

Synergy and Sensory Harmonization in Essential Oil Blends of *Zanthoxylum limonella*, *Z. bungeanum*, and *Z. simulans*: A Chemical and Bioactive Evaluation

Sarita Sangthong^{1,2,*}, Tinnakorn Theansungnoen^{1,2},
Punyawatt Pintathong^{1,2} and Phanuphong Chaiwut^{1,2}

¹School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

²Green Cosmetic Technology Research Group, Mae Fah Luang University, Chiang Rai 57100, Thailand

(*Corresponding author's e-mail: sarita.san@mfu.ac.th)

Received: 20 March 2026, Revised: 22 April 2026, Accepted: 29 April 2026, Published: 5 July 2026

Abstract

Essential oils (EOs) from the genus *Zanthoxylum* are widely valued for their traditional medicinal and culinary uses. There is limited research on the synergistic effects of their combinations. This study investigated the volatile profile and antioxidant activity of EOs from *Z. limonella* (ZL), *Z. bungeanum* (ZB), and *Z. simulans* (ZS) at different extraction times. GC-MS analysis identified key constituents, including: D-limonene (45.9%) and sabinene (22.76%) in ZL, Eucalyptol (15.46%) and terpinen-4-ol (12.44%) for ZB, and Sabinene (17.21%) and xanthoxylin (16.68%) in ZS. The highest antioxidant activity was observed at 3 h for ZB and at 7 hours for ZL and ZS. Importantly, the ZL:ZB:ZS combinations exhibited significant synergistic antioxidant effects, reaching 1.78-fold at the 2:3:1 ratio. The inhibition of *C. acnes* was observed by a clear zone measurement. ZL has the highest activity, followed by ZB and ZS, respectively. The 2:3:1 ratio remained the highest synergized combination (1.27 times higher than expected). The combinations showed lower cytotoxicity against NIH/3T3 cell lines than ZS alone. These findings demonstrate that blending enhances bioactivity while achieving sensory harmonization by masking pungent odors with citrus notes. This could underscore their potential as versatile bioactive agents for multiple applications.

Keywords: *Z. bungeanum*, *Z. limonella*, *Z. simulans*, Antioxidant, *C. acnes* inhibition, Synergistic effect

Introduction

The genus *Zanthoxylum* (Rutaceae) comprises more than 200 species widely distributed in tropical and subtropical regions and recognized for their culinary and ethnobotanical significance, particularly in Asia [1]. Among them, *Zanthoxylum limonella*, *Zanthoxylum bungeanum*, and *Zanthoxylum simulans* are especially valued for their characteristic aroma profiles and documented biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects [2]. These species are traditionally used as spices and medicinal ingredients, reflecting their dual sensory and functional relevance. A distinctive feature of *Zanthoxylum* pericarps is the characteristic tingling and numbing sensation frequently described as a “buzzing” effect. This perception is primarily attributed to non-

volatile alkylamides such as sanshools and their derivatives [3]. This unique trigeminal property is central to the Chinese “Mala” flavor system, in which the numbing effect of *Zanthoxylum* is harmonized with chili heat to create a complex multisensory experience [3]. Such sensory integration highlights the potential for combinatorial interactions not only at the perceptual level but also at the chemical and bioactive levels.

Zanthoxylum species produce essential oils (EOs) rich in volatile terpenoids that define their aromatic identity and contribute to their biological properties [1,2]. These secondary metabolites occur in various plant parts, including pericarps, leaves, stems, and seeds. While seeds contain high levels of unsaturated fatty acids, antioxidant and anti-inflammatory activities

have also been associated with diverse polyphenols identified in these species [4]. The essential oil of *Z. limonella* is dominated by monoterpenes such as sabinene, α -pinene, and β -pinene [5,6]. Ethanolic extracts of *Z. bungeanum* are rich in alkaloids and lignans [1,7], whereas *Z. simulans* leaf essential oil contains limonene (44.3%) and β -caryophyllene (12.5%) as major constituents [8], alongside sanshools and xanthoxylins contributing to sensory characteristics. Despite these compositional insights, most previous studies have focused on single-species extracts with limited investigation of their combinatorial effects. Shared monoterpenes such as limonene exhibit antimicrobial activity and are widely applied in flavor and consumer products, with reported anti-inflammatory, antioxidant, antinociceptive, anticancer, antidiabetic, antihyperalgesic, gastroprotective, and antiviral properties [5].

The combined use of essential oils has increasingly attracted interest due to potential synergistic interactions that may enhance efficacy, modulate toxicity, and influence sensory profiles. Synergistic antioxidant and antimicrobial effects have been reported in blends of *Thymus fontanesii*, *Artemisia herba-alba*, and *Rosmarinus officinalis* [9], mixtures of *Zanthoxylum bungeanum* and *Capsicum annuum* [11], and combinations of *Amomum verum* with *Zanthoxylum limonella* [5]. Similarly, citrus leaf oil blends (*Citrus meyeri*, *Citrus paradise*, *Citrus sinensis*) demonstrated antimicrobial activity with selective cytotoxicity [12], while cosmetic blends of citronella, lemongrass, and patchouli showed enhanced inhibition of acne-causing bacteria [13]. Essential oil combinations have also been proposed for respiratory tract infections, where synergy may reduce required dosages and associated toxicity [14]. Collectively, these studies suggest that multi-oil systems can produce additive or synergistic effects through complementary chemical interactions.

Despite the traditional co-application of *Z. limonella*, *Z. bungeanum*, and *Z. simulans* in flavor systems such as “Mala,” systematic evaluation of their essential oil blends remains limited. Given their overlapping yet distinct volatile profiles and established sensory compatibility, it is hypothesized that blending these three *Zanthoxylum* essential oils may result in synergistic bioactivity alongside sensory harmonization driven by terpene interactions. In particular,

enhancement of antioxidant capacity and inhibition of *Cutibacterium acnes* are of interest for flavor, cosmetic, and dermatological applications.

Therefore, the objectives of this study were: (i) to optimize hydrodistillation times (3, 5 and 7 h) for essential oils extracted from the pericarps of *Z. limonella*, *Z. bungeanum*, and *Z. simulans*; (ii) to characterize their chemical composition using gas chromatography-mass spectrometry (GC-MS); and (iii) to evaluate antioxidant activity, anti-*C. acnes* effects, and cytotoxicity of individual and blended oils at different ratios. By integrating chemical profiling with bioactivity assessment, this study aims to elucidate synergistic interactions and provide a scientific foundation for the rational design of harmonized *Zanthoxylum* essential oil blends for flavor, fragrance, and related applications.

Materials and methods

Plant material

Z. limonella fruits (ZL) were harvested and dried under shade at room temperature in Pua, Nan province, Thailand, in February 2024. *Z. bungeanum* (ZB) and *Z. simulans* (ZS) were grown in Sichuan, China, and fruits were purchased via a herbal shop, Chiang Rai, Thailand, in March 2024. The seedless pericarp was selected for the extraction.

Plant extraction

Plant samples were extracted by using the hydrodistillation method [15]. The ratio of the dried plants to deionized water is 1:10 (w/w). The extraction was separated into 3-time courses for all of the dried plants, including 3, 5, and 7 h at 150 °C (heating mantle temperature; actual distillation temperature of the water-plant mixture was approximately at water boiling point, 100 °C) using a Clevenger apparatus and heating mantle (MS-E series, MTOP, Korea). The EOs were collected and the contaminant water was removed with the addition of anhydrous salt. The percentage of yields was calculated. The primary odor characteristics were evaluated via sniffing by trained researchers to assess the impact of extraction time on the scent profile. The obtained essential oils were stored at 2 - 8 °C in a dark place for further analysis. The three extraction durations (3, 5 and 7 h) were selected based on published hydrodistillation protocols for *Zanthoxylum* species

[15,16] and pilot experiments. Short durations (3 h) preferentially capture early-eluting, more volatile fractions (e.g., linalool, α -terpineol), while longer durations (7 h) allow progressive accumulation of less volatile constituents such as D-limonene, sabinene, and xanthoxylin, enabling a systematic comparison of time-dependent compositional and bioactivity changes.

GC-MS analysis

Essential oils of ZL, ZB, and ZS extracted for 3, 5, and 7 hours were diluted at a ratio of 1:100 (essential oil: absolute ethanol) and transferred into GC vials. GC-MS analysis was performed using an Agilent 6890 GC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a HP-5MS capillary column (Agilent 19091S-433; 30 m $\times$ 0.25 mm i.d., 0.25 μ m film thickness). The injector and detector temperatures were both set at 220 °C. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min with a split ratio of 100:1. The column oven temperature was initially held at 60 °C, then programmed to increase to 240 °C, following the method described earlier [17]. Compounds were identified by comparing their mass spectra with those in the NIST08 library, with a similarity threshold of higher than 90%. Relative area percentages were used to track dynamic shifts in composition.

DPPH radical scavenging assay

DPPH scavenging activities of the oils were determined with the DPPH radical protocol [17]. The DPPH reagent and trolox standard solution were prepared by dissolved in 95% ethyl alcohol. The single oil and the combination of three oils in ten different ratios were tested. The mixture of ethanolic diluted EO samples and DPPH reagent was incubated in dark conditions for 30 min. The absorbance at 517 nm was measured by a microplate reader (BMG LABTECH/SPECTROstar). The results of antioxidant activity (AOA) were expressed in milligram trolox equivalent antioxidant capacity per gram essential oil (mg TEAC/g EO) (Eq. (1)).

$$\text{DPPH radical scavenging activity (mg TEAC/g EO)} = \frac{\% \text{Inhibition} \times \text{dilution} \times \text{Total volume in reaction tube (mL)} \times \text{volume of EO (mL)}}{\text{Slope} \times \text{Volume of EO (mL)} \times \text{Weight of EO (g)}} \quad (1)$$

$$\text{where, \% Inhibition} = \frac{\text{control} - \text{sample}}{\text{control}} \times 100\%$$

Cutibacterium acnes growth inhibition

The *C. acnes* growth inhibition activity of essential oils was evaluated by the disc diffusion method modified from the previous study [18]. The single selected EOs of ZL, ZB, and ZS based on the most efficient antioxidant activity were determined using the agar disc-diffusion method. EOs were diluted in DMSO and sterilized by filtration through 0.2 μ m syringe filters (Sigma-Aldrich, St. Louis, MO, USA). Bacterial suspensions of *C. acnes* at an optical density of 0.5 at OD_{600 nm} were spread on RCM agar plates. The samples (20 μ L) were loaded in sterile filter-paper discs (6 mm in diameter) and placed on the surface of the agar, then incubated under anaerobic conditions. Standard antibiotics, clindamycin (2 μ g/disc), were used as positive controls, while DMSO was used as a negative control. The antibacterial activity of each EO was evaluated by measuring the diameter of the zone of inhibition expressed in millimetres (mm). Each assay was performed in triplicate.

Study of the antioxidant and *C. acnes* growth inhibition synergistic effect

To determine the type of combination interaction, the antioxidant and *C. acnes* inhibitory activities of the mixtures were evaluated using their DPPH radical scavenging activity as mentioned earlier. Ten different ratios of essential oils from the most optimum extraction times of *Z. limonella*, *Z. bungeanum*, and *Z. simulans* pericarp were combined in symmetric and asymmetric mixtures. The calculation of the interaction index was determined by dividing the experimental value of the antioxidant activity (AOA, mg TEAC/g EO) obtained by DPPH by the theoretical value or expected value (Eq. (2)). Theoretical or expected values were obtained by considering the proportion of each essential oil and its DPPH radical scavenging activity in terms of Trolox equivalent when tested alone. The values were then categorized into antagonistic effect (<0.5), additive effect (0.5 < 1.0), or synergistic effect (> 1.0) [19].

$$\text{Interaction index} = \frac{\text{Experimental value}}{\text{Expected value}} \quad (2)$$

where, expected value = $[(AOA_{ZL} \times \% (v/v)_{ZL}) + (AOA_{ZB} \times \% (v/v)_{ZB}) + (AOA_{ZS} \times \% (v/v)_{ZS})]$

Statistical analysis

All data were presented in the mean value with the standard deviation (mean $\pm$ SD). The parameters were compared and analyzed using One-way ANOVA (Duncan) test with a significant level of $p < 0.05$. Data normality was assessed using the Shapiro-Wilk test prior to ANOVA; all datasets met the normality assumption. Duncan's multiple range test was selected as it is appropriate for balanced experimental designs with equal group sizes.

Results and discussion

Plant material and extraction

The essential oil physical characteristics were shown in **Figure 1**. The ZB and ZL oils were colorless, clear solutions that evaporated easily on paper strips.

Where ZS essential oils are present with the presence of waxy particles when stored at 25 ± 2 °C, and essential oils are retained more on the paper strips (**Figure 1**). The odor characteristic of the essential oils was evaluated by sniffing as in **Table 1**. ZL essential oils possess outstanding top notes of citrus and spicy. The citrusy level was highest at 3 h, and instead the spicy level was raised and lowered along the extraction time. The ZB essential oil at 3 h of extraction was herbaceous with a hint of citrusy. The citrus character faded until it disappeared at 7 h of extraction. The main character of herbaceous and spicy was clear at 5 h, where the curry-like character was present at 7 h of extraction. The ZS essential oils were significantly noted for their curry-like characteristics with a sweet and warm hint. The waxy smell was higher with the extraction time, which related to the physical character of the essential oils, which were not a clear solution but with some waxy particles at 25 ± 2 °C.



Figure 1 Physical appearance of *Z. limonella* (Demst.) Alston, *Z. bungeanum* Maxim., and *Z. simulans* fruits (upper) and essential oils (lower).

Table 1 Characteristics and extraction yields of *Z. limonella*, *Z. bungeanum*, and *Z. simulans* essential oils at different extraction times.

Sample	Extraction time (h)	Characteristics	Extraction yield (%w/w)
<i>Z. limonella</i>	3	Clear pale yellow, Fresh, sweet citrusy	8.36 ± 0.04^b
	5	Clear pale yellow, Citrusy and spicy	8.45 ± 0.03^b
	7	Clear pale yellow, Spicy and citrusy	10.42 ± 1.29^a
<i>Z. bungeanum</i>	3	Clear pale yellow, Warm citrusy herbaceous	5.10 ± 0.40^b
	5	Clear pale yellow, Warm spicy herbaceous	5.58 ± 0.06^a
	7	Clear pale yellow, Curry-like herbaceous	5.62 ± 0.47^a

Sample	Extraction time (h)	Characteristics	Extraction yield (%w/w)
<i>Z. simulans</i>	3	Clear pale yellow with wax, Curry-like	4.61 ± 0.37 ^b
	5	Clear pale yellow with wax, Curry-like	4.47 ± 0.19 ^b
	7	Clear pale yellow with wax, Strong curry-like	5.32 ± 0.31 ^a

Values are given as mean ± S.D. from triplicate.

*Different letters in the same column indicate significant differences in each plant sample ($p < 0.05$).

The essential oils of ZL, ZB, and ZS from the hydrodistillation at different extraction times were compared by their yield percentage (**Table 1**). ZL essential oils yields were increased along the extraction time, and the highest yield among the three extractions was $10.42 \pm 1.29\%$ (w/w) at 7 h. This time-dependent trend was also found in ZB and ZS. The highest percentage of yields was found at 7 h of distillation of $5.62 \pm 0.47\%$ (w/w) and $5.32 \pm 0.31\%$ (w/w) for ZB and ZS, respectively. From the previous studies, *Zanthoxylum limonella* (ZL) fruit essential oil yield was $6.32 \pm 0.40\%$ (hydrodistillation) and 0.79% by enzyme-assisted microwave [19]. *Zanthoxylum bungeanum* (ZB) essential oil from hydrodistillation was 2.9% [16] and $10.0 \pm 0.83\%$ by steam extraction [4]. Lastly, *Zanthoxylum simulans* (ZS), the lowest-yielding essential oil, was 0.153% by steam distillation [20]. These can be seen that the different techniques and plant sources affected the %yield of essential oils.

Notably, the unique odor characteristic of each oil affected to the utilization in certain application. The idea of mixing 3 *Zanthoxylum* essential oils helps in odor modifying, whether blend in an acceptable fresh citrusy, warm herbaceous, and sweet floral, or blend out the pungency curry-like odor.

Determination of chemical components of essential oil by Gas chromatography Mass spectroscopy analysis (GC-MS)

The GC-MS analysis provides the chemical fingerprint of the essential oils (EOs) from three distinct *Zanthoxylum* species—*Z. limonella* (ZL), *Z. bungeanum* (ZB), and *Z. simulans* (ZS). Compounds were tentatively identified based on mass spectral matching against the NIST08 library with a similarity threshold of > 90%. Retention index (KI) confirmation was not performed in this study due to the unavailability of a suitable alkane

reference series under the current GC conditions. The identifications should therefore be considered tentative, and future work employing KI verification on both polar and non-polar columns is recommended to confirm compound assignments.

The data clearly demonstrates that the chemical composition is not only species-specific but also dynamically influenced by the duration of hydrodistillation, with notable shifts occurring at the 3, 5, and 7-hour. The overall profiles are dominated by monoterpenes, but the profile of each plant dictates its individual aroma and potential applications. The previous studies have reported eucalyptol, β -myrcene, and limonene as major components in related *Zanthoxylum* species [21]. From **Table 2**, the essential oil of *Z. limonella* is highly composed of monoterpenes, accounting for more than 94% of the total oil. This high concentration explains the species and oriental citrus characteristics. The most significant finding is the dominance of D-limonene, which at 3 hours already constitutes 42.84% of the oil and increases to nearly 46% after 7 h. This sustained increase suggests that D-limonene, a highly stable and major component, is continually extracted throughout the hydrodistillation process. In parallel, β -ocimene also shows a steady rise, from 7.74% to 8.42% which contributes to the sweet, freshly cut grass, and herbaceous notes that complement the primary citrus aroma [22]. In contrast, other major components such as sabinene (21.82% - 22.76%) and terpinen-4-ol (5.52% - 6.84%) remain relatively constant, indicating that these compounds are likely released early and at a consistent rate. The decreasing trend of other compounds, such as linalool, α -terpineol, and capryl acetate, is particularly interesting. For example, the concentration of linalool is halved from 2.74% at 3 h to 1.34% at 7 h. This suggests that these more volatile compounds are released and may either

degrade or be lost over prolonged extraction, consequently altering the odor profile. Limonene imparts a fresh, bright, citrusy (orange-like) aroma that is often described as uplifting and clean [23]. (+)-Sabinene contributes a spicy, woody, and slightly peppery scent with citrus undertones [24]. Terpinen-4-

ol adds a fresh, herbal, and mildly woody aroma [18], while linalool and α -terpineol provide soft floral and sweet herbaceous nuances. Collectively, the overall odor profile of *Z. limonella* EOs is fresh and citrus-dominant with spicy and herbal undertones, presenting a complex and moderately warm scent.

Table 2 Chemical profile of the volatile compounds in *Z. limonella*, *Z. bungeanum*, and *Z. simulans* essential oils at different extraction times by gas chromatography.

Detected compound Common name	Area percentage (%)								
	<i>Z. limonella</i>			<i>Z. bungeanum</i>			<i>Z. simulans</i>		
	3 h	5 h	7 h	3 h	5 h	7 h	3 h	5 h	7 h
<i>Monoterpenes</i>									
Thujene	0.25	0.28	0.30	0.51	0.55	0.66	0.75	0.74	0.81
α -Pinene	0.44	0.41	0.59	2.48	2.71	3.37	2.57	2.50	2.86
Camphene	-	-	-	-	-	-	0.04	0.04	0.04
Sabinene	22.57	21.82	22.76	6.00	5.60	6.15	30.41	31.41	35.55
β -Pinene	-	-	-	0.36	0.38	0.45	0.87	0.90	0.97
β -Myrcene	1.63	1.84	1.98	7.01	7.49	8.12	3.71	3.70	4.21
α -Phellandrene	2.06	1.89	2.60	0.93	1.09	1.29	1.06	1.05	1.17
(+)-4-Carene	0.49	0.59	0.55	-	-	-	2.30	2.27	2.28
α -Terpinene	1.14	1.41	1.30	5.46	5.92	5.89	1.73	1.70	1.81
o-Cymene	0.08	0.09	-	1.20	1.16	1.10	0.67	0.68	0.78
D-Limonene	42.84	44.51	45.90	17.11	18.68	20.43	-	-	-
1,8-Cineole	-	-	-	15.46	13.62	11.38	2.89	2.69	-
trans- β -Ocimene	0.31	0.29	0.37	3.65	4.15	4.78	2.24	2.15	2.41
β -Ocimene	7.74	7.79	8.42	1.56	1.78	2.03	1.11	1.07	1.21
γ -Terpinene	1.73	2.10	1.92	3.14	3.41	3.45	3.63	3.55	3.60
Sabinol	0.31	0.20	0.24	0.54	0.42	0.37	0.39	0.35	0.16
Linalool	2.74	1.94	1.34	4.41	3.94	3.28	-	-	-
cis-Menthol	0.46	0.51	0.39	0.92	0.85	0.71	0.88	0.86	0.70
allo-Ocimene	-	-	-	0.16	0.19	0.27	0.06	-	0.05
Citronellal	-	-	-	0.08	0.08	0.06	-	-	-
Terpinen-4-ol	5.52	6.84	5.60	12.44	11.98	9.92	8.18	8.34	7.48
α -Terpineol	4.11	2.93	1.99	4.24	3.89	3.23	1.88	1.81	1.47
Isopulegol	0.16	0.14	0.10	0.30	0.25	0.22	8.40	7.66	6.32
trans-Isopulegol	0.16	0.17	0.14	0.43	0.40	0.37	0.42	0.39	0.38
cis-Carveol	0.08	-	-	0.12	0.09	0.10	-	-	-
Nerol	-	-	-	0.23	0.24	0.18	0.16	0.15	0.13
Eugenol	-	-	-	-	-	-	0.33	0.30	0.25

Detected compound	Area percentage (%)								
	<i>Z. limonella</i>			<i>Z. bungeanum</i>			<i>Z. simulans</i>		
Common name	3 h	5 h	7 h	3 h	5 h	7 h	3 h	5 h	7 h
Geranyl acetate	0.10	0.09	0.09	0.28	0.32	0.34	0.34	0.34	0.37
α -Terpinyl acetate	-	-	-	0.30	0.31	0.29	1.766	1.811	1.830
Terpinyl acetate	-	-	-	0.09	0.10	0.09	-	-	-
Menthyl acetate	-	-	-	-	-	0.06	-	-	-
D-Carvone	0.055	-	-	0.10	0.08	0.07	-	-	-
Piperitone	-	-	-	3.85	3.28	2.74	0.53	0.50	0.38
<i>Sesquiterpenes</i>									
α -Cedrene	0.10	0.16	-	-	-	-	-	-	-
β -caryophyllene	0.18	0.12	0.15	0.19	0.16	0.18	0.26	0.20	0.21
Longifolene	0.18	0.10	0.14	0.12	0.10	0.12	0.21	0.15	0.16
α -Cadinene	-	-	-	0.15	0.15	0.16	0.10	0.08	0.07
Globulol	-	-	-	0.11	0.13	0.13	0.09	0.09	0.10
Caryophyllene oxide	-	-	-	0.17	0.17	0.19	0.13	0.13	0.13
γ -Cadinene	-	-	-	0.15	0.14	0.18	-	-	-
α -Cadinol	-	-	-	0.14	0.19	0.23	-	-	0.09
<i>Esters</i>									
Isobutyl butyrate	-	-	-	0.05	0.05	-	-	-	-
Hexyl acetate	0.16	0.13	0.10	-	-	-	-	-	-
Octyl acetate	-	-	-	0.09	0.11	0.11	-	-	-
Capryl acetate	1.59	1.52	1.39	-	-	-	-	-	-
Neryl acetate	-	-	-	0.09	0.11	0.10	0.10	0.10	0.11
Decyl acetate	0.10	-	-	-	-	-	-	-	-
<i>Aldehydes</i>									
Capryl aldehyde	0.59	0.39	0.25	-	-	-	-	-	-
Capric aldehyde	0.94	0.87	0.82	-	-	-	-	-	-
Cuminaldehyde	-	-	-	0.11	0.09	0.07	0.12	0.10	-
Perillaldehyde	-	-	-	0.10	0.14	0.15	0.11	0.11	0.10
Lauryl Aldehyde	0.08	0.06	0.01	-	-	-	-	-	-
<i>Alcohols</i>									
Capryl Alcohol	0.55	0.36	0.21	-	-	-	-	-	-
Lauryl Alcohol	0.18	0.15	0.11	-	-	-	-	-	-
<i>p</i> -Methylbenzyl alcohol	-	-	-	0.06	0.06	0.06	0.07	0.05	-
<i>Carboxylic ester</i>									
Xanthoxylin	-	-	-	3.68	4.11	5.86	14.81	16.00	16.68
<i>Phenylpropanoid</i>									
Anethole	-	-	-	0.21	0.20	0.17	0.92	0.85	0.90

Detected compound	Area percentage (%)								
Common name	<i>Z. limonella</i>			<i>Z. bungeanum</i>			<i>Z. simulans</i>		
	3 h	5 h	7 h	3 h	5 h	7 h	3 h	5 h	7 h
Total monoterpenes (%)	94.98	95.84	96.57	93.35	92.96	91.36	77.30	76.95	77.20
Total sesquiterpenes (%)	0.454	0.38	0.28	1.03	1.04	1.18	0.79	0.63	0.76
Total esters (%)	1.85	1.64	1.50	0.23	0.26	0.21	0.10	0.10	0.11
Total aldehydes (%)	1.62	1.32	1.08	0.21	0.22	0.22	0.23	0.21	0.10
Total alcohols (%)	0.73	0.50	0.32	0.06	0.06	0.06	0.07	0.05	0.00
Total identified (%)	99.63	99.68	99.76	99.89	99.92	99.96	94.23	94.80	95.76

The chemical profile of *Z. bungeanum* is characterized by D-limonene (17.10%), 1,8-cineole (15.45%), and terpinen-4-ol (12.44%) at 3 h extraction. These observations are consistent with previous identifying linalool and limonene as signature volatiles in *Z. bungeanum* essential oils. This initial composition explains its characteristic floral-citrus scent with a strong camphoraceous note from the high concentration of 1,8-cineole. However, the oil's profile undergoes a significant transformation with prolonged extraction. While D-limonene increases to 20.43%, the concentrations of 1,8-cineole and terpinen-4-ol sharply decrease, with 1,8-cineole decreasing to 11.38% and terpinen-4-ol to 9.92%. This decline is in direct contrast to the behavior of these compounds in *Z. limonella* and highlights the species-specific differences in compound retention and volatility. The steady increase in xanthoxylin from 3.68% to 5.85%, is responsible for the transition of the oil from a sweet floral-citrus aroma to a more herbaceous, pungent character.

The essential oil of *Z. simulans* stands out due to its high concentration of sabinene and xanthoxylin, which define its aroma profile. Sabinene is the most abundant compound, showing a marked increase from 30.41% at 3 h to a significant 35.55% at 7 h. The persistence of Sabinene contributes a warm, spicy, woody aroma with subtle peppery and citrusy undertones, reminiscent of spices commonly found in curry blends. Concurrently, xanthoxylin is found at a much higher concentration in this species than in *Z. bungeanum*, and it also increases over time (14.81% to 16.68%). Xanthoxylin is associated with the characteristic tingling or numbing sensation of *Zanthoxylum* species with a distinctive spicy note that enriches the curry-like complexity of the aroma. The

decline of terpinen-4-ol (8.18% to 7.47%) and isopulegol (8.40% to 6.32%) was found along the extraction time, resulting in a loss of fresh, herbal, and minty notes, with the overall aroma shifting towards a more intense, spicy, and woody profile [18]. The low content of D-limonene is a key distinguishing feature of *Z. simulans* and explains the lack of a prominent citrus note. The hint of waxy smells is also presented in ZS EOs, which can be told by the physical characteristics of waxy particles.

The results from the three *Zanthoxylum* species reveal a clear trend: the chemical composition of essential oils is not static but changes significantly depending on extraction time. While the total percentage of identified compounds remains consistently high (~94% - 99%), the relative abundance of individual components undergoes key shifts. D-limonene consistently increases with longer extraction times in *Z. limonella* and *Z. bungeanum*, suggesting it is a less volatile compound that is continually released. In contrast, the more volatile alcohols and aldehydes, such as linalool, α -terpineol, and capryl aldehyde, tend to decrease over time, indicating they are either more susceptible to degradation or are completely extracted in the early stages.

The major constituents identified in these essential oils are well documented for diverse bioactivities. Limonene, particularly abundant in *Z. limonella*, is a well-characterized ROS scavenger contributing to neuroprotective, anti-inflammatory, and anticancer effects [22]. Eucalyptol (1,8-cineole), abundant in *Z. bungeanum*, demonstrates notable anti-inflammatory activity and is utilized in wound healing and respiratory treatments [9]. Xanthoxylin and piperitone, unique to *Zanthoxylum* species, contribute not only to their

characteristic tingling sensation but also exhibit antimicrobial and anti-inflammatory properties. Terpinen-4-ol exerts potent antioxidant, anti-inflammatory, and antibacterial properties, often mediated via modulation of reactive oxygen species (ROS) and inflammatory cytokines [18]. Sabinene is recognized for its antifungal, antioxidant, and anti-inflammatory activities, with evidence suggesting its role in preventing muscle atrophy through ROS-mediated pathways [29]. β -Myrcene enhances endogenous antioxidant defenses by elevating superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) levels and has been linked to neuroprotective, analgesic, and anti-aging effects [8].

The presence of common compounds like terpinen-4-ol and sabinene in all three species supports their close biochemical relationship within the *Zanthoxylum* genus. However, the unique dominant components—D-limonene in *Z. limonella*, 1,8-cineole in *Z. bungeanum*, and sabinene/xanthoxylin in *Z. simulans*—are what truly distinguish each species' essential oil profile. This underscores the importance of not just the presence, but the relative abundance of key compounds in defining the overall therapeutic and aromatic properties. The observed variations across species and extraction times highlight a crucial point for commercial and research applications: the optimization of extraction parameters is essential to obtain an oil with a desired chemical profile. For example, if a high concentration of the pungent xanthoxylin is required for a specific application, a longer extraction time is preferable for both *Z. bungeanum* and *Z. simulans*. Conversely, if a fresh, floral scent is desired from *Z. limonella* or *Z. bungeanum*, a shorter extraction time may be more beneficial. These results emphasize that the final product is a direct function of the botanical source and the specific processing conditions, and that future studies should focus on optimizing these parameters for targeted functional applications.

Antioxidant activity of the single selected *Z. limonella*, *Z. bungeanum*, and *Z. simulans* essential oils and their combinations

Our recent aim is to assess antioxidant activity, which will be used to identify the most effective extraction time for each *Zanthoxylum* essential oil. Radical-scavenging activities are critical for preventing the harmful effects of free radicals, including oxidative damage and various diseases. The results in **Table 3** showed that ZB possessed the highest activity in 3 hours of extraction of 253.82 ± 10.02 mg TEAC/g EO. Meanwhile, both ZL and ZS provided the highest activities at 7 hours of extraction (469.29 ± 20.79 and 732.86 ± 5.43 mg TEAC/g EO, respectively).

Previous research on *Zanthoxylum* species has extensively explored the antioxidant activity of their extracts and essential oils in single-use applications. The *Z. limonella* fruit essential oil has been reported to exhibit promising antioxidant activity, with IC₅₀ values ranging from 5.66 mg/mL to 5.764 mg/mL [24,25], underscoring its potent DPPH scavenging action. For instance, the essential oil from the fruits of *Zanthoxylum mantaro* exhibited moderate antioxidant activity in the ABTS assay, with an SC₅₀ value of 274.14 ± 1.06 μ g/mL [26]. Similarly, essential oil from *Zanthoxylum alatum* demonstrated significant free radical scavenging activity and antioxidant potential in various *in vitro* assays, including DPPH scavenging [28]. Studies on *Zanthoxylum nitidum* also showed that essential oils from different parts of the plant, particularly the leaves, possessed considerable *in vitro* antioxidant activity [27]. Furthermore, flavonoids isolated from *Zanthoxylum armatum* have been reported to exhibit strong antioxidant activity, with IC₅₀ values of 0.03 mg/mL for DPPH radical and 0.062 mg/mL for ABTS radical scavenging [28]. These studies confirm the inherent antioxidant properties of various *Zanthoxylum* species when used individually.

Table 3 Antioxidant activity of the single *Z. limonella*, *Z. bungeanum*, and *Z. simulans* essential oils and their combinations.

Sample			Extraction time (h)	DPPH scavenging activity (mg TEAC/ g EO)	
<i>Z. limonella</i>			3	285.56 ± 4.39 ^b	
			5	150.55 ± 4.18 ^c	
			7	469.29 ± 20.79 ^a	
<i>Z. bungeanum</i>			3	253.82 ± 10.02 ^a	
			5	76.52 ± 0.23 ^b	
			7	45.72 ± 4.16 ^b	
<i>Z. simulans</i>			3	676.22 ± 12.79 ^b	
			5	670.56 ± 5.49 ^b	
			7	732.86 ± 5.43 ^a	
			Expected value**	Actual value	Synergistic index
<i>Symmetric combination (v/v)</i>					
1	1	1	302.44	385.71 ± 2.48 ^{cd}	1.28
1	1	2	469.10	540.73 ± 3.55 ^b	1.15
1	2	1	239.77	382.96 ± 7.37 ^{cd}	1.60
2	1	1	244.83	353.46 ± 12.65 ^d	1.44
<i>Symmetric combination (v/v)</i>					
1	2	3	443.67	488.86 ± 10.22 ^b	1.10
1	3	2	346.40	396.04 ± 9.28 ^{cd}	1.14
2	1	3	702.37	439.73 ± 18.17 ^c	0.63
2	3	1	158.13	281.77 ± 28.64 ^e	1.78
3	1	2	436.92	409.58 ± 13.18 ^c	0.94
3	2	1	155.17	252.59 ± 10.97 ^e	1.63

Values are given as mean ± S.D. from triplicate.

**Expected value was calculated by the sum of additive activity of the combination.

*Different letters in the same column indicate significant differences ($p < 0.05$).

Considering the earlier chemical profile of each essential oil in section 3.2, the antioxidant activity varied significantly with extraction time. *Z. bungeanum* exhibited the highest antioxidant capacity at 3 hours, likely reflecting the early extraction of monoterpene alcohols of terpinen-4-ol, linalool, and α -terpineol, and eucalyptol, which are known for contributing to radical

scavenging activity [23]. In contrast, *Z. limonella* showed the highest antioxidant activity at 7 hours, possibly due to cumulative or synergistic effects of more stable compounds such as cyclic monoterpenes of D-limonene and α -phellandrene, and bicyclic monoterpenes of sabinene and α -pinene, as well as β -ocimene. For *Z. simulans*, at 7 h of extraction,

xanthoxylin as well as bicyclic monoterpenes of sabinene and α -pinene were increased in relative concentration with prolonged distillation.

Ten different combination ratios of *Z. bungeanum* (ZB), *Z. limonella* (ZL), and *Z. simulans* (ZS) essential oils (EOs) were prepared, and their DPPH radical scavenging activities were evaluated, as detailed in **Table 3**. This investigation was premised on the hypothesis that combinations of EOs could exhibit enhanced antioxidant activity through synergistic effects, surpassing the activity of single oils. The antioxidant activities of these combinations were statistically compared to those of the single EOs (*Z. limonella* at 469.29 ± 20.79 mg TEAC/g EO, *Z. bungeanum* at 253.82 ± 10.02 mg TEAC/g EO, and *Z. simulans* at 732.86 ± 5.43 mg TEAC/g EO). The first impression after preparing the combinations is the harmonization of odor characteristics. The limitation of the curry-like odor of ZS was blended in with the citrusy of ZL and the herbaceous scent of ZB.

The results indicated that most combination ratios (ZL:ZB:ZS) exhibited significantly different antioxidant activities ($p < 0.05$) compared to the single EOs. However, the 1:1:1 and 3:1:2 ratios showed no significant difference ($p > 0.05$) when compared to the lowest antioxidant of ZB oil. Upon detailed analysis of the actual DPPH scavenging activity, the combination at a ratio of 1:1:2 demonstrated the highest activity among all tested combinations, reaching 540.73 ± 3.55 mg TEAC/g EO. This value is notably higher than the individual essential oils of *Z. limonella* and *Z. bungeanum*, although it is lower than the activity of single *Z. simulans* EO. This combination also exhibited a synergistic index (SI) of 1.15, indicating a synergistic enhancement of antioxidant activity beyond a simple additive effect. Following this, the 2:1:3 ratio showed a substantial actual DPPH scavenging activity of 439.73 ± 18.17 mg TEAC/g EO. However, it is crucial to note that despite this high actual value, its interaction index was 0.63, which signifies an antagonistic effect. The index is less than 1, suggesting that this specific ratio might lead to a reduction in the combined antioxidant potential compared to what would be linearly predicted (expected value). Conversely, the lowest actual DPPH scavenging activities among the combinations were observed in the ratios of 3:2:1 and 2:3:1, with values of 252.59 ± 10.97 mg TEAC/g EO and 281.77 ± 28.64 mg

TEAC/g EO, respectively. Interestingly, despite these lower actual values, both combinations exhibited strong synergistic indices: 1.63 for 3:2:1 and the highest observed synergistic index of 1.78 for 2:3:1. This indicates that, while their absolute antioxidant capacity was lower than that of other combinations, their combined effect was significantly greater than the sum of their individual parts.

A general trend observed from the results is that increasing the proportion of *Z. simulans* (ZS) in the combination tends to correlate with higher DPPH scavenging activity. For instance, the single ZS EO showed the highest activity (732.86 ± 5.43 mg TEAC/g EO), and combinations with a relatively higher ZS proportion, such as 1:1:2, also demonstrated high actual values. Conversely, increasing the proportion of *Z. bungeanum* (ZB) or *Z. limonella* (ZL) in the combination, particularly in ratios like 2:3:1 and 3:2:1, appeared to reduce the overall DPPH scavenging activity. This might be suspected to be due to the influence of specific compounds, such as β -Ocimene, although a quantitative analysis of the combined essential oil compositions is needed to confirm the structure-activity relationships. These observations strongly suggest that *Z. simulans* EOs significantly contribute to the antioxidant activities. It is to be noted that lowering the use of the lower yield ZS EO and also decrease a distinct pungency curry-like odor. Hence, enhances the feasibility in the applications.

Our findings align with previous reports on the synergistic effects observed in combinations of essential oils. It was previously reported that the combination of *Thymus fontanesii*, *Artemisia herba-alba*, and *Rosmarinus officinalis* essential oils demonstrated a significantly higher antioxidant activity compared to their individual components, achieving an IC₅₀ value of 2.6 μ g/mL, which was almost equivalent to the synthetic antioxidant butylated hydroxytoluene (BHT) [9]. The synergism in that study was attributed to the combined action of key chemical compositions, including thymol, carvacrol, 1,8-cineole, camphene, and α -pinene.

Regarding combination studies involving *Zanthoxylum*, research on the interaction between *Zanthoxylum bungeanum* extract and *Capsicum annuum* L. (chili pepper) revealed varied effects depending on the ratio. A significant synergism in scavenging DPPH radicals was observed at a 3:1 (w/w) ratio of *Z.*

bungeanum to *C. annuum*, which was potentially attributed to the synergistic action of catechin and capsaicin [16]. However, the same mixture could show a distinct antagonistic effect in scavenging ABTS radicals at lower ratios, highlighting the complex nature of combinatorial effects.

Therefore, our results show both synergistic and antagonistic effects depending on the ratio of *Zanthoxylum* EOs. The chemical profile of the combinations is the key consideration that impacts the bioactivities. It should be noted that antioxidant activity in this study was assessed solely by the DPPH radical scavenging assay. While DPPH is a widely accepted and reproducible method for comparing EO antioxidant capacity, it captures only hydrogen atom transfer mechanisms. Future studies should validate these findings using complementary assays such as ABTS, FRAP, and ORAC to capture the full spectrum of

radical-scavenging mechanisms and provide a more comprehensive antioxidant profile.

***C. acnes* growth inhibition activity of the single selected *Z. limonella*, *Z. bungeanum*, and *Z. simulans* essential oils and their combinations**

Individually, *Z. limonella* EO yields the largest zone of inhibition (15.00 ± 1.00 mm), indicating the strongest action against *C. acnes*. *Z. bungeanum* (13.67 ± 1.15 mm) and *Z. simulans* (12.33 ± 1.15 mm) are slightly less potent (**Table 4**). Combinations at optimized ratios (e.g., 2:1:1, 1:3:2, 2:3:1, 3:2:1) exceeded additive expectations. The yielding synergistic indices were > 1.0 (maximum 1.27 for 2:3:1), with clear zones surpassing single oils by 10% - 20%. Clindamycin was used as a standard antibiotic resulting in 12.33 ± 0.58 mm. inhibition zone.

Table 4 Preliminary *C. acnes* inhibition activity (represented by clear zone) of *Z. limonella*: *Z. bungeanum*: *Z. simulans* essential oils combinations and their synergistic index.

Sample				Clear zone against <i>C. acnes</i> (mm)	
Clindamycin				12.33 ± 1.00^e	
<i>Z. limonella</i>				15.00 ± 1.00^e	
<i>Z. bungeanum</i>				13.67 ± 1.15^d	
<i>Z. simulans</i>				12.33 ± 1.15^e	
				Expected value**	Synergistic index
<i>Symmetric combination (v/v)</i>					
1	1	1	13.67	10.67 ± 0.58^f	0.78
1	1	2	13.33	14.33 ± 1.15^d	1.08
1	2	1	13.67	12.67 ± 1.15^e	0.93
2	1	1	14.00	16.33 ± 1.53^b	1.17
<i>Asymmetric combination (v/v)</i>					
1	2	3	13.22	13.67 ± 1.53^d	1.03
1	3	2	13.45	16.67 ± 0.58^b	1.24
2	1	3	13.44	15.33 ± 1.15^e	1.14
2	3	1	13.89	17.67 ± 1.15^a	1.27
3	1	2	13.89	15.33 ± 1.15^e	1.10
3	2	1	14.11	17.67 ± 0.58^a	1.25

Values are given as mean $\pm$ S.D. from triplicate.

**Expected value was calculated by the sum of additive activity of the combination.

*Different letters in the same column indicate significant differences ($p < 0.05$) among all samples.

Synergy in *Z. limonella*-dominant blends are agreed with the report of *Amomum verum* and *Z. limonella* combinations showing fractional inhibitory concentration indices < 0.5 against Gram-positive skin pathogens like *S. aureus* and *P. aeruginosa* [5]. Essential oils from *Citrus meyeri*, *Citrus paradise*, and *Citrus sinensis* leaves were studied to perform better when blended [12]. The citronella, lemongrass, and patchouli blends strongly inhibited acne-causing bacteria with lower minimum inhibitory concentrations than single oils [13]. The combination of oils was found to synergize in microbial inhibition, not only the skin-related but also a wide range of gram-positive and gram-negative bacteria [14]. Synergism in essential oil blends against *C. acnes* arises from multi-target mechanisms where hydrophobic terpenoids (e.g., limonene, 1,8-cineole from *Z. limonella*) disrupt cell membranes, inhibit efflux pumps, and perturb metabolic pathways like glycolysis and ATP synthesis, amplifying effects beyond single oils. Complementary compounds facilitate enhanced penetration—monoterpenes increase permeability while sesquiterpenes induce oxidative stress and enzyme inhibition (e.g., pyruvate kinase, malate dehydrogenase) [9]. In our *Zanthoxylum* blends, *Z. limonella*-dominant ratios (2:3:1) achieve synergy indices up to 1.27, which might be concluded by combining lipophilic disruption with biofilm matrix degradation, reducing required doses and resistance risk. These findings reinforce the value of blending *Zanthoxylum* essential oils to enhance antimicrobial

efficacy for skin-care applications, with certain ratios delivering marked synergy that could be leveraged for improved product performance. It should be noted that the current study employed disc diffusion as a preliminary screening method; the synergistic indices reported here (e.g., 1.27-fold for the 2:3:1 ratio) should therefore be regarded as indicative of preliminary synergy. Quantitative confirmation through minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and fractional inhibitory concentration (FIC) index determinations is required and will be addressed in future work.

Cytotoxicity of the single selected *Z. limonella*, *Z. bungeanum*, and *Z. simulans* essential oils and their combinations

The decrease in cell viability was observed for all essential oils and their mixtures in dose-dependent manner (Figure 2). Interestingly, the early concentration of three essential oils also exhibited the slightly cell proliferation (0.0829 $\mu\text{g/mL}$). *Z. bungeanum* consistently exhibited the highest cell viability, confirming it as the least cytotoxic among the three oils. In contrast, *Z. limonella* and *Z. simulans* showed more pronounced reductions in viability. The non-toxic level where cell viability is not less than 80% was at concentrations of 41.45 $\mu\text{g/mL}$ for ZS and 2 times higher for ZB (82.90 $\mu\text{g/mL}$). ZL showed no toxicity against NIH/3T3 cell lines at the maximum tested concentration (414.50 $\mu\text{g/mL}$).

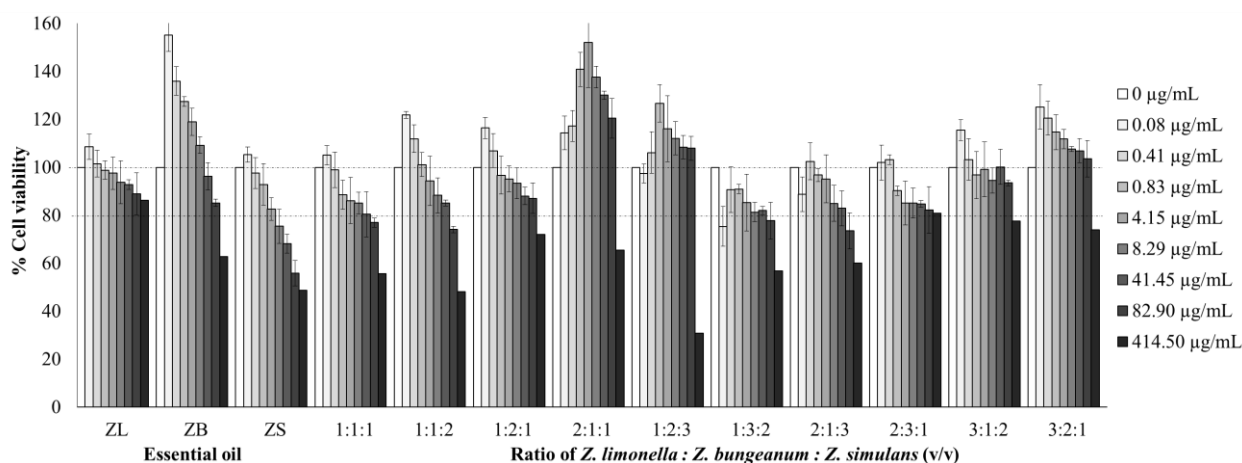


Figure 2 Cytotoxicity of *Z. limonella*, *Z. bungeanum*, and *Z. simulans* essential oils and their combinations on NIH/3T3 fibroblast cell lines.

Several oil blends, particularly those with higher proportions of *Z. limonella* and *Z. bungeanum* (1:2:1, 2:1:1, 1:2:3, 2:3:1, 3:1:2 and 3:2:1), maintain cell viability above the 80% threshold at 82.90 µg/mL, suggesting reduced cytotoxicity compared to ZS alone (4.15 µg/mL). The identification of blend ratios that outperform the more cytotoxic single oils highlights a practical formulation strategy to achieve safer cosmetic ingredients. Studies have shown that *Z. bungeanum* extracts can induce apoptosis while maintaining comparatively higher cell viability [29]. This supports prior findings where essential oil blending optimized biocompatibility and maintained functionality, indicating the practical formulation strategy of adjusting blend ratios to mitigate cytotoxicity [14].

The observed cell viability above an 80% threshold for certain blends at low-to-moderate concentrations further demonstrates the feasibility of blending to achieve safer cosmetic ingredient profiles. Thus, the evidence supports that mixing essential oils, particularly increasing the proportion of *Z. limonella* and *Z. bungeanum*, can reduce the cytotoxicity risks associated with more potent antioxidant single oils like *Z. simulans*, enabling safer formulations with effective bioactivity retained. Nevertheless, the less cytotoxic combinations of 2:1:1, 1:2:3, 2:3:1, and 3:2:1 possessed the synergistic effect in both antioxidant and *C. acnes* inhibitory effect. These cytotoxicity findings should be interpreted as preliminary *in vitro* evidence. Only NIH/3T3 mouse fibroblast cells were used as a screening model; extrapolation of safety conclusions to other cell types, primary human cells, or *in vivo* conditions requires further investigation.

Conclusions

This study provides a comprehensive evaluation of the volatile chemical fingerprints and biological synergies of three *Zanthoxylum* species—ZL, ZB, and ZS—and validates the traditional “Mala” spice combination through modern analytical and sensory lenses. Our findings demonstrate that hydrodistillation duration is a critical parameter for tailoring aromatic profiles; specifically, a 7-hour extraction maximizes the retention of key compounds like D-limonene and xanthoxylin, while shorter durations preserve delicate floral notes such as linalool.

The most significant advancement of this work is the identification of a 2:3:1 (ZL:ZB:ZS) blend ratio, which serves as a dual-purpose optimization for both performance and perception. This specific ratio achieved a 1.78-fold synergistic increase in antioxidant capacity and a 1.27-fold enhancement in *C. acnes* inhibition, surpassing the predicted additive effects of the single oils.

From a fragrance and formulation perspective, this study highlights the utility of odor harmonization. The inclusion of ZL and ZB not only introduces uplifting citrus and herbaceous top notes that mask the pungent “curry-like” odor of ZS but also significantly mitigates the cytotoxicity associated with high-potency ZS oil. By maintaining cell viability above 80% while amplifying bioactivity, these blends present a safer, more organoleptically pleasing alternative to single-oil applications. These results offer a clear scientific framework for the food, cosmetic, and pharmaceutical industries to leverage *Zanthoxylum* blends as potent, standardized bioactive agents. Future research should focus on the structure-activity relationships of these synergistic interactions to further refine the use of these “Mala” volatiles in complex industrial formulations

Acknowledgements

The authors would like to thank the Scientific and Technological Instrument Center, Mae Fah Luang University, Thailand for research infrastructures and instruments.

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., Google NotebookLM) in the preparation of this manuscript, specifically for language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

CRedit Author Statement

Sarita Sangthong: Conceptualization; Investigation; Methodology; Visualization; Writing - Original draft preparation; Reviewing and Editing. **Tinnakorn Theansungnoen:** Supervision; Writing - Reviewing and Editing. **Punyawatt Pintathong:** Supervision; Data curation; Writing - Original draft preparation.

Phanuphong Chaiwut: Conceptualization; Supervision; Writing - Reviewing and Editing.

References

- [1] J Wen, Q Xiang, J Guo, J Zhang, N Yang, Y Huang, Y Chen, T Hu and C Rao. Pharmacological activities of *Zanthoxylum* L. plants and its exploitation and utilization. *Heliyon* 2024; **10(12)**, e33207.
- [2] JO Ombito. Phytochemistry and pharmacology of the genus *zanthoxylum* (Rutaceae): A review. *The Natural Products Journal* 2021; **11(1)**, 21-43.
- [3] X Cai, Y Zeng, K Zhu, Y Peng, P Xv, P Dong, M Qiao and W Fan. Characterization of the quality and flavor in Chinese sausage: Comparison between Cantonese, five-spice, and Mala sausages. *Foods* 2025; **14(11)**, 1982.
- [4] S Liang, W Hu, W Cheng, S Zhang and R Zou. *Zanthoxylum bungeanum* essential oil: Extraction and component analysis for α -glucosidase inhibitory activity and the underlying mechanism based on molecular docking. *Applied Science* 2023; **13(4)**, 2627.
- [5] S Khruengsai, T Sripahco and P Pripdeevech. Antibacterial activity and synergic effects of the essential oils of *Amomum verum* Blackw and *Zanthoxylum limonella* (Dennst.) Alston. *Archives of Microbiology* 2023; **205(3)**, 102.
- [6] B Salehi, S Upadhyay, I Erdogan Orhan, A Kumar Jugran, SLD Jayaweera, DA Dias, F Sharopov, Y Taheri, N Martins, N Baghalpour, WC Cho and J Sharifi-Rad. Therapeutic potential of α - and β -pinene: A miracle gift of nature. *International Journal of Molecular Sciences* 2019; **20(24)**, 6238.
- [7] M Zhang, J Wang, L Zhu, T Li, W Jiang, J Zhou, W Peng and C Wu. *Zanthoxylum bungeanum* Maxim. (Rutaceae): A Systematic Review of Its Traditional Uses, Botany, Phytochemistry, Pharmacology, Pharmacokinetics, and Toxicology. *International Journal of Molecular Sciences* 2017; **18(10)**, 2172.
- [8] K Fidy, A Fiedorowicz, L Strzdała and A Szumny. β -caryophyllene and β -caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Medicine* 2016; **5(10)**, 3007-3017.
- [9] A Touati, A Mairi, NA Ibrahim and T Idres. Essential oils for biofilm control: Mechanisms, synergies, and translational challenges in the era of antimicrobial resistance. *Antibiotics* 2025; **14(5)**, 503.
- [10] S Khruengsai, N Promhom, T Sripahco, P Siriwat and P Pripdeevech. Optimization of enzyme-assisted microwave extraction of *Zanthoxylum limonella* essential oil using response surface methodology. *Scientific Reports* 2023; **13(1)**, 12872.
- [11] Q Peng, Y Lu, R Mo and Q He. Antioxidant and nitrite-scavenging activities of *Zanthoxylum bungeanum* Maxim. and *Capsicum annuum* L.: A synergistic, additive or antagonistic effect of the extracts? *European Food Research and Technology* 2021; **247**, 2877-2885.
- [12] M Hamdan, N Jaradat, N Al-Maharik, S Ismail and M Qadi. Chemical composition, cytotoxic effects and antimicrobial activity of combined essential oils from *Citrus meyeri*, *Citrus paradise*, and *Citrus sinensis* leaves. *Industrial Crops and Products* 2024; **210**, 118096.
- [13] S Bunrathep, P Thongphasuk, S Settharaksa and N Kamkaen. Effect of an essential oil blend of citronella, lemongrass, and patchouli on acne-causing bacteria. *Songklanakarin Journal of Science and Technology* 2020; **42(5)**, 1106-1112.
- [14] SR Leigh-De, A Viljoen and SV Vuuren. S. Essential oil blends: The potential of combined use for respiratory tract infections. *Antibiotics* 2021; **10(12)**, 1517.
- [15] S Sangthong, I Promputtha, P Pintathong and P Chaiwut. Chemical constituents, antioxidant, anti-tyrosinase, cytotoxicity, and anti-melanogenesis activities of *Etlingera elatior* (Jack) leaf essential oils. *Molecules* 2022; **27(11)**, 3469.
- [16] Y Lan, H Li, YY Chen, YW Zhang, N Liu, Q Zhang and Q Wu. Essential oil from *Zanthoxylum bungeanum* Maxim. and its main components used as transdermal penetration enhancers: A comparative study. *Journal of Zhejiang University Science B* 2014; **15(11)**, 940-952.
- [17] S Sangthong, S Phosri, N Thitilertdech, N Yaowiwat, P Chaiwut, M Wilai and T Theansungnoen. Phytochemicals and antimicrobial properties of Thai edible plant

- extracts and their prebiotic-like effects. *Journal of Agriculture and Food Research* 2024; **16**, 101152.
- [18] FM Abdelhamed, NF Abdeltawab, MT ElRakaiby, RN Shamma and NA Moneib. Antibacterial and anti-inflammatory activities of *Thymus vulgaris* essential oil nanoemulsion on acne vulgaris. *Microorganisms* 2022; **10(9)**, 1874.
- [19] N Khodaei, M Houde, S Bayen and S Karboune. Exploring the synergistic effects of essential oil and plant extract combinations to extend the shelf life and the sensory acceptance of meat products: Multi-antioxidant systems. *Journal of Food Science and Technology* 2023; **60(2)**, 679-691.
- [20] H Qi, WX Wang, JL Dai and L Zhu. *In vitro* anthelmintic activity of *Zanthoxylum simulans* essential oil against *Haemonchus contortus*. *Veterinary Parasitology* 2015; **211(3-4)**, 223-227.
- [21] R Supabphol and J Tangjitjareonkun. Chemical constituents and biological activities of *Zanthoxylum limonella* (Rutaceae): A review. *Tropical Journal of Pharmaceutical Research* 2014; **13(12)**, 2119-2130.
- [22] J Sun. D-Limonene: Safety and clinical applications. *Alternative Medicine Review* 2007; **12(3)**, 259-264.
- [23] F Bakkali, S Averbeck, D Averbeck and M Idaomar. Biological effects of essential oils: A review. *Food and Chemical Toxicology* 2008; **46(2)**, 446-475.
- [24] S Nanasombat and P Wimuttigosol. Antimicrobial and antioxidant activity of spice essential oils. *Food Science and Biotechnology* 2011; **20**, 45-53.
- [25] J Tangjitjareonkun, R Supabphol and W Chavasiri. Antioxidant effect of *Zanthoxylum limonella* Alston. *Journal of Medicinal Plants Research* 2012; **6(8)**, 1407-1414.
- [26] V Morocho, O Eras, T Rojas, B Jiménez, MF Roa and L Cartuche. Biological activity and chemical composition of essential oil from leaves and fruits of *Zanthoxylum mantaro* (J.F. Macbr.). *Antibiotics* 2025; **14(3)**, 216.
- [27] Y Yang, Y Li, H He, L Yang, J Zeng, M Bai and H Wu. Comparison of essential oil components and *in vitro* antioxidant activity of *Zanthoxylum nitidum* from different parts. *Plants* 2025; **14(8)**, 1194.
- [28] MJ Zhou, XZ He, CJ Li and XF Liang. Study on antioxidant activity and synergistic effect of flavonoids from *Zanthoxylum armatum* DC. *Food Machinery* 2023; **39(5)**, 23.
- [29] Y Du, S Duan, Y Yang, JJ Tibenda, S Huang, Y Nan, Z Zhang and L Yuan. Antitumor components and mechanisms of *Zanthoxylum bungeanum* Maxim with medicine and food homology. *Frontier in Pharmacology* 2025; **16**, 1525313.