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Development and evaluation of antioxidant and antibacterial herbal cream with *Elateriospermum tapos* Blume seed oil

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Abstract

In this study, an herbal cream incorporating *Elateriospermum tapos* Blume seed oil was developed and evaluated, highlighting the oil's unique composition of omega-3, omega-6, and omega-9 fatty acids confirmed by GC-MS. This study presents *E. tapos*, a locally plentiful but underutilized Southeast Asian seed oil, as a novel bioactive ingredient, distinguishing itself from previous herbal cream formulations that predominantly utilize common plant oils due to its unique profile of polyunsaturated fatty acids and phytochemicals. The cream's antioxidant capacity, measured by DPPH, ABTS, and FRAP tests, showed that it was better at scavenging radicals and reducing ferric ions than the base cream. The formulation also exhibited notable antibacterial activity, producing inhibition zones of 27.25 ± 0.87 mm against *Cutibacterium acnes* and 25.17 ± 1.20 mm against *Staphylococcus epidermidis*, both Gram-positive skin-associated bacteria. The *E. tapos* oil cream was developed, and it showed the strongest antioxidant activity against DPPH and ABTS radicals with an IC_{50} of 32.67 ± 1.34 mg L^{-1} and 17.97 ± 0.04 mg L^{-1}, respectively. The resulting formulation possessed desirable physicochemical properties, including skin-compatible pH, homogeneity, and stability. Overall, this study provides the first scientific validation of *E. tapos* seed oil as a high-value cosmetic ingredient and demonstrates its potential for developing natural anti-acne and antioxidant topical products, supporting future dermatological applications and value-added utilization of a regional botanical resource.

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1. Introduction

Tex Biologically active chemical constituents of herbal plants are used in cosmetic products to enhance beauty and attractiveness. As they have several health benefits and applications, bioactive chemicals might be used in cosmetics made from natural sources [1]. For example, cosmetics for the skin, hair, nails, teeth, and mouth are available in various forms, including creams, powders, soaps, solutions, etc. [2–4]. Herbal cosmetics are extremely suitable as they are plant-based products. They contain various chemical components or crucial elements that have therapeutic effects. These chemicals have antioxidant activity that fights bacteria and other pathogenic microbes. The natural components of herbal remedies have no negative health effects [5]. These herbs provide nutrients and other beneficial minerals. Several studies have investigated the value of herbs in cosmetic formulations and showed that creams may be formulated with herbs like *Nardostachys jatamansi* [6], *Syzygium aromaticum* oil [7], *Curcuma longa* L., *Citrus grandis* (L.) Osbeck [8], *Aloe barbadensis* [9], *Cassia tora* L., *Brassica nigra* L., and *Sesamum indicum* L. [10].

Plants contain different types of bioactive ingredients, such as terpenes, fatty acids, saponins, and flavonoids [11–13]. Fatty acids and their derivatives play a key role in the manufacture of cosmetics and personal care goods. They influence product stability, functionality, and aesthetics [14]. Polyunsaturated fatty acids (PUFAs), especially

omega-6 (linoleic acid), omega-3 (α-linolenic acid), and monounsaturated fatty acids omega-9 (oleic acid), gained attention when fatty acids were found to be important for human health [15, 16]. The application of fatty acids, especially omega-3 and omega-6 fatty acids, has increased in the cosmetics sector due to their benefits to the skin. Omega-fatty acids can moisturize the skin due to their emollient properties, which are found in cosmetic creams. Omega-fatty acids maintain the integrity of the skin barrier. They strengthen and protect the top layer of the skin, and reduce the damage that irritants and toxins in the environment might cause [17]. The balance between omega-3 and omega-6 fatty acids is crucial, and maintaining an optimal ratio provides several benefits.

Sources of omega-fatty acids include fish and other types of seafood, nuts, seeds, and plant oils [18]. Plant oils can be extracted from various plant parts, including leaves, barks, and stems, but seeds are especially useful [19, 20] because they can biosynthesize and store fatty acids (mostly as triacylglycerol) [21]. Recent studies have recognized clear links between high linoleic acid (omega-6) content and improved skin barrier function in acne-prone skin [22]. Linoleic acid is an essential fatty acid that plays a key role in acne management. Linder (2008) observed several linoleic-acid-rich botanical oils, particularly borage seed, grape seed, sunflower seed, wheat germ, apricot seed, pumpkin seed, and rose hip seed oils, for their potential to reduce microcomedones and inflammation in acne-prone skin. These plant oils were emphasized as key natural sources through which the anti-acne role of linoleic acid has been investigated [23].

Elateriospermum tapos Blume (*E. tapos*) is a monoecious tropical canopy species found in the tropical rainforests of Southeast Asia (Peninsular Thailand, Peninsular Malaysia, Sumatra, Java, and Borneo) [24]. In Thailand, *E. tapos* is commonly known as look-pra. This species dominates the Khao Luang and Khao Nan National Park, Nakhon Si Thammarat province, Thailand [25]. The most abundant fatty acids in *E. tapos* seeds oil are oleic, linoleic, α-linolenic, palmitic, and stearic fatty acids. Thus, *E. tapos* seeds oil can be considered to be oleic-linoleic oil [26].

In this study, we evaluated skin creams produced from *E. tapos* seed oils extracted from native plants in the Nakhon Si Thammarat region because they include omega-fatty acids, which facilitate skin nourishment. Preliminary phytochemical screening of *E. tapos* seed oil was screened. The type of omega-fatty acid in *E. tapos* seed oil content was evaluated using the gas chromatography-mass spectrometry technique. Furthermore, the antibacterial activity against *Staphylococcus epidermidis* and *Cutibacterium acnes* was examined, and the antioxidant activity of *E. tapos* oil and its formulated cream was evaluated by DPPH, ABTS, and FRAP assays. The growing interest in natural and locally sourced cosmetic ingredients is a significant factor. The highlights the potential of *E. tapos* as a bioactive resource for skincare applications and establishes how scientific validation can elevate an underutilized local plant into a value-added product that supports community-based utilization and regional economic development.

2. Materials and Methods

Elateriospermum tapos Blume (*E. tapos*) specimens were collected from the Tambon Krungching, Amphoe Nopitum, Nakhon Si Thammarat Province, Thailand, and the surrounding area of Nakhon Si Thammarat Rajabhat University. All analytical reagents (analytical grade) used in this study were acquired from Merck. The formulation cream used was of cosmetic grade. Hi-Media (Mumbai, India) provided nutrient agar for bacterial culture, Mueller–Hinton broth, and antimicrobial activity agar.

Preparation of E. tapos seeds oil and formulation cream

The seeds of *E. tapos* were collected from Tambon Krungching, Amphoe Nopitum, and Nakhon Si Thammarat provinces in Thailand. The seeds were separated, washed, and softly powdered. Then, a Soxhlet apparatus was used to extract seed oil for 8 h. The ethanol extraction process had three replicates. The extracted oil was weighed after the solvent was eliminated. The initial chemical composition of the *E. tapos* seed was assessed by the Fourier-transform infrared spectrophotometry technique to determine the important functional groups. Additionally, total fatty acid content was determined by the gas chromatography technique. In the oil phase (Phase B), the emulsifier (stearic acid) and other oil-soluble components were dissolved and heated to 50 °C. In the aqueous phase (Phase A), the preservatives and other water-soluble components were dissolved and heated to 50 °C. The aqueous phase was added in parts to the oil phase after heating and with continuous stirring until the oil phase cooled. Composition of phase C is an added ingredient. The composition of base cream (without *E. tapos* seeds oil) and the cream produced cooperating with *E. tapos* seeds oil are described in Table 1. The oil-in-water (O/W) emulsion was prepared using a high-speed homogenizer (Ultra-Turrax T25) at a speed of 10,000 rpm for 20 minutes to ensure uniform dispersion of the oil phase within the aqueous base. The homogenization process was performed at a controlled temperature of 50–60 °C, maintaining stability and consistency of the final cream formulation. The emulsion was then cooled to room temperature under continuous stirring at 500 rpm to prevent phase separation and achieve a smooth texture.

Preliminary Phytochemical Analysis

Qualitative phytochemical tests were conducted to identify alkaloids, flavonoids, polyphenols, steroids, terpenoids, glycosides, and saponins, in *E. tapos* seeds oil [27–29]. These assays were performed with different concentrations of samples, and triplicate experiments were conducted for each sample concentration.

Test for alkaloids

A small volume of *E. tapos* seeds oil was dissolved in 5 mL of 1% hydrochloric acid, filtered, and tested with Dragendorff's reagent and Mayer's reagent separately. The presence of alkaloids was indicated by the formation of a precipitate in the solution or when it turned turbid after the reagents were added.

Test for flavonoids

A few drops of concentrated hydrochloric acid and 1–2 magnesium turnings were added to 1 mL of *E. tapos* seed oil. The appearance of pink or magenta-red color indicated the presence of flavonoids.

Test for polyphenols

About 0.25 g of the ethanol extract was treated with a few drops of 5% neutral ferric chloride solution. The appearance of a greenish precipitate indicated the presence of phenol.

Test for Steroids and Terpenoids

First, trace amounts of *E. tapos* seeds oil were dissolved in 1 mL of chloroform and filtered. To the filtrate on ice, 1 mL of acetic acid was added, and then, a few drops of concentrated sulfuric acid was gradually added to the side of the test tube. The appearance of a pink or pinkish-brown ring indicated the presence of terpenoids. The appearance of a blue or bluish-green color or a rapid change from pink to blue indicated the presence of steroids. A combination of pink and these colors indicated the presence of steroids and terpenoids.

Test for glycosides

Borntrager's reagent was used to determine whether glycosides were present. To 2 mL of the filtrate hydrolysate, 3 mL of chloroform was added and shaken, the chloroform layer was separated, and 10% ammonia solution was added to it. The formation of a pink color indicated the presence of glycosides.

Test for saponins

The oil extract (50 mg) was diluted with distilled water and made up to 20 mL. Then, the suspension was shaken well in a test tube for 15 min. Formation of stable froth (foam) indicated the presence of saponin.

Composition analysis of E. tapos seed oil via GC-MS technique

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the composition of *Elateriospermum tapos* seed oil. The analysis was performed using an Agilent CP9205 VF-WAXms column (30 m × 250 μm × 0.25 μm), which operates within a temperature range of 20°C to 250 °C. The oven temperature was programmed to start at 110 °C (held for 5 min), gradually increasing to 240°C, following a multi-step ramping process to ensure optimal separation of volatile compounds.

The injection was performed using an Agilent GC ALS autosampler, with a split ratio of 50:1, injecting 1 μL of the sample. The carrier gas used was helium, with a constant flow rate of 1 mL min^{-1}. The mass spectrometer operated in scan mode, detecting masses in the range of 35–500 m/z, with a solvent delay of 2 min to prevent interference from non-volatile components. The identification of chemical constituents was achieved by comparing the obtained mass spectra with standard reference databases, such as the NIST Mass Spectral Library matching (≥90%) and Wiley Database. Peak retention times and fragmentation patterns were also matched against known compounds in these libraries to ensure accurate identification. Additionally, relative abundances of identified compounds were analyzed to determine the major bioactive constituents of *E. tapos* seed oil.

Table 1 Composition of the cream produced from *E. tapos* seed oil.

Ingredients	Formulation Amount (g)	
	Base cream	***E. tapos* seeds oil cream**
Phase A		
DI water	Up to 100 g	Up to 100 g
EDTA	0.50	0.50
Carbomer (Carbopol 940)	1.00	1.00
Glycerin	2.00	2.00
Phase B		
Stearic Acid	6.00	6.00
Squalane	3.00	3.00
Cetearyl alcohol	4.00	4.00
Eumulgin B1	3.00	3.00
Vitamin E di-α tocopherol	1.00	1.00
E. tapos seeds oil	-	3.00
Phase C		
Triethanolamine	1.00	1.00
Fragrance (Rice milk smell)	1.00	1.00
Phenoxyethanol	2.00	2.00

DPPH radical scavenging assay

The free-radical scavenging ability of *E. tapos* seed oil and its formulated cream was evaluated using the DPPH radical scavenging assay. The stable DPPH radical in an ethanol solution was made to react with the samples (*E. tapos* seeds oil and formulation creams) at a concentration of 30, 40, 50, and 60 mg L^{-1}. The reaction mixture consisted of 1 mL of sample, and 2 mL of DPPH radical solution in 0.2 mM ethanol. DPPH can contribute hydrogen when it interacts with an antioxidant, which reduces DPPH. The change in color (from deep violet to light yellow) was measured (Absorbance) at 518 nm after 5 min of reaction using a UV-VIS spectrophotometer (Thermo Scientific, Evolution 201). Then, 1 mL of control solution was prepared by mixing ethanol and 2 mL of DPPH radical solution. Ascorbic acid was used as a standard.

ABTS assay

Initially, 7.0 mM ABTS solution was mixed with 2.45 mM K_2SO_4 at a ratio of 1:0.5 and incubated for 12–16 h, after which it was diluted with ethanol, and the absorbance was recorded at 0.7–0.9 at 734 nm. Then, 0.0125 g of the sample was dissolved in ethanol. The volume was adjusted to 25 mL, and a solution of 500 mg L^{-1} was obtained. Next, 0.25, 0.50, 0.75, 1, and 1.25 mL of the solution was pipetted, and the volume was adjusted to 25 mL with ethanol to obtain solutions of different concentrations (5, 10, 15, 20, and 25 mg L^{-1}). Then, 1 mL of the sample was mixed with 2 mL of the ABTS solution and incubated in the dark for 10 min. The absorbance of the sample was measured at 734 nm using ascorbic acid as a standard. Standardized graphs were used to evaluate antioxidant capacity.

The percentage of DPPH and ABTS radical scavenging activity was calculated using the following equation:

$$\% \text{ Radical scavenging activity} = [(Ac - As)/Ac] \times 100$$

In this study, Ac represents the absorbance of the control, while As denotes the absorbance of the sample or standard. The percentage of inhibition was plotted as a function of concentration, and the IC_{50} value was obtained from the resulting graph. Each concentration was analyzed in triplicate to ensure accuracy and reproducibility.

Ferric-reducing antioxidant power assay

The total antioxidant potential of a sample was determined by the ferric-reducing antioxidant power assay (FRAP assay). The assay was based on the reducing power of a compound (antioxidant). The ferrous ion (Fe^{2+}) produces a blue complex (Fe^{2+}/TPTZ), which increases the absorbance at 593 nm when an antioxidant reduces the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). Briefly, the FRAP reagent was prepared by mixing an acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at a ratio of 10:1:1 by volume. The reagent of 3 mL and sample solutions of 100 mL were added to each well and mixed thoroughly. The absorbance was recorded at 593 nm after 15 min. The standard curve was prepared using different concentrations of ascorbic acid. All solutions were prepared and used on the same day. The standard curve of ascorbic acid was linear between 200 and 1,600 μM $FeSO_4$. All results were expressed in μmol g^{-1} DW.

Analysis of the antibacterial activity

The antibacterial efficacy of 5 mg mL^{-1} of each sample, including *E. tapos* seed oil, basis cream (formulation cream devoid of *E. tapos* seed oil), and formulation cream, against *Cutibacterium acnes* (*C. acnes*) and *Staphylococcus epidermidis* (*S. epidermidis*) was assessed using the agar well diffusion method. Bacteria suspension were prepared by inoculating bacteria into the sterile 0.85% NaCl. The turbidity of the suspension was adjusted to the 0.5 McFarland standard solution, which was equivalent to 1.5 x 10^8 CFU mL^{-1}. The organism was incubated under aerobic (*S. epidermidis*) and anaerobic conditions (*C. acnes*) at 37 °C. The isolates were seeded on Mueller Hinton agar plates using sterilized cotton swabs at pH 7.4 for 8 h. The agar surface was bored using a sterilized cork borer no. 2 (6 mm in diameter) to make wells. Then, 100 μL of samples concentration of 10 mg mL^{-1} and 100 μL of DMSO (negative control) were poured into separate wells. DMSO is solvent in various biological and antimicrobial tests. DMSO used as control because it's can interact with both polar and nonpolar substance. A standard antibiotic disc (chloramphenicol 30 mg/disc) was placed on the surface of the agar as the positive control. The plates were incubated at 37 °C for 48 h. Three plates were maintained for each organism. The diameter of the zone of inhibition around the sample was measured and compared to those around commercial standard antibiotics.

Physical evaluation of formulation creams

The physical properties of the creams produced from *E. tapos* seed oil, such as the pH, color, homogeneity, and odor, were examined. The pH of the cream was determined at 32 °C using a digital pH meter. The color and homogeneity were examined based on visual appearance and touch. The scent or odor of the cream was assessed by sniffing.

3. Results and Discussion

Preliminary phytochemical analysis

The seeds of *E. tapos*, collected from Tambon Krungching, Amphoe Nopitum, and Nakhon Si Thammarat provinces in Thailand, are about 1.68 inches long and shining brown with a faint ridge on each side. The inside of *E. tapos* seeds has the appearance of white seeds. When oil is extracted, it will produce yellow-brown oil (Fig. 1). The list of identified phytochemicals is presented in Table 2. The phytochemical components of *E. tapos seeds oil* were qualitatively analyzed using well-defined laboratory methods. Our results indicated that alkaloids, flavonoids, terpenoids, glycosides, and phenolic compounds were present in the seed oil of *E. tapos*.

Steroids were not present in the phytochemical test. Steroidal compounds are important in pharmacy due to their relationship with compounds used for treating burns, stings, stretch marks, skin thinning, and infections [30].

Furthermore, there is a research report on the study of the composition of *E. tapos* oil, which found that it contains important omega-fatty acid compounds [26]. There were the key components that should be used as the ingredients of the cream [31, 32]. Therefore, we have studied the composition of the samples extracted by the GC method, which will be discussed in the next section.

Fig. 1 The appearance of (a) *E. tapos* seeds with the outer shell intact, (b) *E. tapos* seeds after the outer shell removal, and (c) seed oil of *E. tapos*.

Table 2 Preliminary phytochemical analysis of *E. tapos* seed oil extract.

Sample	Phytochemical Screening						
	alkaloid	flavonoid	steroid	terpenoid	saponins	glycosides	phenolic
E. tapos oil	+	+	-	-	-	+	+

Note: In the table, the + sign denotes the presence of the compound, while the – sign indicates its absence or presence in trace amounts.

Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography coupled with mass spectrometry (GC-MS) was used to identify and quantify the omega-fatty acids present in *E. tapos* seed oil. The chromatographic profiles of these fatty acids showed that the *E. tapos* seeds oil contains significant amounts of the omega-6 (n-6) fatty acid 9,12-Octadecadienoic acid (Z,Z) methyl ester, also known as methyl linoleate. It is produced from linoleic acid and is a member of the polyunsaturated fatty acid (PUFA) family. The GC chromatogram of *E. tapos* seeds oil (Fig. 2) showed excellent resolution. The retention times of 31.44 min, 32.63 min, and 34.85 min were observed for 9-octadecenoic acid (Z) methyl ester (omega-9; oleic acid), 9,12-octadecadienoic acid (Z,Z) methyl ester (omega-6; linoleic acid), and 9,12,15-octadecatrienoic acid (Z,Z,Z) methyl ester (omega-3; α-linolenic acid), respectively. Their corresponding relative percentages were 45.28%, 100%, and 92.61%, as shown in Table 3. Additionally, 9-Hexadecanoic acid, methyl ester, (Z)- appeared at a retention time of 25.43 min and covered an area of 47.47%. This compound is also known as methyl palmitate and is a saturated fatty acid methyl ester. By directly comparing the retention times and mass spectral data of these compounds with those of library matching with minimum similarity index ≥ 90%.

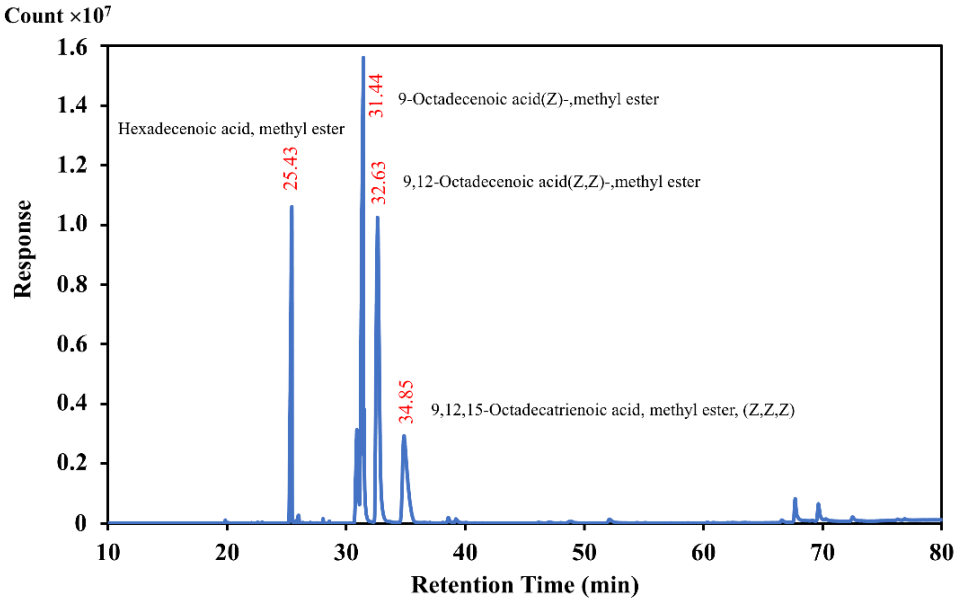

Fig. 2 GC chromatogram of analyzing omega-fatty acids from *E. tapos* seed oil.

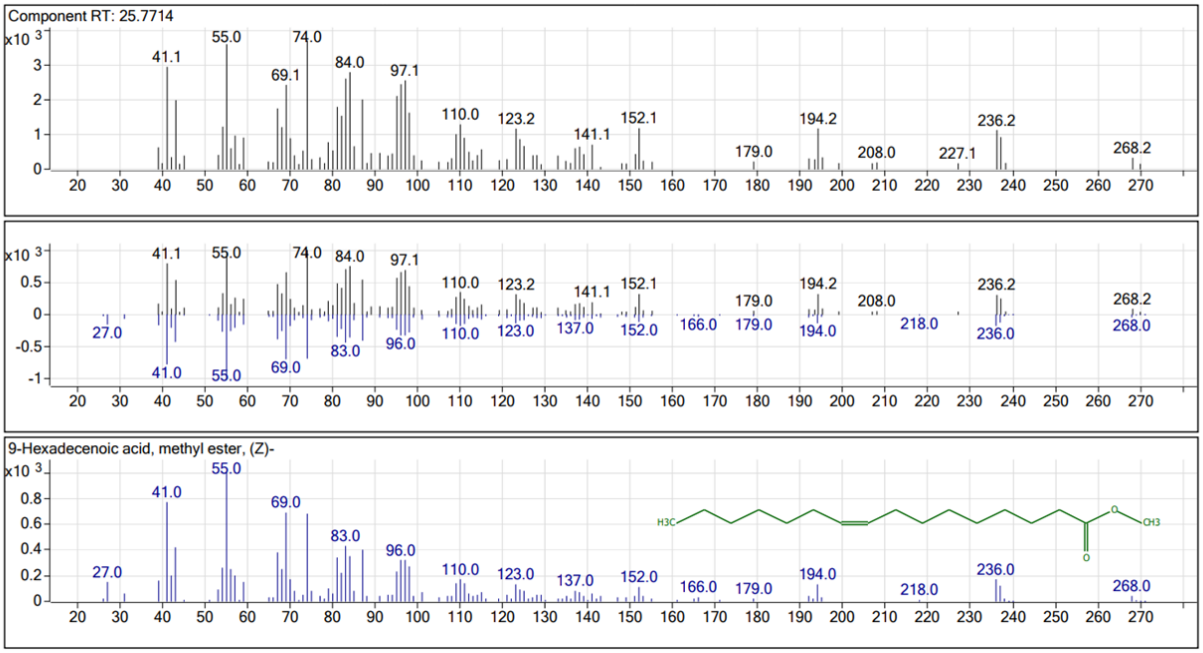

Fig. 3 Mass spectrum of the most abundant saturated fatty acid in *E. tapos* seeds oil.

Table 3 The fatty acid profile of *E. tapos* seeds oil interpreted from the GC spectrum.

RT (min)	Area % (max.)	Estimated (Conc.)	Compound identified
25.43	47.47	14.16	9-Hexadecanoic acid, methyl ester, (Z)-
31.44	92.61	27.63	9-Octadecenoic acid (Z)-, methyl ester
32.63	100.00	29.83	9,12-Octadecadienoic acid (Z,Z) methyl ester
34.85	45.28	13.51	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)

The three major fatty acids identified (omega-3, omega-6, and omega-9 methyl esters) are biologically significant because they are well-established skin-beneficial lipids. omega-6 (linoleic acid) restores barrier function and benefits acne-prone skin; omega-3 (α-linolenic acid) provides anti-inflammatory and antioxidant effects; and omega-9 (oleic acid) enhances absorption and emollience. Their combined presence explains the strong antioxidant and antibacterial activities of *E. tapos* seed oil observed in our study [22]. Relevant supporting literature has been added to the Results section under GC-MS analysis.

However, the mass spectra obtained from the analysis provided valuable information about the fragmentation pattern and characteristic ions of the saturated fatty acid 9-Hexadecanoic acid, methyl ester, (Z)- (Fig. 3). The mass spectrum showed the partial ionization of saturated fatty acids because saturated fatty acids are often quite stable. In the mass spectrum of saturated fatty acids, this can result in a greater abundance of intact molecular ions (sometimes referred to as $[M]^+$ ions). Without fragmentation, the whole fatty acid molecule is represented by intact molecular ions [33].

Fourier-transform infrared spectroscopy analysis

The Fourier Transform Infrared (FT-IR) spectroscopy technology is widely used to study the functional groups and chemical bonds present in fatty acids. The FT-IR spectrum of *E. tapos* seed oil, which is rich in fatty acids, is shown in Fig. 4. The most prominent peak in the FT-IR spectrum of fatty acids is often seen in the region of 1,700–1,725 cm^{-1}. This is known as carboxylic acid group (C=O) stretching. This peak is associated with the carbonyl (C=O) group stretching vibration that is found in the carboxylic acid functional group of fatty acids [34].

Long hydrocarbon chains structure the stretching vibrations of the methylene ($–CH_2–$) group and the methyl ($–CH_3$) group in fatty acid chains. They often appear as a chain of peaks in the 2,800–3,000 cm^{-1} range. These peaks are associated with the stretching vibrations of these groups in both symmetric and asymmetric ways. Between the carboxylic acid groups of adjacent molecules, fatty acids can form intermolecular hydrogen bonds [35].

The hydroxyl (–O–H) stretching area, which is caused by this interaction, appears as a broad and powerful peak between 2,500 and 3,600 cm^{-1}. The strength and shape of this peak can provide information about the extent of hydrogen bonding in the fatty acid sample. The –OH stretching peak of *E. tapos* seeds oil at 3,431 cm^{-1} showed a prominent peak, which indicated that the structure contained many –OH groups. A small peak was found at 1,033 cm^{-1}, which corresponded to C–C and C =O stretching vibrations of ester fatty acids.

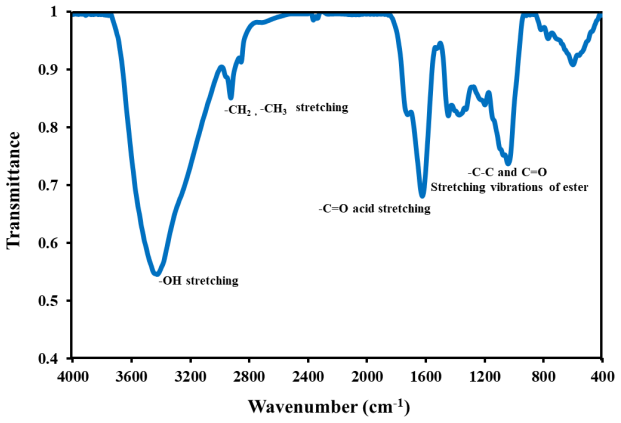

Fig. 4 FT-IR spectra of *E. tapos* oil.

Antioxidant capacity

The free-radical scavenging potential of *E. tapos* seed oil and its formulated cream was investigated using the DPPH, ABTS, and FRAP assays. DPPH and ABTS are reagents composed of free-radical molecules. The ferric-reducing antioxidant power (FRAP) assay was performed to evaluate the antioxidant capacity to ascertain the ferric-reducing activity of the compounds in the seed oil. The concentration of *E. tapos* seeds oil that inhibited 50% (IC_{50}) of DPPH free radicals and ABTS free radicals were 32.67 ±1.34 mg L^{-1} and 19.32 ±0.02 mg L^{-1}, respectively (Table 4). L-ascorbic acid was used as the positive control. It inhibited DPPH and ABTS free radicals at a concentration of 29.72 ±0.95 mg L^{-1} and 15.28 ±0.03 mg L^{-1}, respectively. The cream base without *E. tapos* seeds oil showed an IC_{50} of only 40.45 ±1.80 mg L^{-1} for the scavenging of DPPH and ABTS, and the results suggested that the antioxidant activity of *E. tapos* seeds oil cream depended on the presence of *E. tapos* seed oil. The FRAP assay primarily assesses the capacity of antioxidants to convert ferrous ions (Fe^{2+}) into ferric ions (Fe^{3+}) in a redox reaction. Various fatty acids, some of which may have antioxidant effects, are frequently present in fatty acid oils.

The performance for the reduced electron of *E. tapos* seeds oil and base cream was 5.63 ±0.09 µmol g-1 DW and 3.36 ±0.12 µmol g^{-1} DW, respectively. The antioxidant activity of the formulated cream containing *E. tapos* seeds oil was higher than the antioxidant activities of the seed oil of *E. tapos* and the base cream. Other components might be responsible for this. Improving the antioxidant effect of the cream. The antioxidant activity of *E. tapos* seeds oil and its formulation cream (which was rich in omega-fatty acids, especially omega-6), used for inhibiting the DPPH and ABTS radicals, showed that a solution of $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ was in contact with a substance. It can donate a hydrogen atom or, with another radical ($R^{\bullet}$), the reduced form (such as DPPH–H or DPPH–R) can be produced with the consequent loss of color. This can lead to a decrease in or loss of absorbance [36].

The enhanced antioxidant activity of the *E. tapos* oil cream arises from its high content of omega-3, omega-6, and omega-9 fatty acids, along with phenolic and flavonoid compounds, all of which possess strong hydrogen-donating and radical-scavenging capacities that effectively neutralize free radicals [22]. These bioactive constituents act synergistically to produce lower IC_{50} values in the oil-based cream compared to the base cream, confirming their key contribution to the overall antioxidant performance.

Antibacterial activity

The antibacterial efficacy of *E. tapos* seed oil and its cream formulation against *C. acnes* and *S. epidermidis* was evaluated using the agar well diffusion method. Chloramphenicol antibiotics were used as the positive control in this experiment, and DMSO was used as the negative control. Information on the zone of inhibition of the samples and the images of the Petri dish with clear zones showing the antimicrobial activity of *E. tapos* seeds oil in DMSO, base cream, and formulation oil cream are presented in Fig. 5.

Information on the zone of inhibition was obtained by measuring the center diameter of the zone (in mm). The largest zones of inhibition associated with the antibacterial activity of *E. tapos* seeds oil against *S. epidermidis* and *C. acnes* were 22.51 ±1.40 mm and 24.67 ±0.90 mm, respectively. The formulation cream showed stronger antibacterial activity than the formulation base cream without *E. tapos* seeds oil as determined by the size of the inhibition zone for *S. epidermidis* (25.17 ±1.20 mm) and *C. acnes* (27.25 ±0.87 mm). This greater inhibition occurred because seed oils may contain natural antimicrobial compounds, such as fatty acids, terpenoids, flavonoids, and alkaloids [37,38]. These compounds may exhibit direct antibacterial effects by inhibiting bacterial growth or interfering with bacterial metabolism.

Table 4 The antioxidant activity of *E. tapos* seeds oil.

Sample	Antioxidant activity		
	DPPH IC_{50} (mg L^{-1})	ABTS IC_{50} (mg L^{-1})	FRAP (µmol g^{-1} DW)
Ascorbic acid	29.72 ±0.95	15.28 ±0.03	9.84 ±0.18
E. tapos seed oil	34.65 ±1.31	19.32 ±0.02	5.63 ±0.09
Base cream	40.45 ±1.80	23.15 ±0.01	3.36 ±0.12
E. tapos seeds oil cream	32.67 ±1.34	17.97 ±0.04	6.82 ±0.16

The physical properties of the cream produced from *E. tapos* seeds oil are presented in Table 5. The cream was smooth, homogeneous, and light-yellow in appearance. The pH of the cream was 6–7, which was similar to the pH of the skin (pH 6.8 ±0.5), and thus, it was suitable for topical application [39]. The cream had a homogeneous texture. The *E. tapos* seeds oil was uniformly distributed in the cream, as determined by visual appearance and touch. Both creams had a smell of rice milk because a substance that smelled like rice milk was added. The results indicated that the extracted oils, which had saturated and unsaturated fatty acids, showed good antioxidant and antibacterial efficiency when used as ingredients in the cream.

The antibacterial and antioxidant processes were predicted to occur as indicated in Fig. 6. The structure of omega-6 fatty acids consists of carboxylic functional groups and methyl groups, which affect the electron transport process, accompanied by proton (H^+) transfer from the cytosol to outside the cell, creating a proton gradient across the cell membrane. When oxidative phosphorylation and one of the steps in the electron transport chain are disrupted, bacterial cells have insufficient energy to function which inhibits cell growth and results in their death. The cytoplasmic membrane and increasing the membrane potential, which acts as an energy source for ATP synthase to use in the production of ATP [40]. Fatty acids, especially those with unsaturated bonds (such as omega-3 and omega-6 fatty acids), can also scavenge and neutralize free radicals. They can stabilize the radicals by donating electrons or hydrogen atoms, which prevents the radicals from interacting with biological components and reduces oxidative stress [41].

This mechanistic explanation illustrated, particularly the roles of omega-3, omega-6, and omega-9 fatty acids in membrane disruption, oxidative stress modulation, and electron or proton transfer, is presented as a supportive interpretation based on established biochemical pathways reported in previous literature, rather than mechanisms directly verified in this study. While the experimental results confirm biological activities, the mechanistic pathways remain proposed hypotheses that align with the known behavior of polyunsaturated fatty acids and warrant further targeted investigation [42, 43]. The results indicated that the cream produced from *E. tapos* seed oil exhibited acceptable consistency, homogeneity, appearance, pH, and odor.

Table 5 Evaluation parameters of the formulation cream.

Formulation	color	odor	pH	homogeneity
Base cream	White	Rice milk smell	7.2 ±0.4	homogenous
E. tapos seeds oil cream	Light-yellow	Rice milk smell	6.8 ±0.5	homogenous

4. Conclusion

This study successfully developed and evaluated an herbal cream containing *E. tapos* seed oil, which is a local natural oil, addressing key concerns regarding safety, efficacy, and methodological rigor. The findings suggest that the raw materials of the herbal cream were the active components. The result shows the oil of *E. tapos* seed with the high antioxidant activity of the cream produced from the oil for DPPH and ABTS, as well as its good electron-reducing activity toward ferric ions. They also had a favorable effect on Gram-positive (*S. epidermidis*) and (*C. acnes*) bacteria. This product holds potential for skincare applications, provided that further clinical studies validate its safety and effectiveness in human subjects.

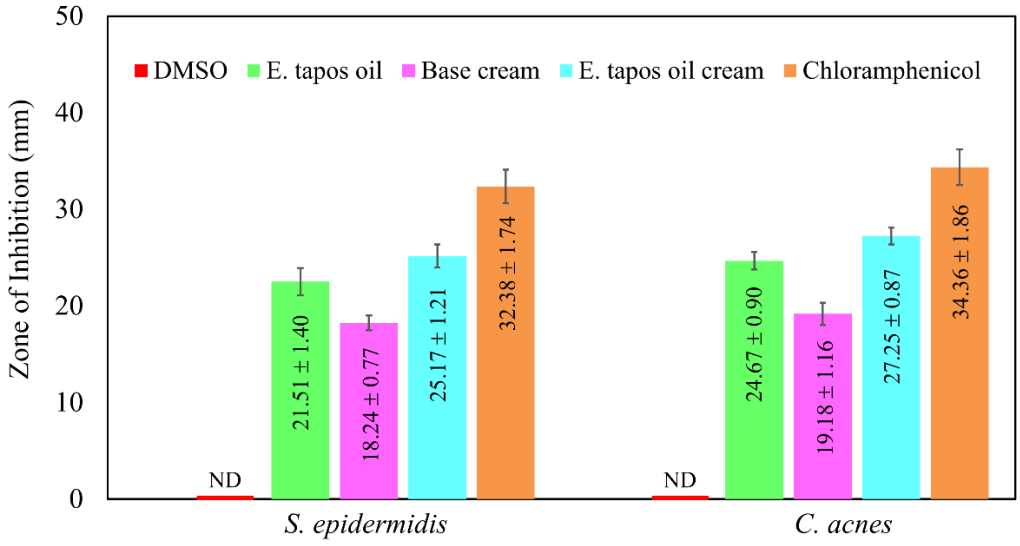

Fig. 5 Antibacterial activity of *E. tapos seeds oil* and its cream formulation against *S. epidermidis* and *C. acnes*.

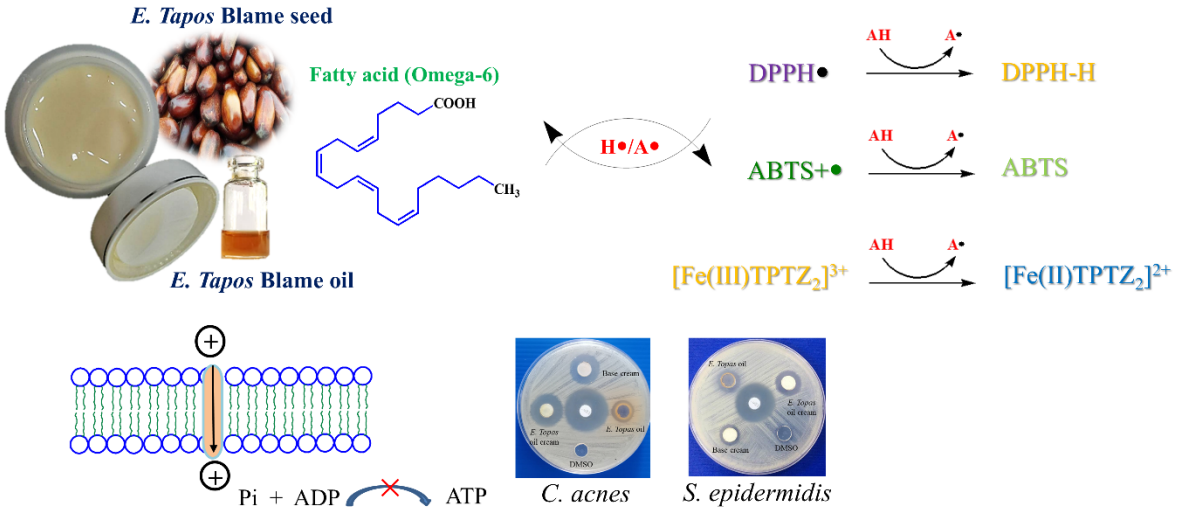

Fig. 6 Schematic illustration of the antioxidant and antibacterial mechanism of *E. tapos* seed oil.

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6. Declaration of generative AI in scientific writing

During the preparation of this manuscript, ChatGPT and QuillBot were utilized to assist with English language editing. All text was thoroughly reviewed, corrected, and refined by the authors to ensure clarity, accuracy, and coherence. The authors assume full responsibility for the integrity and reliability of the published work.

8. CRediT author statement

Naengnoi Saengsane: Conceptualization, Methodology, Data analysis, Writing –Original Draft, Review, Editing.

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9. Research involving human and animals rights

This study did not involve any research on human participants or animals and therefore does not pertain to research involving human or animal rights.

10. Ethics Approval and Consent to Participate

Not applicable

11. Declaration of Competing Interest

The authors confirm that they have no conflicts of interest to declare.

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