

Article

Nanoemulsions Using Sustainable NaDES-Based Tobacco Waste Extracts for Cosmetic Applications

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Abstract

Demand for cosmetics based on green production and the circular economy is growing. The inflorescences and apical leaves of *Nicotiana tabacum* (tobacco) after blunting, deflowering, or topping are considered pre-harvest waste biomass. Using green and ecofriendly solvents such as natural deep eutectic solvents (NaDESs) offers a sustainable way to make use of this biomass for incorporation in cosmetic formulations. The inflorescence and apical leaves of tobacco var Virginia were therefore dried, powdered, and extracted using a NaDES composed of choline-chloride, urea, and distilled water (NaDES CU). The resulting inflorescence and apical leaves extracts showed high concentrations of phenolic compounds and flavonoids. Both extracts demonstrated significant biological activity and effectively inhibited tyrosinase, the enzyme responsible for melanin regulation and skin aging ($IC_{50} = 50 \mu\text{g GAE/mL}$), as well as showing antioxidant capacity ($ABTS^{\bullet+}$; $SC_{50} = 1.7\text{--}7 \mu\text{g GAE/mL}$). Ten nanoemulsions containing tobacco leaf- and inflorescence extract-based NaDES CU, formulated using different polysorbates, deionized water and glycerin, were produced. A low-energy emulsification technique at a constant temperature was applied. Considering the droplet size and polydispersity index, only the nanoemulsions containing inflorescence and leaf extracts based on NaDES CU and containing 5% or 10% polysorbate 85 were selected for further stability assessment and characterization. This study highlights the potential of NaDES combined with tobacco waste extracts as a sustainable and non-toxic ingredient in anti-aging and antioxidant cosmetics.

Keywords: cosmetic formulation; nanoemulsion; tobacco; waste; antityrosinase; NaDES



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1. Introduction

According to current knowledge, ultraviolet radiation and oxidative stress are the main causes of premature aging and some cutaneous damage and diseases, including

skin cancer. In recent years, there has been growing scientific interest in anti-aging strategies, particularly those involving natural antioxidant compounds such as polyphenols, which have shown potential in modulating biological processes related to aging. Several tyrosinase inhibitors have also been identified for their ability to treat skin conditions such as age spots, sagging, wrinkles, freckles, and hyperpigmentation [1–3]. The distinction between the cosmetics industry and topical pharmaceutical products is becoming increasingly blurred due to the ongoing rise in consumer demand for improved product efficacy. Although the active ingredient should not be systemically absorbed after topical application, significant penetration of the active ingredient into the skin is necessary for a cosmetic effect. Several parameters affect how well the active ingredient penetrates the skin, including the molecular size, degree of ionization, lipophilicity, the synergy between the active compound and the base component of the formula, and the synergy between the formulation and skin [3].

In this context, nanoemulsions are a suitable choice for cosmetic applications due to their small droplet size and similarity to the composition and barrier properties of the skin structure, suggesting that lipid-based formulations are among the most suitable for the topical delivery of active chemicals [4–8]. These colloidal dispersions are thermodynamically unstable; however, kinetic stability is reached due to the small droplet size, which differs from that of conventional emulsions. They are composed of two immiscible liquids, which are often mixed with emulsifying agents (e.g., surfactants and co-surfactants) to form a dispersed phase in an external medium. Nanoemulsions have emerged as drug delivery systems with cosmetic applications due to their characteristic sub-micron scale properties (in the range 20–200 nm), which include a high interfacial area, a transparent or translucent appearance, high solubilization capacity, a low viscosity, and high kinetic stability against sedimentation, flocculation, coalescence and Ostwald ripening [4–8]. Nanoemulsions can be generated using two different approaches: high-energy and low-energy methods [7,8].

The use of plant components, such as extracts and essential oils, has increased notably in cosmetic formulations due to their bioactive chemical components [7]. Chemical studies have revealed that tobacco leaves and inflorescences (Figure 1) are rich in sterols, terpenes, alkaloids, and phenolic compounds [9–26]. Phenolic compounds, including flavonols such as rutin, quercetin and isoquercitrin, kaempferol and quercetin derivatives, and anthocyanins such as cyanidin, delphinidin, peonidin, and petunidin, as well as phenolic acids such as phenylacetic acid, quinic acid derivatives, chlorogenic acid, gallic acid, caffeic acid, ferulic acid, sinapic acid, and others, were described in the inflorescence [9,11,15,19,20,22,25] and leaves [9,12–14,16,19,21,23,24,26] of tobacco. One might expect the general public to have a negative perception regarding the tobacco industry and its products. In fact, the World Health Organization report discusses crop transition in tobacco cultivation due to a decline in cigarettes and other non-health tobacco by-products. However, tobacco is a versatile plant with additional advantages and potential applications [27]. In fact, according to Popova et al. (2020) [20], tobacco derivatives are referenced in the Cosmetic Ingredient Database (CosIng) [28] and are included in the International Nomenclature of Cosmetic Ingredients (INCI).

Before the tobacco harvest, the inflorescences and apical leaves are traditionally discarded in the field in a process known as ‘topping’ or ‘trimming’ (Figure 1). This involves cutting the flowers and some lateral buds by hand to prevent the production of new leaves and promote the growth of the remaining leaves for use in the cigarette industry. Therefore, tobacco inflorescences and apical leaves are considered pre-harvest waste or biomass. Given the diversity of specialized metabolites previously described in these organs, this waste could be used for different purposes. Recently, polyphenolic extracts with antioxidant activity were obtained from tobacco inflorescences and apical leaves, using natural deep

eutectic solvents (NaDESs) for the first time [24–26]. The inhibitory activity of elastase and collagenase enzymes was also reported for apical leaf extracts based on NaDES [26]. These solvents (NaDES) are considered “green solvents” and have several advantages over traditional solvents. They are easy to prepare using renewable/nontoxic natural components, such as organic base and acid, sugar, choline chloride, urea, amino acids, besides being low-cost and biodegradable [29,30]. Furthermore, NaDES have demonstrated both chemical and thermal stability during storage, facilitating their subsequent incorporation into pharmaceutical and/or cosmetic products [31,32].



Figure 1. *Nicotiana tabacum* var. Virginia, (a) flowers and apical leaves, pre-harvest waste produced during topping in the field; (b) pre-harvest waste discarded in the field (Perico, Jujuy, Argentina).

Tobacco inflorescences and leaves, extracts, and oils are referenced in the Cosmetic Ingredient Database, CosIng [28], as cosmetic ingredients. Several cosmetic products have been developed using tobacco-derived materials, including tobacco flower concrete [2its0], gels containing phytosomes [33], and a cosmetic gel formulated with resinoid from tobacco obtained with ethanol [34]. In this context, polyphenol-rich extracts derived from tobacco waste and obtained using green solvents [24–26] are promising candidates for the formulation of potentially non-toxic cosmetic and dermatological products, such as nanoemulsions.

Based on the previous analysis, this study aimed to evaluate the antioxidant and anti-tyrosinase activities of *N. tabacum* extracts obtained from inflorescences and apical leaves (considered pre-harvest waste) using a NaDES composed of choline chloride and urea (NaDES CU), to develop stable nanoemulsions as a prototype for cosmetical applications.

2. Materials and Methods

2.1. Reagents

Choline chloride (Sigma-Aldrich, Beijing, China), urea, ABTS, quercetin, Folin and Ciocalteu’s phenol reagent, kojic acid, tyrosinase, and gallic acid (Sigma Aldrich, St. Louis, MO, USA), aluminum chloride (Sigma-Aldrich, Taufkirchen, Germany), double-distilled glycerin (Farmax, Niterói, Brazil), polysorbate 20 (HLB = 16.7), polysorbate 80 (HLB = 15.0), and polysorbate 85 (HLB = 11) (Sigma-Aldrich, USA).

2.2. Plant Material

Apical leaves and inflorescences of *Nicotiana tabacum* var. Virginia that had been recently discarded were collected from a tobacco crop field in Perico, Jujuy, Argentina (26°18' S, 65°37' W, 1700 m a.s.l.). The samples were dried at 60 °C up to constant weight in a forced-air oven. Then, the plant material was ground in a screw mill (Metvisa®, Mod MP-200-Power ½ HP-0.75 Kw, Brusque, Brazil) and conserved in vacuum-sealed bags until use at room temperature.

2.3. NaDES Preparation

The NaDES composed of choline chloride, urea, and distilled water (NaDES CU) was prepared by combining the components in the appropriate mole ratios (1:2:1.5) according to the method described by Leal et al. (2023a,b) [24,25].

2.4. Extraction Procedure

The inflorescences and apical leaves were dried at 60 °C at constant weight in a forced air stove and ground in a Helix mill (Metvisa®, Mod MP-200-Power 1/2 HP-0.75 Kw, Brusque, Brazil) to obtain powder. The plant material was extracted by maceration in NaDES CU 1:10 (*w/v*) for 30 min and shaking at 100 rpm at 25 °C. The extracts were filtered through Whatman No. 1 filter paper and stored at −20 °C until further use [24,25]. Two extracts, named NaDES CU-based inflorescence extract (CUF) and NaDES CU-based leaves extract (CUL), were obtained. The extraction procedures were performed in triplicate.

2.5. Chemical Characterization of Extracts

Total Polyphenol and Flavonoid Quantification

The total phenolic content (TPC) of CUF and CUL was determined using the Folin–Ciocalteu reagent [35]. Different volumes of extracts were mixed with distilled water up to 1 mL, 100 µL of Folin–Ciocalteu reagent, and 400 µL of 15.9% sodium carbonate. The mixture was kept for 20 min at room temperature. The blue color developed was read at 765 nm in a UV/visible spectrophotometer (Jasco v-630, Thermo Fisher Scientific, Tokyo, Japan). Total flavonoid content (TF) was quantified following the method of Woisky and Salatino [36], using AlCl₃ as the complexing reagent. Different volumes of extracts were mixed with distilled water to a total of 2.45 mL. Then, 50 µL of 5% AlCl₃ solution was added. The mixture was kept for 30 min at room temperature. The absorbance was measured at 425 nm. NaDES CU was used as a blank control to correct any background interference. The determinations were performed in triplicate, and the results were expressed as µg of gallic acid equivalent per mL (µg GAE/mL) and quercetin equivalents per mL (µg QE/mL) to TPC and TF, respectively.

2.6. Biological Activities of Extracts

2.6.1. Tyrosinase Inhibition Assay

The inhibitory effect of CUF and CUL on tyrosinase activity was assayed. Tyrosinase activity was spectrophotometrically determined by measuring the hydrolyzed L-tyrosine according to Momtaz et al. (2008) [37], with modifications. Briefly, 5 µL of tyrosinase (333 Units/mL in sodium potassium phosphate buffer 50 mM, pH 6.5), different CUF and CUL concentrations (1–15 µg GAE/mL), 110 µL of substrate (0.54 mg/mL L-tyrosine) in a final volume of 210 µL were incubated for 30 min at 25 °C. The solvent control (NaDES CU) was also performed to determine any possible interference. Substrate control was also performed. Kojic acid was used as a positive control, and distilled water as a negative control. The absorbance was measured at 492 nm using a Microplate Reader (Thermo Scientific Multiskan GO, Vantaa, Finland). Results are expressed as IC₅₀ values. The IC₅₀

value ($\mu\text{g GAE/mL}$) was defined as the concentration of phenolic compounds necessary to inhibit 50% of tyrosinase activity.

2.6.2. Antioxidant Activity Assays Using ABTS Radical Cation ($\text{ABTS}^{\bullet+}$)

The antioxidant capacity of CUF and CUL was assessed by the improved $\text{ABTS}^{\bullet+}$ spectrophotometric method [38]. In this method, an $\text{ABTS}^{\bullet+}$ solution was mixed with various concentrations of the extracts ($1.9\text{--}13.8\ \mu\text{g GAE/mL}$) and kept at $25\ ^\circ\text{C}$. After 6 min, the absorbance was recorded with a UV/visible spectrophotometer (Jasco v-630, Thermo Fisher Scientific, Tokyo, Japan) at 734 nm. The solvent control (NaDES CU) was also performed to determine any possible interference. Quercetin was used as a reference compound. The results are expressed as SC_{50} values, which are defined as the concentration of phenolic compounds necessary to scavenge 50% ABTS free radicals ($\mu\text{g GAE/mL}$).

2.7. Nanoemulsification of Tobacco Extracts

2.7.1. Preparation

Nanoemulsions containing CUF and CUL were prepared using a low-energy emulsification technique at a constant temperature [39]. The CUF and CUL were individually mixed for 1 min in a vortex stirrer at 500 rpm with a mixture of deionized water and surfactant (5% or 10%) or glycerin and surfactant (5% or 10%) at a ratio of 1:20. A formulation containing NaDES CU as a solvent control without tobacco was also obtained in the same conditions. Water and glycerin were used as controls in the experiments. Emulsified NaDES without surfactant was used as a blank. The non-ionic surfactants used were polysorbate 20 (PS20), polysorbate 80 (PS80), and polysorbate 85 (PS85). All nanoemulsions were preserved in screw-capped vials at ambient temperature ($25 \pm 2\ ^\circ\text{C}$) protected from light exposure.

2.7.2. Characterization of Nanoemulsions

Macroscopic Characterization

The nanoemulsions were maintained at room temperature ($25 \pm 2\ ^\circ\text{C}$) and characterized according to macroscopical parameters, such as pH, color, visual appearance, phase separation, creaminess, and sedimentation over a 28-day period. Visual inspection was performed to ensure that the nanoemulsions met not only the physicochemical criteria, such as droplet stability, but also acceptable sensory attributes. Attention was given to any signs of precipitation, phase separation, color changes, or the appearance of bluish reflections.

Size and Polydispersity

The hydrodynamic size and the polydispersity of the formulation particles were evaluated. The dynamic light scattering (DLS) analysis was performed on a Zetasizer NanoZS (Malvern, UK) to determine the droplet size distribution, in terms of droplet size and polydispersity index (Pdl). The equipment has a 10 mW red laser ($\lambda = 632.8\ \text{nm}$), and samples were measured at a $173\ ^\circ\text{C}$ scattering detector angle for size measurements. The results were expressed as mean $\pm$ standard deviation, and the analyses were performed immediately after the nanoemulsification process and after 1 day and every 7 days for a period of 28 days of storage. Before the DLS measurements, all the nanoemulsions were diluted with deionized water at a 1:40 ratio and a 1:60 ratio for the formulations prepared with deionized water and glycerin, respectively. The droplet variation (DV) in terms of droplet size diameter was determined and expressed as follows:

$$\text{DV}_{x,y} = 100 \times [\text{mean size diameter (dy)} - \text{mean size diameter (dx)}] / \text{mean size diameter (dx)}$$

where $\text{DV}_{x,y}$ is the variation in the droplet size in time [39].

2.7.3. Stability Tests

For use in subsequent research, the optimum nanoemulsion was determined by selecting the system with the best overall properties (droplet size less than 200 nm and stability over time) [7].

Centrifugation Test

The centrifugation test was performed on each sample at 3000 rpm for 30 min at room temperature. At the end of the centrifugation, the cosmetic formulations were examined for phase separation and particle size [7].

Thermal Stress

A linear temperature ramp was used to examine the thermal stress of formulations using Zetasizer NanoZS (Panalytical Ltd., Malvern, UK). The temperature started at 25 ± 2 °C and increased to 65 ± 2 °C at 5 °C intervals, returning to 25 ± 2 °C. In each interval, particle size and polydispersity were evaluated. Zeta potential, measured through electrophoretic light scattering, was evaluated at the beginning and end of temperature ramp analysis. After thermal stress, the samples were subjected to a centrifugation test [7].

2.8. Statistical Analysis

For the statistical analysis of the data, the Tukey test was applied, with a level of significance $p < 0.05$, by using the statistical package InfoStat V1.1 [40].

3. Results and Discussion

Both CUF and CUL were prepared and subsequently characterized chemically and biologically for their antioxidant and anti-tyrosinase activities (Figure 2). Then, nanoemulsions were formulated using tobacco waste extracts as the active principle and were further characterized to evaluate their physicochemical properties and stability.

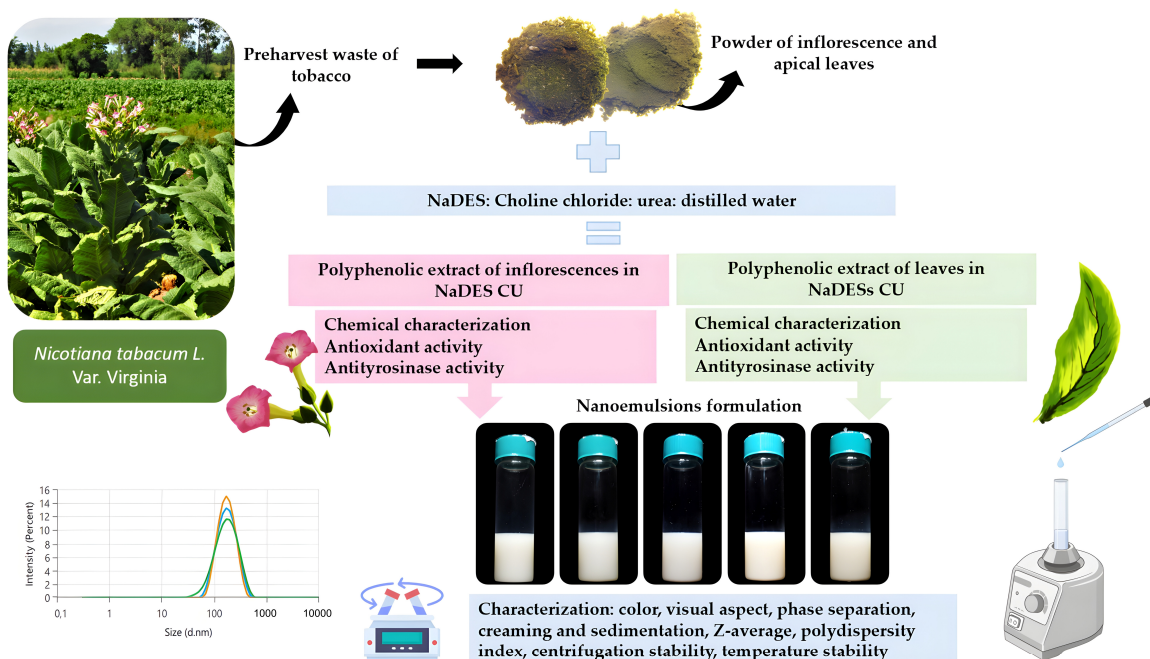


Figure 2. Flowchart of procedure to obtain inflorescences and apical leaves extracts of *Nicotiana tabacum* var. Virginia using choline chloride: urea (NaDES CU) as a solvent, including chemical and functional characterization, and subsequent formulation and characterization of nanoemulsions.

3.1. Characterization of Tobacco Extracts Based on NaDES CU

NaDES CU is a natural deep eutectic solvent consisting of choline chloride as a hydrogen bond acceptor and urea as a hydrogen bond donor. Based on previous work [24,25], NaDES CU was identified as one of the most effective for the extraction of TF and TPC from preharvest tobacco waste. In this paper, NaDES CU was selected to obtain extracts from tobacco leaves and inflorescences. Since each extract is a phytocomplex, to characterize them, it is valid to quantify a group of active compounds, such as phenolic compounds or flavonoids. The TPC and TF content of *N. tabacum* var. Virginia in CUF was higher than in CUL (Table 1). Phenolic-enriched extracts are currently considered to be among the most effective for the development of antioxidant and anti-aging cosmetics [41–43].

Table 1. Total phenolic compounds, total flavonoids, antioxidants and tyrosinase inhibition assays of tobacco waste extracts based on NaDES.

<i>N. tabacum</i> Extracts in NaDES CU	TPC μg GAE/mL	TF μg QE/mL	ABTS Scavenging (SC ₅₀ μg GAE/mL)	Inhibition of Tyrosinase (IC ₅₀ μg GAE/mL)
CUF	2892.7 ± 9.7	218.5 ± 3.2	6.5 ± 0.3	50.0 ± 0.8
CUL	2079.7 ± 3.7	137.2 ± 3.7	1.70 ± 0.2	50.0 ± 1.4
Quercetin μg/mL	-	-	1.40 ± 0.1	-
Kojic acid μg/mL	-	-	-	6.8 ± 0.7

TPC: Total phenolic compounds; TF: total flavonoids; SC₅₀: Scavenger concentration of 50% ABTS free radicals. IC₅₀: Inhibitory concentration of 50% of enzyme activity; GAE: gallic acid equivalent; QE: quercetin equivalent; CUF: NaDES CU based tobacco inflorescence extract; CUL: NaDES CU based tobacco leaves extract; NaDES CU: NaDES of choline chloride: urea.

3.2. Effect of Tobacco Extracts Based on NaDES CU on Tyrosinase Activity

The biosynthetic process known as melanogenesis is responsible for producing the melanin pigment in human skin. A key enzyme in this process is tyrosinase, which catalyzes the conversion of L-tyrosine to L-DOPA, followed by its oxidation to DOPA-quinone, ultimately leading to the formation of melanin. In this context, tyrosinase inhibitors are considered valuable skin-whitening agents, making them particularly relevant for the cosmetic industry [2,3,44,45].

Tyrosinase activity was significantly inhibited by polyphenol-enriched extracts from preharvest waste of tobacco (inflorescence and leaves) obtained in NaDES. The inhibitory potency of both extracts was similar, with IC₅₀ values of 50.0 ± 5.0 μg GAE/mL (Table 1). Both tested extracts containing a mixture of polyphenolic compounds exhibited higher IC₅₀ than those observed for pure kojic acid (reference compound). Phenolic compounds present in NaDES extracts may structurally resemble kojic acid and, as a result, enable them to inhibit tyrosinase activity [26]. In contrast, the NaDES CU itself did not exhibit tyrosinase activity at the assayed concentration. Consequently, both tobacco polyphenol-enriched extracts obtained with NaDES CU could be considered suitable for cosmetic applications without the need to remove the solvent (NaDES CU). This is the first report on the inhibitory activity of tyrosinase in *N. tabacum* extracts made with green solvents (NaDESs). However, the antityrosinase activity of an ethanolic extract of *Nicotiana tabacum* leaves was previously reported [23,46]. The anti-tyrosinase activity shown by the NaDES CU tobacco leaves extracts could be related to the presence of nicotiflorin, azelaic acid, rutin, and phenylacetic acid, as demonstrated recently by Leal et al. (2025) [26]. For example, rutin, one of the flavonoids reported in the NaDES CU tobacco leaf extracts, has demonstrated antioxidant [47], anti-elastase, anti-tyrosinase, and anti-hyaluronidase [48]

activities. Nicotiflorin has been reported to possess antioxidant, anti-inflammatory, and anti-hyaluronidase activities [49]. Azelaic acid is widely used in cosmetics and has demonstrated anti-inflammatory, anti-acne, and antibacterial [50–52] activities. Flavonoids, such as rutin, kaempferol, quercetin, and phenolic acids, such as quinic acid, among others, demonstrate anti-tyrosinase properties in tobacco flower ethanolic extracts [47,48,53,54].

3.3. Effect of Tobacco Extracts Based on NaDESs on Antioxidant Activity

Based on their function, antioxidants can be divided into primary or natural and secondary or synthetic antioxidants. Primary antioxidants comprise mineral antioxidants (such as selenium, copper, iron, zinc, and manganese), vitamins (C and E), and phyto-antioxidants. Natural antioxidants can be a single pure compound/isolate, a mixture of compounds, or plant extracts; these antioxidants are now widely used in cosmetic products. Several spectrophotometric methods can be used for measuring antioxidant capacity *in vitro*. According to recent research, polyphenols that were recovered from tobacco leaves and inflorescence waste using NaDESs scavenge free radicals by different mechanisms [24–26]. In this paper, the antioxidant activity of CUF and CUL on ABTS scavenging was confirmed (Table 1). The results showed that CUL was 3.8-fold more active than CUF. The potency of CUL was like that of the quercetin control.

3.4. Nanoemulsions Formulation

Based on the demonstrated antioxidant and anti-tyrosinase activities of the extracts obtained with NaDES CU from the inflorescences and apical leaves of pre-harvest tobacco waste, and the absence of toxicity previously demonstrated by Leal et al. 2025 [26], cosmetic nanoemulsion prototypes were formulated. The nanoemulsions incorporated each extract (CUL or CUF) with non-ionic surfactants (polysorbate 20, polysorbate 80, or polysorbate 85) and were prepared using a low-energy emulsification method. Also, a nanoemulsion containing NaDES CU was prepared with and without surfactants as a control. In previous studies, nanoemulsions containing ethanolic extracts of tobacco leaves were developed using high-energy techniques for agricultural applications, such as the control of citrus aphids (*Aphis gossypii*) and as biolarvicides against *Aedes aegypti* larvae [55,56]. However, to the best of our knowledge, this is the first report of a nanoemulsion formulated with tobacco industry waste extracts using a green solvent such as NaDES CU and a low-energy preparation method for potential cosmetic use.

The nanoemulsions prepared with NaDES CU and PS20 or PS80 in deionized water showed a transparent appearance and did not show organoleptic irregularities over time. The droplet size analysis revealed a very small diameter (<20 nm) (Figure S1a–d and Table 2). In contrast, nanoemulsions of NaDES CU prepared with PS85 (Figure S1e,f) showed an opaque appearance. Nanoemulsions of NaDES CU in glycerin with PS20, PS80 and PS85 (Figure S1g–l) exhibited an initial opaque appearance, and the particle size was outside the expected optimal range (20–200 nm), and/or high standard deviation, and/or high PDI was observed.

Nanoemulsions obtained from CUL (Figure S2a–d) and CUF (Figure S3a–d), formulated with PS20 and PS80 using deionized water, showed translucent appearance with high diameter (Table 2) and clear destabilization after 1 day of preparation. In contrast, nanoemulsions made with PS85 and deionized water, both from CUL (Figure S2e,f) and CUF (Figure S3e,f), showed remarkable stability, with a bluish reflection and optimal organoleptic characteristics that remained without visible changes during the 28 days of storage. The formulations made with glycerin (Figures S2g–l and S3g–l) exhibited evident opacity with phase separation and high PDI index or particle size (Table 2).

The nanoemulsions obtained from CUF and polysorbate 85, in deionized water and glycerin, showed a trend towards smaller particle sizes (Table 2). Despite being initially categorized as a nanoemulsion, the system prepared with PS85 at 10% in glycerin showed droplet growth. Consequently, only nanoemulsions of CUF in PS85, at 5 and 10% in deionized water, were selected for further stability evaluation. Regarding the nanoemulsions of CUL obtained with PS85 prepared with deionized water at both concentrations of surfactant, they also induced reduced particle diameter.

Dias et al. (2012) [57] revealed that some secondary metabolites (specialized metabolites) can be adsorbed at the interface of nanoemulsions. According to this evidence, the smaller particle size determined is probably explained by a higher content of phenolic compounds in the nanoemulsions of CUL and CUF (Table 2) compared to the blank formulation. Considering the possible presence of glycosylated phenolics, the reduction in surface tension may have contributed to better generation of nanodroplets. The stability of the CUL and CUF nanoemulsions in PS85 for 28 days is shown in Table 3. Based on the particle size and PDI values, the nanoemulsions remained stable for up to 28 days; however, it is worth mentioning that further stability studies are required for the human/commercial exploitation of such systems.

3.5. Nanoemulsions Characterization

3.5.1. Thermal Stability

For both nanoemulsions with CUL and CUF at 5% PS85, no change in droplet size was observed when the temperature increased from 25 °C to 45 °C (Figure 3a,b,e,f; Table 4), followed by a trend of growing droplet size up to 65 °C. However, when the temperature returned to its initial value, the particle size reverted almost to its original state. By analyzing the size intervals, their overall DV was less than 10%. This result, together with the findings that PDI varied only in a narrow range (0.2828–0.2324 for CUL and 0.2674–0.2282 for CUF), suggests that this nanoemulsion has adequate stability. For nanoemulsions of CUF and CUL at 10% PS85, no change in droplet size was observed from 25 °C to 45 °C (Figure 3c,d,g,h), and then a trend of increasing droplet size was seen up to 65 °C. At 25 °C, the particle size does not return to the original size with DV > 70%. This differs from the PDI, which varied only in a narrow range (0.2500–0.2649 for CUL and 0.2452–0.2714 for CUF). Although DV is high, the maximum particle size achieved is still within the optimal parameters.

The zeta potential of the nanoemulsions at room temperature (25 °C) was −12.61 to −15.98 mV, while after the thermal stress was −13.47 to −16.42 mV; there was no statistical significance ($p > 0.05$) at room temperature before and after the thermal stress for CUL and CUF nanoemulsions, suggesting relative stability after brief heating conditions. Even though the NaDES contain the cationic component choline, the negative surface charge of the nanodroplets is likely associated with the adsorption of chloride ions from the NaDES at the oil–water interface, leading to preferential anionic ionization of the external layer. The NaDES tobacco extract is rich in polyphenolic compounds that can act as weak conjugate bases, establishing electrostatic interactions with the positively charged choline moieties. This interaction likely reduces the association between choline and chloride ions in the aqueous external phase, leaving the chloride ions more available to migrate toward and interact at the oil–water interface. As a result, a partial anionic enrichment at the interface may occur, contributing to the negative zeta potential values observed in the nanoemulsions. Rather than the absolute magnitude of the zeta potential values, the preservation of their electrokinetic characteristics after the thermal exposure, since the stability of the system is not solely governed by electrostatic interactions, but also by

the steric stabilization provided by polysorbate 85 used as the non-ionic surfactant [58]. Altogether, it suggests that the evaluated nanoemulsions have adequate thermal stability.

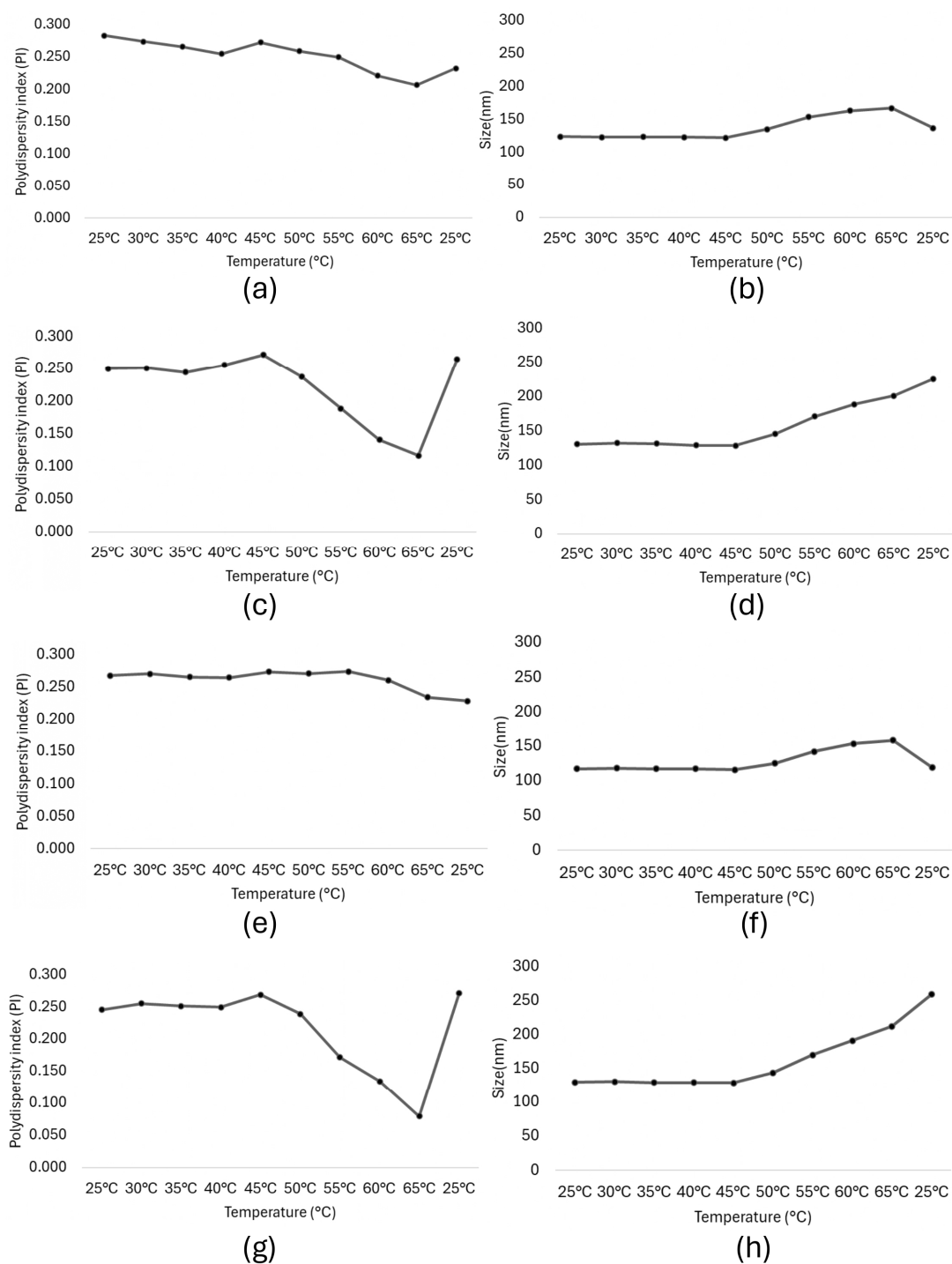


Figure 3. Effect of temperature on the nanoemulsions of CUL and CUF as a function of (a) droplet diameter and (b) polydispersity index in CUL water/PS85 5%; (c) droplet diameter and (d) polydispersity index in CUL water/PS85 10%; (e) the droplet diameter and (f) the polydispersity index in CUF water/PS85 5%; (g) the droplet diameter and (h) the polydispersity index in CUF water/PS85 10%.

Table 2. Droplet size and polydispersity index (PdI) of nanoemulsions recently prepared (Day 0).

Surfactant	Nanoemulsion Containing CUF				Nanoemulsion Containing CUL				Nanoemulsion Containing NaDES CU			
	Deionized Water		Glycerin		Deionized Water		Glycerin		Deionized Water		Glycerin	
	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI
Blank	>1000	0.772 ± 0.238	584.5 ± 321.1	0.518 ± 0.209	473.8 ± 110.1	0.485 ± 0.040	347.3 ± 254.6	0.377 ± 0.132	178.1 ± 74.2	0.315 ± 0.091	>1000	0.594 ± 0.352
PS85 5%	142.9 ± 2.0	0.204 ± 0.012	226.6 ± 2.3	0.416 ± 0.003	154.2 ± 0.8	0.223 ± 0.014	254.7 ± 2.1	0.450 ± 0.006	126.5 ± 2.4	0.199 ± 0.005	288.3 ± 5.6	0.245 ± 0.260
PS85 10%	151.0 ± 1.9	0.151 ± 0.011	196.9 ± 1.2	0.340 ± 0.005	160.4 ± 0.6	0.174 ± 0.027	270.7 ± 3.6	0.396 ± 0.018	155.2 ± 0.5	0.164 ± 0.004	218.8 ± 1.8	0.407 ± 0.009
PS80 5%	>1000	1.0 ± 0.000	294.0 ± 263.1	0.301 ± 0.181	39.7 ± 12.1	0.568 ± 0.029	>1000	0.723 ± 0.362	9.1 ± 0.1	0.035 ± 0.008	21.1 ± 12.1	0.224 ± 0.085
PS80 10%	>1000	0.935 ± 0.059	182.2 ± 133.8	0.254 ± 0.104	450.2 ± 318.6	0.668 ± 0.120	73.0 ± 49.1	0.304 ± 0.175	8.6 ± 0.2	0.078 ± 0.003	13.0 ± 0.1	0.206 ± 0.017
PS20 5%	>1000	0.871 ± 0.223	330.2 ± 101.1	0.422 ± 0.044	>1000	1.000 ± 0.000	74.1 ± 64.4	0.738 ± 0.453	8.2 ± 0.1	0.095 ± 0.008	121.8 ± 120.4	0.646 ± 0.322
PS20 10%	>1000	0.748 ± 0.265	79.3 ± 65.5	0.689 ± 0.350	652.5 ± 231.0	0.887 ± 0.118	273.9 ± 207.4	0.511 ± 0.221	8.0 ± 0.1	0.088 ± 0.030	14.9 ± 0.5	0.373 ± 0.033

noindentCUF: NaDES CU based tobacco inflorescence extract; CUL: NaDES CU based tobacco leaves extract; NaDES CU: NaDES of choline chloride:urea; PS: polysorbate.

Table 3. Stability of nanoemulsions with polysorbate 85 (5% and 10%) in deionized water after 28 days of preparation.

Day	Nanoemulsion Containing CUF				Nanoemulsion Containing CUL				Nanoemulsion Containing NADES CU			
	PS85 5%		PS85 10%		PS85 5%		PS85 10%		PS85 5%		PS85 10%	
	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI	Size (nm)	PdI
0	163.7 ± 1.9	0.182 ± 0.015	156.4 ± 1.6	0.097 ± 0.007	158.6 ± 1.7	0.116 ± 0.014	156.8 ± 0.9	0.090 ± 0.025	161.2 ± 0.5	0.138 ± 0.024	166.6 ± 1.2	0.101 ± 0.017
1	160.2 ± 1.3	0.169 ± 0.011	159.2 ± 0.2	0.081 ± 0.027	159.2 ± 0.6	0.128 ± 0.008	165.3 ± 3.0	0.100 ± 0.025	158.2 ± 0.9	0.133 ± 0.020	164.9 ± 1.0	0.088 ± 0.014
7	165.0 ± 0.5	0.133 ± 0.036	165.4 ± 1.6	0.066 ± 0.036	161.6 ± 3.2	0.110 ± 0.030	170.0 ± 0.1	0.068 ± 0.016	159.5 ± 2.3	0.138 ± 0.048	172.6 ± 0.6	0.122 ± 0.020
14	144.8 ± 1.3	0.188 ± 0.013	146.9 ± 0.9	0.080 ± 0.011	159.9 ± 2.1	0.116 ± 0.008	169.6 ± 4.2	0.094 ± 0.026	160.9 ± 0.6	0.136 ± 0.015	171.4 ± 0.8	0.081 ± 0.030
21	142.9 ± 2.0	0.165 ± 0.046	134.1 ± 1.7	0.139 ± 0.016	161.2 ± 1.4	0.120 ± 0.009	145.2 ± 0.2	0.127 ± 0.025	159.7 ± 0.8	0.115 ± 0.020	180.3 ± 2.7	0.096 ± 0.031
28	-	-	131.1 ± 2.4	0.125 ± 0.019	161.0 ± 1.1	0.116 ± 0.014	133.6 ± 0.5	0.140 ± 0.003	158.3 ± 2.1	0.132 ± 0.031	133.6 ± 0.5	0.140 ± 0.003

CUF: NaDES CU based tobacco inflorescence extract; CUL: NaDES CU based tobacco leaves extract; NaDES CU: NaDES of choline chloride: urea; PdI: polydispersity index.

Table 4. Average droplet size (nm), average polydispersity index (PdI), and average zeta potential (mV) after thermal stability assay of *Nicotiana tabacum* nanoemulsions with polysorbate 85.

Nanoemulsion Containing CUL							Nanoemulsion Containing CUF					
Deionized Water 10%			Deionized Water 5%				Deionized Water 10%			Deionized Water 5%		
°C	Size (nm)	PdI	ZP (mv)	Size (nm)	PdI	ZP (mv)	Size (nm)	PdI	ZP (mv)	Size (nm)	PdI	ZP (mv)
25	130.2 ± 0.6	0.250 ± 0.008	−14.63 ± 0.83	123.6 ± 0.5	0.282 ± 0.004	−12.61 ± 0.41	129.6 ± 1.4	0.245 ± 0.002	−12.78 ± 0.38	117.2 ± 1.4	0.267 ± 0.001	−15.98 ± 0.17
30	131.9 ± 1.5	0.251 ± 0.004	-	122.7 ± 0.7	0.274 ± 0.001	-	130.7 ± 2.0	0.254 ± 0.012	-	118.2 ± 1.0	0.27 ± 0.009	-
35	131.0 ± 0.7	0.244 ± 0.005	-	123.2 ± 2.3	0.266 ± 0.010	-	129.4 ± 2.3	0.251 ± 0.010	-	117.0 ± 0.1	0.265 ± 0.002	-
40	128.8 ± 1.3	0.256 ± 0.018	-	122.7 ± 0.7	0.254 ± 0.013	-	129.5 ± 0.3	0.249 ± 0.006	-	117.2 ± 0.7	0.265 ± 0.005	-
45	128.3 ± 1.1	0.271 ± 0.005	-	121.7 ± 1.5	0.272 ± 0.004 **	-	128.8 ± 0.3	0.269 ± 0.017	-	115.7 ± 1.4	0.273 ± 0.012	-
50	145.2 ± 3.2 *	0.237 ± 0.011	-	134.5 ± 0.7 *	0.259 ± 0.005	-	143.8 ± 4.4	0.239 ± 0.010	-	125.1 ± 0.8	0.271 ± 0.005	-
55	170.6 ± 1.9 **	0.188 ± 0.009	-	153.1 ± 0.7 ***	0.250 ± 0.004	-	170.0 ± 4.4 *	0.171 ± 0.012 *	-	142.0 ± 0.5 **	0.274 ± 0.009	-
60	188.2 ± 2.9 **	0.141 ± 0.015 *	-	163.0 ± 0.5 ***	0.221 ± 0.006 *	-	191.2 ± 4.4 **	0.134 ± 0.008 **	-	153.2 ± 0.1 **	0.260 ± 0.009	-
65	200.8 ± 2.9 **	0.117 ± 0.009 **	-	166.6 ± 1.4 **	0.206 ± 0.005 **	-	212.0 ± 1.2 ***	0.080 ± 0.013 **	-	158.0 ± 0.5 **	0.234 ± 0.006 *	-
25	136.4 ± 0.8	0.232 ± 0.009	−15.34 ± 0.47	225.3 ± 3.3	0.264 ± 0.013	−13.47 ± 0.31	118.9 ± 0.4	0.228 ± 0.015	−13.83 ± 0.73	258.8 ± 3.3	0.271 ± 0.012	−16.42 ± 0.68

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ZP: zeta potential; CUF: NaDES CU based tobacco inflorescence extract; CUL: NaDES CU based tobacco leaves extracts; NaDES CU: NaDES of choline chloride: urea.

3.5.2. Centrifugation Stability

Gravitational centrifugation was used to evaluate the samples and assess the stability of the formulations under physical stress. This enabled potential changes in stability to be predicted, such as phase separation and sediment formation. No phase separation was observed in CUF and CUL in PS85 at 5 and 10% in deionized water during storage. According to Brazil's National Health Surveillance Agency (ANVISA) in its Cosmetic Product Stability Guide (Guia de Estabilidade de Produtos Cosméticos) [59], since the prototype remained stable and did not show any irregularity, which is evidence that it should be reformulated, it can be submitted to stability tests.

4. Conclusions

In this study, we demonstrated the remarkable efficacy of Natural Deep Eutectic Solvents (NaDES), particularly the choline chloride: urea (CU) system, in extracting antioxidant and anti-tyrosinase compounds from tobacco preharvest waste, such as inflorescences and apical leaves. For the first time, nanoemulsions containing these extracts were innovatively developed using a mixture of deionized water and polysorbate 85 and specifically characterized for their potential applications in antioxidant and anti-aging cosmetic formulations. Notably, all components employed in the proposed method are cost-effective, easy to produce, and biocompatible at the concentrations used, supporting their feasibility for sustainable cosmetic development.

5. Limitations

While this study provides a comprehensive fundamental analysis of tobacco nanoemulsions, certain limitations should be acknowledged. Stability experiments were conducted over a relatively short term, and rheological properties were not evaluated. These aspects may limit the extrapolation of the results to long-term storage conditions and processing scenarios. Therefore, future studies should focus on long-term stability and rheological characterization to better assess the potential application of tobacco nanoemulsions in different technological and cosmetic contexts.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cosmetics13020051/s1>, Figure S1: Macroscopic characteristics of *Nicotiana tabacum* nanoemulsions using NADES CU (CU) prepared with (a) water and PS20 at 5%, (b) water and PS20 at 10%, (c) water and PS80 at 5%, (d) water and PS80 at 10%, (e) water and PS85 at 5%, (f) water and PS85 at 10%, (g) glycerin and PS20 at 5%, (h) glycerin and PS20 at 10%, (i) glycerin and PS80 at 10%, (j) glycerin and 10% PS80, (k) glycerin and PS85 at 5%, (l) glycerin and PS85 at 10% on the day of preparation (day 0) and after 1 day of preparation (day1). Figure S2. Macroscopic characteristics of *Nicotiana tabacum* nanoemulsions using leaves (CUL) prepared with (a) water and PS20 at 5%, (b) water and PS20 at 10%, (c) water and PS80 at 5%, (d) water and PS80 at 10%, (e) water and PS85 at 5%, (f) water and PS85 at 10%, (g) glycerin and PS20 at 5%, (h) glycerin and PS20 at 10%, (i) glycerin and PS80 at 10%, (j) glycerin and 10% PS80, (k) glycerin and PS85 at 5%, (l) glycerin and PS85 at 10% on the day of preparation (day 0) and after 1 day of preparation (day1). Figure S3. Macroscopic characteristics of *Nicotiana tabacum* nanoemulsions using inflorescences (CUF) prepared with (a) water and PS20 at 5%, (b) water and PS20 at 10%, (c) water and PS80 at 5%, (d) water and PS80 at 10%, (e) water and PS85 at 5%, (f) water and PS85 at 10%, (g) glycerin and PS20 at 5%, (h) glycerin and PS20 at 10%, (i) glycerin and PS80 at 5%, (j) glycerin and PS80 at 10%, (k) glycerin and PS85 at 5%, (l) glycerin and PS85 at 10% on the day of preparation (day 0) and after 1 day of preparation (day 1).

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References

1. Hoang, H.T.; Moon, J.Y.; Lee, Y.C. Natural antioxidants from plant extracts in skincare cosmetics: Recent applications, challenges and perspectives. *Cosmetics* **2021**, *8*, 106. [\[CrossRef\]](#)
2. Hassan, M.; Shahzadi, S.; Kloczkowski, A. Tyrosinase inhibitors naturally present in plants and synthetic modifications of these natural products as anti-melanogenic agents: A review. *Molecules* **2023**, *28*, 378. [\[CrossRef\]](#)
3. Baber, M.A.; Crist, C.M.; Devolve, N.L.; Patrone, J.D. Tyrosinase inhibitors: A perspective. *Molecules* **2023**, *28*, 5762. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Rachman, E.S.; Soeratri, W.; Erawati, T. Characteristics and Physical Stability of Nanoemulsion as a Vehicle for Anti-Aging Cosmetics: A Systematic Review. *J. Farm. Ilmu Kefarmasian Indones.* **2023**, *10*, 62–85. [\[CrossRef\]](#)
5. Tayal, S.; Tiwari, P.; Singh, U.P.; Dubey, S.; Vishwakarma, R. A review on formulation, characterization and applications of nanoemulsion. *Asian J. Appl. Sci. Technol.* **2024**, *8*, 67–84. [\[CrossRef\]](#)
6. Chuo, S.C.; Setapar, S.H.M. Application of Nanoemulsion in Cosmetics. In *Micro and Nanotechnologies; Nanotechnology for the Preparation of Cosmetics Using Plant-Based Extracts*; Setapar, S.H.M., Ahmad, A., Jawaid, M., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 355–371. [\[CrossRef\]](#)
7. Iskandar, B.; Liu, T.W.; Mei, H.C.; Kuo, I.C.; Surboyo, M.D.C.; Lin, H.M.; Lee, C.K. Herbal nanoemulsions in cosmetic science: A comprehensive review of design, preparation, formulation, and characterization. *J. Food Drug Anal.* **2024**, *32*, 428–458. [\[CrossRef\]](#)
8. Zhang, M.; Cao, Q.; Yuan, Y.; Guo, X.; Pan, D.; Xie, R.; Ju, X.; Liu, Z.; Wang, W.; Chu, L. Functional nanoemulsions: Controllable low-energy nanoemulsification and advanced biomedical application. *Chin. Chem. Lett.* **2024**, *35*, 108710. [\[CrossRef\]](#)
9. Gui, Z.Q.; Yuan, X.L.; Yang, J.; Du, Y.M.; Zhang, P. An updated review on chemical constituents from *Nicotiana tabacum* L.: Chemical diversity and pharmacological properties. *Ind. Crops Prod.* **2024**, *214*, 118497. [\[CrossRef\]](#)
10. Zhang, W.; Pan, X.; Fu, J.; Cheng, W.; Lin, H.; Zhang, W.; Huang, Z. Phytochemicals derived from *Nicotiana tabacum* L. plant contribute to pharmaceutical development. *Front. Pharmacol.* **2024**, *15*, 1372456. [\[CrossRef\]](#)
11. Schwingel, L.; Schwingel, G.; Storch, N.; Barreto, F.; Bassania, V. 3-O-Methylquercetin from organic *Nicotiana tabacum* L. trichomes: Influence of variety, cultivation and extraction parameters. *Ind. Crops Prod.* **2014**, *55*, 56–62. [\[CrossRef\]](#)
12. Shang, S.; Shi, J.; Tang, J.; Jiang, J.; Zhao, W.; Zheng, X.; Lei, P.; Han, J.; Wang, C.; Yuan, D.; et al. New isolates from leaves of *Nicotiana tabacum* and their biological activities. *Nat. Prod. Res.* **2019**, *33*, 1577–1583. [\[CrossRef\]](#)
13. Zou, X.; Bk, A.; Rauf, A.; Saeed, M.; Al-Awthan, Y.S.; Al-Duais, M.; Bahattab, O.; Khan, M.H.; Suleria, H.A.R. Screening of polyphenols in tobacco (*Nicotiana tabacum*) and determination of their antioxidant activity in different tobacco varieties. *ACS Omega* **2021**, *6*, 25361–25371. [\[CrossRef\]](#)
14. Sifola, M.I.; Carrino, L.; Cozzolino, E.; del Piano, L.; Graziani, G.; Ritieni, A. Potential of pre-harvest wastes of tobacco (*Nicotiana tabacum* L.) crops, grown for smoke products, as source of bioactive compounds (phenols and flavonoids). *Sustainability* **2021**, *13*, 2087. [\[CrossRef\]](#)

15. Xu, C.P.; Xiao, Y.; Mao, D.B. Antioxidant activities of polysaccharide fractions isolated from burley tobacco flowers. *Croat. J. Food Sci. Technol.* **2013**, *5*, 46–52. Available online: <https://hrcak.srce.hr/113687> (accessed on 1 May 2025).
16. Banožić, M.; Babić, J.; Jokić, S. Recent advances in extraction of bio-active compounds from tobacco industrial waste—a review. *Ind. Crops Prod.* **2020**, *144*, 112009. [[CrossRef](#)]
17. Kapetanović, S. Results of extraction of tobacco flowers and inflorescences with volatile solvents with regard to yield and quality of essential oils. *Tobacco* **1989**, *39*, 91–102.
18. Xu, C.; Zhao, S.; Li, M.; Dai, Y.D.; Tan, L.; Liu, Y. Chemical composition, antimicrobial and antioxidant activities of essential oil from flue-cured tobacco flower bud. *Biotechnol. Biotechnol. Equip.* **2016**, *30*, 1026–1030. [[CrossRef](#)]
19. Putri, D.A.; Solihah, R.; Oktavia, R.; Anggraini, D.A.; Fatmawati, S. Secondary metabolites of *Nicotiana tabacum* and their biological activities: A review. *J. Pure Appl. Chem. Res.* **2022**, *11*, 149–165. [[CrossRef](#)]
20. Popova, V.; Ivanova, T.; Stoyanova, A.; Nikolova, V.; Hristeva, T.; Zheljazkov, V. GC-MS Composition and olfactory profile of concretes from the flowers of four *Nicotiana* species. *Molecules* **2020**, *25*, 2617. [[CrossRef](#)] [[PubMed](#)]
21. Zou, X.; Bk, A.; Abu-Izneid, T.; Aziz, A.; Devnath, P.; Rauf, A.; Mitra, S.; Bin Emran, T.; Mujawah, A.A.; Lorenzo, J.M.; et al. Current advances of functional phytochemicals in *Nicotiana* plant and related potential value of tobacco processing waste: A review. *Biomed. Pharmacother.* **2021**, *143*, 112191. [[CrossRef](#)] [[PubMed](#)]
22. Xiao, Q.; Zhu, Y.; Cui, G.; Zhang, X.; Hu, R.; Deng, Z.; Lei, L.; Wu, L.; Mei, L. A comparative study of flavonoids and carotenoids revealed metabolite responses for various flower colorations between *Nicotiana tabacum* L. and *Nicotiana rustica* L. *Front. Plant Sci.* **2022**, *13*, 828042. [[CrossRef](#)]
23. Zhang, M.; Sun, J.Y.; Qiu, H.M.; Zhang, P.; Deng, L.L.; Li, J.; Hao, X.J.; Su, X.K.; Mu, S.Z. Active constituents with tyrosinase inhibitory activities from waste tobacco leaves. *Chem. Biodivers.* **2024**, *21*, e202400463. [[CrossRef](#)]
24. Leal, M.; Moreno, M.A.; Albornoz, P.L.; Mercado, M.I.; Zampini, I.C.; Isla, M.I. *Nicotiana tabacum* leaf waste: Morphological characterization and chemical-functional analysis of extracts obtained from powder leaves by using green solvents. *Molecules* **2023**, *28*, 1396. [[CrossRef](#)] [[PubMed](#)]
25. Leal, M.; Moreno, M.A.; Albornoz, P.L.; Mercado, M.I.; Zampini, I.C.; Isla, M.I. Morphological characterization of *Nicotiana tabacum* inflorescences and chemical-functional analysis of extracts obtained from its powder by using green solvents (NaDESs). *Plants* **2023**, *12*, 1554. [[CrossRef](#)]
26. Leal, M.; Moreno, M.A.; Orqueda, M.E.; Simirgiotis, M.; Isla, M.I.; Zampini, I.C. Green extract from pre-harvest tobacco waste as a non-conventional source of anti-aging ingredients for cosmetic applications. *Plants* **2025**, *14*, 2189. [[CrossRef](#)] [[PubMed](#)]
27. Manoharlal, R.; Saiprasad, G.V.S. *Tobacco and Its Alternative Uses*; StoryMirror Infotech Pvt Ltd.: Mumbai, India, 2023.
28. Cosmetic Ingredient Database (CosIng) of the European Commission. Available online: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022D0677> (accessed on 2 November 2025).
29. Dai, Y.; van Spronsen, J.; Witkamp, G.J.; Verpoorte, R.; Choi, Y.H. Natural deep eutectic solvents as new potential media for green technology. *Anal. Chim. Acta* **2013**, *766*, 61–68. [[CrossRef](#)]
30. Choi, Y.H.; Verpoorte, R. Green solvents for the extraction of bioactive compounds from natural products using ionic liquids and deep eutectic solvents. *Curr. Opin. Food Sci.* **2019**, *26*, 87–93. [[CrossRef](#)]
31. Villa, C.; Caviglia, D.; Robustelli della Cuna, F.S.; Zuccari, G.; Russo, E. NaDES application in cosmetic and pharmaceutical fields: An overview. *Gels* **2024**, *10*, 107. [[CrossRef](#)]
32. Bencresciuto, G.F.; Carnevale, M.; Paris, E.; Gallucci, F.; Santangelo, E.; Migliori, C.A. A sustainable alternative for cosmetic applications: NADES extraction of bioactive compounds from Hazelnut by-products. *Sustainability* **2025**, *17*, 1516. [[CrossRef](#)]
33. Chittasupho, C.; Chaobankrang, K.; Sarawungkad, A.; Samee, W.; Singh, S.; Hemsuwimon, K.; Okonogi, S.; Kheawfu, K.; Kiattisin, K.; Chaiyana, W. Antioxidant, anti-inflammatory and attenuating intracellular reactive oxygen species activities of *Nicotiana tabacum* var. Virginia leaf extract phytosomes and shape memory gel formulation. *Gels* **2023**, *9*, 78. [[CrossRef](#)]
34. Popova, V.; Tumbarski, Y.D.; Ivanova, T.; Hadjikinova, R.; Stoyanova, A. Tobacco resinoid (*Nicotiana tabacum* L.) as an active ingredient of cosmetic gels. *J. Appl. Pharm. Sci.* **2019**, *9*, 111–118. [[CrossRef](#)]
35. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [[CrossRef](#)]
36. Woisky, R.G.; Salatino, A. Analysis of propolis: Some parameters and procedures for chemical quality control. *J. Apic. Res.* **1998**, *37*, 99–105. [[CrossRef](#)]
37. Momtaz, S.; Mapunya, B.M.; Houghton, P.J.; Edgerly, C.; Hussein, A.; Naidoo, S.; Lall, N. Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening. *J. Ethnopharmacol.* **2008**, *119*, 507–512. [[CrossRef](#)]
38. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [[CrossRef](#)]

39. Ortiz-Zamora, L.; Bezerra, D.C.; de Oliveira, H.N.S.; Duarte, J.L.; Guisado-Bourzac, F.; Chil-Núñez, I.; da Conceição, E.; Barroso, A.; Veras Mourão, R.H.; Maciel da Faria Mota Oliveira, A.E.; et al. Preparation of non-toxic nano-emulsions based on a classical and promising Brazilian plant species through a low-energy concept. *Ind. Crops Prod.* **2020**, *158*, 112989. [\[CrossRef\]](#)
40. Di Rienzo, J.A.; Casanoves, F.; Balzarini, M.G.; Gonzalez, L.; Tablada, M.; Robledo, C.W. *InfoStat*, versión 2015; Grupo InfoStat, FCA, Universidad Nacional de Córdoba: Córdoba, Argentina, 2015.
41. Rocha, D.; Freitas, D.S.; Magalhães, J.; Fernandes, M.; Silva, S.; Noro, J.; Ribeiro, A.; Cavaco-Paulo, A.; Martins, M.; Silva, C. NADES-Based cork extractives as green ingredients for cosmetics and textiles. *Processes* **2023**, *11*, 309. [\[CrossRef\]](#)
42. Bildik, F. Application of choline chloride as natural deep eutectic solvents for the green extraction of phenolic compounds from *Rheum ribes* leaves. *Eur. J. Tech.* **2021**, *11*, 140–143. [\[CrossRef\]](#)
43. Ratz-Lyko, A.; Arct, J.; Pytkowska, K. Methods for evaluation of cosmetic antioxidant capacity. *Ski. Res. Technol.* **2012**, *18*, 421–430. [\[CrossRef\]](#)
44. Panzella, L.; Napolitano, A. Natural and bioinspired phenolic compounds as tyrosinase inhibitors for the treatment of skin hyperpigmentation: Recent advances. *Cosmetics* **2019**, *6*, 57. [\[CrossRef\]](#)
45. Vaezi, M. Structure and inhibition mechanism of some synthetic compounds and phenolic derivatives as tyrosinase inhibitors: Review and new insight. *J. Biomol. Struct. Dyn.* **2023**, *41*, 4798–4810. [\[CrossRef\]](#)
46. Prommaban, A.; Kheawfu, K.; Chittasupho, C.; Sirilun, S.; Hemsuwimon, K.; Chaiyana, W. Phytochemical, antioxidant, anti-hyaluronidase, antityrosinase, and antimicrobial properties of *Nicotiana tabacum* L. leaf extracts. *Evid. Based Complement. Altern. Med.* **2022**, *1*, 5761764. [\[CrossRef\]](#)
47. de Macêdo, I.D.S.V.; Cunha, K.G.; Alves, A.T.V.; Martins, R.M.; da Silva Simões, M.O. Atividade antioxidante da rutina: Uma revisão. *BIOFARM J. Biol. Pharm. Agric. Manag.* **2017**, *13*, 14–19.
48. Girsang, E.; Lister, I.N.E.; Ginting, C.N.; Sholihah, I.A.; Raif, M.A.; Kunardi, S.; Million, H.; Widowati, W. Antioxidant and antiaging activity of rutin and caffeic acid. *Pharmaciana* **2020**, *10*, 147–156. [\[CrossRef\]](#)
49. Patel, D.K. Medicinal importance, pharmacological activities and analytical aspects of a flavonoid glycoside ‘Nicotiflorin’ in the medicine. *Drug Metab. Lett.* **2022**, *15*, 2–11. [\[CrossRef\]](#)
50. Sauer, N.; Oślizło, M.; Brzostek, M.; Wolska, J.; Lubaszka, K.; Karłowicz-Bodalska, K. The multiple uses of azelaic acid in dermatology: Mechanism of action, preparations, and potential therapeutic applications. *Adv. Dermatol. Allergol.* **2023**, *40*, 716–724. [\[CrossRef\]](#)
51. Singh, S.K.; Chaubey, S.; Bansal, A.; Kaur, G.; Malik, D.S. Cosmeceutical aptitudes of azelaic acid. *Curr. Drug Res. Rev.* **2021**, *13*, 222–229. [\[CrossRef\]](#)
52. Petrovici, A.G.; Spennato, M.; Bîtcă, I.; Péter, F.; Cotarcă, L.; Todea, A.; Ordodi, V.L. A comprehensive review of azelaic acid pharmacological properties, clinical applications, and innovative topical formulations. *Pharmaceuticals* **2025**, *18*, 1273. [\[CrossRef\]](#)
53. Gómez-Molina, M.; Albaladejo-Marico, L.; Yepes-Molina, L.; Nicolas-Espinosa, J.; Navarro-León, E.; Garcia-Ibañez, P.; Carvajal, M. Exploring phenolic compounds in crop by-products for cosmetic efficacy. *Int. J. Mol. Sci.* **2024**, *25*, 5884. [\[CrossRef\]](#)
54. Choi, J.Y.; Lee, J.W.; Jang, H.; Kim, J.G.; Lee, M.K.; Hong, J.T.; Lee, M.S.; Hwang, B.Y. Quinic acid esters from *Erycibe obtusifolia* with antioxidant and tyrosinase inhibitory activities. *Nat. Prod. Res.* **2021**, *35*, 3026–3032. [\[CrossRef\]](#)
55. Wuryantini, S. The Prospect of Nanoinsecticide in Controlling Citrus Aphids (*Aphis gossypii*). In Proceedings of the International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019), Bogor, Indonesia, 24–26 September 2019; pp. 182–187.
56. Ekapratiwi, Y.; Rachmadiva; Virgine, K.A.; Fauzantoro, A.; Gozan, M.; Jufri, M. The Effect of Tobacco Extracts Based Biolarvicide Emulsion Formulation Against *Aedes aegypti* Larvae. *AIP Conf. Proc.* **2019**, *2092*, 030009. [\[CrossRef\]](#)
57. Dias, D.D.O.; Colombo, M.; Kelmann, R.G.; De Souza, T.P.; Bassani, V.L.; Teixeira, H.F.; Veiga, V.F., Jr.; Limberger, R.P.; Koester, L.S. Optimization of headspace solid-phase microextraction for analysis of β -caryophyllene in a nanoemulsion dosage form prepared with copaiba (*Copaifera multijuga* Hayne) oil. *Anal. Chim. Acta* **2012**, *721*, 79–84. [\[CrossRef\]](#)
58. Marhamati, M.; Ranjbar, G.; Rezaie, M. Effects of emulsifiers on the physicochemical stability of Oil-in-water Nanoemulsions: A critical review. *J. Mol. Liq.* **2021**, *340*, 117218. [\[CrossRef\]](#)
59. Agência Nacional de Vigilância Sanitária (ANVISA). *Guia de Estabilidade de Produtos Cosméticos*, 1st ed.; Agência Nacional de Vigilância Sanitária: Brasília, Brazil, 2004. Available online: <https://www.gov.br/anvisa/pt-br/centraisdeconteudo/publicacoes/cosmeticos/manuais-e-guias/guia-de-estabilidade-de-cosmeticos.pdf> (accessed on 15 October 2025).

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