Mosquito Larvicidal Activity of the Essential Oil of Zingiber collinsii against Aedes albopictus and Culex quinquefasciatus

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Abstract: The chemical composition and larvicidal activity of essential oils from the leaves and rhizomes of Zingiber collinsii Mood & Theilade (Zingiberaceae) were reported. The main compounds in the leaf oil were α-pinene (25.6%), β-caryophyllene (16.8%), β-pinene (16.1%) and bicyclogermacrene (6.9%) while the rhizome oil consist mainly of camphene (22.5%), β-pinene (16.3%), α-pinene (9.0%) and humulene oxide II (9.0%). The rhizome oil demonstrated larvicidal effects towards fourth instant larvae of mosquito vectors. The highest mortality (100%) was observed at 24 h exposure against Aedes albopictus (concentration 100 μg/mL) and 48 h (concentration of 50 and 100 μg/mL), while the highest mortality (100%) was observed for Culex quinquefasciatus at 24 h and 48 h at concentration of 100 μg/mL. The 24 h mosquito larvicidal activity of the rhizome oil against Aedes albopictus were LC50 = 20.03 μg/mL; LC90 = 40.22 μg/mL and towards Cx. quinquefasciatus with LC50 = 50.11 μg/mL and LC90 = 71.53 μg/mL. However, the 48 h larvicidal activity were LC50 = 25.51 μg/mL and LC90 = 40.22 μg/mL (Ae. albopictus), as well as LC50 = 36.18 μg/mL and LC90 = 55.11 μg/mL (Cx. quinquefasciatus). On the other hand, no appreciable mortality and larvicidal activity was observed for the leaf oil. The larvicidal activity of the essential oils of Z. collinsii was being reported for the first time.

Key words: Zingiber collinsii, essential oil composition, monoterpenes, sesquiterpenes, larvicidal activity

1 Introduction

Vietnam is classified as a hyperendemic dengue country with present throughout the year and dengue fever epidemics have increased in frequency³,⁴. Mosquitoes have been and continue to be the most deadly creatures on earth. Culex quinquefasciatus Say (Diptera: Culicidae), the southern house mosquito, is a vector of lymphatic filariasis and other arboviruses including West Nile virus, St. Louis encephalitis, chikungunya viruses, filarial nematodes⁸. The Asian tiger mosquito, Aedes albopictus (Skuse) (Diptera Culicidae) is ranked among the most invasive mosquito species in the world⁴.⁵. Besides its aggressive daytime biting behaviour, the medical importance of Aedes albopictus is due to its ability to transmit many human pathogens and parasites (e.g. yellow fever, dengue fever, West Nile, Japanese encephalitis, St. Louis encephalitis, chikungunya viruses, filarial nematodes). The control of adult mosquitoes commonly relies on the use of synthetic insecticides and repellents, but treatments with such chemicals are expensive, show scarce efficacy and have a strong environmental impact associated to relevant human health risks⁶,⁷. For these reasons, alternative natural insecticides and repellents are now very appreciated by consumers. Essential oils of aromatic plants are con-

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Accepted December 7, 2019 (received for review June 30, 2019)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jocs
sidered among the most promising alternative to synthetic chemicals\(^8\). Essential oils are generally recognized as environmental friendly, easily biodegradable, minimally toxic to mammals and have shown repellent activities against different mosquito species.

*Zingiber* Miller (*Zingiberaceae*) is distributed in tropical to warm-temperate Asia with the highest diversity in the monsoonal parts of Asia. It is considered the largest genus in subfamily Zingiberoidae with more than 200 names corresponding to approximately 100-150 species\(^9\). *Zingiber collinsii* Mood & Theilade is a newly described species in the genus. The plant is known to repel insect pests. A chemical investigation of the rhizomes of *Z. collinsii* resulted in the isolation and identification of zerumbone, acoumarin (scopoletin), quercetin, rutin, 5- (hydroxymethyl) furfural, bisdemethoxycurcumin and demethoxycurcumin\(^{10}\). The monoterpene hydrocarbons, α-pinene (50.2%), β-pinene (23.6%) and limonene (5.3%) were reported previously as the main constituents of the rhizome oil\(^11\). Till moment the authors are not aware of any report describing the biological activity of *Z. collinsii* essential oils.

Many essential oils from *Zingiber* plants have been used as insecticides for controlling many insect pests including mosquito vectors. The essential oils of *Z. cassumunar* displayed a high larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*\(^{12,13}\), while *Z. ottenssii* and *Z. zerumbet* have also displayed similar actions\(^14\). The oils of *Z. officinale* exhibited toxicity towards *Ae. albopictus* and *Cx. quinquefasciatus*\(^15\). The larvicidal action of several *Zingiber* essential oils against mosquito vectors of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* have been reported\(^16\). However, such report and activity was never reported for the essential oil of *Z. collinsii*.

In this paper we report the volatile components of the leaves and rhizome oils, as well as the larvicidal action of the rhizome oil against the fourth-instant larvae of vector mosquitoes, *Ae. albopictus* and *Cx. quinquefasciatus*. This is in continuation of our extensive research aimed at identifying the chemical composition and sourcing for potential larvicides from natural sources\(^17,18\).

### 2 Experimental Procedures

#### 2.1 Plant collection

The leaves and rhizome of *Z. collinsii* were collected from Thượng Nhật commune, Nam Động District, Thừa Thiên Huế Province (GPS 16° 20’ N 107° 35’ E), Vietnam in September 2018. Voucher specimens THH 748 was deposited at the Botany Muse um, Vinh University, Vietnam.

#### 2.2 Hydrodistillation of the essential oils

Two kilogram of each of the air-dried leaves and rhizomes of *Z. collinsii* was used for the experiment at different time. The samples were carefully and separately introduced into the flask, after which distilled water was added to cover the surface of the sample. Essential oils were obtained by hydrodistillation for 3 h at normal pressure, according to the Vietnamese Pharmacopoeia\(^19\) conducted in Clevenger-type apparatus. The distilled oils were recovered into previous weighed sample bottle through the receiver arm of the distillation unit. The oils were kept under refrigeration till moment of analysis. All the oil samples were light yellow coloured.

#### 2.3 Analysis of the essential oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas He (1 mL/min), injector temperature (PTV) 250°C, detector temperature 260°C, column temperature programmed from 60°C (2 min hold) to 220°C (10 min hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μL. Inlet pressure was 6.1 kPa. The relative amounts of individual components were calculated based on the GC peak area (FID response), as previously described\(^17,18\).

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the gas chromatography-mass spectrometry (GC/MS) analysis. The same conditions described above was also used for GC. The mass spectrum operating conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-550 amu at a sampling rate of 1.0 scan/s.

#### 2.4 Identification of the constituents

The identification of constituents was performed on the basis of retention indices (RI) determined with reference to a homologous series of n-alkanes (–C<sub>n</sub>-C<sub>40</sub>), under identical experimental conditions, co-injection with standards or known essential oil constituents, and by comparing with MS literature data as described previously\(^17,18\).

#### 2.5 Larvicidal activity

##### 2.5.1 Mosquito larvae

Adults of *Culex quinquefasciatus* and *Aedes albopictus* collected in Hoa Khanh Nam ward, Lien Chieu district, Da Nang city (16° 03’ 14.9” N, 108° 09’ 31.2” E). Adult mosquitoes were maintained in entomological cages (40 × 40 × 40 cm) and fed a 10% sucrose solution and were allowed to blood feed on mice. Eggs hatching were induced with tap water. Larvae were reared in plastic trays (24 × 35 × 5 cm).
The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. All stages were held at 25 ± 2°C, 65-75% relative humidity, and a 12:12 h light: dark cycle at the Center for Entomology and Parasitology Research, Duy Tan University, Vietnam.

2.5.2 Larvicidal test

The larvicidal activity bioassay was performed according to the method proposed by the World Health Organization with slight modifications. For the assay, aliquots of the essential oil of *Z. collinsii* dissolved in EtOH (1% stock solution) was placed in a 200-mL beaker and added to water that contained 20 larvae (fourth instar). With each experiment, a set of controls using EtOH was also run for comparison. Mortality was recorded after 24 h and again after 48 h of exposure during which no nutritional supplement was added. The experiments were conducted with four replicates with concentrations (100, 50, 25 and 12.5 µg/mL).

The mortality rate was calculated according to the formula:

\[ \text{Mc} = \frac{(\text{Mo-Mt})}{(100-\text{Mt})} \times 100 \]

Mo = mortality in the treated groups, Mt = mortality in the control group and Mc = calculated mortality.

2.5.3 Statistic analysis

The data obtained were subjected to log-probit analysis to obtain Median lethal concentrations namely LC₅₀ values, LC₉₀ values, and 95% confidence limits using XLSTAT v. 2018.5 (Addinsoft, Paris, France).

3 Results

3.1 Chemical constituents

The yields of essential oils were 0.22% and 0.35% (v/w) respectively, for the leaf and rhizome, calculated on a dry weight basis. The samples of oils obtained from the hydrodistillation were light yellow coloured. Thirty-five compounds representing 98.1% of the oil contents were identified in the leaf of *Z. collinsii* (Table 1). Also, monoterpenic hydrocarbons (49.0%) and sesquiterpenic hydrocarbons (36.3%) represent the main classes of compounds present in the oil. The major constituents of the leaf oil were mostly terpene hydrocarbons represented by α-pinene (25.6%), β-caryophyllene (16.8%) and β-pinene (16.1%). On the other hand, 51 constituents accounting for 97.7% of the total oil content were identified in the rhizome oil. The representative classes of compounds present in the oil were monoterpenic hydrocarbons (58.3%), oxygenated sesquiterpenes (21.1%) and oxygenated monoterpenes (10.9%). The significant compounds of the rhizome oil were camphene (22.5%), β-pinene (16.3%), α-pinene (9.0%) and humulene oxide II (9.0%).

3.2 Larvicidal test

The 24 h and 48 h mosquito larvicidal activities of the essential oil were determined against the mosquito vectors at concentrations of 12.5, 25, 50 and 100 µg/mL. The percentage mortality as well as the minimum lethal concentration is shown in Table 2. Assessment of larvicidal efficacy demonstrated good larvicide effects by the rhizome oil towards mosquito vectors. The highest mortality (100%) was observed at 24 h exposure for *Ae. albopictus* (concentration 100 µg/mL) and 48 h (concentration of 50 and 100 µg/mL), while the highest mortality (100%) was observed for *Cx. quinquefasciatus* at 24 h and 48 h at concentration of 100 µg/mL. There was no mortality in the EtOH controls. The 24 h mosquito larvicidal activities of the rhizome oil were *Ae. albopictus* (LC₅₀ = 25.51 µg/mL, LC₉₀ = 40.22 µg/mL) and *Cx. quinquefasciatus* (LC₅₀ = 50.11 µg/mL and LC₉₀ = 71.53 µg/mL). However, the 48 h mosquito larvicidal activities were LC₅₀ = 20.03 µg/mL and LC₉₀ = 24.51 µg/mL (*Ae. albopictus*), as well as LC₅₀ = 36.18 µg/mL and LC₉₀ = 55.11 µg/mL (*Cx. quinquefasciatus*). Moreover, no significant mortality and larvicidal activity was observed for the leaf oil. Permethrin, the standard drug used as control displayed larvicidal activity against *Cx. quinquefasciatus* with LC₅₀ of 2.19 µg/mL and 1.91 µg/mL at 24 h and 48 h respectively. The LC₅₀ values of 1.60 µg/mL and 1.12 µg/mL were obtained against *Ae. albopictus* at 24 h and 48 h respectively.

4 Discussion

The constituents of essential oils of *Z. collinsii* leaf are being reported for the first time. The abundance of α-pinene and β-pinene in the rhizome oil was in agreement with a previous report on the same part. As usual, ubiquitous terpenoids were identified in both essential oils, consistent with most data obtained for the essential of *Zingiber* genus analysed from Vietnam and other parts of the world. However, the identities of these terpene compounds may vary from one species to another depending on several factors such as environmental and climatic conditions, age and nature of the plants, parts of the plant being analysed, handling procedure etc. For example, recently, β-pinene and β-caryophyllene were identified as the main constituents in the leaf oil and stem oil of *Z. vuquangensis*, while the root oil contained bornyl acetate, zerumbone and α-humulene, with β-pinene, 1,8-cineole, α-pinene and β-caryophyllene making up the significant compounds of the fruit oil. The same authors reported that the leaf oil *Z. castaneum* was dominated by β-pinene, α-pinene, β-caryophyllene and bicycloelemene, while identifying larger amounts of β-caryophyllene, δ-cadinene and bicycloelemene in the stem. However, while camphene, 1,8-cineole and linalool constituted the bulk of the root oil,
Table 1  Constituents of essential oils from *Z. collinsii*.

| S/N | Compound | RI<sup>a</sup> | RI<sup>b</sup> | Percent composition |
|-----|----------|----------------|----------------|---------------------|
|     |          | Leaf | Rhizome | Leaf | Rhizome |
| 1   | Tricyclene | 928  | 921   | -    | 0.8    |
| 2   | α-Thujene  | 930  | 921   | -    | 0.1    |
| 3   | α-Pinene   | 939  | 932   | 25.6 | 9.0    |
| 4   | Camphene   | 956  | 946   | 1.7  | 22.5   |
| 5   | Sabinene   | 976  | 956   | 0.6  | 0.5    |
| 6   | β-Pinene   | 984  | 978   | 16.1 | 16.3   |
| 7   | β-Myrcene  | 993  | 988   | 1.2  | 0.9    |
| 8   | α-Phellandrene | 1010 | 1004 | -    | 0.5    |
| 9   | δ-3-Carene | 1016 | 1008  | -    | 0.8    |
| 10  | Limonene   | 1035 | 1030  | 1.2  | 4.1    |
| 11  | β-Phellandrene | 1036 | 1032 | 0.3  | 2.5    |
| 12  | 1,8-Cineole | 1038 | 1034  | -    | 0.6    |
| 13  | γ-Terpinene | 1064 | 1056  | -    | 0.1    |
| 14  | Terpinolene | 1095 | 1098  | 0.3  | 0.3    |
| 15  | Linalool   | 1104 | 1100  | 1.0  | 1.7    |
| 16  | Perillene  | 1105 | 1104  | 2.0  | -      |
| 17  | n-Nonanol  | 1106 | 1110  | 1.2  | -      |
| 18  | Camphor    | 1155 | 1148  | -    | 0.4    |
| 19  | Pinocarvone| 1173 | 1170  | -    | 0.1    |
| 20  | Borneol    | 1178 | 1180  | -    | 1.8    |
| 21  | Terpinen-4-ol | 1187 | 1189  | -    | 0.2    |
| 22  | Cryptone   | 1197 | 1193  | -    | 0.2    |
| 23  | Myrtenal   | 1208 | 1210  | -    | 0.2    |
| 24  | Neral      | 1247 | 1248  | 0.3  | -      |
| 25  | Cumaldehyde| 1251 | 1254  | -    | 0.3    |
| 26  | Geranial   | 1275 | 1278  | 0.5  | -      |
| 27  | Bornyl acetate | 1294 | 1293  | -    | 5.2    |
| 28  | Isobornyl acetate | 1297 | 1297  | -    | 0.2    |
| 29  | 2-Undecanol| 1304 | 1300  | 0.5  | -      |
| 30  | cis-β-Elemene | 1404 | 1404  | 4.9  | 0.5    |
| 31  | β-Caryophyllene | 1419 | 1417  | 16.8 | 2.3    |
| 32  | β-Gurjunene| 1436 | 1434  | -    | 0.1    |
| 33  | Aromadendrene| 1455 | 1452  | 0.6  | -      |
| 34  | α-Humulene | 1457 | 1454  | 2.0  | 1.3    |
| 35  | 9-epi-(E)-caryophyllene | 1479 | 1476  | 0.7  | 0.3    |
| 36  | β-Chamigrene| 1480 | 1482  | 0.5  | 0.2    |
| 37  | Germacrene D | 1485 | 1484  | 0.6  | 0.1    |
| 38  | Aristolochene| 1502 | 1502  | 0.4  | -      |
| 39  | β-Selinene | 1505 | 1505  | 1.6  | 0.3    |
| 40  | (E, E)-α-Farnesene | 1513 | 1508  | -    | 0.7    |
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Table 1  Continued.

| S/N | Compounda | RIb | RIC | Percent composition |
|-----|-----------|-----|-----|---------------------|
|     |           | Leaf | Rhizome |
| 41  | Bicyclogermacrene | 1514 | 1510 | 6.9 | 1.4 |
| 42  | β-Bisabolene | 1518 | 1512 | 0.9 | – |
| 43  | δ-Cadinene | 1537 | 1535 | 0.5 | 0.2 |
| 44  | Elemol | 1565 | 1561 | – | 0.1 |
| 45  | (E)-Nerolidol | 1571 | 1568 | 0.5 | – |
| 46  | Germacrene D-4-ol | 1595 | 1594 | – | 0.7 |
| 47  | Spathulenol | 1599 | 1590 | 1.1 | 1.0 |
| 48  | Caryophyllene oxide | 1605 | 1598 | 1.7 | 3.2 |
| 49  | Humulene oxide I | 1621 | 1618 | – | 0.6 |
| 50  | 10-epi-γ-Eudesmol | 1643 | 1644 | – | 0.2 |
| 51  | Humulene oxide II | 1653 | 1650 | – | 9.0 |
| 52  | α-Muurolol | 1662 | 1660 | – | 0.8 |
| 53  | δ-cadinol | 1665 | 1664 | – | 0.1 |
| 54  | α-Eudesmol | 1674 | 1672 | 0.5 | 2.4 |
| 55  | ar-Turmerone | 1678 | 1676 | 1.6 | 0.2 |
| 56  | neo-Intermedeol | 1680 | 1678 | – | 2.1 |
| 57  | Intermedeol | 1684 | 1685 | – | 0.2 |
| 58  | 14-Hydroxy-9-epi-(E)-Caryophyllene | 1691 | 1690 | – | 0.2 |
| 59  | Curlone | 1716 | 1720 | 0.6 | 0.1 |
| 60  | Z-Lanceol | 1794 | 1790 | 0.9 | 0.1 |
| 61  | 6,10,14-Trimethylpentadecan-2-one | 1848 | 1846 | 0.7 | – |
| 62  | Phytol | 2125 | 2124 | 1.6 | – |

Total 98.1 97.7

Monoterpene hydrocarbons (Sr. No. 1-11, 13, 14, 16) 49 58.3

Oxygenated monoterpenes (Sr. No. 12, 15, 18-28) 1.8 10.9

Sesquiterpene hydrocarbons (Sr. No. 30-43) 36.3 7.4

Oxygenated sesquiterpenes (Sr. No. 44-61) 7.6 21.1

Diterpenes (Sr. No. 62) 1.6 –

Non-terpenes (Sr. No. 17, 29) 1.7 –

a Elution order on HP-5MS column; b Retention indices on HP-5MS column; c Literature retention indices; Sr. No, Serial Number; - Not identified.

(E)-nerolidol, (Z)-9-octadecenamide and β-caryophyllene were the main constituents in the fruit oil. The main constituents of the leaves oil of Z. nudicarpum were α-cedrol (14.8%), β-eudesmol (13.8%), β-pinene (11.7%), while β-pinene (27.6%), α-pinene (8.5%) were obtained in the root with (E)-nerolidol (30.0%), β-caryophyllene (9.4%) and sabinene (8.5%) making up the composition of the fruit oil.

The larvicidal activity was found to be concentration dependent. Thus, total and complete inhibition of mosquito larvae was observed in the 100 mg/L compared to other concentrations. The larvicidal activity of some Zingiber oils has been documented in the literature. For example, Z. zerumbet showed the most effective extract against Cx. quinquefasciatus larvae with LC₅₀ = 49.28 mg/L and LC₉₀ = 83.87 mg/L, while Z. officinale var. rubrum, Z. montanum and Z. spectabile displayed larvicidal action against Ae. albopictus with LC₅₀ = 96.86, 99.04 and 93.35 mg/L; LC₉₀ = 168.65, 153.77, and 168.65 mg/L respectively. The volatile oil from Z. officinalis was shown to have larvicidal activity against Cx. quinquefasciatus with a LC₅₀ value of 50.78 ppm. The essential oils from the rhizome of Z. nim-
several authors have developed individual criteria to characterize the potency of mosquito larvicides developed from natural products. LC\textsubscript{50} ≤ 750 mg/L to be effective, and LC\textsubscript{50} ≤ 50 mg/L to be inactive. Kiran et al. have previously shown to demonstrate larvicidal activities of the major compounds namely α-pinene, β-pinene, β-caryophyllene, bicyclogermacrene and humulene epoxide II. At times, minor compounds present in the oil sample may impact synergistic effect on the observed activities. Compounds such as α-pinene\textsuperscript{18, 28–30}, β-pinene\textsuperscript{18, 28–30}, β-caryophyllene\textsuperscript{18} and bicyclogermacrene\textsuperscript{29}, and humulene epoxide II\textsuperscript{26}, seem to play an important role in increasing the potential toxicity of essential oils. These compounds have previously shown to demonstrate larvicidal activities against mosquito vectors.

### Table 2 Larvicidal activity of *Z. collinsii* rhizome oil.

| Concentration (µg/mL) | 24 h | 48 h | 24 h | 48 h |
|-----------------------|------|------|------|------|
| 12.5                  | 0    | 1.2 ± 0.500 | 0    | 4.6 ± 0.957 |
| 25                    | 67.5 ± 1.732 | 73.7 ± 0.500 | 7.5 ± 1.291 | 16.3 ± 1.893 |
| 50                    | 70.0 ± 1.891 | 100.0 ± 0.000 | 38.7 ± 2.217 | 66.3 ± 2.500 |
| 100                   | 100.0 ± 0.000 | 100.0 ± 0.000 | 100.0 ± 0.000 | 100.0 ± 0.000 |

### Minimum lethal concentration µg/mL.

| Treatment time | 24 h | 48 h | 24 h | 48 h |
|----------------|------|------|------|------|
| LC\textsubscript{50} | 25.51 | 20.03 | 50.11 | 36.18 |
| LC\textsubscript{90} | (23.329-28.095) | (18.619-21.262) | (46.103-55.617) | (33.274-38.486) |
| LC\textsubscript{95} | (36.568-45.312) | (23.257-26.057) | (64.230-83.322) | (50.496-61.409) |

Regression Equation: \( y = -1.91 + 0.075x \) \( y = -5.73 + 0.286x \) \( y = -3.00 + 0.600x \) \( y = -2.45 + 0.680x \)

\( X^2 \) 11.34 8.73 7.61 10.78

\( P \) < 0.001 < 0.001 < 0.001 < 0.001

\(^{a}\) (n= 4); There was no mortality in the EtOH controls.

The larvicidal activity of *Z. collinsii* is likely caused by the wide variety of phytochemicals and volatile composites present in the essential oils. These compounds have previously shown to demonstrate larvicidal activities against mosquito vectors.

### 5 Conclusion

In this study, the chemical composition of essential oil of the leaf (realized for the first time in Vietnam) and rhizome of *Z. collinsii* by GC-MS were evaluated. This allowed the identification of 62 compounds in both oil samples. The major compounds are: α-pinene\textsuperscript{18, 28–30}, β-pinene\textsuperscript{18, 28–30}, β-caryophyllene\textsuperscript{18} and bicyclogermacrene\textsuperscript{29}, and humulene epoxide II\textsuperscript{26}. The rhizome oil exhibited larvicidal property against larvae of *Ae. albopictus* and *Cx. quinquefasciatus* after 24 h of exposure.
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Acknowledgments
This research was funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.03-2017.328.

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