

# Thermal degradation kinetics of phenylbutanoids in *Zingiber montanum* extract and stability improvement by oil extraction

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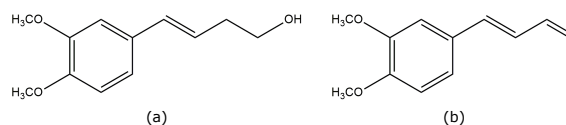
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**ABSTRACT:** (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) and (E)-4-(3',4'-dimethoxyphenyl)-but-3-en-1-ol (Compound D), both phenylbutanoids, are recognized as potent anti-inflammatory agents predominantly found in the essential oil or rhizome extract of *Zingiber montanum*. The instability of phenylbutanoids plays a pivotal role in determining the efficacy of formulations containing extracts or essential oils derived from *Z. montanum* rhizomes. This current study aimed to demonstrate the impact of temperature on the Compound D and DMPBD present in *Z. montanum* extract (ZME) and *Z. montanum* oil (ZMO) obtained by hot oil extraction. The stability of Compound D and DMPBD is assessed by investigating thermal degradation kinetics and predicting shelf life using Arrhenius plot models. The findings unveiled that Compound D and DMPBD exhibit degradation following a first-order kinetic model. Under this model, the predicted shelf life of ZME at 25 °C for Compound D and DMPBD amounts to 12.14 days and 10.18 days, respectively. In contrast, the anticipated shelf life of these compounds in ZMO extends to 480.31 days and 188.57 days, respectively. Consequently, Compound D and DMPBD within ZMO hold significant promise for further advancement in product development.

**KEYWORDS:** anti-inflammatory agents, rhizome extract, rhizome essential oil

## INTRODUCTION

Phenylbutanoids are bioactive compounds that are prominently found in the rhizome of *Zingiber montanum* (J. Koenig, Link ex A. Dietr), which is traditionally used in recipes for treating muscle or bone inflammation. The main phenylbutanoids present in *Z. montanum* rhizome are (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD) and (E)-4(3',4'-dimethoxyphenyl)-but-3-en-1-ol (Compound D), which exhibits potent anti-inflammatory activity (Fig. 1). An essential oil extracted from rhizomes is also used as an ingredient in spa products due to the bioactivity and scent. Previous studies have shown that DMPBD effectively reduces inflammation and edema *in vivo* experiments [1]. An *in vitro* study of DMPBD demonstrated its effectiveness in inhibiting cyclooxygenase-2 [2]. Compound D has also been identified as an effective anti-inflammatory agent, acting through a pharmacological mechanism similar to that of DMPBD [3]. Additionally, Compound D may play a role in reducing the synthesis of interleukin-1 $\beta$  (IL-1 $\beta$ ) protein, both in its pro-form and active form, which is crucial in the up-regulation of matrix metalloproteinases (MMPs) in synovial fibroblast



**Fig. 1** Chemical structure of (a) (E)-4-(3', 4'-dimethoxyphenyl)-but-3-en-1-ol (Compound D) and (b) (E)-1-(3,4-dimethoxyphenyl) butadiene (DMPBD).

cells [4].

Additionally, the rhizomes of *Z. montanum*, which are thought to be components of functional foods that exhibit antihyperlipidemic activity in addition to a hepatoprotective effect, were considered for future health food development, according to recent studies that also showed promising activities for motivating others to develop health products [5]. There was evidence that the essential oil had cytotoxic effects on cancer cell lines [6]. Although the rhizome extracts and essential oil of *Z. montanum* have been developed as ingredients for anti-inflammatory applications and others due to the bioactivity of phenylbutanoids, the stability of these compounds has not been fully clar-

ified. A few studies indicate that Compound D in *Z. montanum* extract degrades more when solubilized in an acidic solution and kept at temperatures higher than 50 °C [7], while the stability of Compound D is improved by using the niosome encapsulation technique [8]. The Arrhenius plot model is one of the mathematical models that has been used for estimating the stability of compounds by examining how reaction rates change with temperature. It is primarily used to predict chemical degradation rates, estimate product shelf life, and understand the thermal stability of compounds. The relationship among parameters associated with chemical reactions is expressed in Eqs. (1) and (2), where  $k$  is the reaction rate constant,  $A$  is the Arrhenius frequency factor ( $A$ ) for the accelerated breakdown over the tested temperature of each compound,  $E_a$  is the activation energy,  $R$  is the gas constant (8.3145 J/mol kelvin), and  $T$  is absolute temperature.

$$k = Ae^{(-E_a/RT)}. \quad (1)$$

The slope of the semi-logarithmic curve multiplied by the gas constant ( $R$ ) represented the activation energy ( $E_a$ ). The natural logarithm of each rate reaction constant ( $\ln k$ ) was plotted against the inverse of absolute temperature ( $1/T$ ).

$$\ln k = \ln A - E_a/RT. \quad (2)$$

The reaction rate constants of the compound at the designed temperature were calculated from the linear equation; then their shelf lives were estimated by Eq. (3) [9].

$$\text{Shelf life } (T_{90\%}) = 0.105/k. \quad (3)$$

The current study aimed to prepare *Z. montanum* oil (ZMO) and extract (ZME) to represent the degradation profile of Compound D and DMPBD. The shelf life of both compounds in the extract and oil was determined and compared using Arrhenius's equation that is usually used for evaluating the stability behavior and estimated expiry date of herbal applications [10–12].

## MATERIALS AND METHODS

### Materials

Denatured 95% ethanol was purchased from the Liquor Distillery Organization (Chachoengsao, Thailand). Acetonitrile (HPLC grade), methanol (HPLC grade), ethyl acetate and hexane (Analytical grade) were purchased from Labscan Asia Co. Ltd. (Bangkok, Thailand). Sodium chloride and potassium hydrogen orthophosphate were purchased from EMD Chemicals Inc. (New Jersey, USA).

### *Z. montanum* extract preparation by hexane extraction

*Z. montanum* rhizomes were purchased from Khaokhoherbary organic farm (Phetchaboon, Thailand). The

plant collection method and experimental use were in accordance with all the relevant guidelines. The specimens were identified by Peangkamon Sornpuang, the botanist of the Botanical Garden of Sirindhorn College of Public Health, Phitsanulok. The voucher specimen, collection number 04293, was deposited at the Thai traditional medicine herbarium, also located at the Sirindhorn College of Public Health, Phitsanulok. Five hundred grams of rhizomes were chopped into tiny pieces (approximately 2 cm × 2 cm) and then dried at 50 °C for 24 h. The dried rhizomes were extracted using a Soxhlet apparatus with hexane for 4 h. Extraction temperature was approximately 70 °C. The extract solution was filtered through Whatman No. 1 filter paper before undergoing rotary evaporation at 40 °C for 2 h. The *Z. montanum* extract (ZME) was stored in vials at –20 °C and protected from light until use.

### *Z. montanum* oil preparation by hot oil extraction

*Z. montanum* oil (ZMO) was prepared according to the previous report [13]. The preparation was slightly modified and can be described briefly as follows: the fresh rhizomes of *Z. montanum* were chopped into tiny pieces (approximately 2 cm × 2 cm) and then fried with refined coconut oil at 120 °C for 1 h. The ratio of fresh rhizomes to oil was 1:3 (weight by weight). The preparing temperature and ratio were modified from the National List of Essential Medicines recommendation for increasing the active compounds [14]. The resulting yellowish oil was purified using paper filtration, and the filtered oil was stored in a cool place, protected from light.

### Isolation of Compound D and DMPBD by column chromatography

Flash column chromatography was performed on a pure chromatography system (Buchi C-810/815, Flawil, Switzerland). The system was equipped with a binary pump with 4 solvent selections, an ultraviolet (UV), evaporating light scattering detector (ELSD), and a fraction collector. The pre-packed Select silica cartridge (120 g, 40 g, and 25 g) with a particle size of 15 µm and Flash Pure Select C18 (120 g, 40 g, and 25 g) with a particle size of 30 µm were employed as chromatographic column. NMR spectra were acquired using a Bruker Avance 600 MHz spectrometer. Analytical plates for thin-layer chromatography (TLC) were silica gel 60 F254 plates (20 × 20 cm<sup>2</sup>) for the normal phase (Merck, Darmstadt, Germany).

The crude *Z. montanum* extract (PI) (9.39 g) was subjected to flash chromatography (Selected silica cartridge, 120 g, 5% EtOAc-n-hexane) to afford 8 subfractions (PI-1-PI-8). Fr. PI-1 (1.51 g) was subjected to flash chromatography (Selected silica cartridge, 25 g, 3–5% EtOAc-n-hexane) to afford DMPBD (436.9 mg). Fr. PI-7 (971.0 mg) was subjected to flash chromatog-

raphy (Selected silica cartridge, 120 g, 10–50% EtOAc-n-hexane) to afford Compound D (54.9 mg).

#### Determination of Compound D and DMPBD by High Performance Liquid Chromatography (HPLC)

Compound D and DMPBD in *Z. montanum* oil or extract were analyzed by the RP-HPLC system using an Agilent 1260 Infinity system (Agilent Technologies, USA) with detection at 260 nm. A Luna Phenomenex analytical column (250 mm × 7 mm, 5- $\mu$ m particle size) (Phenomenex, Deerfield, IL, USA) with a flow rate of 1 ml/min, and injection volume of 10  $\mu$ l was used for this experiment. The mobile phase was a gradient elution of 2% acetic acid in ultrapure water (A) and methanol (B) of 60 to 50% of A, 50 to 30% of A, 30 to 20% of A, 20 to 50% of A, 50 to 60% of A, and 60% of A for 0–5 min, 5–15 min, 15–25 min, 25–30 min, 30–32 min, and 32–40 min, respectively. The analytical process was done at room temperature.

#### Thermal degradation kinetic study of Compound D and DMPBD

One milliliter of ZMO or 10 mg of ZME was separately placed into microcentrifuge tubes and sealed with parafilm tapes. These tubes were then placed in environments containing a saturated sodium chloride solution in an incubator (Memmert GmbH, Selecta Biosciences, Germany) to maintain 75% relative humidity at temperatures ranging from 50 to 90 °C for 12 or 28 days. ZME samples were collected on days 0, 3, 6, 9, and 12, while ZMO samples were collected on days 0, 7, 14, 21, and 28. After collection, all samples were stored at –20 °C for further determination of the remaining Compound D and DMPBD using the HPLC method. The remaining Compound D and DMPBD were used to calculate the reaction order, rate of reaction constant ( $k$ ), and shelf life ( $T_{90\%}$ ).

## RESULTS

ZME and ZMO were successfully prepared. Then ZME was used to create the isolated Compound D and isolated DMPBD by using column chromatography. Isolated compounds were used as standard references in bioactive compound measurements using the HPLC.

#### Isolated Compound D and DMPBD from *Z. montanum* extract

DMPBD and Compound D isolated from *Z. montanum* rhizomes were pale yellow oil. The NMR spectra are presented similarly to those in other reports [15, 16]; (Fig. 2 and Fig. 3).

#### Determination of Compound D and DMPBD by HPLC method

The amount of Compound D and DMPBD isolated from *Z. montanum* rhizomes was measured using the HPLC.

The validation parameters presenting the accuracy and precision of this method are shown in Table 1.

The method validation indicated that this HPLC method was accurate and reliable for the determination of the amount of Compound D and DMPBD. The chromatogram of isolated Compound D and DMPBD as the standard compounds for HPLC is presented in Fig. 4.

After using hot oil extraction, ZMO produced a yellowish solution, while ZME generated a brownish semi-solid. We measured the amount of Compound D and DMPBD present in both extract forms using HPLC; those quantities are presented in Table 2.

ZME and ZMO contained varying quantities of Compound D and DMPBD. It is evident that ZME is enriched in Compound D and DMPBD when compared to ZMO, with the content of Compound D and DMPBD in ZME being approximately 10 times greater than that in ZMO.

#### Stability of Compound D and DMPBD in ZME and ZMO

ZME and ZMO were kept in various temperatures. The remaining amounts of Compound D and DMPBD were quantified at designated times for determining the thermal degradation reactions. The order of reaction was predicted by graphical method [9]. The reaction orders of both Compound D and DMPBD were first order reactions presenting the linear correlation between natural logarithm of remaining Compound D or DMPBD against time. The reaction rate constant ( $k$ ) of chemical reaction at each temperature was calculated from slope of linear equation. The estimates of shelf life of Compound D and DMPBD in extract and oil are needed for the further development of *Z. montanum* products. This portion of the current study was important; we evaluated the thermal chemical kinetics stability for Compound D and DMPBD in ZME and ZMO using Arrhenius's theory. The remaining amounts of Compound D and DMPBD in all sample forms after being stored at various temperatures for 12–28 days are presented in Fig. 5 and Fig. 6.

The graphs indicated that the rate of Compound D and DMPBD decomposition increased at elevated temperatures. The correlation between the natural logarithm of remaining Compound D or DMPBD in each sample form versus time was linear. The degradation rate constant ( $k$ ) of Compound D or DMPBD at each tested temperature was calculated from the slope of the curve plotted by natural logarithm of remaining each compound concentration versus time elapsed. The reaction rate constants are presented in Table 3.

The results indicated that elevated temperature obviously affected the reaction rate constant; the reaction rate constant tended to increase at elevated temperatures. In addition, the rate reaction constants of Compound D and DMPBD in ZME were higher

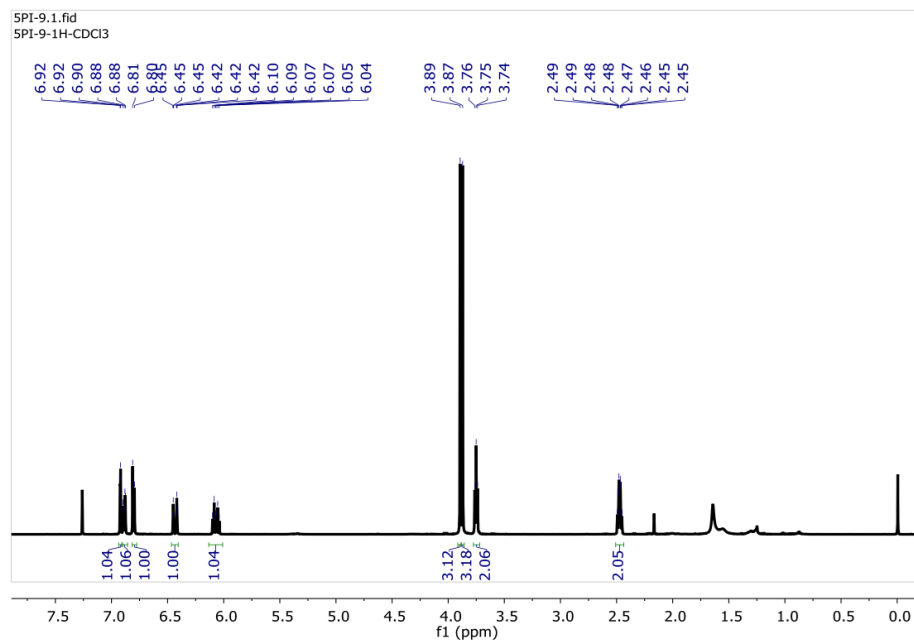


Fig. 2  $^1\text{H}$  NMR (600 MHz) spectra of Compound D in  $\text{CDCl}_3$ .

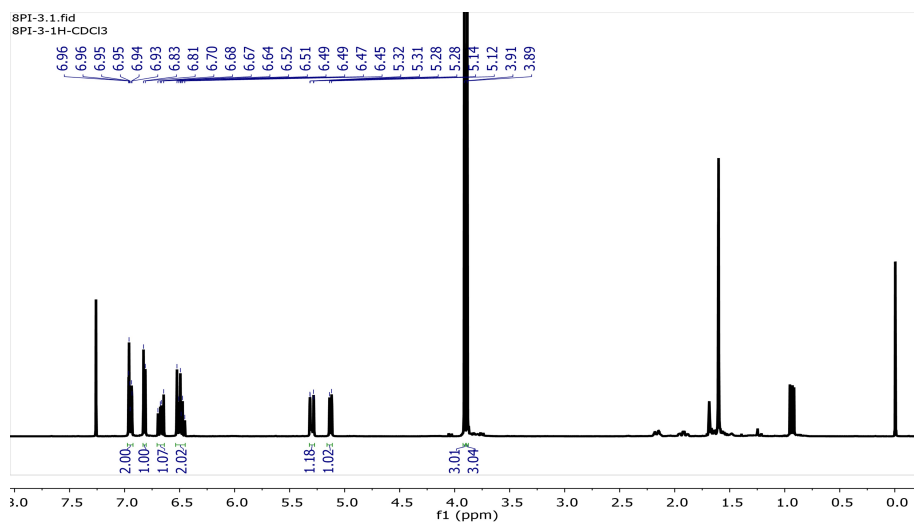


Fig. 3  $^1\text{H}$  NMR (600 MHz) spectra of DMPBD in  $\text{CDCl}_3$ .

**Table 1** Data for HPLC method validation according to Association of Official Analytical Cooperation International (AOAC) recommendations.

Parameter	Compound D	DMPBD
Range ( $\mu\text{g}/\text{ml}$ )	3.91–500.00	3.91–500.00
Linearity ( $r^2$ )	0.9995	0.9999
Accuracy (% recovery $\pm$ SD)	102.44 $\pm$ 0.78	101.89 $\pm$ 1.34
Reproducibility (% RSD $\pm$ SD)		
Inter-day	98.78 $\pm$ 1.45	105.45 $\pm$ 3.22
Intra-day	104.78 $\pm$ 1.78	99.77 $\pm$ 2.45
Limit of quantitation (LOQ) ( $\mu\text{g}/\text{ml}$ )	13.44	22.19
Limit of detection (LOD) ( $\mu\text{g}/\text{ml}$ )	4.43	7.32

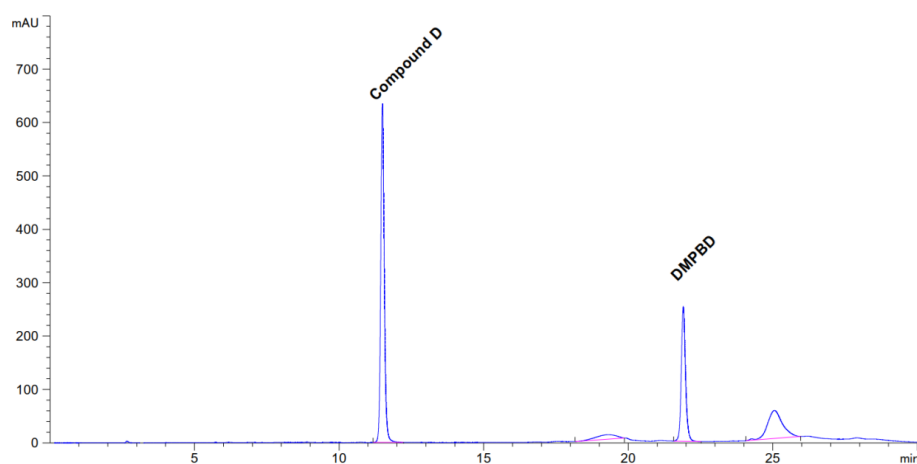


Fig. 4 HPLC chromatogram of isolated Compound D and DMPBD.

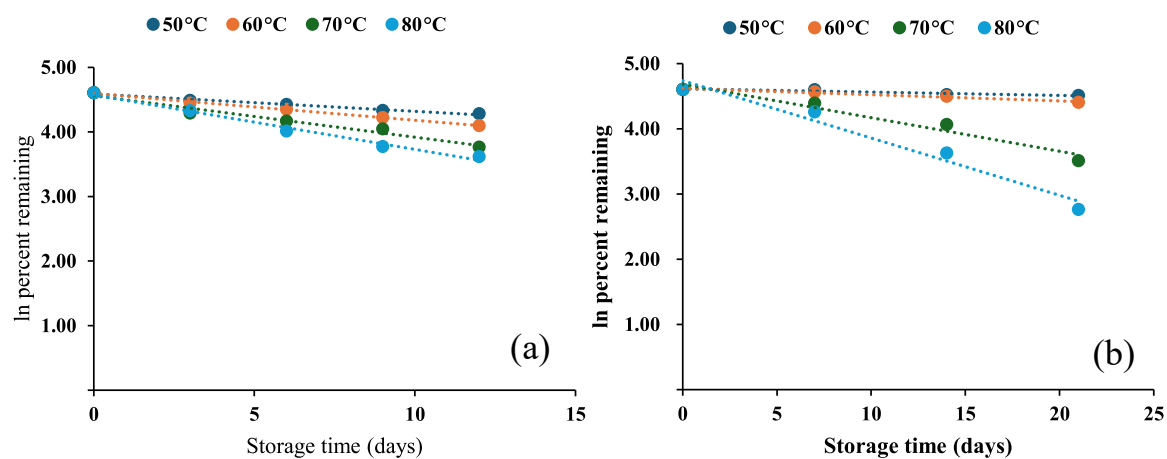


Fig. 5 Thermal degradation kinetics for Compound D in ZME (a) and ZMO (b) as fitted to a first-order reaction model;  $n \geq 3$ .

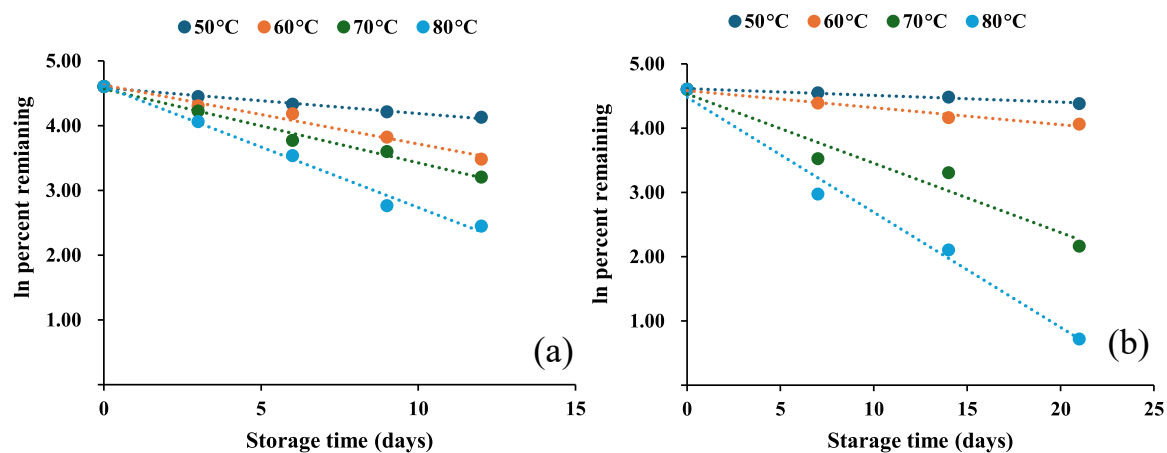


Fig. 6 Thermal degradation kinetics for DMPBD in ZME (a) and ZMO (b) as fitted to a first-order reaction model;  $n \geq 3$ .

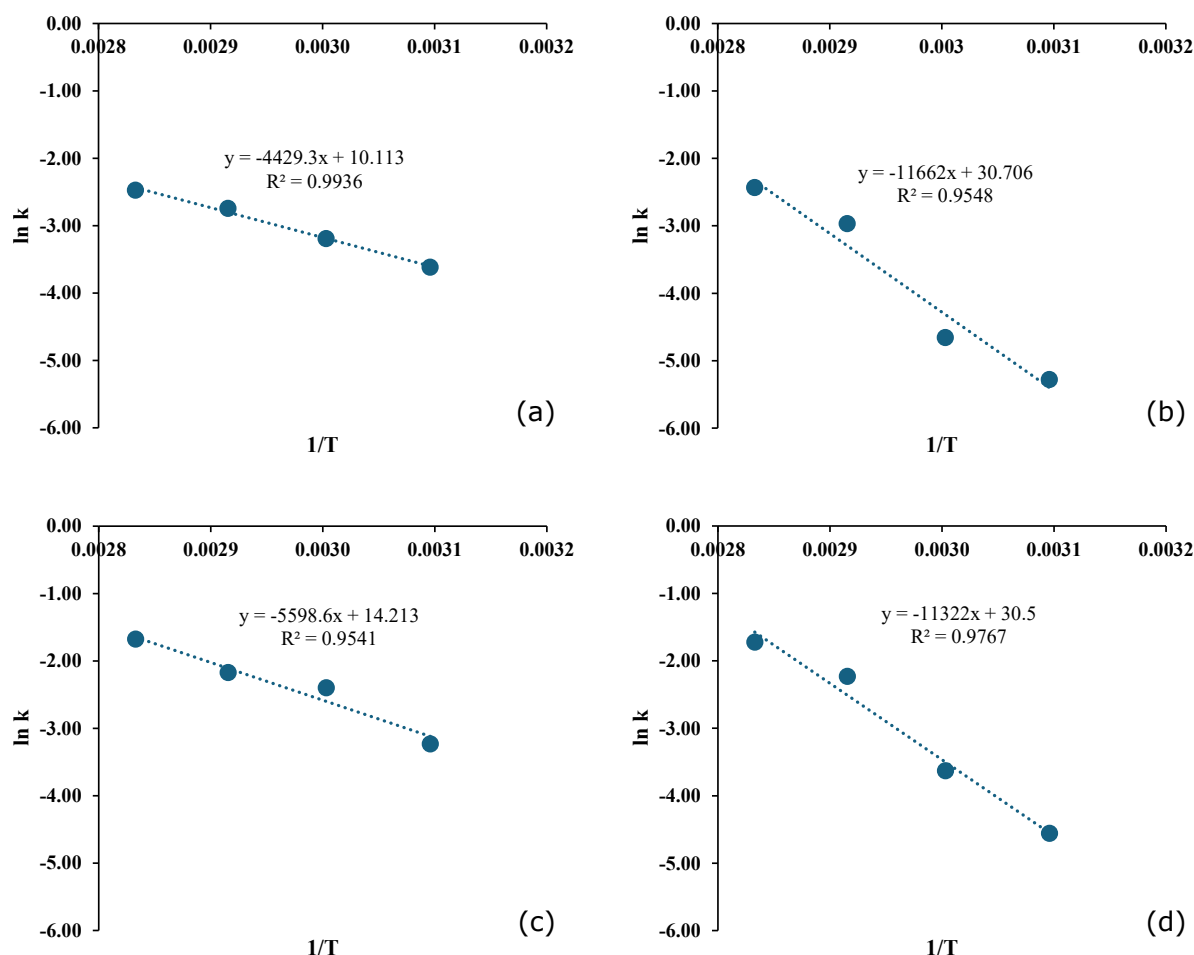


Fig. 7 Arrhenius plot for thermal degradation of Compound D in ZME (a) and ZMO (c) and DMPBD in ZME (b) and ZMO (d).

**Table 2** Quantities of Compound D and DMPBD in ZME and ZMO measured by using HPLC;  $n \geq 3$ .

Sample	Compound D (Mean $\pm$ SD, %w/w)	DMPBD (Mean $\pm$ SD, %w/w)
ZME	$6.36 \pm 0.78$	$8.57 \pm 0.08$
ZMO	$0.68 \pm 0.01$	$0.62 \pm 0.11$

than those in ZMO when compared at the same temperature. This result implied that the degradation rates of Compound D and DMPBD in ZME were faster than those in ZMO. The reaction rate constants of Compound D and DMPBD in ZME and ZMO at 25 °C were calculated from the linear equation; then their shelf lives were estimated.

The shelf lives of Compound D and DMPBD at 25 °C were predicted by using Eq. (3). The reaction rate constant ( $k$ ) for this temperature was obtained by utilizing the linear equation expressing the relationship between the natural logarithm of rate constant ( $\ln k$ )

and the inverse of absolute temperature ( $1/T$ ) (Fig. 7). The estimated reaction rate constants and shelf life at 25 °C of Compound D and DMPBD in ZME and ZMO, including activation energy ( $E_a$ ), are expressed in Table 4.

With regard to Table 4, the shelf lives of Compound D and DMPBD in ZMO (480 days and 188.57 days, respectively) were obviously higher than those in ZME (12.14 days and 10.18 days, respectively). Moreover, Compound D in both ZME and ZMO also had higher shelf lives than DMPBD in both forms.

## DISCUSSION

*Z. montanum* is a medicinal plant known for its anti-inflammatory properties. The rhizome extract and essential oil of this plant contain bioactive compounds, namely Compound D and DMPBD, which are potent anti-inflammatory agents [1–3, 17]. While these compounds have demonstrated their bioactivity through *in vitro* and *in vivo* studies, their thermal stability has not been fully established. Previous studies have



**Table 3** Effect of temperature on the reaction rate constant ( $k$ ) of Compound D and DMPBD in ZME and ZMO ( $n \geq 3$ ).

Temperature (°C)	$k^\dagger$ (day <sup>-1</sup> ) $\times 10^2$			
	Compound D		DMPBD	
	ZME	ZMO	ZME	ZMO
50	2.69 $\pm$ 0.46	0.51 $\pm$ 0.03	3.95 $\pm$ 1.87	1.05 $\pm$ 0.77
60	4.11 $\pm$ 0.79	0.95 $\pm$ 0.01	9.10 $\pm$ 0.45	2.65 $\pm$ 0.44
70	6.43 $\pm$ 1.54	5.14 $\pm$ 1.11	11.41 $\pm$ 3.45	10.78 $\pm$ 0.41
80	8.44 $\pm$ 0.34	8.79 $\pm$ 1.34	18.71 $\pm$ 2.78	17.90 $\pm$ 1.34

<sup>†</sup> Mean of degradation rate constants  $\pm$  standard deviation.

**Table 4** Comparison of the reaction rate constants and shelf lives of Compound D and DMPBD at 25 °C between ZME and ZMO.

Parameter	Compound D		DMPBD	
	ZME	ZMO	ZME	ZMO
$k$ (day <sup>-1</sup> ) $\times 10^{-3}$	8.60	0.22	10.30	0.56
$E_a$ (KJ/mol)	36.82	96.96	46.54	94.13
Shelf life (days)	12.14	480.31	10.18	188.57

indicated that the stability of Compound D decreases when it is solubilized in strong acid solutions or exposed to high temperatures [7, 8], but the stability of DMPBD has not been reported. The current study isolated and identified Compound D and DMPBD from *Z. montanum* rhizomes. These compounds were used as markers to describe their stability profiles in ZME and ZMO. Compound D and DMPBD were properly extracted from the rhizomes using both hexane and hot oil extraction methods. However, the contents of Compound D and DMPBD in ZMO were lower than those in ZME due to the advantage of the semi-solid form being more concentrated than the liquid form [18]. Moreover, elevated temperatures during hot oil extraction might have caused active compound degradation [19]. When the ZME and ZMO were stored at various temperatures, it became evident that elevated temperatures significantly affected the stability of Compound D and DMPBD. The decreased degradation rate of Compound D and DMPBD in ZMO was evidenced by the estimated activation energy and reaction rate constant. The rate constant is a parameter that determines the potential reaction progression rate; it quantifies how rapidly a reaction occurs under specific conditions. Activation energy, conversely, acts as a kinetic barrier that directly controls the chemical reaction rate. Lower activation energy corresponds to faster reaction rates, whereas higher activation energy indicates slower reaction rates [9]. Their instability can be attributed to oxidation reactions, which are accelerated by high temperatures [20, 21]. At these elevated temperatures, the reaction rate constant tends to increase because the induced oxidation reaction and degradation of Compound D and DMPBD were evident aligning with the first-order reaction model [9]. There

is a study that attempted to improve the stability of Compound D by encapsulating it into a niosome gel. The results showed that the niosome significantly enhanced the stability of Compound D compared to the free form [8]. The improved stability of Compound D may be attributed to the ability of the niosome to prevent oxidative reactions [22], as indicated by the decrease in the degradation rate constant at the same temperature point [8]. However, the study of the degradation kinetics of Compound D in niosomes and gel revealed that Compound D was degraded following a zero-order reaction. The discrepant results were due to differences in dosage forms and temperature variations. Niosome and gel represent hydrophilic formulations that might affect the chemical degradation of Compound D differently from hydrophobic systems; moreover, the difference in temperature testing ranges also influences the reaction order. There were reports that have indicated that individual compounds could exhibit order variations due to the effects of different temperatures, test concentration ranges, and environmental conditions [23, 24]. In addition, there was a study that recommended that the stability of sensitive compounds might be evident by dissolving them in an oil vehicle. The herbal extract macerated with oil is one extraction method for stabilizing active compounds against oxidative reactions [25]. In this current study, we prepared *Z. montanum* extract in oil form by using hot oil extraction. The results revealed that Compound D and DMPBD in ZMO were more stable than those in ZME. The shelf life of Compound D in ZME and ZMO at 25 °C was 12.14 days and 406 days, respectively; while for DMPBD, the shelf life was 10.18 days in ZME and 229.74 days in ZMO. Compound D and DMPBD in oil form were more stable due

to oil-mediated oxidative stabilization. The presence of oil could prevent oxidation reactions by limiting the exposure between oxygen and the active chemical compounds. Regarding this mechanism, Compound D and DMPBD solubilized in the oil phase exhibited more advantages than those in the aqueous phase, which contains a higher concentration of oxygen molecules [26–28]. Additionally, the high temperature during oil extraction also reduces the solubility of oxygen in the oil [29,30]. These factors contribute to the improvement of Compound D and DMPBD stability in ZMO. Regarding the degradation profile of both compounds at elevated temperatures, instability was also evident, although the oil could stabilize the oxidation reaction. Degradation was also induced by other factors such as external oxygen species, lipid peroxidation, and changes in temperature [30,31]. Moreover, DMPBD also demonstrated more pronounced degradation when subjected to elevated temperatures in comparison to Compound D. The instability of DMPBD can be attributed to its chemical properties, including the presence of a terminal double bond that is more susceptible to degradation at high temperatures [32]. Therefore, in order to decrease the degradation reaction of both Compound D and DMPBD, it is imperative to consider critical factors such as preventing exposure to oxygen or avoiding high-temperature conditions.

## CONCLUSION

Compound D and DMPBD represent bioactive compounds isolated from *Z. montanum* rhizomes, demonstrating significant potential as therapeutic agents due to their anti-inflammatory properties. The thermal degradation kinetic study reveals that the degradation reaction order of Compound D and DMPBD conforms to a first-order reaction. In accordance with Arrhenius's theory, the chemical reaction parameters indicate their susceptibility to instability when exposed to elevated temperatures. However, the stability of both compounds marked improvement through the hot oil extraction, effectively extending the stability of Compound D and DMPBD. The predicted shelf life of ZME at 25 °C for Compound D and DMPBD amounts to 12.14 days and 10.18 days, respectively, with activation energies of 36.82 kJ/mol and 46.54 kJ/mol, respectively. In contrast, the anticipated shelf life of these compounds in ZMO extends obviously to 480.31 days and 188.57 days, respectively, with activation energies of 96.96 kJ/mol and 94.13 kJ/mol, respectively. Consequently, the extraction of Compound D and DMPBD from *Z. montanum* rhizomes in oil form offers substantial advantages for the development of herbal products. Nevertheless, further investigation through *in vitro* and clinical studies is imperative to comprehensively evaluate their safety and efficacy.

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