# The protective effects of Zingiber zerumbet rhizome against pyrexia in rats

**Haslinda Mohd Salleh**
Universiti Malaya
**Abdulwali Ablat**
Universiti Malaya
**Soon-Lim Chong**
Universiti Malaya
**Hazrina Hazni**
Universiti Malaya
**Norsita Tohar**
Universiti Malaya
**Nurulwahidah Fauzi**
Universiti Sains Islam Malaysia
**Habibah A. Wahab**
Universiti Sains Malaysia
**Muhamad Aqmal Othman**
Universiti Malaya
**Sook Yee Liew**
Universiti Malaya
**Khalijah Awang** ( khalijah@um.edu.my )
Universiti Malaya

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## Abstract

# Background

The *Zingiber zerumbet* rhizomes are traditionally used to treat fever, and *in vitro* inhibitory effect of ethyl acetate extract from *Zingiber zerumbet* rhizomes (ZZEA) against DENV2 NS2B/NS3 has been reported early. This study was carried out to establish an acute toxicity profile and evaluate the anti-pyretic activities of ZZEA in yeast-induced pyrexia in rats.

# Methods

The toxicity of ZZEA was evaluated using OECD Test Guideline 425 (tg-425) with minor modifications at concentrations of 2000 mg/kg, 3000 mg/kg, and 5000 mg/kg of ZZEA. Antipyretic effect was determined by yeast-induced pyrexia in rats.

# Results

The acute toxicity study showed that ZZEA is safe at the highest 5000 mg/kg body weight dose in Sprague Dawley rats. Rats treated with ZZEA at doses of 125, 250, and 500 mg/kg exhibited a significant reduction in rectal temperature ($T_R$) in the first 1 hour. ZZEA at the lower dose of 125 mg/kg showed substantial potency against yeast-induced pyrexia up to 2 hours compared to 0 hours. A significant reduction of $T_R$ was observed in rats treated with standard drug aspirin (ASA) at the 3rd and 4th hours.

# Conclusions

Based on the present findings, ethyl acetate extract of *Zingiber zerumbet* rhizomes could be considered safe up to the dose of 5000 mg/kg, and active ingredients of *Zingiber zerumbet* rhizomes may be a novel target for the treatment of fever with dengue virus infection.


## Background

Fever is the first indicator of illness or infection detected by patients or physicians. Elevation in body temperature occurs with increasing prostaglandin E2 ($PGE_2$) concentration within certain brain parts. These elevations alter the rate of neuron firing that controls thermoregulation of the hypothalamus. Increases in body temperature can be due to biological stress, such as the ovulation phase, increased thyroid secretion, excessive exercise, abrasions of the central nervous system, and microbial infections [1].

Antipyretic agents have been used to reduce the body's high temperature. Common anti-pyretic agents used globally are acetaminophen, aspirin, and other non-steroidal anti-inflammatory drugs (NSAIDs) [2].

These synthetic antipyretic agents are prone to cause adverse side effects such as gastrointestinal bleeding, dyspepsia, perforated ulcer, and renal toxicity [3]. In India and Thailand, many natural anti-pyretic agents have been used as alternative medicines to cure fever, such as *Azadirachta indica*, *Ocimum sanctum*, *Kaempferia galanga*, *Myristica fragrans*, and *Citrus aurantiifolia* [4, 5]. Today, medicinal plant-based drugs are becoming more acceptable. They are used as primary healthcare remedies, especially in rural African, Asian, and Central and South American communities [6]. These natural remedies also gained attention in other countries like China and Germany, where China aimed to integrate traditional Chinese medicine into its healthcare system in the year 2020 [7, 8].

*Zingiber zerumbet* (L.) Roscoe ex Sm. (*Z. zerumbet*), also known as *lempoyang* in Malaysia, it is one of the most used wild ginger species in traditional Malay medicine. In addition to being commonly consumed as appetizers, its rhizomes are also used to treat fever [9], stomachache [10], and worm infestation [11]. The young stems, rhizomes, and inflorescences are also used as a poultice for topical application to treat muscle sprain and swelling sores [11]. On top of that, *Z. zerumbet* has been reported to possess anti-oxidant [10], anti-inflammatory [12], anti-pyretic [13], anti-cancer [14], anti-protease activity [15, 16] and antinociceptive activities [11].

Recently, we have reported that the ethyl acetate extract of *Z. zerumbet* has potent anti-protease activity against dengue 2 NS2B/NS3 protease [16]. Since dengue infection is always accompanied by fever and *Z. zerumbet* has been reported to be used to treat fever traditionally, we have embarked on a study of anti-pyretic activity towards ethyl acetate extract of *Z. zerumbet* (ZZEA). In this communication, we shall report the analysis of acute toxicity and anti-pyretic effects of ZZEA. The presence of the major compound in ZZEA, zerumbone, was proven by its isolation, structural characterization, and HPLC and LCMS profiling studies.

## Methods

# Chemicals and reagents

Corn oil was purchased from the local market. Acetonitrile liquid chromatography grade was used in High Performance Liquid Chromatography (HPLC) and Liquid Chromatography Mass Spectrometry (LCMS) analysis. Solvents such as *n*-hexane, ethyl acetate (EA), and ethanol were distilled prior to use for extraction. All other chemicals were of analytical grade.

# Plant material

*Zingiber zerumbet* (L.) Roscoe ex Sm. (lempoyang, shampoo ginger) was purchased from the local traditional herb's supplier in Temerloh, Pahang (Kizaherbs Sdn. Bhd.) and was identified by the botanist, Mr. Teo Eng Leong, at the Department of Chemistry, Faculty of Science, Universiti Malaya, where the voucher specimens (KL5835) were deposited. This plant name was accepted in The Plant List [17].

# Sample preparation

The extract preparation was performed using the method previously described by Salleh *et al*. (2019), which involved the maceration technique of the rhizomes with ethyl acetate [16].

# Isolation and purification of the major compound

An amount of 9.0 g of ZZEA was fractionated using column chromatography over silica gel 60 (0.043–0.060 mm) which eluted with gradient system of *n*-hexane:EA:MeOH (100:0:0, v/v) to *n*-hexane:EA:MeOH (0:0:100, v/v) to afford eight fractions. Subsequent purification of second fraction (0.3324 g) using glass Preparative Thin Layer Chromatography (PTLC) silica gel 60 $F_{254}$ (20 cm × 20 cm) with *n*-hexane and EA as the eluents (90:10, v/v) resulted a pure compound; zerumbone (83.4 mg).

# Structural elucidation of the major compound

The structural elucidation of the major compound was established by various spectroscopic methods such as Infrared (IR), Nuclear Magnetic Resonance (NMR), Liquid Chromatography Mass Spectrometer – Ion Trap – Time of Flight (LCMS-IT-TOF) and by comparison with reported data [18].

# Zerumbone

White crystal needle; IR $\nu_{max}$ = 2962, 2921, 2858, 1652, 1385, 1364, 964, 827 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) = 244 nm; LCMS-IT-TOF *m/z* 219.2364 $[M + H]^+$ (calcd. for $C_{15}H_{23}O$, 218.1671); $^1H$ NMR (400 MHz, $CDCl_3$) : δ 6.02 (d, 12.0, 1H, H-6), 5.99 (d, 16.4, 1H, H-9), 5.87 (d, 16.4, 1H, H-10), 5.24 (dd, 12.0, 4.0, 1H, H-2), 2.44 (dd, 12.0, 4.0, 1H, H-5b), 2.34 (d, 12.0, overlapping, 2H, H-1b, H-4b), 2.23 (dd, 12.0, 4.0, overlapping, H-5a), 2.11 (dd, 12.0, 4.0, overlapping, 1H, H-4a), 1.91 (d, 12.0, 1H, H-1a), 1.79 (s, 3H, H-13), 1.53 (s, 3H, H-12), 1.19 (s, 3H, H-14), 1.06 (s, 3H, H-15). $^{13}C$ NMR (100 MHz, $CDCl_3$) : δ 11.8 (C-13), 15.2 (C-12), 24.2 (C-14), 24.4 (C-5), 29.4 (C-15), 37.8 (C-11), 39.4 (C-4), 42.4 (C-1), 124.9 (C-2), 127.2 (C-9), 136.2 (C-3), 137.9 (C-7), 148.8 (C-6), 160.7 (C-10), 204.4 (C-8).

# HPLC profiling of ethyl acetate extract (ZZEA)

ZZEA profile was performed with high performance liquid chromatography (HPLC) by Shimadzu equipped with Pump LC-20AT, an autosampler SIL-20A HT, DAD detector SPD-M20A and column oven CTO-10AS VP. The isocratic eluents used were acetonitrile (A) and water with 0.1% formic acid (B) with the ratio of 60 (A) to 40 (B) at a flow rate of 1.0 mL/min for 40 minutes. The column used was ZORBAX Eclipse Plus C18, 4.6 × 150 mm (3.5 µm) by Agilent. An amount of 1 µL of each sample (ZZEA, pure zerumbone, and ZZEA co-injected with pure zerumbone) was injected into the instrument.

# LCMS profiling of ethyl acetate extract (ZZEA)

The ZZEA and the pure zerumbone were subjected to liquid chromatography quadruple time of flight (Agilent 1260–6530 Infinity) attached with a 1260 DAD detector for profiling. The isocratic eluents used were acetonitrile (A) and water with 0.1% formic acid (B) in a ratio of 60 (A) to 40 (B) at a flow rate of 1.0 mL/min for 40 minutes. The column used was ZORBAX Eclipse XDB-C18, 2.1 × 100 mm (1.8 µm) by Agilent. An amount of 1 µL of the sample was injected into the instrument.

# Evaluation of the anti-pyretic effect

# Animals

Fifty-two Sprague Dawley female rats (190–230 g) were purchased from Universiti Malaya Animal Experimental Unit. The animals were housed in cages under standard laboratory conditions with free access to drinking water and a standard rat diet. A temperature of 22.0 ± 0.9°C with a relative humidity of 65 ± 5% and a 12 hours light/dark cycle was maintained. All animal procedures were carried out according to the principles outlined in the guidelines for the 'Animal Use Protocol' prepared by the Animal Care and Use Committee of the Universiti Malaya (Ethic No: 2017-180309/IBS/R/AAB).

# Acute oral toxicity

The oral acute toxicity test was performed by OECD Test Guideline 425 (tg-425) with minor modifications. A limit test of 2000 mg/kg concentration of the extract was applied as the low dosage and 5000 mg/kg concentration as the maximum dosage. Sixteen Sprague Dawley female rats have fasted for approximately 16 hours, and initial body weight was weighed before oral administration of ZZEA at doses of 2000, 3000, and 5000 mg/kg body weight. ZZEA was dissolved in corn oil, and the total volume administered for each rat was 10 mL/kg body weight. A control group was given an equivalent amount of corn oil. Animals were observed for signs of toxicity during the first 30 min, then 2 hours intervals at least 3 times on the first day, and daily for 14 days after dosing. At the end of the experiment, animals were euthanized by carbon dioxide ($CO_2$) asphyxiation and were disposed of by the UM Registered Bio-disposable contractor.

# Induction of pyrexia

Pyrexia was induced in both 15 male and 15 female rats according to the method as described previously by Hassan *et al*. (2015) with minor modifications [19]. Prior to the experiment, rectal temperatures of each animal were measured by a digital thermometer, and rats with constant temperature were selected for the study. The rats were fasted for approximately 16 hours, and pyrexia in the rats was induced by subcutaneous injection of Brewer's yeast (25% w/v) in 0.9% saline solution at a volume of 20 mL/kg of body weight. A group of 6 rats received an equal amount of 0.9% saline solution and was defined as untreated control. After 18 hours of the yeast injection, the rectal temperature of all rats was measured, and rats with a temperature rise of at least 0.9°C were selected for the anti-pyretic activity.

# Anti-pyretic activity

The method described earlier by Pingsusaen *et al*. (2015) was used for the anti-pyretic activity of ZZEA [20]. Untreated rats (3 males and 3 females) with constant rectal temperatures in the normal range (35.0–36.0°C) were defined as a control group (Group I). All rats that showed an increase in temperature of at least 0.9°C after the 18 hours of yeast injection (30 rats) were divided into 5 subgroups (Group II, III, IV, V, and VI) of 6 rats each. Group II served as a negative control. Rats in Groups I and II received corn oil at a volume of 10 ml/kg body weight. The rats in Group III served as the standard control and received ASA

dissolved in distilled water at a dose of 100 mg/kg body weight. In contrast, rats in Group IV, V, and VI were given ZZEA suspended in corn oil at doses of 125, 250, and 500 mg/kg body weight, respectively. All samples were administered orally. Rectal temperatures were recorded before yeast injection (initial), 18 hours after the injection (0 hours), and 1, 2, 3, and 4 hours after treatment. After evaluation, the animals were euthanized by $CO_2$ asphyxiation and disposed of by the registered biodisposable contractor of UM.

# Statistical analysis

The results were reported as mean ± standard deviation (M ± SD). Statistical analysis was performed using the GraphPad Prism (6) software. Changes in body temperatures within groups at different time points were assessed by analysis of variance (two-way ANOVA), followed by Tukey's multiple comparison test. Statistical significance was defined as $p < 0.05$.

## Results

# Isolation and structural elucidation of the major compound

The major compound from the ZZEA was isolated and identified as zerumbone. The zerumbone was obtained as a white crystal needle from ZZEA after several separations and fractionation processes. The structural elucidation was established through various spectroscopic methods and comparison with previously reported data [18, 21]. The infrared experiment revealed a strong absorption at 1652 $cm^{-1}$ which indicated stretching vibration of an α,β unsaturated ketone and ethylenic linkage [18]. Two strong absorption bands that appeared at 964 $cm^{-1}$ and 827 $cm^{-1}$ described the out-of-plane *trans-sym*-disubstituted ethylenic linkage and out-of-plane trisubstituted ethylenic linkage, respectively [22]. The infrared spectrum and molecular structure of zerumbone were presented in Fig. 1 and Fig. 2, respectively.

# HPLC profiling

In this study, HPLC profiling was performed for three samples: ZZEA, pure zerumbone, and ZZEA co-injected with zerumbone. The HPLC profile of ZZEA showed two major peaks at a retention time of 1.993 min and 10.219 min, while the HPLC profile of zerumbone appeared as a single peak at 10.207 min with a 97.83% peak area. This suggested that the significant peak at 10.219 min in the ZZEA profile belongs to zerumbone. To confirm the results, the ZZEA was co-injected with pure zerumbone, and the result showed the intensity increment of peak number 2 (retention time of 10.165 min) compared to the ZZEA profile. The three HPLC profiles are shown in Figs. 3A, B, and C.

# LCMS profiling

The ZZEA LCMS chromatogram is shown in Fig. 4A. A significant peak of pseudo-molecular ion, $[M + H]^+$ was observed at a retention time of 7.008 min with *m/z* 219.2355 (Fig. 4B). The LCMS profile for pure zerumbone showed a single peak at a retention time of 7.005 min (Fig. 4C), and its fragmentation pattern

(Fig. 4D) was similar to the peak at a retention time of 7.008 min of ZZEA (*m/z* 219.2364). Therefore, it can be concluded that the significant peak that appeared in ZZEA was zerumbone.

# Acute oral toxicity study

ZZEA with a dose of up to 5000 mg/kg body weight did not cause mortality or any clinical signs of toxicity in experimental rats during the 16 days of the study of acute oral toxicity. A similar absence of toxic effect was observed in the previously reported case of ethanol extract from doses of *Z. zerumbet* of 1000, 2000, or 3000 mg/kg [23]. Since the main compound in ZZEA is zerumbone, a single injected dose of zerumbone at 100–200 mg/kg was also reported not to show any ill effect on the kidney and liver tissues of female Sprague Dawley rats' kidney and liver tissues [24]. Thus, for evaluating the antipyretic effect of ZZEA in rats, the maximum safe starting dose (500 mg/kg body weight) was chosen based on (1/10th of highest dose of 5000 mg/kg). Hence, doses of 125 and 250 mg/kg body weight were selected as a comparison to the maximum dose, respectively.

# Effect of ZZEA on Brewer’s yeast induced pyrexia in rats

The anti-pyretic effect of ZZEA was evaluated using yeast-induced pyrexia in a rat model. As shown in Fig. 5, all experimental rats have normal body temperatures (36.07 ± 0.29°C), and subcutaneous injection of yeast caused a significant ($p < 0.0001$) rise in the rectal temperatures of rats after 18 hours compared with the standard control group (Group I) indicating that pyrexia model was successfully established in rats. After one hour of treatment, the ZZEA at the doses of 125 mg/kg body weight (group IV) and 250 mg/kg body weight (group V) exhibited a significant ($p < 0.05$) decrease in rectal temperature in rats, where reduction of body temperature was 1.97% and 1.99% compared with 0 hours, respectively. At this hour, ZZEA at the dose level of 500 mg/kg body weight (group VI) lowered the rectal temperature by 1.82%. In comparison, the standard drug aspirin (ASA) 100 mg/kg body weight (group III) decreased fever by only 1.29%, and both groups showed no significant ($p > 0.05$) differences compared with 0 hours in the respective groups (Fig. 5).

In the 2nd hour, ZZEA at 125 mg/kg body weight dose level maintained the significantly ($p < 0.05$) highest anti-pyretic effect with 2.23% of fever reduction compared to 0 hours, while at the doses of 250 and 500 mg/kg body weight, it displayed no significant ($p > 0.05$) decrease in pyrexia levels where reduction of body temperature was 1.35% and 0.94%, respectively. Similarly, the standard drug ASA at the dose of 100 mg/kg body weight (group III) showed no significant ($p > 0.05$) fever reduction (1.47%) at this hour (Fig. 5).

In the third hour, ZZEA at dose levels of 125, 250, and 500 mg/kg body weight (groups IV, V, and VI) showed a slight reduction in pyrexia of 1.21%, 0.32%, and 0.21% compared to 0 hours, respectively. At this time, there was no significant ($p > 0.05$) variation in the anti-pyretic effect of ZZEA at all doses treated compared with 0 hours in respective groups, and it failed to control rats’ pyrexia in the following hour (in the 4th hour). In comparison, the reference drug ASA at 100 mg/kg dose level (group III) exhibited

statistically significant ($p < 0.05$) anti-pyretic potential ($p < 0.05$) in rats at the third and fourth hour compared to 0 hours, and pyrexia levels at these times reduced by 2.30% and 1.96%, respectively (Fig. 5).

# Discussion

Fever is affiliated with various diseases and is a physiological immune response to pathogenic infections such as viruses, bacteria, and fungi that eventually cause various endogenous pyrogens [25]. One of these pyrogens, called prostaglandin $E_2$ ($PGE_2$), is the primary fever mediator in animals and humans. Yeast-induced pyrexia in animals is associated with the increased release of $PGE_2$ and is regarded as a useful model for identifying anti-pyretic agents *in vivo* [25].

In the present study, the effect of ZZEA has been assessed on yeast-induced pyrexia in rats. The yeast injection remarkably elevated the pyrexia in rats after 18 hours of administration. This study revealed that the treatment of ZZEA at the doses of 125, 250, and 500 mg/kg body weight was effective in lowering yeast-induced pyrexia in rats for up to 3 hours. On the other hand, the duration of anti-pyretic effect is reduced during the following hour (in the 4th hour).

The most potent anti-pyretic effect of ZZEA was with the lowest dose (125 mg/kg body weight) in 1st and 2nd hour and was significant ($p < 0.05$), while medium dose (250 mg/kg body weight) showed significant ($p < 0.05$) anti-pyretic potential only in the 1st hour of treatment. Inversely, the standard drug, ASA at the dose of 100 mg/kg body weight, showed no significant ($p > 0.05$) anti-pyretic effect during 1st and 2nd hours of the experiment, but it exhibited a significant ($p < 0.05$) anti-pyretic potential in the 3rd and 4th hour. Thus, the evidence of a rapid onset of action of ZZEA at lowest dose (125 mg/kg body weight) was demonstrated through a significant anti-pyretic effect as of the first two hours of treatment which is better than ASA.

However, this study revealed that the antipyretic effect of ZZEA at a higher dose (500 mg/kg body weight) was not as effective as lower (125 mg/kg body weight) and medium (250 mg/kg body weight) doses, and its potency was dose-dependently decreased. This result indicates that the malfunction of ZZEA at a higher dose is possibly due to high levels of phytochemicals that antagonize the anti-pyretic activity observed in this study [26].

Fever preventive treatment of ethanol and water extracts of rhizomes of *Z. zerumbet* was previously studied by Somchit *et al*. (2005) [13]. The extract was given 30 minutes prior to the administration of the yeast to induce fever, which is contrary to our current treatment procedure, where pyrexia in rats was confirmed after 18 hours of yeast administration prior to the treatment with the *Z. zerumbet* extracts. Our results showed that treatment with *Z. zerumbet* exhibited significant antipyretic properties compared to that of the control group. Hence, this suggests that *Z. zerumbet* can both prevent and reduce fever.

The fever up-regulated body temperature and pain are two significant signs of the body against inflammation [27]. Usually, an anti-inflammatory drug will also exhibit anti-pyretic and analgesic properties [28]. ASA, the non-steroidal anti-inflammatory drug (NSAID), which also possesses anti-pyretic

and analgesic properties, was chosen as the positive control in this study. The mechanisms of action of ASA that are responsible for all those effects are believed to be involved with both central and peripheral mechanisms [2]. Previous studies have reported that *Z. zerumbet* extract has anti-inflammatory and analgesic properties [12, 13]. Therefore, we suggest that the anti-inflammatory effect could be involved in the mechanism of the anti-pyretic activity.

As reported by many researchers, the main constituent of *Z. zerumbet* is zerumbone [18, 29]. The current study agreed with previous findings, although it was carried out with ethyl acetate extract. The HPLC and LCMS profiles of the ZZEA showed the presence of zerumbone, which eluted at 7.008 min (Fig. 4A). The chemical structure of zerumbone was elucidated and confirmed by nuclear magnetic resonance, infrared, and mass spectrometry analyses, which was in agreement with previous studies [18, 21, 22].

Somchit *et al*. (2012) had demonstrated zerumbone has inhibitory activities on $PGE_2$ and λ–Carrageenan, which induced inflammation [30]. Thus, the anti-pyretic activity observed in this investigation might be in part due to the major presence of zerumbone in ZZEA.

Our finding indicates that the anti-pyretic effect of ZZEA at a dosage of 125 mg/kg is significant and may be used as supplementary for the treatment of pyrexia. In addition, our previous study has shown that the ZZEA exhibited potent anti-protease activity at $IC_{50}$ value 2.39 ± 0.86 µg/mL [16]. Since dengue infection is always associated with fever, therefore, this result may suggest that *Z. zerumbet* could be a potential candidate to be developed as a treatment for dengue fever.

# Conclusions

In summary, the rapid onset of action of ZZEA at lowest dose was demonstrated through a significant reduction of yeast-induced pyrexia in rats, suggesting that zerumbone as a major ingredients of *Z. zerumbet* rhizome could potentially be used as a fever prevention and treatment.

# Abbreviations

| | |
|---|---|
| ASA | aspirin |
| $CO_2$ | carbon dioxide |
| HPLC | High Performance Liquid Chromatography |
| IR | infrared |
| LCMS | Liquid Chromatography Mass Spectrometry |
| MeOH | Methanol |
| NMR | nuclear magnetic resonance |

| | |
|---|---|
| NSAIDs | non-steroidal anti-inflammatory drugs |
| $PGE_2$ | prostaglandin $E_2$ |
| SD | standard deviation |
| ZZEA | ethyl acetate extract of *Zingiber zerumbet* |

# Declarations


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### Authors' contributions

HMS and KA participated in research design and interpretation data. HMS, SYL, SLC and AA conducted the experimental works, performed the statistical analysis and drafted the manuscript. NT, NF, HAW, MAO, SYL and KA participated in edited and revised the manuscript. All authors read and approved the final manuscript.


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### Availability of data and materials

The supporting materials can be obtained upon request via email to the corresponding author.

### Ethics approval and consent to participate

Animals' ethic for this study was approved by Faculty of Medicine Institutional Animal Care and Use Committee, Universiti Malaya with the reference number 2017-180309/IBS/R/AAB. This study was reported in accordance with ARRIVE guidelines and all the methods involving the plant and its material complied with relevant institutional, national, and international guidelines and legislation.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Author information

### Affiliations

*Department of Chemistry, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*

Haslinda Mohd Salleh, Abdulwali Ablat, Soon-Lim Chong, Norsita Tohar, Muhamad Aqmal Othman & Khalijah Awang

*Centre for Natural Products Research and Drug Discovery (CENAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*

Hazrina Hazni, Muhamad Aqmal Othman, Sook Yee Liew & Khalijah Awang

*Faculty of Quranic and Sunnah Studies, Universiti Sains Islam Malaysia, 71800 Nilai, Negeri Sembilan, Malaysia.*

Nurulwahidah Fauzi

*School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.*

Habibah A. Wahab

*Chemistry Division, Centre for Foundation Studies in Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.*

Sook Yee Liew

# References

1. Shah M, Parveen Z, Khan MR. Evaluation of antioxidant, anti-inflammatory, analgesic and antipyretic activities of the stem bark of *Sapindus mukorossi*. BMC Complement Altern Med. 2017;17(1):526.
2. Aronoff DM, Neilson EG. Antipyretics: mechanisms of action and clinical use in fever suppression. Am J Med. 2001;111(4):304-315.
3. Greisman L, Mackowiak P. Fever: Beneficial and detrimental effects of antipyretics. Curr Opin Infect Dis. 2002;15:241-245.
4. Prommee N, Itharat A, Panthong S, Makchuchit S, Ooraikul B. Ethnopharmacological analysis from Thai traditional medicine called Prasachandaeng remedy as a potential antipyretic drug. J Ethnopharmacol. 2020:113520.
5. Afiffudden SKN, Alwi H, K.H. Ku Hamid. Potential antipyretic agents from plants: An Overview. Adv Mat Res. 2015;1113:382-387.

6. World Health O. WHO traditional medicine strategy: 2014-2023. Geneva: World Health Organization; 2013;2013.
7. Joos S, Glassen K, Musselmann B. Herbal medicine in primary healthcare in Germany: The patient's perspective. Evid Based Complement Alternat Med. 2012;2012:294638.
8. Wang W-Y, Zhou H, Wang Y-F, Sang B-S, Liu L. Current Policies and Measures on the Development of Traditional Chinese Medicine in China. Pharmacol Res. 2021;163:105187.
9. Hamid A, Ibrahim FW, Ming TH, Nasrom MN, Eusoff N, Husain K, et al. *Zingiber zerumbet* L. (Smith) extract alleviates the ethanol-induced brain damage via its antioxidant activity. BMC Complement Altern Med. 2018;18(1):101.
10. Ruslay S, Abas F, Shaari K, Zainal Z, Maulidiani, Sirat H, et al. Characterization of the components present in the active fractions of health gingers (*Curcuma xanthorrhiza* and *Zingiber zerumbet*) by HPLC–DAD–ESIMS. Food Chem. 2007;104(3):1183-1191.
11. Khalid MH, Akhtar MN, Mohamad AS, Perimal EK, Akira A, Israf DA, et al. Antinociceptive effect of the essential oil of *Zingiber zerumbet* in mice: Possible mechanisms. J Ethnopharmacol. 2011;137(1):345-351.
12. Somchit M, Mohamad Hazir NS. Anti inflammatory property of ethanol and water extracts of *Zingiber zerumbet*. Indian J Pharmacol. 2003;35:181-182.
13. Somchit M, Shukriyah M, Bustamam A, Zuraini A. Anti-pyretic and analgesic activity of *Zingiber zerumbet*. Int J Pharmacol. 2005;1(3):277-280.
14. Sakinah S, Handayani ST, Hawariah L. Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. Cancer Cell Int. 2007;7(4):1-11.
15. Tan SK, Pippen R, Yusof R, Rahman NA, Ibrahim H, Khalid N. Screening of selected Zingiberaceae extracts for dengue-2 virus protease inhibitory activities. Sunway Academic Journal. 2006;3:1-7.
16. Salleh HM, Chong S-L, Othman R, Hazni H, Ahmad K, Mohd Yusof MYZ, et al. Dengue protease inhibition activity of selected Malaysian medicinal herbs. Trop Biomed. 2019;36(2):357-66.
17. The Plant List [Internet]. The Plant List. 2013 [cited 1 Feb 2021]. Available from: http://www.theplantlist.org/.
18. Baby S, Dan M, Thaha ARM, Johnson AJ, Kurup R, Balakrishnapillai P, et al. High content of zerumbone in volatile oils of *Zingiber zerumbet* from southern India and Malaysia. Flavour Fragr J. 2009;24(6):301-308.
19. Hassan FI, Zezi AU, Yaro AH, Danmalam UH. Analgesic, anti-inflammatory and antipyretic activities of the methanol leaf extract of *Dalbergia saxatilis* Hook.F in rats and mice. J Ethnopharmacol. 2015;166:74-78.
20. Pingsusaen P, Kunanusorn P, Khonsung P, Chiranthanut N, Panthong A, Rujjanawate C. Investigation of anti-inflammatory, antinociceptive and antipyretic activities of *Stahlianthus involucratus* rhizome ethanol extract. J Ethnopharmacol. 2015;162:199-206.

21. Rana VS, Ahluwalia V, Shakil NA, Prasad L. Essential oil composition, antifungal, and seedling growth inhibitory effects of zerumbone from *Zingiber zerumbet* Smith. J Essent Oil Res. 2016:Ahead of Print.
22. Oliveros MB, Cantoria MC. Pharmacognostic studies on *Zingiber zerumbet* (Linné) Smith and its proposed variety (family Zingiberaceae). Int J Crude Drug Res. 1982;20(3):141-153.
23. Chang CJ, Tzeng T-F, Liou S-S, Chang Y-S, Liu I-M. Acute and 28-day subchronic oral toxicity of an ethanol extract of *Zingiber zerumbet* (L.) Smith in rodents. Evid Based Complement Alternat Med. 2012;2012:11.
24. Ibrahim MY, Abdul AB, Ibrahim TAT, Abdelwahab SI, Elhassan MM, Syam M. Evaluation of acute toxicity and the effect of single injected doses of zerumbone on the kidney and liver functions in Sprague Dawley rats. Afr J Biotechnol. 2010;9(28):4442-4450.
25. Muhammad A, Khan B, Iqbal Z, Khan AZ, Khan I, Khan K, et al. Viscosine as a Potent and Safe Antipyretic Agent Evaluated by Yeast-Induced Pyrexia Model and Molecular Docking Studies. ACS Omega. 2019;4(10):14188-14192.
26. Minkah PAB, Danquah CA. Anti-infective, anti-inflammatory and antipyretic activities of the bulb extracts of Crinum jagus (J. Thomps.) Dandy (Amaryllidaceae). Sci Afr. 2021;12:e00723.
27. Meli R, Antonelli E, Cirino G. Analgesia and cyclo-oxygenase inhibitors. Dig Liver Dis. 2001;33(Suppl 2):S8-11.
28. Perianayagam JB, Sharma SK, Joseph A, Christina AJ. Evaluation of anti-pyretic and analgesic activity of *Emblica officinalis* Gaertn. J Ethnopharmacol. 2004;95(1):83-85.
29. Singh CB, Nongalleima K, Brojendrosingh S, Ningombam S, Lokendrajit N, Singh LW. Biological and chemical properties of Zingiber zerumbet Smith: a review. Phytochem Rev. 2012;11(1):113-125.
30. Somchit MN, Mak JH, Ahmad Bustamam A, Zuraini A, Arifah AK, Adam Y, et al. Zerumbone isolated from *Zingiber zerumbet* inhibits inflammation and pain in rats. J Med Plants Res. 2012;6(2):177-180.

# Figures

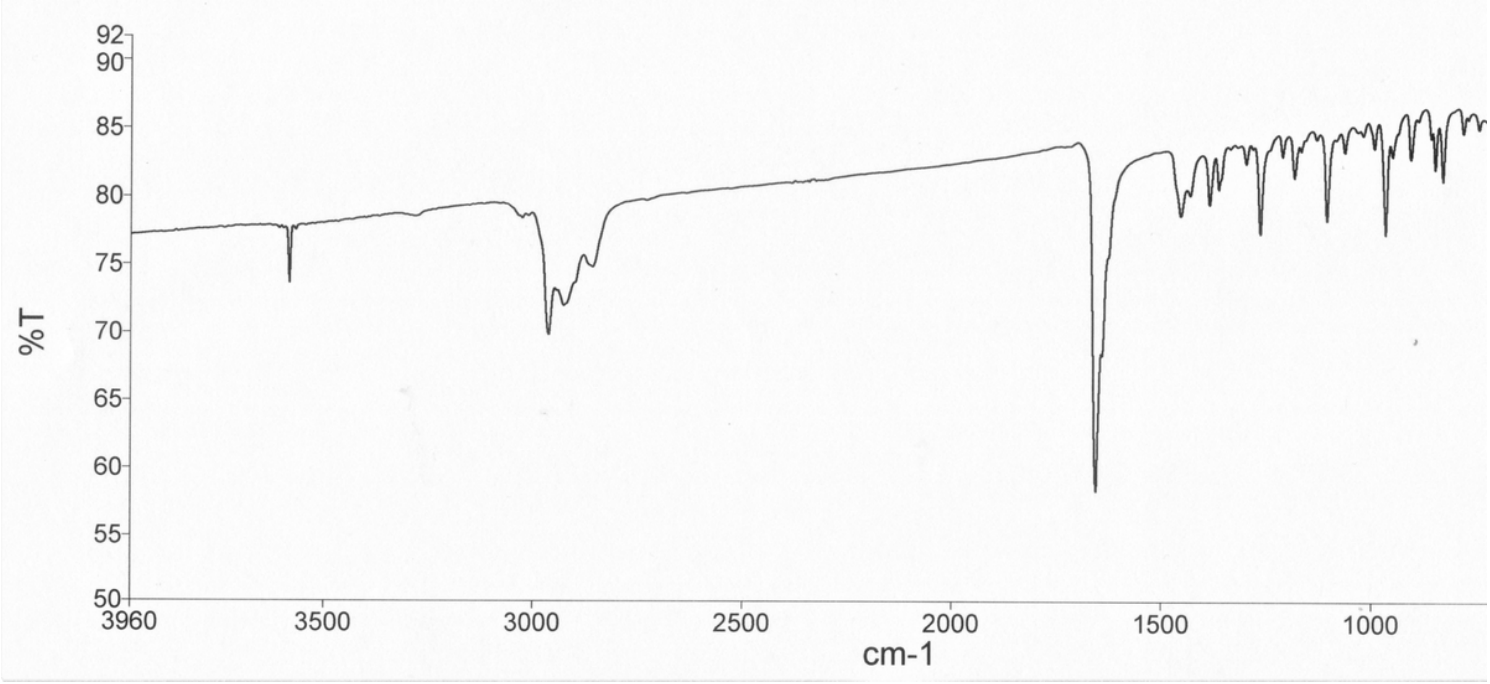



**Figure 1**

Infrared spectrum of zerumbone

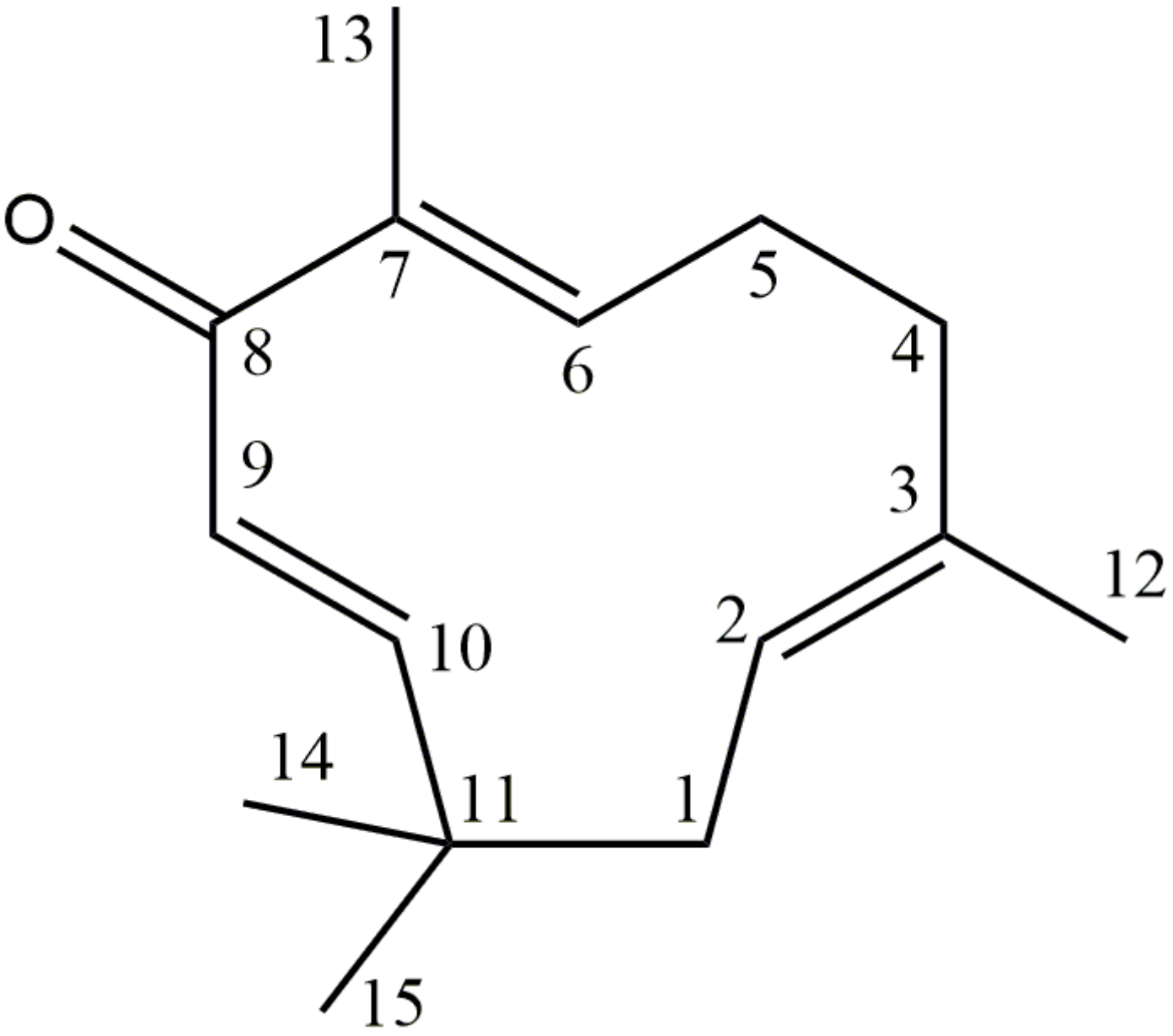
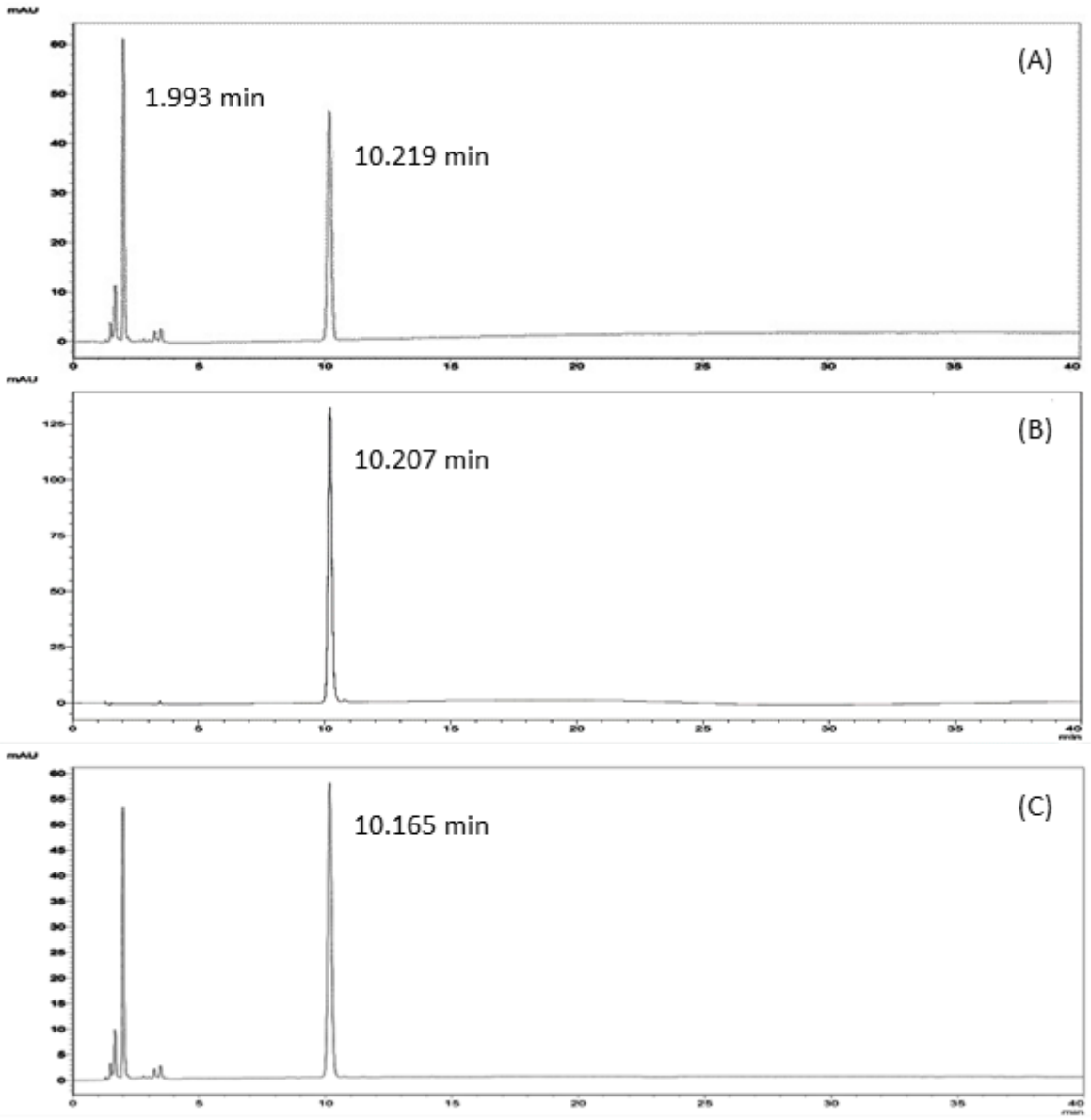


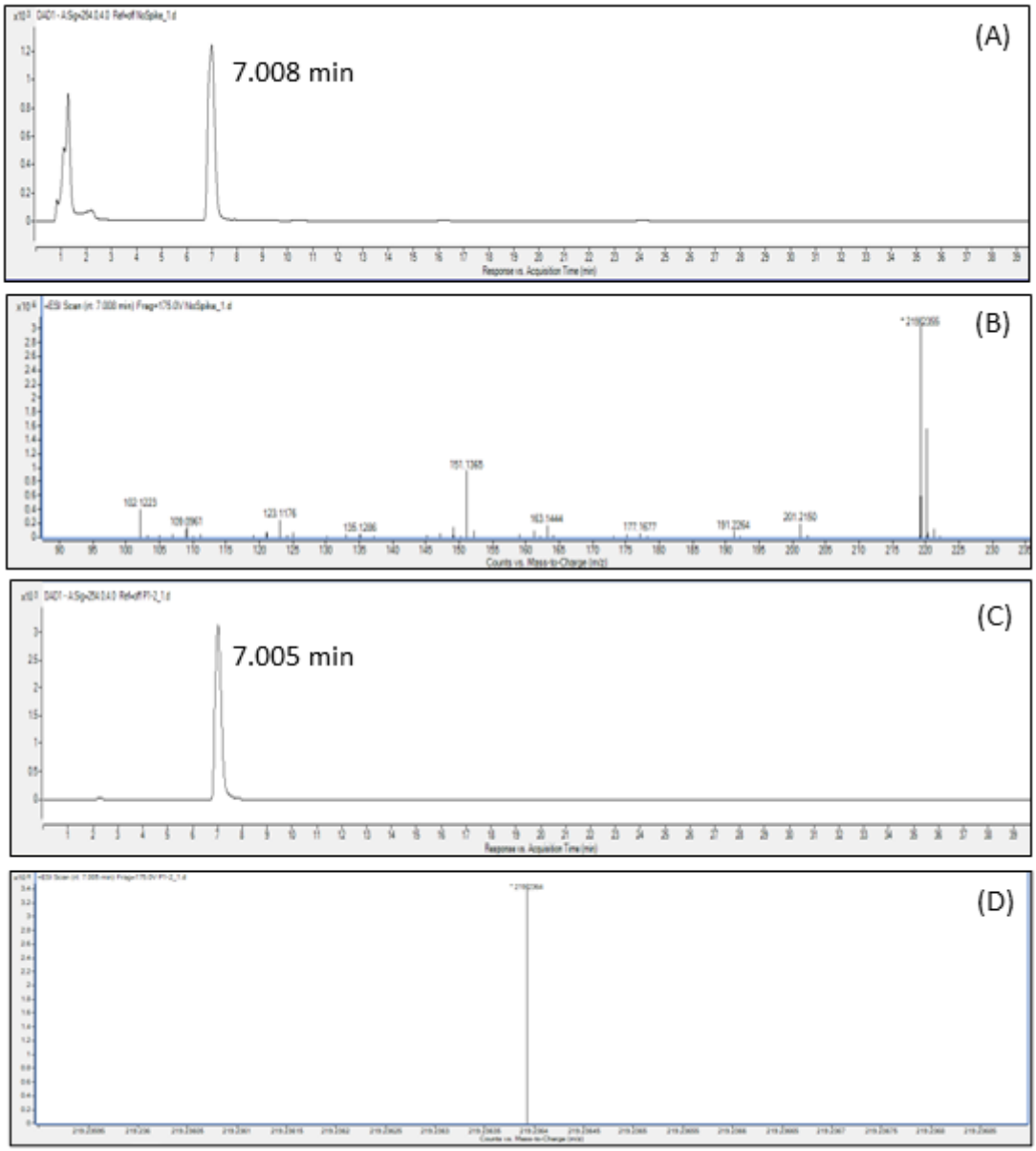

**Figure 2**

Structure of zerumbone.



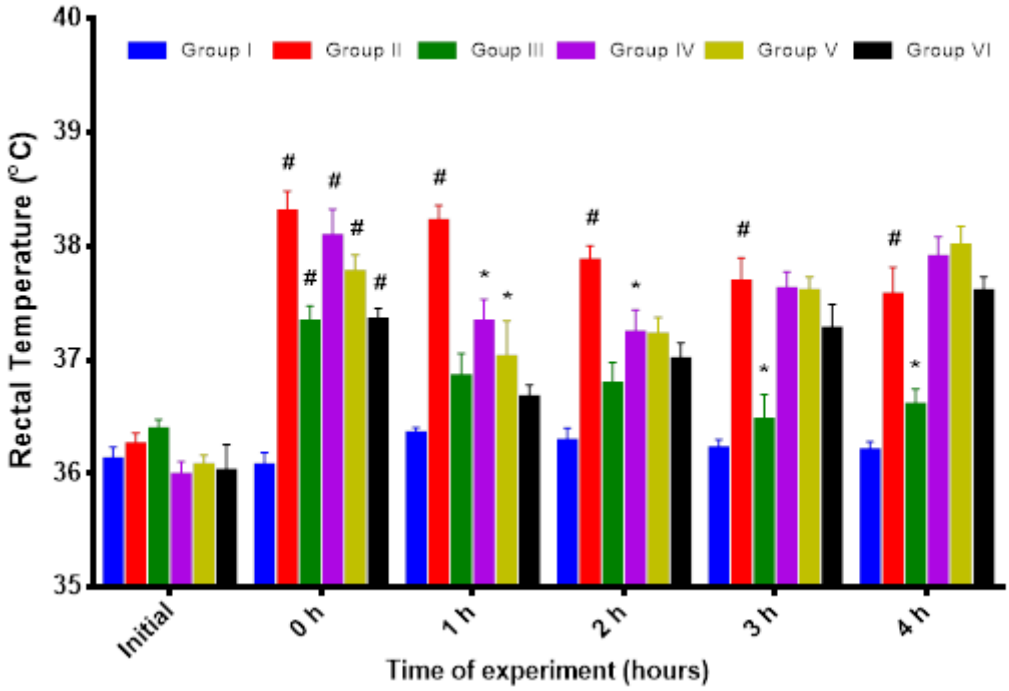

## Figure 3

HPLC-DAD chromatograms of (A) ZZEA, (B) pure zerumbone and (C) ZZEA spiked with zerumbone.




## Figure 4

LCMS profile and MS data of ZZEA and zerumbone at 254 nm. (A) LCMS profile of ZZEA, (B) Mass spectrum of pseudomolecular ion peak $[M+H]^+$ at 7.008 min (C) LCMS profile of zerumbone, (D) mass spectrum of zerumbone at 7.005 min.




## Figure 5

Anti-pyretic effect of ethyl acetate extract of *Zingiber Zerumbet* (ZZEA) in yeast-induced pyrexia. Group I: normal control, Group II: Yeast, Group III: yeast + aspirin (ASA) 100 mg/kg body weight, Group IV: yeast + ZZEA 125 mg/kg body weight, Group V: yeast + ZZEA 250 mg/kg body weight, Group VI: yeast + ZZEA 500 mg/kg body weight. $^{\#}p<0.0001$ compared to normal control within the group, $^{*}p<0.05$ compared to 0 hour within the group.