioactivity, an important aspect of this lichen extract in KO is its physicochemical compatibility. Usnic acid is lipophilic; it is nearly insoluble in water but dissolves easily in oils and organic solvents [52]. Karanja oil, rich in oleic acid, naturally solubilizes lipophilic polyphenols. The oily matrix of KO could serve as an ideal carrier for usnic acid, potentially enhancing its skin bioavailability by promoting penetration, as demonstrated with *U. barbata* extract in canola oil [40]. Lipid-based systems address the instability and poor solubility of lipophilic actives. Nanostructured lipid carriers co-encapsulating flavonoids can achieve synergistic antimicrobial effects against multidrug-resistant pathogens. Furthermore, Karanja oil's emollient fatty acids can help counteract the potential drying or irritating effects of usnic acid at higher concentrations, improving tolerability — especially in sensitive-skin formulations.

5. Conclusions

The results of this complex approach suggest that *Usnea barbata* lichen extract in *Pongamia pinnata* (Karanja) seed oil may form a synergistic combination with antimicrobial and photoprotective properties for skincare. Further research should focus on testing usnic acid plus karanjin against *S. aureus* and other bacteria and fungi responsible for skin infections to accurately assess their synergistic interactions. Additional studies are also needed to verify whether Karanja oil improves usnic acid delivery compared to an aqueous system, and to conduct a clinical pilot trial in inflammatory skin conditions using a combined formulation versus each ingredient alone.

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Abbreviations

The following abbreviations are used in this manuscript:

AFM	Atomic Force Microscopy
FTIR	Fourier Transform Infrared spectroscopy
GFAAS	Graphite Furnace Atomic Absorption Spectrophotometry
KO	Karanja oil
UBKO	*Usnea barbata* extract in KO
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
UHPLC	Ultra High-Performance Liquid Chromatography
OD	Optical density
ΔOD	Decrease in optical density
SPF	Sun Protection Factor
AC	Adhesion capacity
MIC	Minimum inhibitory concentration
ROS	Reactive oxygen species
TRPM8	Transient Receptor Potential Cation Channel Subfamily M Member 8
TNF	Tumor Necrosis Factor-Alpha

References

1. Rybczyńska-Tkaczyk, K.; Grenda, A.; Jakubczyk, A.; Kiersnowska, K.; Bik-Małodzińska, M. Natural Compounds with Antimicrobial Properties in Cosmetics. *Pathogens* 2023, *12*, 320, doi:10.3390/pathogens12020320.
2. Pop, R.-A.; Săplăcan, Z.; Alt, M.-A. Social Media Goes Green—The Impact of Social Media on Green Cosmetics Purchase Motivation and Intention. *Information* 2020, *11*, 447, doi:10.3390/info11090447.
3. Gălbău, C.-Ş.; Irimie, M.; Neculau, A.E.; Dima, L.; Pogačnik da Silva, L.; Vârciu, M.; Badea, M. The Potential of Plant Extracts Used in Cosmetic Product Applications—Antioxidants Delivery and Mechanism of Actions. *Antioxidants* 2024, *13*, 1425, doi:10.3390/antiox13111425.
4. Lyss, G.; Schmidt, T.J.; Merfort, I.; Pahl, H.L. Helenalin, an Anti-Inflammatory Sesquiterpene Lactone from Arnica, Selectively Inhibits Transcription Factor NF-KB. *Biol. Chem.* 1997, *378*, doi:10.1515/bchm.1997.378.9.951.
5. Araújo, L.U.; Reis, P.G.; Barbosa, L.C.O.; Saúde-Guimarães, D.A.; Grabe-Guimarães, A.; Mosqueira, V.C.F.; Carneiro, C.M.; Silva-Barcellos, N.M.S. In Vivo Wound Healing Effects of Symphytum Officinale L. Leaves Extract in Different Topical Formulations. *Pharmazie* 2012, *67*, 355–360, doi:10.1691/ph.2012.1563.
6. Prateeksha; Paliya, B.S.; Bajpai, R.; Jadaun, V.; Kumar, J.; Kumar, S.; Upreti, D.K.; Singh, B.R.; Nayaka, S.; Joshi, Y.; et al. The Genus Usnea: A Potent Phytomedicine with Multifarious Ethnobotany, Phytochemistry and Pharmacology. *RSC Adv.* 2016, *6*, 21672–21696, doi:10.1039/c5ra24205c.

 doi:10.20944/preprints202605.0159.v1

7. Thakre, R.; Jamane, S.; Yeole, S.; Kathale, P.; Tiple, R. FORMULATION AND EVALUATION OF KARANJA-BASED HERBAL SUNSCREEN. *World Journal of Pharmaceutical Research www.wjpr.net* / 2023, *12*.
8. Sharma, A.; Kaushik, N.; Rathore, H. Karanja (Milletia Pinnata (L.) Panigrahi): A Tropical Tree with Varied Applications. *Phytochemistry Reviews* 2020, *19*, 643–658, doi:10.1007/s11101-020-09670-z.
9. Gore, V.K.; Satyamoorthy, P. Determination of Pongamol and Karanjin in Karanja Oil By Reverse Phase Hplc. *Anal. Lett.* 2000, *33*, 337–346, doi:10.1080/00032710008543056.
10. Salgado, F.; Albornoz, L.; Cortéz, C.; Stashenko, E.; Urrea-Vallejo, K.; Nagles, E.; Galicia-Virviescas, C.; Cornejo, A.; Ardiles, A.; Simirgiotis, M.; et al. Secondary Metabolite Profiling of Species of the Genus Usnea by UHPLC-ESI-OT-MS-MS. *Molecules* 2018, *23*, 54, doi:10.3390/molecules23010054.
11. Popovici, V.; Bucur, L.; Popescu, A.; Caraiane, A.; Badea, V. Determination of the Content in Usnic Acid and Polyphenols from the Extracts of Usnea Barbata L. And the Evaluation of Their Antioxidant Activity. *Farmacia* 2018, *66*, 337–341.
12. Sangwan, S.; Rao, D.V.; Sharma, R.A. A Review on Pongamia Pinnata (L.) Pierre: A Great Versatile Leguminous Plant. *Nat. Sci. (East. Lansing).* 2010, *8*, 1.
13. Baswa, M.; Rath, C.C.; Dash, S.K.; Mishra, R.K. Antibacterial Activity of Karanj (Pongamia Pinnata) and Neem (Azadirachta Indica) Seed Oil: A Preliminary Report. *Microbios* 2001, *105*, 183–189.
14. Dwivedi, D.; Dwivedi, M.; Malviya, S.; Singh, V. Evaluation of Wound Healing, Anti-Microbial and Antioxidant Potential of Pongamia Pinnata in Wistar Rats. *J. Tradit. Complement. Med.* 2017, *7*, 79–85, doi:10.1016/j.jtcme.2015.12.002.
15. Živković, M.; Kukić-Marković, J.; Milenković, M.; Nikolić, G.; Savić, I.; Zlatković, S.; Kundaković, T. The Antimicrobial Activity of Usnea Barbata Ethanol Extract. *Planta Med.* 2009, *75*, PJ77, doi:10.1055/s-0029-1234882.
16. Basiouni, S.; Fayed, M.A.A.; Tarabees, R.; El-Sayed, M.; Elkhatam, A.; Töllner, K.R.; Hessel, M.; Geisberger, T.; Huber, C.; Eisenreich, W.; et al. Characterization of Sunflower Oil Extracts from the Lichen Usnea Barbata. *Metabolites* 2020, *10*, 1–16, doi:10.3390/metabo10090353.
17. Popovici, V.; Bucur, L.A.; Schröder, V.; Gherghel, D.; Mihai, C.T.; Caraiane, A.; Badea, F.C.; Vochița, G.; Badea, V. Evaluation of the Cytotoxic Activity of the Usnea Barbata (L.) F. H. Wigg Dry Extract. *Molecules* 2020, *25*, 1865, doi:10.3390/molecules25081865.
18. Engel, K.; Schmidt, U.; Reuter, J.; Weckesser, S.; Simon-Haarhaus, B.; Schempp, C.M. Usnea Barbata Extract Prevents Ultraviolet-B Induced Prostaglandin E2 Synthesis and COX-2 Expression in HaCaT Keratinocytes. *J. Photochem. Photobiol. B* 2007, *89*, doi:10.1016/j.jphotobiol.2007.08.002.
19. Al Muqarrabun, L.M.R.; Ahmat, N.; Ruzaina, S.A.S.; Ismail, N.H.; Sahidin, I. Medicinal Uses, Phytochemistry and Pharmacology of Pongamia Pinnata (L.) Pierre: A Review. *J. Ethnopharmacol.* 2013, *150*, 395–420, doi:10.1016/j.jep.2013.08.041.
20. Thierry Bernoud; Parfait Ramiandrasoa Photoprotective Composition WO2014016349A1. *Google Patents* 2014.
21. Mohini Shekhar Dhule, M.S.D.; Sukanya Vilas Lad, S.V.L.; Mr. Krishna Khamkar, Mr.K.K.; Dr.Rajnikant Kakade., Dr.R.Kakade. A Study on the Preparation and Evaluation of an Herbal Antifungal Spray. *International Journal of Pharmaceutical Research and Applications* 2025, *10*, doi:10.35629/4494-1003669679.
22. Devidas, T.B.; Vyas, A.; Sridhar, K.; Chawla, P.; Bains, A.; Sharma, M. Valorization of Pongame Oiltree (Millettia Pinnata) Seed and Seed Oil: A Promising Source of Phytochemicals and Its Applications. *Waste Biomass Valorization* 2024, *15*, 5705–5717, doi:10.1007/s12649-023-02352-9.
23. Shiven, A.; Alam, A.; Dewangan, H.K.; Shah, K.; Alam, P.; Kapoor, D.N. Optimisation and in-Vivo Evaluation of Extracted Karanjin Loaded Liposomal Topical Formulation for Treatment of Psoriasis in Tape-Stripped Mouse Model. *J. Microencapsul.* 2024, *41*, 345–359, doi:10.1080/02652048.2024.2354249.
24. Ansari, S.A.; Qadir, A.; Warsi, M.H.; Mujeeb, Mohd.; Aqil, Mohd.; Mir, S.R.; Sharma, S. Ethosomes-Based Gel Formulation of Karanjin for Treatment of Acne Vulgaris: In Vitro Investigations and Preclinical Assessment. *3 Biotech* 2021, *11*, 456, doi:10.1007/s13205-021-02978-3.
25. Popovici, V.; Matei, E.; Cozaru, G.; Bucur, L.; Gîrd, C.E.; Schröder, V.; Ozon, E.A.; Sarbu, I.; Musuc, A.M.; Atkinson, I.; et al. Formulation and Development of Bioadhesive Oral Films Containing Usnea Barbata (L

 doi:10.20944/preprints202605.0159.v1

.) F . H . Wigg Dry Ethanol Extract (F-UBE-HPC) with Antimicrobial and Anticancer Properties for Potential Use in Oral Cancer Complementary Therapy. *Pharmaceutics* 2022, *14*, 1808.

26. Araújo, E.; Pereira, E.; da Costa, M.; da Silva, N.; de Oliveira, H. Bactericidal Activity of Usnic Acid-Loaded Electrospun Fibers. *Recent Pat. Nanotechnol.* 2016, *10*, 252–257, doi:10.2174/1872210510666160517160144.
27. Zhao, X.; Shi, Y.; Niu, S.; Wei, X.; Liu, T.; Yang, M.; Wu, M.; Gao, G.; Ma, T.; Li, G. Enhancing Wound Healing and Bactericidal Efficacy: A Hydrogel Membrane of Bacterial Cellulose and Sanxan Gel for Accelerating the Healing of Infected Wounds. *Adv. Healthc. Mater.* 2024, *13*, doi:10.1002/adhm.202303216.
28. Anwar, F.; Tariq, M.; Nisar, J.; Ali, G.; Kanwal, H. Optimization of Biodiesel Yield from Non-Food Karanja Seed Oil: Characterization and Assessment of Fuel Properties. *Sustainable Chemistry for the Environment* 2023, *3*, 100035, doi:10.1016/j.scenv.2023.100035.
29. FAO/WHO, 1995 GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD AND FEED (CODEX STAN 193-1995) Adopted in 1995 Revised in 1997, 2006, 2008, 2009 Amended in 2010, 2012, 2013, 2014, 2015. *GENERAL STANDARD FOR CONTAMINANTS AND TOXINS IN FOOD AND FEED (CODEX STAN 193-1995)* 2015, *1*.
30. Assubaie, F.N. Assessment of the Levels of Some Heavy Metals in Water in Alahsa Oasis Farms, Saudi Arabia, with Analysis by Atomic Absorption Spectrophotometry. *Arabian Journal of Chemistry* 2015, *8*, 240–245, doi:10.1016/j.arabjc.2011.08.018.
31. Commission Regulation (EC) Commission Regulation (EC) No 118/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs. *Official Journal of the European Union* 2006, *364*, 5–24.
32. Pawar, R. V.; Hulwan, D.B. Rheological and Tribological Evaluation of Sustainable Biolubricants Derived from Jatropha and Karanja Oils for Crankshaft Bearing Applications. *Tribol. Int.* 2025, *211*, 110875, doi:10.1016/j.triboint.2025.110875.
33. Gobi, R.; Ravichandiran, P.; Babu, R.S.; Yoo, D.J. Biopolymer and Synthetic Polymer-Based Nanocomposites in Wound Dressing Applications: A Review. *Polymers (Basel).* 2021, *13*, 1962, doi:10.3390/polym13121962.
34. Tsao, C.-H.; Chang, C.-W.; Ho, Y.-C.; Chuang, Y.-K.; Lee, W.-J. Application of OXITEST for Prediction of Shelf-Lives of Selected Cold-Pressed Oils. *Front. Nutr.* 2021, *8*, 763524, doi:10.3389/fnut.2021.763524.
35. Ranković, B.; Kosanić, M.; Stanojković, T.; Vasiljević, P.; Manojlović, N. Biological Activities of Toninia Candida and Usnea Barbata Together with Their Norstictic Acid and Usnic Acid Constituents. *Int. J. Mol. Sci.* 2012, *13*, 14707–14722, doi:10.3390/ijms131114707.
36. Popovici, V.; Bucur, L.; Calcan, S.I.; Cucolea, E.I.; Costache, T.; Rambu, D.; Schröder, V.; Gîrd, C.E.; Gherghel, D.; Vochita, G.; et al. Elemental Analysis and In Vitro Evaluation of Antibacterial and Antifungal Activities of Usnea Barbata (L.) Weber Ex F.H. Wigg from C ă Limani Mountains, Romania. *Plants* 2022, *11*, 32.
37. Popovici, V.; Bucur, L.; Popescu, A.; Schröder, V.; Costache, T.; Rambu, D.; Cucolea, I.E.; Gîrd, C.E.; Caraiane, A.; Gherghel, D.; et al. Antioxidant and Cytotoxic Activities of Usnea Barbata (L.) F.H. Wigg. Dry Extracts in Different Solvents. *Plants* 2021, *10*, 909, doi:10.3390/plants10050909.
38. Popovici, V.; Bucur, L.; Costache, T.; Gherghel, D.; Vochita, G.; Mihai, C.T.; Rotinberg, P.; Schroder, V.; Badea, F.C.; Badea, V. Studies on Preparation and UHPLC Analysis of the Usnea Barbata (L) F.H.Wigg Dry Acetone Extract. *Revista de Chimie* 2019, *70*, 3775–3777, doi:10.37358/rc.70.19.11.7645.
39. Popovici, V.; Bucur, L.; Gîrd, C.E.; Popescu, A.; Matei, E.; Caraiane, A.; Botnarciuc, M. Phenolic Secondary Metabolites and Antiradical and Antibacterial Activities of Different Extracts of Usnea Barbata (L .) Weber Ex F . H . Wigg from C ă Limani Mountains , Romania. *Pharmaceuticals* 2022, *15*, 829, doi:https://doi.org/10.3390/ph15070829.
40. Popovici, V.; Bucur, L.; Gîrd, C.E.; Rambu, D.; Calcan, S.I.; Cucolea, E.I.; Costache, T.; Ungureanu-Iuga, M.; Oroian, M.; Mironeasa, S.; et al. Antioxidant , Cytotoxic , and Rheological Properties of Canola Oil Extract of Usnea Barbata (L .) Weber Ex F . H . Wigg from Călimani Mountains , Romania. *Plants* 2022, *11*, 854, doi:10.3390/plants11070854.
41. Popovici, V.; Matei, E.; Cozaru, G.C.; Aschie, M.; Bucur, L.; Rambu, D.; Costache, T.; Cucolea, I.E.; Vochita, G.; Gherghel, D.; et al. Usnic Acid and Usnea Barbata (L.) F.H. Wigg. Dry Extracts Promote Apoptosis and

 doi:10.20944/preprints202605.0159.v1

DNA Damage in Human Blood Cells through Enhancing ROS Levels. *Antioxidants* 2021, *10*, 1171, doi:10.3390/antiox10081171.

42. Wati, M.; Rani, P.; Charan Singh, C.; Khabiruddin, M. Studies on Pharmacology and Phytochemistry of Pongamia Pinnata. *Int. J. Chem. Stud.* 2018, *6*, 2555–2559.
43. Păduraru, D.N.; Coman, F.; Ozon, E.A.; Gherghiceanu, F.; Andronic, O.; Ion, D.; Stănescu, M.; Bolocan, A. The Use of Nutritional Supplement in Romanian Patients – Attitudes and Beliefs. *Farmacia* 2019, *67*, 1060–1065, doi:10.31925/farmacia.2019.6.18.
44. Conti, M.E.; Tudino, M.B. Lichens as Biomonitors of Heavy-Metal Pollution. *Comprehensive Analytical Chemistry* 2016, *73*, 117–145, doi:10.1016/bs.coac.2016.02.005.
45. Paoli, L.; Vannini, A.; Monaci, F.; Loppi, S. Competition between Heavy Metal Ions for Binding Sites in Lichens: Implications for Biomonitoring Studies. *Chemosphere* 2018, *199*, doi:10.1016/j.chemosphere.2018.02.066.
46. Sepahvand, A.; Studzińska-Sroka, E.; Ramak, P.; Karimian, V. Usnea Sp.: Antimicrobial Potential, Bioactive Compounds, Ethnopharmacological Uses and Other Pharmacological Properties; a Review Article. *J. Ethnopharmacol.* 2021, *268*, 113656, doi:10.1016/j.jep.2020.113656.
47. Vidhale, D.K.; Vamshi Reddy Mora; Sairam Pasam; Aishwarya Kotha; Konda, U.; Narender Boggula Critical Review on Pongamia Pinnata: A Versatile Medicinal Plant. *Asian Journal of Pharmaceutical Research and Development* 2024, *12*, 138–146, doi:10.22270/ajprd.v12i4.1455.
48. Sajid, Z.I.; Anwar, F.; Shabir, G.; Rasul, G.; Alkharfy, K.M.; Gilani, A.-H. Antioxidant, Antimicrobial Properties and Phenolics of Different Solvent Extracts from Bark, Leaves and Seeds of Pongamia Pinnata (L.) Pierre. *Molecules* 2012, *17*, 3917–3932, doi:10.3390/molecules17043917.
49. Asaiya, A.; Raj, D.; Prajapati, C. Antibacterial Effect of Pongamia Pinnata Leaf Extract against Some Human Pathogenic Bacteria. *Journal of Plant Pathology & Microbiology Research Article 1 J Plant Pathol Microbiol* 2021, *12*.
50. Chithra, B.K.; Anand, P.P.; Pushpalatha, E.; Vardhanan, Y.S.; Shareef, K.P.M. Bio-Augmentative Antibacterial Activity of <I>Pongamia Pinnata</I> (L.) Gall Extract with Lemongrass Oil Formulation against Selected Gram-Negative Bacteria. *Curr. Sci.* 2024, *127*, 1219, doi:10.18520/cs/v127/i10/1219-1226.
51. Wrońska, N.; Szlaur, M.; Zawadzka, K.; Lisowska, K. The Synergistic Effect of Triterpenoids and Flavonoids—New Approaches for Treating Bacterial Infections? *Molecules* 2022, *27*, 847, doi:10.3390/molecules27030847.
52. Ingólfsdóttir, K. Usnic Acid. *Phytochemistry* 2002, *61*, 729–736, doi:10.1016/S0031-9422(02)00383-7.
53. Dan, M.A.; Cioancă, O.; Popovici, V.; Musuc, A.M.; Nițulescu, G.M.; Anastasescu, M.; Ozon, E.A.; Marinas, I.C.; Guțu, C.M.; Baconi, D.L.; et al. Phytochemical Screening and Physicochemical Properties of Oil Extract of Usnea Barbata L. F.H.Wigg from Călimani Mountains, Romania. *Int. J. Mol. Sci.* 2026, *27*, 1903, doi:10.3390/ijms27041903.
54. Ungureanu, A.R.; Musuc, A.M.; Ozon, E.A.; Anastasescu, M.; Atkinson, I.; Mitran, R.-A.; Rusu, A.; Luță, E.-A.; Chițescu, C.L.; Gîrd, C.E. Physicochemical Investigations on Samples Composed of a Mixture of Plant Extracts and Biopolymers in the Broad Context of Further Pharmaceutical Development. *Polymers (Basel).* 2025, *17*, 1499, doi:10.3390/polym17111499.
55. Ungurianu, A.; Şeremet, O.; Gagniuc, E.; Olaru, O.T.; Guțu, C.; Grădinaru, D.; Ionescu-Tîrgovişte, C.; Margină, D.; Dănciulescu-Miulescu, R. Preclinical and Clinical Results Regarding the Effects of a Plant-Based Antidiabetic Formulation versus Well Established Antidiabetic Molecules. *Pharmacol. Res.* 2019, *150*, 104522, doi:10.1016/j.phrs.2019.104522.
56. Nicholson, S.K.; Tucker, G.A.; Brameld, J.M. Physiological Concentrations of Dietary Polyphenols Regulate Vascular Endothelial Cell Expression of Genes Important in Cardiovascular Health. *British Journal of Nutrition* 2010, *103*, 1398–1403, doi:10.1017/S0007114509993485.
57. MARGINĂ, D.; OLARU, O.T.; ILIE, M.; GRĂDINARU, D.; GUȚU, C.; VOICU, S.; DINISCHIOTU, A.; SPANDIDOS, D.A.; TSATSAKIS, A.M. Assessment of the Potential Health Benefits of Certain Total Extracts from Vitis Vinifera, Aesculus Hyppocastanum and Curcuma Longa. *Exp. Ther. Med.* 2015, *10*, 1681–1688, doi:10.3892/etm.2015.2724.

 doi:10.20944/preprints202605.0159.v1

58. Dan, M.A.; Nedea, M.I.; Ozon, E.A.; Ungurianu, A.; Ungureanu, A.R.; Popovici, V.; Musuc, A.M.; Bratan, V.; Cazacincu, R.G.; Arsene, A.L.; et al. Antioxidant, Photoprotective, and Antimicrobial Potential of Oil Extract of Usnea Barbata L. F.H.Wigg from Călimani Mountains, Romania. *Molecules* 2026, *31*, 1324, doi:10.3390/molecules31081324.
59. Nedea (Ilie), M.I.; Bădiceanu, C.D.; Gheorghe-Barbu, I.; Marinaș, I.C.; Pericleanu, R.; Dragomir, R.-I.; Dumbravă, A. Ștefania; Dascălu, A.M.; Șerban, D.; Tudor, C.; et al. Antifungal and Antioxidant Activity of Thiourea Derivatives Against Nosocomial Candida Auris Strains Isolated in Romania. *Molecules* 2025, *30*, 1675, doi:10.3390/molecules30081675.
60. Nagy, S.-R.; Mititelu, M.; Popovici, V.; Bontea, M.G.; Pallag, A.; Jurca, T. Protein and Amino Acid Supplementation Among Recreational Gym Goers and Associated Factors—An Exploratory Study. *J. Funct. Morphol. Kinesiol.* 2025, *10*, 248, doi:10.3390/jfmk10030248.
61. Neagu, R.; Popovici, V.; Ionescu, L.E.; Ordeanu, V.; Popescu, D.M.; Ozon, E.A.; Gîrd, C.E. Antibacterial and Antibiofilm Effects of Different Samples of Five Commercially Available Essential Oils. 2023, *12*, 1191.