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# Volatile signatures and antioxidant potentials of white mugwort (Artemisia lactiflora) extracts in emulsions: The influence of pH on functionality and stability

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### **ABSTRACT**

**Background:** White mugwort (*Artemisia lactiflora*), a medicinal plant native to several Asian countries, is known for its antioxidant-rich compounds and aromatic volatiles, making it a promising natural additive in food and cosmetic emulsions. However, its stability and effectiveness can be influenced by environmental factors, especially pH.

**Objective:** This study investigated the volatile compound composition and antioxidant efficiency of white mugwort in its fresh and dried forms under different pH conditions (3, 5, and 7), with the aim of exploring its potential as a natural antioxidant in oil-in-water (O/W) emulsions.

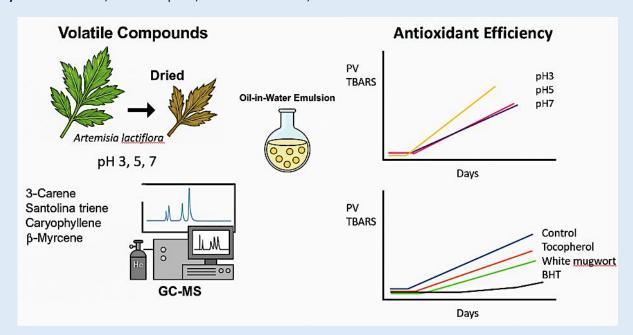
**Methods:** Volatile compounds were analyzed using GC-MS. The antioxidant efficiency of white mugwort extract was evaluated in O/W emulsions at different concentrations (200, 400, and 600 ppm) and compared with BHT and  $\alpha$ -tocopherol (both at 200 ppm) using peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) assays.

Results: Volatile compounds were analyzed by GC-MS, identifying 31 compounds in fresh samples and 34 in dried samples. Drying at 50 °C for 24 hours caused reductions in several key volatiles, including  $\beta$ -Myrcene,  $\beta$ -cis-Ocimene, 3-Carene, and  $\gamma$ -Gurjunene. Conversely, compounds such as Santolina triene, Caryophyllene, and Bicyclosesquiphellandrene increased, indicating thermal degradation and chemical conversion during drying. pH significantly influenced the volatile profiles. These findings highlight the role of pH-driven chemical reactions in aroma stability. The antioxidant study showed that antioxidant efficiency increased with higher extract concentrations. Extracts at pH 3 and 5 demonstrated stronger antioxidant activity than those at pH 7, evidenced by longer lag times in lipid hydroperoxide formation and lower TBARS values. This pH-dependent behavior was not observed with BHT or  $\alpha$ -tocopherol, suggesting a unique interaction between white mugwort phenolics and the emulsion interface.

**Novelty:** This study provides novel insights into the volatile compound dynamics and antioxidant properties of white mugwort under varying processing and environmental conditions, with a particular focus on pH- and dose-dependent antioxidant performance in emulsions. These findings hold significant scientific and industrial relevance.

**Conclusion:** Overall, white mugwort extract demonstrated promising potential as a natural antioxidant, especially under mildly acidic conditions, with superior performance to  $\alpha$ -tocopherol and applicability in clean-label formulations. Additionally, insights into pH effects on volatile stability contribute to improving aroma retention in food and cosmetic products.

Keywords: Aromatic, Medicinal plant, Natural antioxidant, Oil-in-water emulsion



**Graphical Abstract:** Volatile signatures and antioxidant potentials of white mugwort (*Artemisia lactiflora*) extracts in emulsions: the influence of pH on functionality and stability

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## **INTRODUCTION**

White mugwort (*Artemisia lactiflora*), a medicinal plant native to several Asian countries, has long been valued for its roles in traditional medicine and cuisine. Recent scientific studies have confirmed its rich phytochemical content including flavonoids, polyphenols, and terpenoids which contribute to its wide range of biological activities, such as antioxidant, anti-inflammatory, and antidiabetic effects. For instance, it has been shown to inhibit carbohydrate-digesting enzymes [1], scavenge reactive oxygen species in hepatic cells [2], and reduce inflammation in macrophages [3].

Beyond its health benefits, white mugwort is also known as a culinary herb due to its distinctive aroma. While the volatile profiles of related Artemisia species such as A. asiatica, A. princeps, and A. vulgaris have been well-characterized, limited information is available on the aromatic constituents of A. lactiflora. Previous studies on other species have identified major volatiles including 1,8-cineole, borneol,  $\alpha$ -thujone, and terpinen-4-ol [4-6]. These volatile compounds not only contribute to sensory appeal but also exhibit therapeutic properties, making them valuable for both food and pharmaceutical applications [7]. However, the stability of volatile compounds can be affected by processing conditions such as pH and drying, which may lead to degradation or transformation through chemical reactions like oxidation and isomerization [8-11]. To date, no comprehensive studies have examined how pH affects the volatile profile of fresh and dried white mugwort.

Moreover, in modern food and nutraceutical formulations, plant extracts are increasingly incorporated into emulsion systems due to their antioxidant potential. Emulsions, particularly oil-in-water (O/W) emulsions, are widely used as delivery systems for lipophilic bioactive compounds in food, cosmetics, and pharmaceuticals. However, the antioxidant efficacy of plant extracts in emulsions is highly sensitive to

environmental factors, especially pH. Studies have shown that the stability and efficiency of phenolic compounds can be drastically altered under different pH conditions [12-15]. For example, in flaxseed oil-in-water emulsions, EGCG acted as an effective antioxidant at neutral pH (pH 7), helping to maintain oxidative stability. However, at acidic pH (pH 3), EGCG exhibited pro-oxidant behavior, promoting lipid oxidation. This shift is likely related to differences in hydrogen peroxide production and stability of EGCG under varying pH conditions [16].

Given this context, understanding the influence of pH on both the aromatic and antioxidant properties of white mugwort is essential for its practical application in complex formulations. This study aims to address this gap by (1) characterizing the volatile profiles of fresh and dried white mugwort leaves under varying pH conditions and (2) evaluating the antioxidant efficiency of its ethanolic extract in O/W emulsions across different pH levels. The findings will contribute to the effective utilization of white mugwort as a multifunctional ingredient, offering both antioxidant protection and aromatic appeal in food and health-related products [17].

## **MATERIALS AND METHODS**

**Materials:** Fresh white mugwort (*Artemisia lactiflora*) was sourced from a local farm in Prachinburi Province, Thailand. All chemicals used were of analytical grade.

# Study On Volatile Composition Of Fresh And Dried White Mugwort Leaves

Sample preparation for volatile composition analysis: Fresh white mugwort leaves with a moisture content of 88.92% were cut into small pieces and divided into two fractions. One fraction was analyzed as a fresh sample, prepared immediately prior to volatile compounds analysis to preserve its native profile. The second fraction was subjected to drying to a final moisture content of 6.41% using a hot air oven (Binder FD53, USA) at 50°C for

24 h. Dried white mugwort leaves were ground and kept in an aluminium foil bag at room temperature for further volatile composition analysis by GC-MS.

GC-MS analysis: Volatile compounds analysis was performed according to [18, 19] with adaptations. Gas chromatography mass spectrometer (GC/MS) Agilent Technologies 7890B GC System / 7000D Triple Quadrupole GC/MS System equipped with PAL auto sample system (CTC Analytics AG, Switzerland) were used in this experiment. Fresh (1 g) or dried (0.1183 g) mugwort leaves were added into 5 mL of 10 mM phosphate buffer solutions (pH 3, 5, 7) in 20 mL GC vials capped with polytetrafluoroethylene-silicon. Samples were pre-equilibrated at 40°C for 30 min, the headspace volatiles were then extracted by an SPME fiber Divinylbenzene/Carboxen/ Polydimethylsiloxane (CAR/DVB/PDMS) (50/30 µm) for 40 min. Volatiles were thermally desorbed at 250°C for 2 min and separated in a non-polar HP-5 capillary column (30 m length x 0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness). The flow rate of helium, the carrier gas, was 1.2 mL/min. The initial oven temperature was set at 40°C and held for 4 min, then ramped to 100°C and 220°C at a rate of 5°C/min. Total run time was 40 min. Ion source temperature was set at 230°C. The mass of 70 eV ionized compound was scanned between range of 30-550. Compounds were tentatively identified based on the comparison of the mass spectra with the National Institute of Standards and Technology (NIST) MS Library and further confirmed by comparing the experimental retention indices (RI) with the linear retention indices (LRI) calculated using C7-C21 alkane standards. Volatile compounds were relatively quantified using peak areas of the ions extracted for each compound and reported as % relative peak area of total ion chromatogram.

Study On Antioxidant Efficiency Of White Mugwort
Extract In Oil In Water Emulsion

Preparation of white mugwort extract: Fresh white mugwort leaves were washed and dried in a hot air oven (Binder, FD53, USA) at 45°C to obtain a dried sample with a moisture content of less than 10%. The dried samples were ground and passed through a 1 mm sieve. Then, the ground samples were extracted with aqueous ethanol (90%v/v) at solid to solvent ratio of 0.1:1 (w/v). The sample flasks were incubated at 30°C for 6 h in an incubator shaker (N-Biotek, NB-205LF, Germany) for extraction. The extract was filtered, and the filtrate was evaporated at 60 °C, 175 mbar, 55 rpm to dryness in a rotary evaporator (Büchi, R-205, Switzerland). The extract was then dried in a hot air oven (Binder, FD53, USA) at  $60 \pm 5$  °C for 3 hours. The dried extract was stored in an amber glass bottle with a tightly sealed cap and kept at -20 °C until further use in the oil-in-water emulsion system.

Preparation of Stripped Soybean Oil: The preparation method was adapted from [20]. First, 250 grams of powdered silica gel were soaked in 2 liters of distilled water and allowed to settle for 1 hour. The supernatant was then discarded, and the remaining silica gel was dried in a hot air oven at 110 ± 5 °C for 20 hours to obtain activated silica gel. Next, 100 grams of soybean oil were dissolved in 100 mL of n-hexane. A chromatographic column (60 cm in height and 4.5 cm in diameter) was prepared by sequentially loading components, including 87 grams of activated silica gel soaked in 260 mL of n-hexane, 22 grams of activated charcoal soaked in 180 mL of n-hexane, and another 87 grams of activated silica gel soaked in 260 mL of nhexane. The soybean oil solution was passed through the column and eluted with 1,000 mL of n-hexane. The collected stripped soybean oil was wrapped with aluminum foil in a round-bottom flask and kept in an ice bath to minimize lipid oxidation. The solvent was removed using a rotary vacuum evaporator at 37 ± 5 °C, followed by flushing with nitrogen gas to eliminate residual solvent. The stripped oil was stored at

 $-18\pm5\,^{\circ}\text{C}$  until use in the oil-in-water emulsion preparation.

Preparation of oil-in-water emulsions at different ph levels: The method was adapted from [21]. Stripped soybean oil (1% w/w) was dispersed in 10 mM phosphate buffer solutions at pH 3, 5, and 7. Tween 20 was used as the emulsifier at an emulsifier-to-oil ratio of 0.1:1. Sodium azide (0.1% w/w) was added to inhibit microbial growth. All components were mixed in a beaker and homogenized using a homogenizer (IKA, T-25 basic Ultra-Turrax, Germany) for 2 minutes to form a coarse emulsion. The coarse emulsion was then processed using a microfluidizer (Microfluidics, M-110P, USA) at a pressure of 9 kbar for three passes to achieve uniform particle size and improved stability.

The oil-in-water emulsions prepared at pH 3, 5, and 7 were incorporated with white mugwort extract at 200, 400, and 600 ppm. Emulsions with 200 ppm BHT, 200 ppm  $\alpha$ -Tocopherol, and without antioxidants served as comparisons. All samples were stored in amber bottles at 40 °C in the dark, and analyzed over 20 days for peroxide value (PV) and thiobarbituric acid reactive substances (TBARS).

Analysis of antioxidant properties: Antioxidant properties of the white mugwort extract were analyzed, including total phenolic content (TPC), free radical (DPPH and ABTS) scavenging activity, and ferric reducing antioxidant power (FRAP).

The TPC was measured using the Folin–Ciocalteu assay [22]. Briefly, a 0.4 mL sample solution was mixed with 2.0 mL of Folin–Ciocalteu reagent and allowed to react at room temperature for 5 min. Then 1.6 mL of sodium carbonate (7.5% w/v) was added and kept at room temperature for 30 min. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Libra S12, Biochrom, USA). A calibration curve of gallic acid was prepared (y = 0.0115x + 0.0296, R<sup>2</sup> = 0.9992).

The TPC was expressed as mg gallic acid equivalent/g dried extract (mg GAE/ g dried extract).

To evaluate antioxidant activity, multiple assays are typically employed, as no single method can fully capture the complex mechanisms involved. This approach allows for a more comprehensive and accurate assessment of antioxidant potential. In the present study, three widely used in vitro assays including DPPH, ABTS, and FRAP were applied. DPPH and ABTS assays assess free radical scavenging activity, whereas the FRAP assay measures reducing power [23].

The DPPH radical scavenging activity of the white mugwort extract was measured according to the method described by [22]. Briefly, 1 mL of the extract and 3 mL of the DPPH methanolic solution (0.1 mM) were added to a test tube. The mixture was well shaken in a vortex (S0100-230V, Labnet, Mexico) for 1 min and then incubated at room temperature for 30 min in the dark. The absorbance at 517 nm was measured using a spectrophotometer (Biochrom, Libra S12, USA). The DPPH radical scavenging activity was calculated from the calibration curve of trolox (y = -0.0102x + 0.5727,  $R^2 = 0.9979$ ) and expressed the results in mg trolox equivalent antioxidative capacity (TEAC) per g dried extract (mg TEAC/ g dried extract).

The ABTS assay was performed by following the method explained by [22] with some modifications. The 700  $\mu$ M of ABTS<sup>++</sup> solution was produced by mixing 0.096 g of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation and 0.0166 g potassium persulfate, then the volume was adjusted to 250 mL with distilled water. The ABTS radical solution was incubated for 12 h in order to allow the radical generation at room temperature in the dark. Then, the absorbance of the ABTS working solution was adjusted to the value of 0.700  $\pm$  0.020 at 734 nm by diluting with ethanol. For the spectrophotometric assay, 3 mL of the ABTS radical solution and 30  $\mu$ L of the sample extract were mixed and

the absorbance was measured at 734 nm immediately after 6 min incubation using a spectrophotometer (Biochrom, Libra S12, USA) with ethanol as the blank solvent. The TEAC values were calculated from the calibration curve of trolox (y = -0.0041x + 0.6811,  $R^2 = 0.9971$ ) and expressed in mg TEAC/ g dried extract.

FRAP assay was conducted according to the procedure of [22] with slight modifications. The working FRAP reagent was prepared by mixing ferric chloride solution (20 mM), acetate buffer (300 mM, pH 3.6), and TPTZ solution in 40 mM HCl (10 mM) in the proportion of 10:1:1 (v/v), respectively. The FRAP reagent was freshly prepared and incubated at 37°C before analysis. The extract (150  $\mu$ L) was mixed with the FRAP reagent (2.85 mL). The reaction mixture was incubated for 10 min. The absorbance was then recorded at 595 nm using a spectrophotometer (Biochrom, Libra S12, USA). Trolox was used as the standard (y = 0.0307x + 0.1479, R² = 0.9994) and the results were expressed in mg TEAC/ g dried extract.

Analysis of peroxide value (PV): Peroxide value was analyzed in accordance with the method of [24]. A 150  $\mu$ L aliquot of the emulsion sample was mixed with 750 µL of an isooctane/2-propanol solution (3:1, v/v) and vortexed for 10 seconds, repeated three times. The mixture was then centrifuged at 4,000 rpm for 5 minutes to separate the emulsion and extract layers. From the upper layer, 100 µL was collected and mixed with 1.4 mL of methanol/1-butanol (2:1, v/v), 7.5  $\mu$ L of 3.94 M ammonium thiocyanate, and 7.5 µL of ferrous ion solution. The ferrous ion solution was prepared by mixing 0.132 M BaCl<sub>2</sub> in 0.4 M HCl and 0.144 M FeSO<sub>4</sub> in distilled water at a 1:1 ratio. The reaction mixture was incubated at room temperature in the dark for 20 minutes. Absorbance was measured at 510 nm using a microplate reader. The hydroperoxide concentration in the sample was calculated using a cumene hydroperoxide standard

curve.

Analysis of thiobarbituric acid reactive substances (TBARS): A 0.5 mL aliquot of the sample was mixed with 0.45 mL of distilled water and 1.0 mL of TBARS reagent in a test tube. The mixture was thoroughly combined and incubated in a water bath at  $95 \pm 5$  °C for 15 minutes. It was then allowed to cool to room temperature for 10 minutes. The sample was centrifuged at 5,000 rpm for 5 minutes, and the supernatant was collected to measure absorbance at 540 nm using a microplate reader. TBARS reagent was used as the blank. A standard curve was prepared using 1,1,3,3-tetraethoxypropane [25].

Statistical analysis: Data were presented as means  $\pm$  standard deviation of triplicate measurements. Analyses of variance were performed by ANOVA test using IBM SPSS STATISICS 21.0. Duncan's New Multiple Range Test was performed to determine differences between the means with a level of significance of p  $\leq$  0.05.

### **RESULTS AND DISCUSSION**

**Compositional Changes of Volatile Compounds in Fresh** and Dried White Mugwort Leaves under Different pH Conditions: Volatile compound compositions of fresh and dried white mugwort incubated under different pH conditions (3, 5, 7) were identified by GC-MS, with the resulting base peak chromatograms presented in Figure 1. A total of 31 and 34 volatile compounds were identified in fresh and dried white mugwort leaves, respectively (Table 1). The major volatile constituents of fresh white mugwort leaves were 3-Carene, α-(Z,E)-Farnesene, Santolina triene, α-Farnesene, Caryophyllene, Bicyclosesquiphellandrene, β-Myrcene, γ-Gurjunene, βcis-Ocimene, β-Sesquiphellandrene, (E)-3-Hexen-1-ol, 1-Hexanol, β-trans-Farnesene, and 1,2,4-Trimethylbenzene. These volatile constituents have been described as wood, herb, spice, sweet, and green aroma [26, 27].

The compositional changes of volatile compounds were observed after drying at 50°C for 24 h. Dried white mugwort leaves contained Santolina triene,  $\alpha$ -(Z,E)-Farnesene, Caryophyllene,  $\alpha$ -Farnesene, Bicyclosesquiphellandrene, Sesquisabinene,  $\alpha$ -Ocimene,  $\beta$ -Sesquiphellandrene, D-Limonene,  $\beta$ -cis-Ocimene and  $\beta$ -trans-Farnesene as major components. Drying, a common post-harvest process, led to notable reductions in several key volatiles such as  $\beta$ -Myrcene,  $\beta$ -cis-Ocimene, 3-Carene,  $\gamma$ -Gurjunene, compounds associated with the herb's characteristic aroma [26]. This reduction can be attributed to thermal degradation, oxidation, and

evaporation during the drying process [9, 28, 29]. However, some volatile compounds increased after drying including Santolina triene, Caryophyllene, and Bicyclosesquiphellandrene. Among them, Santolina triene, which responsible for a sweet scent [30], were mainly found in both fresh and dried white mugwort leaves. Previous studies have also reported higher levels of volatile compounds in dried samples compared to fresh ones, possibly due to the conversion of other constituents during the drying process, leading to an increase in certain volatile compounds [10].

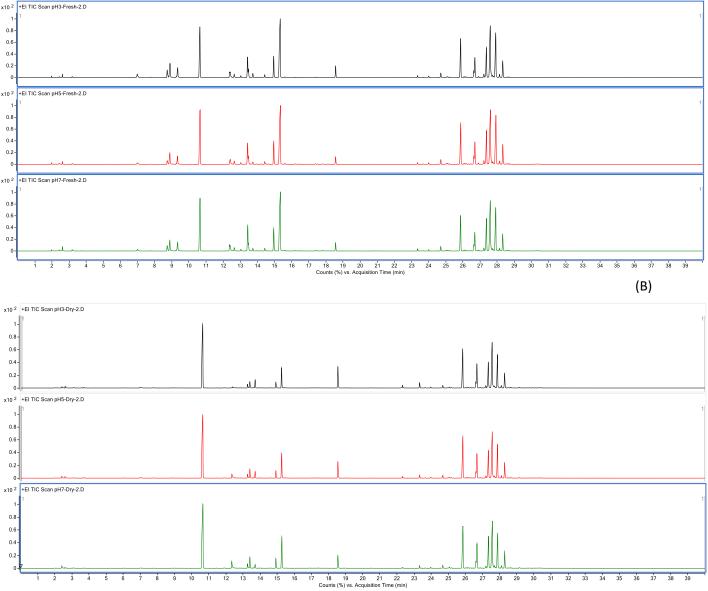


Figure 1. Base peak chromatograms comparing between (A) fresh and (B) dried white mugwort incubated under pH 3, 5 and 7

Furthermore, the change of environmental pH significantly affected the volatile composition in white mugwort leaves. 3-Carene, a major volatile compound commonly found in flowers and fruits, particularly in pine essential oils [31], showed a significant increase with rising pH. Meanwhile, Santolina triene was not significantly different at the pH range of 3-7. There have been reports explaining that the pH caused the degradation of volatile compounds through reactions such as enzymatic hydrolysis, acid-catalyzed cyclization,

redox reaction, and oxidation. *p*-Mentha-1,5-dien-8-ol and *p*-mentha-1,2-dien-8-ol, which are intermediate monoterpene alcohols from the cyclization reaction of citral, can be degraded via the disproportionation and redox reactions under acidic conditions [32]. The interplay between pH and volatile stability is particularly important in food and pharmaceutical formulations, where both aroma retention and antioxidant capacity are critical.

**Table 1.** Volatile compound compositions of fresh and dried white mugwort incubated under different pH conditions.

No.	RI	Volatile compounds	Relative peak area (%)					
			Fresh			Dried		
			рН 3	pH 5	pH 7	pH 3	pH 5	pH 7
1	806	Hexanal	0.82 ± 0.17 <sup>a</sup>	0.55 ± 0.11 <sup>b</sup>	0.30 ± 0.16°	0.21 ± 0.06 <sup>c</sup>	0.19 ± 0.03°	0.16 ± 0.11 <sup>c</sup>
2	850	(E)-2-Hexenal	1.40 ± 0.31 <sup>a</sup>	0.90 ± 0.10 <sup>b</sup>	0.72 ± 0.46 <sup>b</sup>	tr.	tr.	tr.
3	852	(E)-3-Hexen-1-ol	2.77 ± 0.74	2.24 ± 0.23	2.05 ± 0.54	N.D.	N.D.	N.D.
4	860	1-Hexanol	1.77 ± 0.45	1.46 ± 0.10	1.55 ± 0.41	N.D.	N.D.	N.D.
5	908	Santolina triene	11.03 ± 0.66 <sup>b</sup>	12.95 ± 1.25 <sup>b</sup>	12.99 ± 1.25 <sup>b</sup>	24.43 ± 2.45 <sup>a</sup>	25.98 ± 0.96 <sup>a</sup>	26.44 ± 0.61 <sup>a</sup>
6	970	1-Ethyl-2-methylbenzene	0.98 ± 0.19	1.28 ± 0.26	1.16 ± 0.22	N.D.	N.D.	N.D.
7	972	Mesitylene	0.50 ± 0.09	0.49 ± 0.03	0.73 ± 0.34	N.D.	N.D.	N.D.
8	981	β-Myrcene	3.32 ± 0.56 <sup>a</sup>	3.71 ± 0.08 <sup>ab</sup>	4.82 ± 1.01 <sup>b</sup>	N.D.	N.D.	N.D.
9	982	Benzaldehyde	N.D.	N.D.	N.D.	0.45 ± 0.10 <sup>b</sup>	1.52 ± 0.23 <sup>ab</sup>	1.87 ± 0.84°
10	986	Sulcatone	N.D.	N.D.	N.D.	1.01 ± 0.14	1.01 ± 0.14	0.99 ± 0.12
11	990	1,2,4-Trimethylbenzene	1.29 ± 0.20	1.17 ± 0.07	1.18 ± 0.08	N.D.	N.D.	N.D.
12	1004	D-Limonene	N.D.	N.D.	N.D.	1.92 ± 0.28 <sup>b</sup>	2.08 ± 0.08 <sup>ab</sup>	2.34 ± 0.19 <sup>a</sup>
13	1010	(+)-3-Carene	N.D.	N.D.	N.D.	tr.	tr.	tr.
14	1030	Limonene	N.D.	N.D.	N.D.	tr.	tr.	tr.
15	1038	β- <i>cis</i> -Ocimene	3.57 ± 0.42 <sup>b</sup>	3.93 ± 0.05 <sup>b</sup>	4.20 ± 0.29 <sup>ab</sup>	1.58 ± 0.18°	1.67 ±0.03°	1.81 ± 0.15°
16	1047	α-Ocimene	N.D.	N.D.	N.D.	5.60 ± 0.55	6.02 ± 0.15	6.12 ± 0.72
17	1055	3-Carene	16.80 ± 1.48 <sup>b</sup>	18.15 ± 0.22ab	19.29 ± 1.27°	N.D.	N.D.	N.D.
18	1270	(±)-Lavandulol acetate	N.D.	N.D.	N.D.	0.63 ± 0.05 <sup>a</sup>	0.43 ± 0.03 <sup>b</sup>	0.28 ± 0.15 <sup>b</sup>
19	1351	α-Cubebene	0.28 ± 0.01°	0.26 ± 0.02 <sup>a</sup>	0.23 ± 0.01 <sup>a</sup>	0.25 ± 0.16 <sup>a</sup>	0.23 ± 0.00 <sup>a</sup>	0.16 ± 0.08b
20	1368	Cyclosativene	N.D.	N.D.	N.D.	tr.	tr.	tr.
21	1389	β-Cubebene	0.88 ± 0.10 <sup>a</sup>	0.78 ± 0.06 <sup>a</sup>	0.78 ± 0.07 <sup>a</sup>	0.23 ± 0.00 <sup>b</sup>	0.16 ± 0.08 <sup>b</sup>	0.16 ± 0.08 <sup>b</sup>
22	1398	β-Elemene	0.13 ± 0.15 <sup>b</sup>	0.30 ± 0.02a	0.22 ± 0.08ab	0.33 ± 0.03 <sup>a</sup>	0.30 ± 0.01 <sup>a</sup>	0.31 ± 0.04 <sup>a</sup>
23	1411	α-Cedrene	N.D.	N.D.	N.D.	tr.	tr.	tr.
24	1419	Caryophyllene	8.73 ± 0.84 <sup>b</sup>	8.07 ± 0.32 <sup>bc</sup>	7.58 ± 0.09°	12.34 ± 0.59 <sup>a</sup>	12.12 ± 0.23 <sup>a</sup>	11.64 ± 0.61 <sup>a</sup>
25	1432	β- <i>cis</i> -Copaene	tr.	tr.	tr.	0.13 ± 0.02	0.12 ± 0.01	0.11 ± 0.00
26	1435	α-Bergamotene	tr.	tr.	tr.	0.18 ± 0.01 <sup>a</sup>	0.17 ± 0.01 <sup>ab</sup>	0.16 ± 0.01 <sup>b</sup>
27	1440	β-Humulene	tr.	tr.	tr.	tr.	tr.	tr.
28	1457	β- <i>trans</i> -Farnesene	1.38 ± 0.30 <sup>ab</sup>	1.23 ± 0.06ab	1.15 ± 0.12b	1.44 ± 0.06 <sup>a</sup>	1.33 ± 0.04 <sup>ab</sup>	1.27 ± 0.08 <sup>ab</sup>
29	1461	γ-Gurjunene	4.05 ± 0.21 <sup>a</sup>	3.92 ± 0.10 <sup>ab</sup>	3.66 ± 0.13 <sup>b</sup>	N.D.	N.D.	N.D.

No.	RI	Volatile compounds	Relative peak area (%)					
			Fresh		Dried			
			pH 3	pH 5	pH 7	pH 3	pH 5	pH 7
30	1464	Sesquisabinene	N.D.	N.D.	N.D.	6.60 ± 0.39	6.29 ± 0.11	6.03 ± 0.31
31	1471	Acoradiene	N.D.	N.D.	N.D.	tr.	tr.	tr.
32	1477	γ-Muurolene	0.68 ± 0.06 <sup>a</sup>	0.63 ± 0.01 <sup>a</sup>	0.58 ± 0.02 <sup>ab</sup>	0.64 ± 0.11 <sup>a</sup>	0.58 ± 0.02 <sup>ab</sup>	0.52 ± 0.04 <sup>b</sup>
33	1489	Bicyclosesquiphellandrene	6.89 ± 0.65 <sup>b</sup>	6.82 ± 0.42 <sup>b</sup>	6.80 ± 0.12 <sup>b</sup>	8.36 ± 0.29 <sup>a</sup>	8.36 ± 0.22 <sup>a</sup>	8.38 ± 0.43 <sup>a</sup>
34	1491	α-(Z, E)-Farnesene	16.03 ± 1.41 <sup>ab</sup>	15.45 ± 0.49 <sup>ab</sup>	14.37 ± 1.33 <sup>b</sup>	16.83 ± 0.64 <sup>a</sup>	15.95 ± 0.29 <sup>ab</sup>	15.43 ± 0.57 <sup>ab</sup>
35	1499	Eremophilene	0.46 ± 0.05 <sup>abc</sup>	0.38 ± 0.13°	0.39 ± 0.13 <sup>bc</sup>	0.54 ± 0.06 <sup>ab</sup>	0.55 ± 0.02 <sup>a</sup>	0.56 ± 0.04 <sup>a</sup>
36	1503	α-Muurolene	0.19 ± 0.02a	0.13 ± 0.09 <sup>a</sup>	0.11 ± 0.09 <sup>a</sup>	0.17 ± 0.06 <sup>a</sup>	0.14 ± 0.02 <sup>a</sup>	0.14 ± 0.01 <sup>a</sup>
37	1508	α-Farnesene	11.65 ± 1.12 <sup>a</sup>	11.06 ± 0.19 <sup>ab</sup>	10.22 ± 0.92bc	9.76 ± 0.45 <sup>cd</sup>	9.08 ± 0.23 <sup>cd</sup>	8.91 ± 0.35 <sup>d</sup>
38	1513	γ-Cadinene	0.59 ± 0.05 <sup>bc</sup>	0.50 ± 0.02bc	0.46 ± 0.01°	0.67 ± 0.11 <sup>a</sup>	0.57 ± 0.02 <sup>ab</sup>	0.54 ± 0.02 <sup>bc</sup>
39	1524	β-Sesquiphellandrene	3.82 ± 0.52ab	3.65 ± 0.16 <sup>b</sup>	3.34 ± 0.15 <sup>b</sup>	4.22 ± 0.35 <sup>a</sup>	3.79 ± 0.07 <sup>ab</sup>	3.60 ± 0.19 <sup>b</sup>
40	1532	Cubebene	tr.	tr.	tr.	tr.	tr.	tr.
41	1538	α-Cadinene	tr.	tr.	tr.	0.17 ± 0.03 <sup>a</sup>	0.14 ± 0.00 <sup>ab</sup>	0.13 ± 0.01 <sup>b</sup>
42	1564	(+)-Nerolidol	tr.	tr.	tr.	0.19 ± 0.02°	0.15 ± 0.00 <sup>b</sup>	0.16 ± 0.01 <sup>b</sup>

RI = linear retention index from NIST Library; ND = not detected; tr. = trace (below 0.01%)  $^{a, b, c}$  Means with different letters within the same row are significantly different (p < 0.05).

Antioxidant efficiency of white mugwort extract in oil-in-water emulsion at different pH levels: The total phenolic content and antioxidant activity of white mugwort extract are presented in Table 2. In this study, the ethanolic extract of white mugwort was 58.19±0.20 mg GAE/g dried extract, which was relatively high compared with [33] who reported that the crude ethanolic extract of dried white mugwort contained 48.11±1.45 mg GAE/g dry extract. The antioxidant activity of white mugwort ethanolic extract, as assessed by DPPH, ABTS, and FRAP assays, was 26.50±1.14, 35.66±0.02, and 35.41±1.65 mg TEAC/g dried extract, respectively. The results indicate that the extract

possesses strong antioxidant potential across multiple mechanisms. These findings align with previous reports highlighting the richness of *A. lactiflora* in phenolic compounds, flavonoids, and terpenoids, which contribute to its strong antioxidant capacity [2, 3, 33]. The close values between ABTS and FRAP also suggest a consistent and balanced presence of both electron- and hydrogen-donating antioxidants in the extract [34]. This result confirms that white mugwort ethanolic extract can serve as a potent natural antioxidant, supporting its potential applications in products where oxidative stability and health-promoting properties are desired.

Table 2 Total phenolic content and antioxidant activity of white mugwort extract.

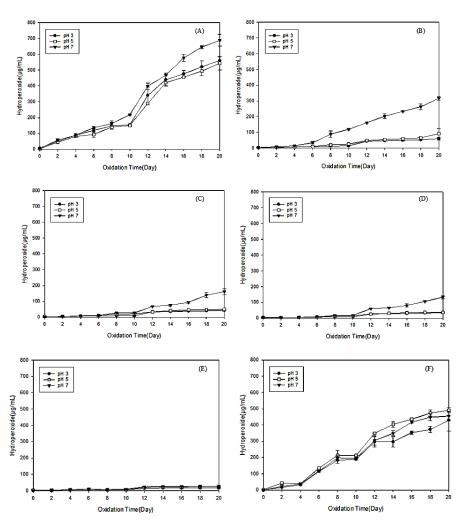
Total phenolic content (mg GAE	Antioxidant activity					
/g extract)	DPPH (mg TEAC / g extract)	ABTS (mg TEAC / g extract)	FRAP (mg TEAC / g extract)			
58.19 ± 0.20	26.50 ± 1.14	35.66 ± 0.02	35.41 ± 1.65			

Oil-in-water (O/W) emulsions are widely used in food, cosmetic, and pharmaceutical products for incorporating hydrophobic compounds in aqueous systems, but their vulnerability to lipid oxidation poses challenges to product quality and shelf life [35]. The use

of natural antioxidants has gained increasing interest as a safer and more sustainable alternative to synthetic preservatives. In this study, the antioxidant efficiency of white mugwort extract at different concentrations (200, 400 and 600 ppm) in O/W emulsions under different pH

conditions (pH 3, 5, 7) were investigated compared with BHT (200 ppm) and  $\alpha$ - Tocopherol (200 ppm) as a synthetic and natural antioxidant, respectively. To monitor lipid oxidation during storage, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were measured as indicators of primary and secondary oxidation products, respectively. Figure 2 and Figure 3 illustrates the changes in PV of all samples during storage. Antioxidant efficiency was assessed based on the lag time of lipid hydroperoxide formation during storage. As shown in Figure 2, the extract at pH 3 and 5 exhibited greater antioxidant efficiency than at pH 7, as indicated by their longer lag times. These results suggest that mildly acidic conditions enhance effectiveness of white mugwort extract in inhibiting lipid oxidation in oil-in-

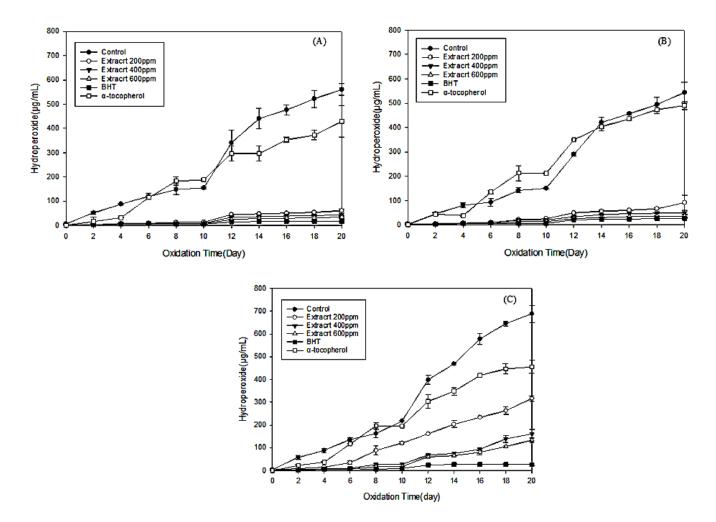
water emulsions. This may be attributed to the increased protonation and solubility of phenolic compounds in acidic environments, which can enhance their interaction at the oil–water interface where oxidation primarily occurs [12]. Interestingly, this pH-dependent behavior was not observed for BHT and  $\alpha\text{-tocopherol},$  which showed relatively stable antioxidant efficiency across the tested pH values. These differences suggest that the antioxidant mechanisms of white mugwort extract are more sensitive to pH, likely due to its complex mixture of polyphenolic compounds whose structure and efficiency may be influenced by protonation states. This highlights the potential advantage of using white mugwort extract in acidic food or cosmetic formulations, where its antioxidant efficacy can be maximized.



**Figure 2** Peroxide value of O/W emulsions containing (A) No extract; (B) 200 ppm white mugwort extract; (C) 400 ppm white mugwort extract; (E) 200 ppm BHT; (F) 200 ppm  $\alpha$ -Tocopherol during storage at 40 °C in the dark.

According to Figure 3, the antioxidant efficiency of white mugwort extract in oil-in-water emulsions increased with concentration, with 600 ppm showing the strongest efficiency, followed by 400 ppm and 200 ppm. This dose-dependent effect suggests that higher levels of bioactive compounds in the extract enhance its ability to inhibit lipid oxidation. When compared to standard antioxidants, white mugwort extract at all concentrations exhibited lower antioxidant efficiency than BHT (200

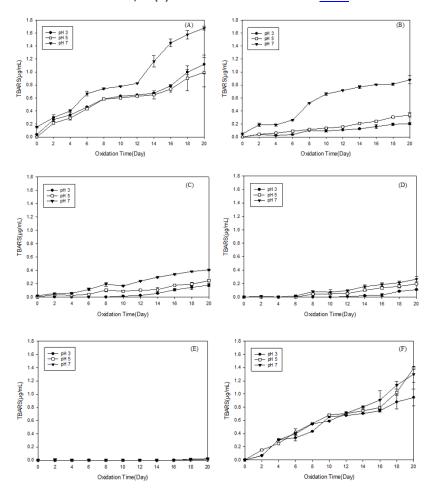
ppm), a potent synthetic antioxidant. However, it outperformed  $\alpha$ -tocopherol (200 ppm), a widely used natural antioxidant. These findings suggest that although white mugwort extract is less effective than the synthetic antioxidant BHT, it offers a promising natural alternative with better performance than  $\alpha$ -tocopherol, particularly at higher concentrations. This highlights its potential as a promising natural antioxidant for use in clean-label formulations.



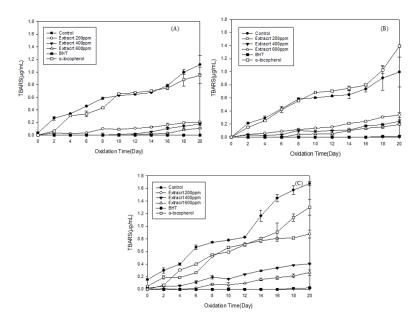
**Figure 3** Peroxide value of O/W emulsions containing white mugwort extract (200, 400, 600 ppm), BHT (200 ppm) and  $\alpha$ -Tocopherol (200 ppm) at (A) pH 3; (B) pH 5; (C) pH 7 during storage at 40 °C in the dark

In addition to PV, TBARS assay was used to evaluate antioxidant efficiency, as it reflects the formation of secondary lipid oxidation products, such as malondialdehyde. As shown in Figure 4 and 5, the TBARS results followed a similar trend to the PV data, further

confirming the effectiveness of white mugwort extract in inhibiting lipid oxidation in oil-in-water emulsions. Specifically, the extract demonstrated stronger antioxidant efficiency at pH 3 and 5 compared to pH 7 in a dose-dependent pattern.



**Figure 4** TBARS of O/W emulsions containing (A) No extract; (B) 200 ppm white mugwort extract; (C) 400 ppm white mugwort extract; (E) 200 ppm BHT; (F) 200 ppm  $\alpha$ -Tocopherol during storage at 40 °C in the dark.



**Figure 5** TBARS of O/W emulsions containing white mugwort extract (200, 400, 600 ppm), BHT (200 ppm) and  $\alpha$ -Tocopherol (200 ppm) at (A) pH 3; (B) pH 5; (C) pH 7 during storage at 40 °C in the dark.

This suggests that mildly acidic conditions enhance the capacity of mugwort extract to prevent both primary and secondary oxidation processes. In contrast, BHT and tocopherol, used as synthetic and natural antioxidant controls respectively, did not exhibit notable differences in TBARS values across the pH range. These results highlight the pH-sensitive nature of mugwort extract's antioxidant compounds, likely due to changes in their chemical structure, solubility, or interfacial activity under different pH conditions. Overall, the TBARS findings support the potential of white mugwort extract as a natural antioxidant, particularly in formulations with acidic to mildly acidic pH.

Scientific Innovation and Practical Implications: The comprehensive profiling of volatile compounds in both fresh and dried white mugwort leaves under different pH conditions highlights the dynamic nature of aroma compound stability and transformation. Notably, the study revealed that drying at 50 °C not only reduced key volatiles such as 3-Carene and β-Myrcene, likely due to thermal degradation and evaporation but also increased others, including Santolina triene, Caryophyllene, and Bicyclosesquiphellandrene. This dual effect illustrates a complex interplay of degradation and conversion reactions, enhancing specific aroma notes even as others diminish. Furthermore, the impact of pH on volatile composition revealed that compounds like 3-Carene is pH-sensitive, whereas Santolina triene remains stable across pH 3-7. This adds to the limited knowledge on how pH modulates the stability and reactivity of sesquiterpenes and monoterpenes in plant matrices, supporting hypotheses of pH-driven redox or cyclization reactions.

On the antioxidant front, this is among the first studies to demonstrate the dose- and pH-dependent antioxidant activity of white mugwort extract in oil-inwater emulsions, a model system widely used in food and cosmetic formulations. The extract showed enhanced

antioxidant efficiency at acidic to mildly acidic pH (3 and 5), an effect not observed with conventional antioxidants like BHT and  $\alpha$ -tocopherol. This pH sensitivity is likely driven by the protonation state and solubility behavior of mugwort's polyphenolic constituents, which could enhance their localization and reactivity at the oil–water interface where oxidation initiates.

Understanding the influence of pH on volatile composition enables better control over aroma retention and flavor optimization during processing and storage. For instance, minimizing losses of key aroma-active compounds or selectively enhancing desirable notes through pH adjustment during drying or formulation could improve sensory quality.

Moreover, the findings of this study have significant implications for the formulation of clean-label, plantbased antioxidant systems. The dose-dependent antioxidant efficiency suggests that higher concentrations (e.g., 600 ppm) of white mugwort extract can effectively suppress lipid oxidation, offering performance superior to  $\alpha$ -tocopherol, a widely accepted natural antioxidant. Although it does not match the strength of synthetic BHT, its efficacy, especially in acidic environments, makes it a strong candidate for use in acidic food products such as dressings, beverages, and sauces. In cosmetics, plant extracts are widely used as active ingredients but often suffer from low solubility and instability. This study elucidates their fragrance and antioxidant functions, especially relevant for emulsions formulated at acidic pH. These insights aid in optimizing extract selection to enhance product efficacy, stability, and sensory qualities.

#### **CONCLUSION**

This study highlights the complex behavior of volatile and antioxidant compounds in white mugwort under varying pH and drying conditions. Drying at 50 °C reduced key volatiles such as  $\beta$ -Myrcene,  $\beta$ -cis-Ocimene, 3-Carene, and  $\gamma$ -Gurjunene through thermal degradation, while

increasing others like Santolina triene, Caryophyllene, and Bicyclosesquiphellandrene. pH also significantly affected volatile stability, with 3-Carene showing pH sensitivity, whereas Santolina triene remained stable across pH 3–7. Antioxidant efficiency of the extract demonstrated both dose- and pH-dependence in oil-inwater emulsions, with stronger performance at acidic to mildly acidic conditions (pH 3 and 5). In contrast, synthetic BHT and natural  $\alpha$ -tocopherol maintained consistent activity across pH levels. Overall, these findings enhance understanding of white mugwort's chemical behavior under different processing conditions and support its application as a natural multifunctional ingredient, providing both aroma and antioxidant benefits in diverse emulsion-based formulations.

**Competing interests:** The authors declare that they have no competing interests.

**Authors' contributions:** KK, PA, PhN, PP designed the research. AK, SP, PN, WP conducted the experiments and collected the data. KK, AK, SP, PN, and WP performed statistical analyses. KK, PP wrote original draft of the manuscript. KK, PA, PhN, PP supervised the project and acquired the funding. All authors read and approved the final version of the manuscript.

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