

Phytochemicals and Antidiabetic, Antioxidant and Anti-inflammatory Activities of Ethanol Flower Extract of *Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae)

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Received: 6 April 2022, Revised: 24 July 2022, Accepted: 31 July 2022, Published: 20 November 2022

Abstract

Syzygium aromaticum (L.) Merr. & Perry is a spice that has been used traditionally for centuries to preserve food and treat for a number of ailments. This research was carried out to analyze phytochemicals and to determine antidiabetic, antioxidant and anti-inflammatory activities of 95 % ethanol flower extract from *S. aromaticum* (FESA). The GC-MS analysis demonstrated the presence of 17 bioactive compounds in FESA, with eugenol (56.17 %) the most active compound. Antidiabetic activity study revealed that the inhibitory activity of the FESA against α -glucosidase enzyme was stronger than the activity of Acarbose, an antidiabetic drug. In addition, the FESA exhibited potent antioxidant activity by inhibiting 2, 2'-diphenyl-1-picrylhydrazylhydrate (DPPH) free radicals higher than butylated hydroxytoluene (BHT), a synthetic antioxidant. Furthermore, the FESA potentially inhibited the nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 264.7 cells but less effective than Diclofenac, a non-steroidal anti-inflammatory drug. The activities of FESA are partly due to the presence of active compounds. The results obtained confirm the antidiabetic, antioxidant and anti-inflammatory properties, and the traditional used of *S. aromaticum* for the treatment of diabetes and oxidative and inflammatory-related diseases.

Keywords: *Syzygium aromaticum*, Phytochemicals, Antidiabetic, Antioxidant, Anti-inflammatory

Introduction

Syzygium aromaticum (L.) Merr. & Perry or clove is a traditional spice that has been used in several Asian countries as food preservative and traditional medicine for treatment of various diseases, such as flatulence, indigestion, diarrhea, dental disorders, respiratory disorders, headache, and sore throat [1-3]. It is rich in phenolic compounds and contains various phytochemicals [4]. Its main bioactive compound is eugenol [5-7]. The n-hexane extract from its buds comprises 16 volatile compounds, with eugenol and eugenol acetate the most and second most dominant compounds [8]. In addition, its supercritical extract of buds and the methanol extract of flowers consist of non-phenolic compounds and phenolic compounds, especially eugenol [9,10]. The dominant phytochemicals in clove oil have also been reported to be eugenol, eugenol acetate and β -caryophyllene [11-14]. *S. aromaticum* has been demonstrated to possess antidiabetic property. Dietary supplementation with its bud powder reduces blood glucose level in streptozotocin (STZ)-induced Type 2 diabetic rats, and inhibits the α -glucosidase activity in rats [15]. Its extract has been shown to exhibit the glucose-lowering effect [16], antidiabetic effect on the STZ-induced diabetes rats [17], lowers the blood glucose levels in Type 2 diabetic KK-Ay mice [18] and reduces the blood glucose in STZ-induced diabetic rats [19]. *S. aromaticum*-derived oleanolic acid also lowers blood glucose in experimental diabetes mellitus [20]. Its polyphenols has been reported to inhibit the α -glucosidase activity as well [21]. The antihyperglycemic effect of *S. aromaticum* is attributable to its inhibitory action against α -glucosidase [22]. *S. aromaticum* is rich in antioxidant compounds [23] and thus could be used as an alternative to chemical preservatives [24,25]. Its extract exhibited the highest antioxidant activity among a variety of extracts from Thai medicinal plants [26]. The extracts and flavonoids from its buds showed strong antioxidant activity against DPPH radical scavenging activity [5,27]. Its essential oil also possesses high antioxidant activity with effective scavenging on DPPH and 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, ABTS [12,28,29]. Its oil [30], ethanol extract [8] and supercritical extract [9] of buds have been reported to display the antioxidant activity comparable to butylated hydroxyl toluene (BHT), a synthetic antioxidant. Its

antioxidant activity higher than BHT has also been reported [31-33]. Furthermore, *S. aromaticum* has also been revealed to have anti-inflammatory [34-36].

Most existing studies on pharmacological activities of *S. aromaticum* have been performed using its essential oil, eugenol and the 50 - 80 % ethanol extracts. This study was therefore carried out to determine the phytochemicals, α -glucosidase inhibitory activity, DPPH radical scavenging activity, and inhibitory activity on NO production of 95 % ethanol extract from *S. aromaticum* flowers. The results obtained from this research can be helpfully confirmed the traditionally used of *S. aromaticum* and elucidate the underlying mechanisms of its activities.

Materials and methods

Plant materials

The plant part used in this study was dried unopened flowers of *Syzygium aromaticum* (L.) Merr. & Perry (Myrtaceae) purchased from Vejapong Osod, a medicinal plant supplier in Bangkok, Thailand. The sample flowers were authenticated by Dr. Panarat Thongpoem of the Royal Forest Department, Thailand's Ministry of Natural Resources and Environment. The specimen under the registration identification code RMUTT/SC/BIO/SA2020-001 has been deposited in the library of the Division of Biology, Faculty of Science and Technology, Rajamangala University of Technology, Pathumthani, Thailand.

Preparation of plant extract

The *S. aromaticum* flowers were cleaned thoroughly with running tap water, dried in the shade and oven-dried. The dried flowers were ground into powder. The maceration extraction was carried out using 95 % ethanol as solvent solution whereby 400 mL of 95 % ethanol was added into 100 g of the powder and retained for 7 days at room temperature with periodic stirring. Afterward, the mixture was filtered using clean muslin cloth, followed by Whatman filter paper No.1. The filtrates were evaporated at 50 °C using a rotary evaporator (Heidolph Laborota 4000, Germany). The concentrated extract was transferred to glass Petri dishes and oven-dried at 40 - 50 °C until the paste-like mass was formed. The crude ethanol flower extract of *S. aromaticum* (*FESA*) was kept in air tight bottles and stored at -20 °C.

Determination of phytochemicals

The phytochemicals of the *FESA* was analyzed using gas chromatography-mass spectrometry (GC-MS). The analysis was carried out using Stabilwax column (30 m \times 0.25 mm and the film thickness of 0.25 μ m) with the inlet temperature and the split ratio of 250 °C and 50:1, the initial oven temperature at 45 °C held for 2 min before ramping up to 250 °C at 7 °C/min, and maintained at 250 °C for 25 min. The carrier gas was helium with a flow rate of 1.0 mL/min. The mass spectrometer was operated in electron ionization with acquisition mode scan of 35 - 500 amu. The temperatures of the ion source and transfer line were maintained at 250 °C. The GC-MS was carried out by injecting 1 μ L of *FESA* in 0.1 % absolute methanol. The chemical compounds in *FESA* were identified by comparison with retention times of standards, and the mass spectra obtained were compared to those recorded in the National Institute of Standards and Technology (NIST) library (NIST11–Mass Spectral Library, 2011 version) with an acceptance criterion of a match above a critical factor of 80 % [45].

Determination of antidiabetic activity

The *in vitro* α -glucosidase inhibition assay of *FESA* was carried out according to Dong *et al.* [46] with slight modifications. Briefly, a volume of 60 μ L of sample solution and 50 μ L of 0.1 M phosphate buffer containing α -glucosidase solution (0.2 U/mL) in 96-well plate was incubated at 37 °C for 20 min. After pre-incubation, 50 μ L of 5 mM p-nitrophenyl- α -D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer solution was added to each well and incubated at 37 °C for another 20 min. A volume of 160 μ L of 0.2 M Na₂CO₃ was added to terminate the reaction. The absorbance reading was recorded at 405 nm by using micro-plate reader and compared to control which had 60 μ L of buffer solution in place of the sample. For blank incubation, enzyme solution was replaced by the buffer solution and absorbance was recorded. The α -glucosidase inhibitory activity was expressed as % inhibition and was calculated using the following equation:

$$\% \text{ inhibition} = [(Abs_{\text{blank}} - Abs_{\text{sample}}) / Abs_{\text{blank}}] \times 100$$

where Abs_{blank} is the absorbance of control without test solution (*FESA* or Acarbose) and Abs_{sample} is absorbance of sample with test solution (*FESA*). Acarbose, a positive control of α -glucosidase inhibitor was assayed as well.

Determination of antioxidant activity

Antioxidant activity of the *FESA* was determined spectrophotometrically using 2, 2-diphenyl-2-picrylhydrazylhydrate (DPPH) radical scavenging assay, estimated by the reduction of the reaction color of DPPH solution and sample extracts. In the assay, the antioxidant compounds reduce the free radicals by donating hydrogen, resulting in color change from deep violet to light yellow. The DPPH radical scavenging activity of the *FESA* was measured according to Nassar *et al.*[8] with slight modifications. The *FESA* 25, 50, 100, 200 or 400 µg/mL prepared by dissolving the *FESA* in ethanol was added to solution of DPPH in ethanol. The mixtures were shaken vigorously and left to stand at room temperature in a dark place for 30 min. After 30 min, the absorbance at 517 nm was recorded as Abs_{sample} using an ultraviolet/visible (UV/VIS) spectrophotometer. The experiment was carried out in triplicate. The synthetic antioxidant agent, BHT was used as positive control, and 95 % ethanol as negative control. The radical scavenging activity was expressed as percentage DPPH radical scavenging and was calculated using the following equation:

$$\text{DPPH radical scavenging (\%)} = [(Abs_{\text{negative control}} - Abs_{\text{sample}})/Abs_{\text{negative control}}] \times 100$$

where Abs_{negative control} is the absorbance of 95 % ethanol and Abs_{sample} is the absorbance of *FESA* or BHT.

Determination of anti-inflammatory activity

The anti-inflammatory activity of *FESA* was determined by measuring nitric oxide (NO), a pro-inflammatory mediator, in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells after treating with *FESA*. To ensure that the RAW264.7 cells were healthy, and the concentrations of the *FESA* were not toxic to the cells (cell viability of the RAW264.7 cells ≥ 80 %), cytotoxicity was determined.

Determination of cytotoxicity

In this study, the determination of cytotoxicity was carried out using 3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay, a colorimetric assay which is based on the conversion of MTT solution into insoluble formazan by living cells. The RAW264.7 macrophage cells, purchased from the American Type Culture Collection (ATCC®TIB-71™ USA) were used. The cells (1×10⁵ cells/well) were cultured in 96-well plate containing Dulbecco's modified Eagle's medium (DMEM; ATCC® 30-2002™ USA), supplemented with 5 % fetal bovine serum and 1 % antibiotics penicillin-streptomycin, and incubated for 24 h at 37 °C in a 5 % CO₂ incubator. After 24 h incubation period, the cultured medium was removed from each well, washed with PBS and replaced with a fresh medium containing LPS (1 µg/mL). The cells were treated with the *FESA* solution (25, 50, 100, 150, 200, 250, 500, 750 and 1000 µg/mL) in a volume of 200 µL/well and incubated in a 5 % CO₂ incubator at 37 °C for 24 h. After 24 h, the cultured medium was replaced with a fresh medium. Subsequently, a volume of 50 µL/well of 5 mg/mL MTT solution in PBS was added into each well and incubated at 37 °C for 24 h. The solutions in the plates were removed and the formazan was solubilized by adding 100 µL of DMSO and shaken gently for 15 min. Finally, an absorbance of the solubilized formazan was measured. The cell viability was determined by absorbance reading at 570 nm using the micro-plate reader (Multiskan Go, Thermo/Scientific, the Netherlands). The percentage of cell viability is calculated by the following equation,

$$\% \text{ cell viability} = (Abs_{\text{sample}} / Abs_{\text{negative control}}) \times 100$$

where Abs_{negative control} is the absorbance of negative control (PBS) and Abs_{sample} is the absorbance of *FESA*.

The *FESA* with non cytotoxic concentrations selected (25-250 µg/mL) was used for subsequent experiments.

Determination of NO production

NO in the biological matrix is very unstable and rapidly oxidizes to nitrite (NO₂⁻), thus the measurement of nitrite is routinely used as an index of NO production. In this research, determination of NO production was performed by measuring nitrite concentrations in the cultured medium using Griess assay, a technique commonly used to quantify NO. The basic reaction involves reacting of the Griess reagent, consisting of sulphanilamide and N-1-naphthylethylenediamine dihydrochloride (NED), to form a stable azo compound.

In the nitrite concentration analysis, the RAW264.7 cells were cultured in DMEM medium with PBS in 96-well plate. LPS (1 µg/mL) was added to each well prior to adding the *FESA* (25 - 250 µg/mL). The

plate was incubated in the 5 % CO₂ incubator at 37 °C for 24 h. After the incubation period, the cultured medium was collected and transferred to a new plate followed by the addition of Griess reagent. Absorbance was measured at 570 nm using the micro-plate reader (Multiskan Go, Thermo/Scientific, the Netherlands). The percentage of NO production is calculated by using the following equation and compared to Diclofenac (50 µg/mL).

$$\% \text{ NO production} = (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{negative control}}) \times 100$$

where Abs_{negative control} is the absorbance of negative control (PBS) and Abs_{sample} is the absorbance of *FESA* or Diclofenac.

Statistical analysis

The results were expressed as mean ± standard error mean (SEM). Analysis of variance (ANOVA) followed by Least Significant Difference (LSD) and Student's t-test were applied to measure the degree of significance for 95 % significance level ($p < 0.05$).

Results and discussion

Phytochemicals

The GC-MS analysis of the *FESA* indicated 17 distinct peaks at the retention time of 15.74, 18.46, 18.82, 19.67, 20.18, 20.66, 22.30, 24.03, 25.77, 30.43, 31.18, 32.90, 33.51, 34.39, 34.96, 37.47, and 37.6. The first compound identified in the shortest retention time (15.74 min) was alpha-copaene (0.73 %), while ethyl linoleate (0.15 %) was the last compound identified with the longest retention time (**Figure 1**). The analysis results showed that eugenol (56.17 %) was the most dominant bioactive compound in the *FESA*, followed by acetugenol (21.40 %), trans-caryophyllene (12.95 %), and α-humulene (1.60 %). **Table 1** tabulates the 17 phytochemicals along with the retention time (RT), compound name, peak area, peak height, and relative abundance (%).

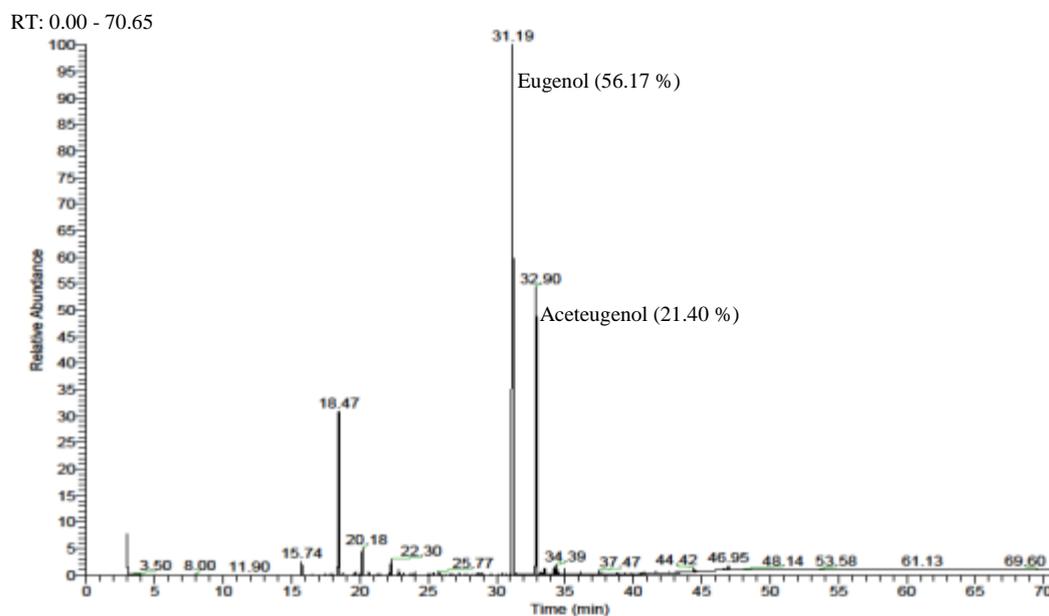


Figure 1 GC-MS chromatogram of the phytochemicals in *FESA*.

Table 1 Phytochemicals identified in the *FESA* by using GC-MS.

No.	RT(min)	Compound Name	Peak Area	Peak Height	Relative Abundance (%)
1	15.74	α -Copaene	176067140.43	59949867.12	0.73
2	18.46	Trans-Caryophyllene	3111273125.74	778974963.03	12.95
3	18.82	Bicyclo [7.2.0] undecane,10,10-dimethy 12,6-bis (methylene)-,	22840293.90	8096465.74	0.10
4	19.67	Humulen-(v1)	35606187.71	11647722.09	0.15
5	20.18	α -Humulene	385234065.59	125031274.85	1.60
6	20.66	α -Amorphene	36187802.22	11842390.37	0.15
7	22.30	δ -Cadinene	224062896.41	70559502.99	0.93
8	24.03	1S, Cis-Calamenen	47959218.19	15973957.19	0.20
9	25.77	4(1H)-Azulenone, octahydro-1-methylene-, trans	45201819.16	14448150.44	0.19
10	30.43	1H-Benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-Octahydro-1,1,4a,7-tetramethyl-, cis	35903339.51	10387917.19	0.15
11	31.18	Phenol, 2-methoxy-4-(2-propenyl)- (Eugenol)	13490389902.45	2582758005.59	56.17
12	32.90	Phenol, 2-methoxy-4-(2-propenyl)-, acetate (Aceteugenol)	5139724330.39	1360450022.68	21.40
13	33.51	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	83587949.74	28751363.68	0.35
14	34.39	Chavicol	143743454.98	43555936.43	0.60
15	34.96	(-)-Caryophyllene oxide	84059257.09	27659168.03	0.35
16	37.47	2',3',4' Trimethoxyaceto-phenone	49470116.92	15149287.35	0.21
17	37.69	Ethyl linoleate	36950236.41	12181237.40	0.15

The GC-MS analysis of the *FESA* revealed eugenol the most dominant bioactive compound. This finding is in line with previous studies [9,10,47,48,50]. Meanwhile, ketones and anthraquinones are the main bioactive compounds in the clove flower bud extract have also been found [2]. Differences phytochemicals in the *FESA* have been reported to depend on the environment, geography of the cultivated area, the storage conditions and extraction methods used [51,52]. Phytochemicals have their own specificity and functions. Phytochemicals such as eugenol, aceteugenol, α -amorphene, α -capaene, ethyl linoleate, and α -humulene, α -caryophyllene to be responsible for antidiabetic, antioxidant and anti-inflammatory activities have been documented [53-63]. According to the biological properties of phytochemicals as above and due to the presence of these phytochemicals in *FESA*, indicate that the *FESA* can be used as a natural source of α -glucosidase inhibitors and anti-inflammatory and antioxidant agents.

Antidiabetic activity

The *in vitro* enzymatic model revealed that the *FESA* significantly inhibited α -glucosidase activity and was concentration dependently, as shown in **Figure 2**. By comparison, given the same concentrations of 0.63, 1.25 and 2.50 mg/mL, the inhibitory activity of the *FESA* were 47.63 ± 3.26 , 55.44 ± 3.57 and 59.27 ± 3.06 %, respectively and higher than those of Acarbose (14.25 ± 3.78 , 29.30 ± 4.14 and 40.64 ± 3.73 %, respectively).

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia and associated with abnormalities in carbohydrate metabolism [64]. The number of diabetic patients globally is estimated to reach 693 million by 2045, with 90 % of them suffering from Type 2 DM [65]. The common practice to regulate blood glucose in patients with Type 2 diabetes mellitus is to inhibit the production of glucose or reduces the rate of glucose absorption in the small intestine. Specifically, α -glucosidase is an enzyme that

breaks disaccharides into absorbable monosaccharides. Inhibiting this enzyme delays the carbohydrate digestion, reduces the rate of glucose absorption, reduces postprandial rise in blood glucose, and attenuates glucose-induced insulin secretion. Due to diminished postprandial hyperglycemia and hyperinsulinemia by α -glucosidase inhibitor, triglyceride uptake into adipose tissue, hepatic lipogenesis, and triglyceride content are reduced. Therefore, α -glucosidase inhibitor treatment may have the potential to delay or possibly prevent the development of diabetic complications [66].

In this research, the *FESA* exhibited the inhibitory effect against α -glucosidase enzyme activity. This finding is in accordance with the previous studies [21,22,65-68]. Thus, the *FESA* can effectively be used as α -glucosidase inhibitor to regulate blood glucose in patients with Type 2 DM.

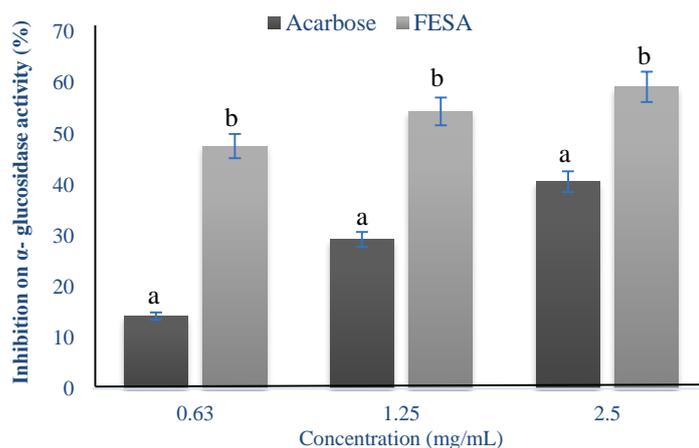


Figure 2 Inhibitory effect on α -glucosidase activity (%). Data are shown as mean \pm SEM (n = 3). Bars having different letters are significantly different at $p < 0.05$.

Antioxidant activity

Antioxidant potential of the *FESA* and BHT against DPPH is presented in **Figure 3**. DPPH radical scavenging activity of the *FESA* and BHT was increased with an increasing concentration. Given the same concentrations of 25, 50, 100, 200, and 400 mg/mL, the scavenging activity of the *FESA* were 11.93 ± 3.86 , 23.78 ± 4.23 , 39.53 ± 3.65 , 54.50 ± 3.29 , and 69.70 ± 3.61 %, respectively and more significant effective than those of BHT (8.42 ± 3.07 , 14.85 ± 4.09 , 21.52 ± 3.58 , 32.16 ± 4.21 , and 49.70 ± 3.77 %, respectively).

The DPPH radical scavenging activity of the *FESA* is positively correlated to the extract concentration and higher than BHT. Similarly, the ethanol extract from *S. aromaticum* buds [8] and flowers [32] exhibited high radical scavenging activity, compared to the synthetic antioxidants such as BHT. Due to the presence of active compounds such as eugenol, eugenol acetate (aceteugenol) and α -cappaene which have been reported to show efficient antioxidant activity [8,53,54, 60], the antioxidant activity of the *FESA* is more likely associated to its active compounds.

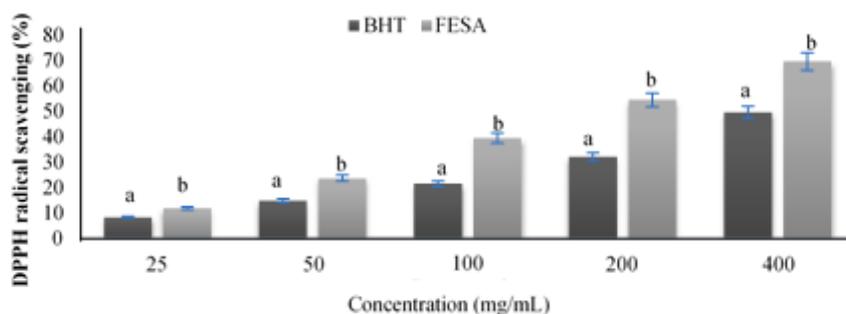


Figure 3 DPPH radical scavenging activity (%). Data were shown as mean \pm SEM (n = 3). Bars having different letters are significantly different at $p < 0.05$.

Anti-inflammatory activity

Cytotoxicity determined using MTT assay revealed that the *FESA* ≤ 250 $\mu\text{g/mL}$ displayed non-cytotoxic to the RAW264.7 cells, with the cell viability of 107.19 ± 3.22 , 105.55 ± 2.88 , 105.62 ± 4.50 , 104.62 ± 3.78 , 104.76 ± 4.85 , and 103.25 ± 3.98 % for the *FESA* at 25, 50, 100, 150, 200, and 250 $\mu\text{g/mL}$, respectively. However, the viability of the RAW264.7 cells was decreased at the concentrations of the *FESA* ≥ 500 $\mu\text{g/mL}$. The viability of the RAW264.7 cells were 60.60 ± 4.32 , 4.42 ± 1.69 , and 2.97 ± 0.06 % for *FESA* at 500, 750 and 1000 $\mu\text{g/mL}$, respectively (**Figure 4**). The half-maximal concentration (IC_{50}) of the *FESA* on cell viability of the RAW 264.7 cells was 520.74 ± 6.88 $\mu\text{g/mL}$.

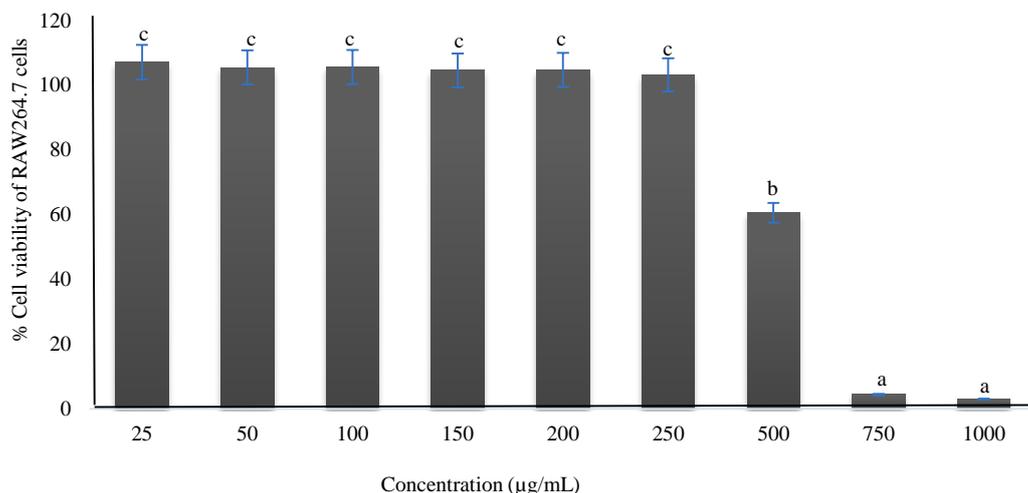


Figure 4 The percentage cell viability of RAW264.7 cells which was treated with various concentrations of *FESA* in the presence of 1 $\mu\text{g/mL}$ LPS for 24 h, using MTT assay. The data were represented as means \pm SEM of three independent experiments. Bars having different letters are significantly different at $p < 0.05$.

The *FESA* ranging from 25 to 250 $\mu\text{g/mL}$ showed the cell viability of RAW264.7 cells higher than 80 %. As a result, they were used further for the determination of NO production. In **Table 2**, at the concentrations of 25 - 150 $\mu\text{g/mL}$, the *FESA* failed to inhibit NO production in the LPS-stimulated RAW264.7 cells. On the other hand, at 200 and 250 $\mu\text{g/mL}$, the *FESA* inhibited the production of NO by 12.12 ± 3.47 and 33.78 ± 0.42 %, respectively, whilst Diclofenac at 50 $\mu\text{g/mL}$ inhibited the NO production in the LPS-stimulated RAW264.7 cells by 27.07 ± 1.81 %.

The *FESA* 200 and 250 $\mu\text{g/mL}$ inhibited the NO production in LPS-stimulated RAW 264.7 cells. The results demonstrate the potential anti-inflammatory activity of the *FESA* and mediates its anti-inflammatory activity through the inhibition of NO production. This result corresponds with the previous studies such as the ethanol extract and eugenol of flower buds from *S. aromaticum* exhibited the anti-inflammatory activity by inhibiting the inflammatory mediator, NO [37], eugenol showed the anti-inflammatory action on macrophages [38] and inhibited the NO production in lipopolysaccharide (LPS)-induced RAW264.7 cells [39]. Moreover, *S. aromaticum* essential oil and eugenol possess the anti-inflammatory properties against murine macrophages by suppressing the production of pro-inflammatory cytokines [40,41]. The anti-inflammatory effects of *S. aromaticum* could be attributed to the dominant bioactive eugenol [42]. *Syzygium* species has been documented to possess anti-inflammatory activity due to the high content of flavonoids [43-44]. Furthermore, α -Amorphene [53], copaene [54], caryophyllene [55,56], ethyl linoleate [57-59], eugenol [53,60], and humulene [62,63] have been demonstrated to possess anti-inflammatory activity. The presence of these active compounds in the *FESA* suggests the anti-inflammatory activity of the *FESA* is due to its active compounds which exhibit the activity by inhibiting NO production. This result provides scientific support for the use of *S. aromaticum* in preventing inflammatory diseases mediated by excessive production of NO.

Table 2 Inhibition of NO production (%) in LPS-stimulated RAW264.7 cells treated with *FESA* or Diclofenac.

Concentration ($\mu\text{g/mL}$)	Inhibition of NO production (%)	
	<i>FESA</i>	Diclofenac
25	NI	ND
50	NI	27.07 \pm 1.81
100	NI	ND
150	NI	ND
200	12.12 \pm 3.47 ^a	ND
250	33.78 \pm 0.42 ^b	ND

The values are expressed as mean \pm SEM of three independent experiments. The values with different superscripts (a, b) in the same column indicate the statistical difference at 5 % significance level ($p < 0.05$). NI denotes no inhibition of nitric oxide production and ND denotes no determination of the nitric oxide inhibition.

Conclusions

The 95 % ethanol flower extract of *S. aromaticum* (*FESA*) composes of 17 phytochemicals with eugenol (56.17 %) as the most predominant chemical. The *FESA* exhibits antidiabetic activity by inhibiting α -glucosidase enzyme, shows antioxidant activity by DPPH radical scavenging and demonstrates anti-inflammatory activity by effectively inhibiting the NO production. The findings of this research clearly provide evidence that supports the traditional use of *S. aromaticum* for the preservation of food and treatment of various ailments. Based on the data obtained, *S. aromaticum* could be adopted as an effective natural alternative to synthetic drugs treating diabetes and inflammatory-related disorders.

Acknowledgements

This study is partially supported by the Research Fund of Rajamangala University of Technology Thanyaburi (RMUTT), Thailand. The author is grateful to the Research Fund of IRF62RMUTT for financial support (Project no: IRF62A0601).

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