Potential Biological Activities of Clausena Essential Oils for the Treatment of Diabetes

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Abstract: Diabetes mellitus constitutes one of the most important public health problems. It is defined as a metabolic disorder characterized by a chronic hyperglycemic condition. The inhibition of α-glucosidase and inflammation effects is still considered a strategy for the treatment of diabetes. The aim of this study was to search for new and effective natural functional foods agents for the complementary treatment of diabetes and other related complications. The essential oils from the leaves of three Clausena plants collected in Thailand: C. harmandiana, C. guillauminii and C. excavata were obtained by hydrodistillation. The chemical compositions, antioxidant, anti-α-glucosidase and anti-inflammatory activities of these essential oils were evaluated. The C. excavata and C. harmandiana essential oils contained seselin and terpinen-4-ol, respectively, as the major components and showed strong inhibition of DPPH free radical, α-glucosidase, lipoxygenase and nitric oxide activities with the IC₅₀ values ranging from 0.031±0.005 to 0.702±0.003 mg/mL. From these results, it may be concluded that seselin and terpinen-4-ol are important for high antioxidant, anti-α-glucosidase, and anti-inflammatory activities. Therefore, these Clausena essential oils may be useful in industrial applications for the treatment of diabetes and other related complications.

Key words: diabetes, Clausena, essential oils, α-glucosidase, anti-inflammatory activity

1 Introduction

Diabetes mellitus (DM) constitutes one of the most important public health problems. It is defined as a metabolic disorder characterized by a chronic hyperglycemic condition and associated with abnormalities of carbohydrate, protein, and lipid metabolism which is caused by a decrease or deficiency of insulin secretion. The International Diabetes Federation reported that this disease is responsible for 4.6 million deaths each year, or one death every seven seconds, yielding enormous expenses at economic, public health, and individual levels. Normally, the synthetic hyperglycemic oral agents are therapeutic options for managing DM but these drugs elicit negative human health effects. Recently, an increase in the use of herbs throughout the world for the maintenance and improvement of health and for the treatment of various human conditions and diseases has been observed because the medicinal plants have lower toxicity and side-effect than synthetic drugs. Therefore, the complementary treatment of diabetes has recently focused on functional foods and bioactive compounds from herbs or medicinal plants because these metabolites act in diverse ways to decrease blood glucose, reduce insulin resistance, elicit antioxidant activity as well as anti-inflammatory action. A number of indigenous plants have been widely used for the treatment of diabetes and several other ailments. Among all these plants, the plants under the genus of Clausena belonging to the citrus family (Rutaceae) have strong therapeutic potentials. This genus has about 30 species of shrubs and trees. The aerial parts of Clausena plants have been used as traditional medicine, food and perfumery ingredients. C. excavata is used for the treatment of snakebite, malaria, dysentery, and HIV infection together with C. indica and C. lansium which produce edible fruit and they are used

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Accepted July 21, 2021 (received for review November 18, 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

1669
as natural preservatives due to their broad spectrum of biological activities including antimicrobial, antioxidant and anti-inflammatory effects\(^{12}\). Gas chromatography-mass spectrometry (GC-MS) analysis of essential oils from *Clausena* species has confirmed the presence of terpenoids, sesquiterpenoids and phenylpropanoids as the major components which include estragole (methyl chavicol), \((E)\)-anethole, \(\beta\)-pinene, sabinene, germacrene-D, linalool, pulegone and 1,8-cineole\(^{13-15}\). Furthermore, the studies have also found a wide spectrum of biological activities such as anticancer, anticholinesterase and insecticidal activities\(^{17,18}\). However, the search for new and effective natural functional foods agents for the complementary treatment of diabetes with less toxicity and side-effects has remained an area of great interest. Therefore, the aim of the current study was to comparatively analyze the chemical compositions of the leaves essential oils of three *Clausena* species: *C. harmandiana*, *C. guillauminii* and *C. excavata* and to investigate the anti-diabetic, anti-inflammatory and related effects. The results suggest that *Clausena* essential oils from Thailand could be potentially used for the treatment of diabetes and related diseases.

### 2 Materials and Methods

#### 2.1 Materials and preparation of essential oils

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. Fresh leaves of *Clausena harmandiana* were collected in November 2016 from Wangsomboon district, Sakaeo province, Thailand. *Clausena guillauminii* and *Clausena excavata* leaves were collected in November 2016 from Khukhan district, Sisakat province, Thailand. The species were identified by the Forest Botany Division, Forest and Plant Conservation Research Office, Department of National Parks, Wildlife and Plant Conservation, Bangkok 10900, Thailand. Voucher specimens (CH-001, CG-001 and CE-001) were deposited at The Research Unit in Synthetic Compounds and Synthetic Analogues from Natural Product for Drug Discovery, Department of Chemistry, Faculty of Science, Burapha University, Bangsaen, Chonburi 20131, Thailand. The preparation and chemical composition of the essential oils of the leaves from three *Clausena* species were studied. Fresh leaves (250 g) of *Clausena* plants were subjected to hydrodistillation using a modified Clevenger-type apparatus for 1 h. The yields were averaged and calculated over three experiments. The essential oils were refrigerated until further use.

#### 2.2 Chemical composition analysis

The GC–MS analysis of *Clausena* essential oils was carried out using a Hewlett–Packard 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA), coupled to a quadrupole mass spectrometer. The GC was equipped with a fused silica capillary DB-5 column (0.25 μm 5% diphenyl dimethyl silicone, 30 m × 0.25 mm, 0.25 μm film thickness). The GC settings were as follows: the initial oven temperature was held at 40°C for 1 min and ramped at 5°C min\(^{-1}\) to 200°C for 2 min. The injector temperature was maintained at 270°C. The samples (1.0 μL) were injected neat, with a split ratio of 1:10. Helium was used as carrier gas at a flow rate of 1 mL/min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.6 s was applied, covering a mass range from 50 to 650 m/z at 2 scans s\(^{-1}\). Quantification was performed by percentage peak area calculations using the GC-MS, and individual components were identified using NIST library spectra and the literature and several authentic reference compounds. Relative contents of the volatile oil components were calculated based on GC peak areas without applying correction factors.

#### 2.3 DPPH free radical scavenging activity

This assay was carried out according to the modified method of Braca *et al.*\(^{19}\). A stock solution of each essential oil (4.0 mg/mL) was prepared in dimethyl sulfoxide (DMSO). After 180 μL of 0.05 mM DPPH methanol solution was mixed with 20 μL of the sample solution at different concentrations, decreases of the absorbance of the reaction mixtures were monitored at 517 nm using a UV–Vis spectrophotometer (EPOCH 2 Microplate Reader Spectrophotometer, BioTek Instruments, Winooski, VT, USA) after incubation at room temperature for 30 min in the dark. The DPPH inhibition (%) was calculated as follows, % Inhibition = [(Ablank – Asample)/Ablank] × 100, where Ablank is the absorbance of the control reaction (containing all reagents except the test compound), and Asample is the absorbance of the test compound-containing reaction mixture. The sample concentration providing 50% inhibition (IC\(_{50}\)) was calculated by plotting the inhibition percentages against the concentrations of the sample. All tests were carried out in triplicate and IC\(_{50}\) values were reported as means. Ascorbic acid was used as the positive antioxidant agent.

#### 2.4 α-Glucosidase inhibitory activity

The inhibitory activity of essential oils towards α-glucosidase was determined as described in the literature with a slight modification\(^{20}\). The sample was dissolved in DMSO to make the different concentrations. Briefly, 20 μL of sample and 20 μL of 10 unit/mL α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) in 50 mM phosphate buffer (pH 6.8) were mixed and incubated at 25°C for 10 min. Then 20 μL of 2.0 mM p-nitrophenyl-α-D-glucopyranoside (pNP) was added as a substrate and incubated further at 25°C for 5 min. The reaction was stopped by adding 20 μL of 1.0 mM sodium carbonate.
The yellow color of p-nitrophenol (pNP) produced was read at 405 nm using a EPOCH 2 Microplate Reader Spectrophotometer. Each experiment was performed in triplicate, along with appropriate blanks. Acarbose was used as a positive control. Negative control without extract (DMSO) was set up in parallel. The percentage of α-glucosidase inhibition was calculated as follows: α-glucosidase inhibition (%) = [(A−B)/A] × 100, where A is the absorbance of the control with the enzyme and B is the absorbance of the sample with the enzyme.

2.5 Lipoygenase inhibitory activity
Lipoygenase (LOX) inhibitory activity was determined as described in the literature with a slight modification. The assays are performed as follows: 30 μL of soybean lipoygenase (enzyme’s final concentration per well: 400 U/mL) in 50 mM phosphate buffer (pH 8.0) were distributed in each well, together with 10 μL of essential oils in DMSO. The plate was incubated in the dark for 5 min. Then, 40 μL of 0.08 μM linoleic acid were distributed in each well. After a further incubation of 50 min, the reaction mixture was measured at 234 nm to assess the percentage of inhibition. All tests were carried out in triplicate and IC50 values were reported as means. Quercetin was used as a positive control.

2.6 Nitric oxide production inhibitory activity
Mouse macrophage cell line (RAW264.7) obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), was plated at a density of 1 × 10⁵ cells/well in 96-well plates and incubation was carried out at 37°C in 5% CO2 atmosphere for 3 h, followed by treatment with LPS (lipopolysaccharide) and different concentrations of the indicated compounds for further 48 h. Nitrite (NO2⁻) accumulation was used as an indicator of nitric oxide (NO) production in the cell culture medium by the Griess reaction. One hundred microliters of each supernatant were mixed with the same volume of Griess reagent (1% sulfanilamide in 2.5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid). The absorbance of each sample after Griess reaction was determined by ELISA plate reader at 540 nm. The nitrite concentration was evaluated by means of a calibration curve, using sodium nitrite as a standard. Apigenin was used as a positive drug.

Cell viability was assessed by the MTT[3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The reaction is catalyzed by mitochondrial succinate dehydrogenase and requires NADH, which must be supplied by living cells. The MTT assay was performed by plating cells in 96-well plates (5 × 10⁴ cells/well) and incubation was carried out at 37°C in 5% CO2 atmosphere overnight. The cells were treated with different concentrations of the indicated compounds for further 48 h. 15 μL of MTT dye solution (0.5% MTT stock dye) was added and the plate was incubated for 2 h. The dye crystal was dissolved by DMSO. The absorbance of each sample after dissolving was determined by ELISA plate reader at 540 nm and reference wavelength at 620 nm. Fractional absorbance was calculated by using the following formula: % Cell survival = [(Mean absorbance in test wells/Mean absorbance in control wells)] × 10⁰².

2.7 Statistical analysis
All analyses data in this study are expressed as mean ± standard deviation (SD) of three independent experiments, which were performed in three replicates. Statistical analysis was carried out using Minitab software version 18 followed by the Tukey’s test for multiple comparisons. Differences at <5% (p<0.05) level were considered significant.

### 3 Results and Discussion

#### 3.1 Extraction yield and physical properties of *Clausena* essential oils
The total extraction yields and physical properties of *Clausena* essential oils are shown in Table 1. The yield of essential oil from *C. guillauminii* leaves (1.28%) was higher than those of *C. harmandiana* (0.08%) and *C. excavata* (0.03%).

#### 3.2 Chemical constituents of *Clausena* essential oils
The chemical components of the essential oils from three *Clausena* species are presented in Table 2. A total of seventeen compounds amounting 88.40% in the *C. harmandiana* essential oil were identified. Among these, 64.41% were oxygenated monoterpenes, 25.57% monoterpenoid hydrocarbons, 8.95% phenolics and 0.87% sesquiterpene hydrocarbons. The major components of the mono-

| *Clausena* species | Extract weight (g) | Total yields (%) | Color of essential oils |
|-------------------|-------------------|-----------------|------------------------|
| *C. harmandiana*   | 0.19              | 0.08            | Brownish yellow        |
| *C. guillauminii*  | 3.19              | 1.28            | Pale brownish yellow   |
| *C. excavata*      | 0.07              | 0.03            | Pale yellow            |

*Total yield (%) = [sample extract weight/sample fresh weight] × 100

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*J. Oleo Sci.* **70**, (11) 1669-1676 (2021)
Table 2  Chemical composition of essential oils from three *Clausena* species.

| No. | Compounds                  | RT<sup>a</sup> | Content (%) | C. harmandiana | C. guillauminii | C. excavata | Identification method |
|-----|----------------------------|----------------|-------------|----------------|-----------------|-------------|-----------------------|
| 1   | α-Sabinene                 | 9.03           | 21.62       |                |                 |             | MS<sup>b</sup>         |
| 2   | γ-Terpinene                | 11.52          | 1.16        |                |                 |             | MS                    |
| 3   | *trans*-4-Thujanol          | 11.79          | 5.13        |                |                 |             | MS                    |
| 4   | *cis*-4-Thujanol            | 12.72          | 8.71        |                |                 |             | MS                    |
| 5   | Linalool                   | 12.76          | 3.00        |                |                 |             | MS                    |
| 6   | *cis*-p-2-Menth-1-ol       | 13.39          | 2.87        |                |                 |             | MS                    |
| 7   | *trans*-p-2-Menth-1-ol     | 13.91          | 1.82        |                |                 |             | MS                    |
| 8   | Terpinen-4-ol              | 15.11          | 34.34       |                |                 |             | MS                    |
| 9   | (-)-α-Terpineol            | 15.42          | 2.04        |                |                 |             | MS                    |
| 10  | (-)-Myrtenol               | 15.50          | 0.49        |                |                 |             | MS                    |
| 11  | *trans*-Piperitol          | 15.55          | 0.61        |                |                 |             | MS                    |
| 12  | Estragole                  | 15.72          | 24.54       | 1.84           |                 |             | MS                    |
| 13  | *cis*-Piperitol            | 15.89          | 0.93        |                |                 |             | MS                    |
| 14  | Coumaran                   | 16.16          | 3.41        |                |                 |             | MS                    |
| 15  | Anethole                   | 18.30          | 62.38       | 14.18          |                 |             | MS                    |
| 16  | 4-Vinylguaiacol            | 18.81          | 1.30        |                |                 |             | MS                    |
| 17  | Vanillin                   | 21.02          | 1.40        |                |                 |             | MS                    |
| 18  | Safrole                    | 20.54          | 11.60       | 3.31           |                 |             | MS                    |
| 19  | β-Elemene                  | 20.91          | 0.88        |                |                 |             | MS                    |
| 20  | Caryophyllene              | 21.64          | 0.77        | 0.72           | 2.24            |             | MS                    |
| 21  | 4-Propylguaiacol           | 22.56          | 1.14        |                |                 |             | MS                    |
| 22  | *trans*-Isoeugenol         | 22.67          | 0.77        | 0.53           |                 |             | MS                    |
| 23  | β-Copaene                  | 23.19          | 6.60        |                |                 |             | MS                    |
| 24  | β-Cyclogermacrane          | 23.56          | 0.88        |                |                 |             | MS                    |
| 25  | *trans*-Nerolidol          | 25.08          | 0.97        |                |                 |             | MS                    |
| 26  | (-)-Globulol               | 25.64          | 0.53        |                |                 |             | MS                    |
| 27  | (+)-Isospathulenol         | 26.65          | 0.41        |                |                 |             | MS                    |
| 28  | Cedrelanol                 | 26.95          | 1.02        |                |                 |             | MS                    |
| 29  | Epicubebol                 | 27.04          | 0.44        |                |                 |             | MS                    |
| 30  | τ-Muurolol                 | 27.24          | 1.90        |                |                 |             | MS                    |
| 31  | 5-Cyclodecen-1-ol          | 27.95          | 0.46        |                |                 |             | MS                    |
| 32  | Seselin                    | 34.47          | 0.66        | 38.49          |                 |             | MS                    |

|                | Monoterpene hydrocarbons (%) | Oxygenated monoterpenes (%) | Sesquiterpene hydrocarbons (%) | Oxygenated sesquiterpenes (%) | Phenolics (%) | Identified components (%) | Unidentified components (%) |
|----------------|------------------------------|-----------------------------|--------------------------------|-------------------------------|---------------|---------------------------|-----------------------------|
|                | 25.77                        | 0.00                        | 0.00                           | 3.59                          | 6.86          | 88.40                     | 11.61                       |
|                | 25.77                        | 0.00                        | 0.00                           | 3.59                          | 6.86          | 88.40                     | 11.61                       |

<sup>a</sup> Retention time (min);

<sup>b</sup> Comparison of mass spectra with those listed in the NIST 05 and Wiley 275 libraries and with published data.
terpenoids were terpinen-4-ol (34.34%) and α-sabinene (21.62%). In the essential oil extracted from C. guillauminii leaves, four phenolics (99.29%) and one sesquiterpene hydrocarbon (0.72%) were obtained. Anethole (62.38%), estragole (24.54%) and safrole (11.60%) were the principal constituents of C. guillauminii essential oil. Moreover, eighteen compounds amounting to 83.50% in the C. excavata essential oil were identified. Among these, 76.19% were phenolics, 13.35% sesquiterpene hydrocarbons, 6.86% oxygenated sesquiterpenes and 3.59% oxygenated monoterpenes. The coumarin, namely seselin (38.49%) and anethole (14.18%) were the major components of C. excavata essential oil. These results indicated that the major compositions of three Clausena essential oils were quite different.

3.3 Antioxidant activity of Clausena essential oils

Diabetes mellitus is defined as a metabolic disorder characterized by high levels of blood sugar (hyperglycemia). Hyperglycemia increases the production of reactive oxygen species (ROS) inside the aortic endothelial cells. Therefore, the antioxidant activity of Clausena essential oils was studied. The antioxidant activity of Clausena essential oils and antioxidant standard ascorbic acid was studied by the DPPH radical scavenging method and the results are presented in Table 3.

The DPPH radical scavenging activity of these essential oils increased as their concentrations increased from 0.03 to 2.0 mg/mL. Scatter diagrams were plotted and linear regression estimated using the equation y = ax + b, where y is percentage of inhibition and x is concentration of the sample (Fig. 1). Three points of inhibition concentrations with values between less than and more than 50% inhibition together with the best-fit slope were selected. IC50 was calculated as the concentration that caused 50% inhibition of DPPH. All tests were carried out in triplicate (Fig. 1: B1-B3 or C1-C3) and IC50 values were reported as mean ± SD. A smaller IC50 means higher antioxidant activity.

From the result in Table 3, ascorbic acid as the positive agent (0.157 ± 0.002 mg/mL) was more active than all essential oils. However, it was found that the highest DPPH radical scavenging activity was found in the C. harmandiana essential oil (IC50 = 0.397 ± 0.004 mg/mL) followed by those of C. excavata (IC50 = 0.631 ± 0.027 mg/mL) and C. guillauminii (IC50 > 2.0 mg/mL) essential oils (Fig. 1).

From these results, it may be concluded that the oxygenated monoterpenes and oxygenated sesquiterpenes found in the essential oils from C. harmandiana and C. excavata leaves were good electron donors to eliminate the unpaired condition of the radical, resulting in stronger DPPH free radical scavenging activity while these phytochemical compounds were not detected in the C. guillauminii essential oil. In addition, the major oxygenated monoterpenoid, terpinen-4-ol, found in Zingiber montanum essential oil showed good DPPH radical scavenging activity, and Nakayama and co-workers reported that terpinen-4-ol inhibited colorectal cancer growth via ROS. Therefore, it may be concluded that the oxygenated monoterpenoids in the C. harmandiana essential oil are important for high radical scavenging activity.

3.4 α-Glucosidase inhibitory activity of Clausena essential oils

Recently, the most viable therapeutic target for symptomatic treatment in hyperglycemic effects is focused on suppressing the glucose production from carbohydrates by inhibiting digestive α-glucosidase enzymes. A number of essential oils from medicinal plants were reported to be α-glucosidase inhibitors such as turmeric, Leucas infulata, Marrubium vulgare, Salvia schimper and Origanum majorana essential oils. IC50 values given in Table 3 were estimated in a similar manner to that shown in Fig. 1. All essential oils showed good α-glucosidase inhibitory activity with higher than 50% inhibition for the substrate.

| Table 3 Biological properties of Clausena essential oils. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Volatile oil    | Sample concentration providing 50% inhibition (IC50: mg/mL) | Anti-oxidant | Anti-α-glucosidase | Anti-lipoxygenase | Anti-NO production |
| C. harmandiana  | 0.397 ± 0.004<sup>a</sup> | 0.464 ± 0.002<sup>b</sup> | 0.916 ± 0.017<sup>b</sup> | 0.087 ± 0.010<sup>b</sup> |
| C. guillauminii | > 2.0<sup>a</sup> | 1.243 ± 0.063<sup>b</sup> | 1.429 ± 0.101<sup>b</sup> | 0.177 ± 0.010<sup>b</sup> |
| C. excavata     | 0.631 ± 0.027<sup>b</sup> | 0.702 ± 0.003<sup>b</sup> | 0.188 ± 0.001<sup>b</sup> | 0.031 ± 0.005<sup>b</sup> |
| Ascorbic acid<sup>b</sup> | 0.157 ± 0.002 |             |             |             |
| Acarbose<sup>b</sup> |             | 0.173 ± 0.001 |             |             |
| Quercetin<sup>b</sup> |             |             | 0.068 ± 0.002 |             |
| Apigenin<sup>b</sup> |             |             |             | 0.082 ± 0.004 |

<sup>a</sup> All values are mean ± SD based on three replicates; <sup>b</sup> Standard drugs

Different letters (a, b, c) in the same row indicate significant differences (p < 0.05) as analyzed by the Tukey’s test.

J. Oleo Sci. 70, (11) 1669-1676 (2021)
The *C. harmandiana* essential oil containing oxygenated monoterpenoids, terpinen-4-ol and α-sabinene, as the major components showed the highest α-glucosidase inhibitory activity with IC$_{50}$ value of 0.464 ± 0.002 mg/mL while the *C. guillauminii* essential oil showed lower inhibitory activity (1.243 ± 0.063 mg/mL). In addition, the essential oil obtained from *C. excavata* leaves showed moderate anti-α-glucosidase activity with IC$_{50}$ value of 0.702 ± 0.003 mg/mL. However, the essential oils showed less activity compared to acarbose (0.173 ± 0.001 mg/mL). From the results of anti-α-glucosidase and antioxidant activities, it may be concluded that the oxygenated monoterpenoid compounds are also important for good α-glucosidase inhibitory activity. Consequently, these results suggested that the essential oils of these *Clausena* plants are able to inhibit α-glucosidase activity as well as hyperglycemic effects.

### 3.5 Anti-inflammatory activity of *Clausena* essential oils

Diabetes mellitus is an inflammatory state since hyperglycemia leads to cell malfunction and elevation of several cytokines and inflammatory mediators such as NO which is known as a biomarker of airway inflammation greatly associated with cysteinyll-leukotrienes, the products of LOX. Therefore, the anti-inflammatory activities of *Clausena* essential oils as well as LOX and NO inhibitory activities were evaluated and IC$_{50}$ values were estimated in a similar manner to that shown in Fig. 1. From the result in Table 3, the highest anti-inflammatory activities against LOX enzyme and NO production in mouse macrophage cell line (RAW264.7) were found in the *C. excavata* essential oil which contained seselin and anethole as the major components (IC$_{50}$ = 0.188 ± 0.001 and 0.031 ± 0.005 mg/mL) followed by those of *C. harmandiana* (IC$_{50}$ = 0.916 ± 0.017 and 0.087 ± 0.010 mg/mL) and *C. guillauminii* (IC$_{50}$ = 1.429 ± 0.101 and 0.177 ± 0.010 mg/mL). In addition, es-
Potential Biological Activities of Clausena Essential Oils for the Treatment of Diabetes

The essential oils showed less LOX inhibitory activity than the positive control (0.068 ± 0.002 mg/mL). Fortunately, C. excavata essential oil also showed stronger inhibitory NO activity than the standard drug (0.082 ± 0.004 mg/mL) whereas the essential oil from C. harmandiana (0.087 ± 0.010 mg/mL) was as active as apigenin. These results were correlated with those previously reported in the literature. A number of essential oils such as seselin and terpinen-4-ol were the major components which also showed good anti-inflammatory activities. From these results, it may be concluded that seselin and terpinen-4-ol, the major components in C. excavata and C. harmandiana essential oils, respectively, are important for high anti-inflammatory activity. These results suggested that the C. excavata and C. harmandiana essential oils which are able to inhibit DPPH free radical, α-glucosidase, LOX and NO activities may be useful in industrial applications for the treatment of diabetes and other related complications.

4 Conclusion

The essential oils from the leaves of three Clausena species in Thailand C. harmandiana, C. guillauminii and C. excavata possessed significant antioxidant, anti-α-glucosidase, and anti-inflammatory activities. These Clausena essential oils may be useful in industrial applications for the treatment of diabetes and other related complications.

Acknowledgments

The authors wish to thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC), The Research Unit in Synthetic Compounds and Synthetic Analogues from Natural Product for Drug Discovery (RSND) and Department of Chemistry, Faculty of Science, Burapha University for providing research facilities. We also gratefully thank Burapha University for Grant No.46/42562 from the National Council of Thailand. We also thank Professor Ronald Beckett for his comments and grammatical suggestions.

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