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Investigation of Antioxidant Extracts from *Etlingera Elatior* Flowers and *Citrus Reticulata Blanco* Peels through Optimized Microwave-Assisted Extraction for Anti-Acne Potential of Skincare Serum and Face Mask Formulation

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Abstract. This study aimed to develop skincare serum and face mask formulations for acne treatment using extracts from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels. Extracts were prepared through Microwave-Assisted Extraction (MAE), and the optimal extraction conditions for phenolic compounds from *Etlingera elatior* flowers were determined. The total phenolic content (TPC) of the extracts was quantified, and their antioxidant activities were evaluated. Furthermore, the antibacterial activity of *Etlingera elatior* flower extract against *Cutibacterium acnes* was assessed using the disc diffusion method. The optimal extraction condition for phenolic compounds from *Etlingera elatior* flower powder was identified as follows: 4 g of flower powder with 40 mL of ethanol as the solvent, under microwave power of 400 W for one 30-second cycle. This condition yielded an extract with a TPC of 0.51 ± 0.03 mg GAE/g extract. For *Citrus reticulata blanco* peel extract, the TPC was 0.24 ± 0.05 mg GAE/g extract. Antioxidant activity analysis revealed that *Etlingera elatior* extract and *Citrus reticulata blanco* peel extract exhibited notable free radical scavenging activity, with IC_{50} values of 56.85 ± 2.25 µg TAE/mL and 1,249.59 ± 78.67 µg TAE/mL, respectively. Additionally, *Etlingera elatior* extract demonstrated significant inhibitory effects against *Cutibacterium acnes*, comparable to standard substances commonly employed in acne treatment. Stability testing confirmed that the prototype skincare serum formulation exhibited favorable stability. These findings indicate that the developed formulations incorporating *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts can be utilized to produce prototype skincare serum and face mask products depending on the users' needs. Such formulations not only offer potential efficacy in acne management but also contribute to value-added utilization of these distinctive local plant resources.

Keywords: *Etlingera Elatior*; *Citrus Reticulata Blanco*; Microwave-Assisted Extraction; Anti-Acne; Skincare

1. Introduction

The use of acne-related skincare among adolescents has been increasingly prevalent in Thailand due to the influence of hormonal changes, genetic factors, personal hygiene behaviors, and the frequent wearing of face masks that may cause skin irritation, rashes, or acne. Reflecting this situation, the overall demand for skincare products has steadily expanded among both males and females. Therefore, the development of effective skincare formulations should emphasize the exclusion of pore-clogging and oil-based substances while incorporating properties that can alleviate acne-related allergic reactions and suppress microorganisms including bacteria and fungi that contribute to acne formation (Phawanthaksa, 2019). Grounded in the concept of innovative cosmetics, the researchers initiated a study and product development utilizing herbal resources derived from local plants in Yala Province, Thailand. This initiative emerged in response to the necessity of creating a novel product capable of addressing the identified problems while remaining cost-effective and competitive in the market.

A review of related literature indicated that the *Etlingera elatior* flower which is abundantly available in Yala Province of Thailand possesses noteworthy bioactive properties. Extracts from this plant have been reported to inhibit *Cutibacterium acnes* (*C. acnes*) which is a principal bacterium responsible for acne pathogenesis. Such extracts not only reduce acne scarring and suppress new acne formation but also enhance skin clarity through the inhibition of tyrosinase activity and melanin biosynthesis. Moreover, they promote collagen production in the dermis, exhibit antioxidant capacity, and mitigate inflammation induced by free radicals (Kaew-on, 2016). In addition, *Citrus reticulata blanco* which refer to a fruit widely consumed by local communities in Yala, Thailand provides valuable peel extracts with dermatological benefits. The bioactive compounds derived from orange peels have been shown to reduce sebum secretion from sebaceous glands and to exert anti-inflammatory effects leading to a reduction in acne symptoms. These properties further assist in dissolving excess oil, preventing pore blockage, and decreasing facial oiliness which ultimately lowers the risk of recurrent acne. Prolonged application also contributes to skin hydration, the recovery of dry and flaky skin, and improvements in overall skin brightness (Tantipaibulvut et al., 2012).

To address acne-related problems through sustainable approaches, this study was designed with the objective of developing skincare serum and face mask formulations enriched with bioactive compounds from local plants in Yala Province, Thailand. The research process commenced with the extraction of *Etlingera elatior* flowers and *Citrus reticulata blanco* peels using the Microwave-Assisted Extraction (MAE) technique. Following this, the experimental work emphasized optimizing the extraction conditions of phenolic compounds from *Etlingera elatior* flowers and determining the total phenolic content present in both *Etlingera elatior* and *Citrus reticulata blanco* peel extracts alongside evaluating their antioxidant activities. The antibacterial potential of *Etlingera elatior* extracts against *Cutibacterium acnes* (*C. acnes*) was subsequently examined through the Disc Diffusion Method. In the final stage, prototype formulations of skincare serum and face mask were developed and subjected to stability testing. Ultimately, the study aims not only to create effective cosmetic products derived from local plant resources but also to promote environmentally friendly innovation that contributes to income generation and commercial value creation.

2. Research Objectives

1. To obtain crude extracts from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels by employing the MAE process as a preparatory step for subsequent analyses.
2. To optimize extraction parameters for phenolic compounds from *Etlingera elatior* flowers using the Microwave-Assisted Extraction (MAE) technique.

3. To quantify the total phenolic content of extracts prepared from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels and assess their antioxidant activities.

4. To investigate the antibacterial efficacy of *Etlingera elatior* extracts against *Cutibacterium acnes* through application of the Disc Diffusion Method.

5. To formulate and develop a skincare serum utilizing bioactive compounds obtained from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels.

6. To evaluate the stability of prototype skincare serum and mask formulations which incorporate extracts derived from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels.

3. Methodology

3.1 Preparation of extracts from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels using MAE.

The plant materials consisting of *Etlingera elatior* flowers and *Citrus reticulata blanco* peels were first subjected to a cleaning process to remove impurities. Following this, the samples were cut into small sections and subsequently dried in a hot-air oven at 50 °C for approximately 48 hours until constant dryness was achieved. Once thoroughly dried, the materials were ground into a fine powder which was then utilized for subsequent extraction procedures.

Figure 1: Preparation of extracts from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels using MAE

3.2 Optimization of extraction conditions for phenolic compounds from *Etlingera elatior* flowers using MAE.

The optimization of extraction conditions for phenolic compounds from *Etlingera elatior* flowers using the Microwave-Assisted Extraction (MAE) technique was carried out through a stepwise experimental design. Initially, different powder-to-solvent ratios were evaluated by extracting 2, 4, 6, and 8 g of flower powder with 40 mL of ethanol. Extractions were performed at 400 W for three microwave cycles, each cycle consisting of 30 seconds on followed by 30 seconds off. After each extraction, the solutions were filtered, the solvent evaporated, and the total phenolic content quantified spectrophotometrically to determine the optimal ratio for subsequent experiments. Based on the identified ratio, the influence of microwave power was then investigated at 200, 400, and 600 W under the same extraction cycle conditions. The extract with the highest phenolic yield was selected to further optimize the number of microwave cycles, which were varied across 1, 2, 3, and 4 repetitions. Finally, to assess the effect of repeated extraction, the residue obtained from the optimized extraction was subjected to re-extraction using fresh solvent at the same ratio for two additional cycles. Each extract was consistently filtered, the solvent evaporated, and the total phenolic content analyzed using a spectrophotometer.

Figure 2: Optimization of extraction conditions for phenolic compounds from *Etlingera elatior* flowers using MAE

3.3 Determination of total phenolic content and antioxidant activity of *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts.

The total phenolic content of the extracts was quantified using the Folin-Ciocalteu colorimetric method (Torres et al., 1987). In this procedure, 0.1 mL of the extract sample was mixed with 1 mL of 1 N Folin-Ciocalteu phenol reagent and allowed to stand for 2–5 minutes before adding 2 mL of 20% (w/v) sodium carbonate. The mixture was kept at room temperature for 10 minutes, after which the absorbance was measured at 760 nm and compared against a gallic acid calibration curve ranging from 0 to 0.12 mg/mL. Antioxidant activity was subsequently evaluated using the DPPH radical scavenging assay. A 2 mM DPPH solution was prepared by dissolving 0.0197 g of 2,2-diphenyl-1-picrylhydrazyl in methanol, adjusting the volume to 25 mL in a volumetric flask, and further diluting 2.5 mL of this stock solution to 25 mL with methanol to obtain a 0.2 mM working solution. A Trolox standard solution was prepared in methanol, and five concentrations of both Trolox and the extract samples were tested. For each measurement, 2.9 mL of the 0.2 mM DPPH solution was transferred to a cuvette followed by the addition of 100 µL of either the standard or extract solution. The mixtures were thoroughly mixed and absorbance readings were taken at 5, 20, 30, 40, and 60 minutes at a wavelength of 517 nm using methanol as the blank. The antioxidant capacity of the samples was expressed as the percentage of DPPH radical scavenging, calculated according to the standard formula.

Figure 3: Quantitative determination of total phenolic content in *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts

3.4 Assessment of antimicrobial activity of *Etlingera elatior* flower extracts against *C. acnes* using the Disc Diffusion method.

The inhibitory activity of *Etlingera elatior* flower extracts against *C. acnes* was evaluated using the Disc Diffusion method with methodological modifications based on Nurul et al. (2019). Extracts obtained under the optimized microwave-assisted extraction conditions were subsequently applied to determine their effectiveness in suppressing the growth of *C. acnes*.

3.5 Formulation of skincare serum and face mask incorporating *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts.

The serum formulation was developed using a pre-formulated serum base derived from the most effective composition identified in previous studies. For skincare

serum, *Etlingera elatior* flower extract was incorporated at a concentration of 10% w/w, while *Citrus reticulata blanco* peel extract was added at 5% w/w. However, *Etlingera elatior* flower extract was incorporated at a concentration of 12% w/w, while *Citrus reticulata blanco* peel extract was added at 1% w/w for face mask. The finished product was expected to exhibit uniform color, absence of phase separation or aggregation, lack of foreign particles, and a pleasant natural fragrance. The extracts were blended with the serum base, left to equilibrate for 24 hours, and subsequently supplemented with essential oils for fragrance enhancement. Following formulation, the serum was subjected to stability testing to evaluate its suitability for further application.

Figure 4: Formulation process of skincare serum and face mask containing bioactive extracts derived from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels

3.6 Stability evaluation of the prototype serum formulated with *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts.

To examine the stability, the finished serum was transferred into 5 mL glass containers with tightly sealed caps and stored at 55 °C. The physical properties of the formulation were then monitored on days 0, 3, 6, and 9, with specific evaluations of color, odor, phase separation, overall physical appearance, application characteristics on the skin, and pH. These assessments were conducted to detect any changes that may influence the long-term stability and suitability of the product for further application.

4. Results

4.1 Results of extract preparation from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels.

Using the Microwave-Assisted Extraction (MAE) method, extracts of *Etlingera elatior* flower powder and *Citrus reticulata blanco* peel powder were successfully obtained. In this process, 20 g of *Citrus reticulata blanco* peel powder were accurately weighed and placed into a 500 mL beaker, then 200 mL of ethanol was added as the extraction solvent. The suspension was exposed to microwave irradiation at 400 W. Applying two cycles in which each cycle consisted of 30 seconds of heating followed by a 30-second rest period. Upon completion of the microwave treatment, the mixture was filtered and the solvent removed through evaporation. The resulting crude extract was then subjected to analysis for total phenolic content and antioxidant properties, and subsequently incorporated as an active ingredient for further skincare serum and face mask formulation development.

4.2 Results of the optimization of phenolic compound extraction from *Etlingera elatior* flowers.

The extraction of total phenolic compounds from *Etlingera elatior* flower powder was optimized under specific conditions. The most effective parameters were identified as 4 g of flower powder extracted with 40 mL of ethanol employing the Microwave-Assisted Extraction (MAE) method at a power of 400 W. The process was conducted using a single microwave cycle of 30 seconds, which was sufficient to obtain the desired extract.

4.3 Results of the determination of total phenolic content and antioxidant activity of *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts.

The total phenolic compounds present in extracts derived from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels were quantified to assess their phytochemical composition and the antioxidant potential of the extracts from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels was investigated using the DPPH radical scavenging assay.

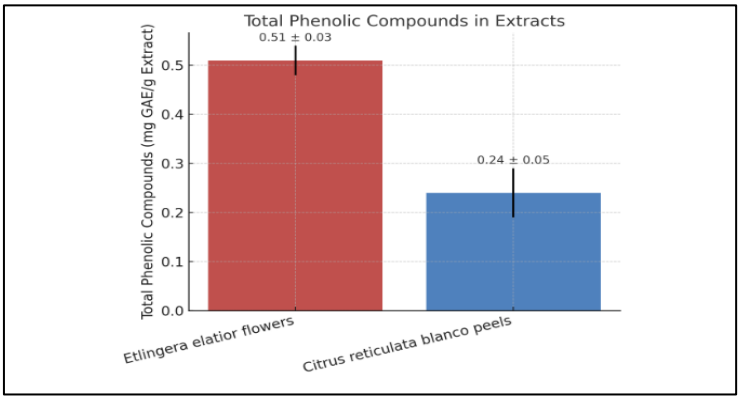

Figure 5: Quantitative analysis of total phenolic content in *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts

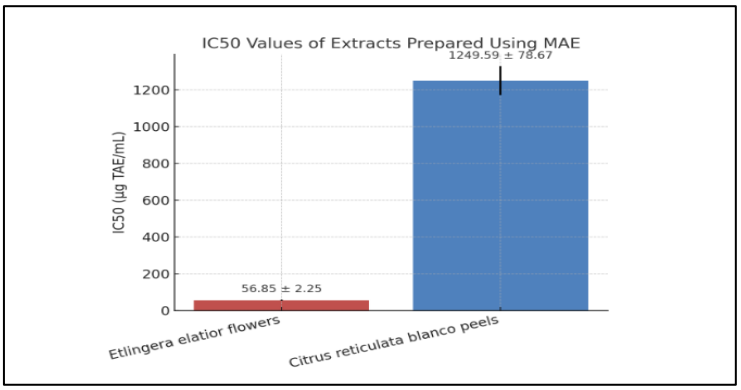

Figure 6: IC_{50} determination of *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts obtained through Microwave-Assisted Extraction (MAE)

4.4 Results of evaluating the inhibitory effect of *Etlingera elatior* flower extract against *C. acnes* using the Disc Diffusion method.

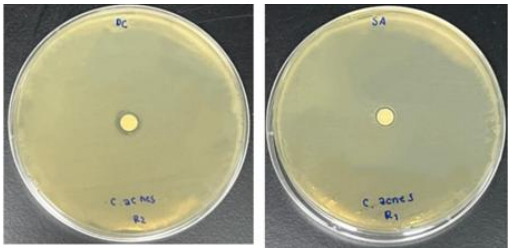

Figure 7: Comparative analysis of inhibition zones against *C. acnes* between *Etlingera elatior* flower extract and the reference compound salicylic acid.

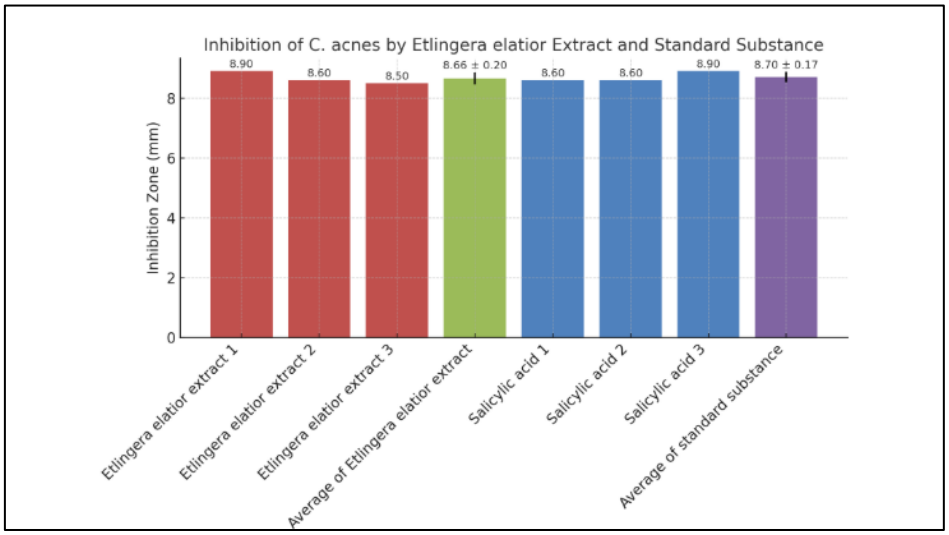

Figure 8: Evaluation of the inhibitory efficacy of *Etlingera elatior* flower extract against *C. acnes*.

4.5 Results of developing skincare serum and face mask formulation incorporating *Etlingera elatior* flower and *Citrus reticulata blanco* peel extracts.

The study involved the development of skincare serum and face mask formulation enriched with extracts derived from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels followed by an evaluation of the prototype's physicochemical stability. The resulting skincare serum and face mask formulation demonstrated promising effectiveness in acne treatment, attributable to the bioactive constituents present in both plant extracts.

Table 1: Formulation of skincare serum and face mask incorporating bioactive extracts derived from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels.

Number	INS	Name of the substance	% w/w Skincare serum	% w/w Facemask
1	7732-18-5	AQUA	34	36
2		ETLINGERA ELATIOR FLOWER EXTRACT	10	12
3	68916-04-1	CITRUS AURANTIUM AMARA FLOWER EXTRACT	5	1
4	84696-19-5	HAMAMELIS VIRGINIANA EXTRACT	10	10
5	85507-69-3	ALOE BARBADENSIS LEAF EXTRACT	20	20
6	79725-98-7	KOJIC ACID DIPALMITATE	10	10
7	97-59-6	ALLANTOIN	3	3
8	98-92-0	NIACINAMIDE	3	3
9	9007-20-9	CARBOMER 940	2	2
10	68647-73-4	TEA TREE OIL	1	1
11	182212-41-5	ALLYL METHACRYLATES CROSSPOLYMER	1	1
12	70445-33-9	ETHYLHEXYLGLYCERIN	0.5	0.5
13	504-63-2	PROPANEDIOL	0.5	0.5

4.6 Results of stability testing on the prototype skincare serum and face mask formulations.

The stability testing on the prototype skincare serum and face mask formulations demonstrated their effectiveness in treating acne and post-acne hyperpigmentation, attributed to the bioactive compounds derived from *Etlingera elatior* flowers and *Citrus reticulata blanco* peels.

Table 2: Stability evaluation of the prototype serum and face mask formulation

Observation Period	Color	Odor	pH	Skin Absorption	Overall Physical Appearance
Day 0	Light pink	Normal	6.0	Good	Normal
Day 3	Light pink	Normal	6.0	Good	Normal
Day 6	Light pink	Normal	6.0	Good	Normal
Day 9	Light pink	Normal	6.0	Good	Normal

5. Discussion and Conclusion

From the extraction of bioactive compounds in *Etlingera elatior* flowers and *Citrus reticulata blanco* peels, the optimal conditions for Microwave-Assisted Extraction (MAE) of *Etlingera elatior* flower powder were determined. The use of 4 g of flower powder with 40 mL of ethanol at 400 W for a single 30-second cycle yielded an extract with a total phenolic content of 0.51 ± 0.03 mg GAE/g extract. In contrast, MAE of *Citrus reticulata blanco* peel produced an extract with 0.24 ± 0.05 mg GAE/g extract. Antioxidant evaluation revealed that both extracts displayed significant activity with IC50 values of 56.85 ± 2.25 and 1,249.59 ± 78.67 µg TAE/mL respectively. Moreover, the *Etlingera elatior* flower extract demonstrated notable antibacterial activity against *C. acnes* comparable to standard anti-acne agents, consistent with findings reported by Nurul et al.

These extracts were subsequently incorporated into a prototype skincare serum and face mask, which exhibited favorable stability and anti-acne efficacy. Given the local abundance of *Etlingera elatior* and *Citrus reticulata blanco* in Yala Province, Thailand, the formulation highlights the potential to increase the economic value of these plants, support community enterprises, and promote commercialization. Additionally, the eco-friendly extraction method adds value to agricultural raw materials and may serve as an alternative to synthetic cosmetic ingredients. Future studies should further optimize MAE conditions for *Citrus reticulata blanco* peels and examine the stability of the prototype serum under diverse storage conditions, including accelerated aging to provide comprehensive data for product development.

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