Phytochemical and bioactive compounds identification of Ocimum tenuiflorum leaves of methanol extract and its fraction with an anti-diabetic potential

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\textbf{ABSTRACT}

Phytochemical screening assay on crude extracts of \textit{Ocimum tenuiflorum} L. leaves and methanol fractions was performed in this study. Phytochemical qualitative assay on aqueous crude extract and aqueous fraction did not show any steroid and terpenoid. However, ethanol and butanol fractions also did not show steroid in the screening test. The result of HPLC identification of active crude extracts and active fractions showed the possible bioactive compounds in this plant extracts, which may control diabetes. These findings showed that the active crude extract (methanol) and its active fractions (ethyl acetate, and butanol) showed the presence of polyphenolic active constituents such as 3,4-dimethoxycinnamic acid, caffeic acid, diosmetin, luteolin, kaempferol, rosmarinic acid, apigenin, and genistein that control the blood glucose in diabetic rats. Furthermore, isolation of the active components may pave the way for the development of new agents for the treatment of diabetes and its complications.

\textbf{ARTICLE HISTORY}

Received 3 June 2018
Accepted 1 August 2018

\textbf{KEYWORDS}

\textit{Ocimum tenuiflorum} L.; phytochemical; HPLC; bioactive compounds

\textbf{Introduction}

In the developing world, plant-based traditional medicines are demanded by approximately 3.4 billion people. This number makes up 88% of the overall inhabitants in the world, where traditional medicine is their main approach for primary health care. As defined by the World Health Organization, a medicinal plant is any plant where substances that can be used for therapy reside in one or more of its organs. They are also the source for chemo-pharmaceutical semi-synthesis. The parts of the plants of this species, such as grains or seeds, fruits, flowers, barks, stems, rhizomes, roots, and leaves, are utilized to regulate and treat diseases, as there are chemical components in them which are medically active. Furthermore, most of the time, these bioactive components or non-nutrient plant chemical compounds are known as phytoconstituents (‘phyto-’ means ‘plant’ in Greek) or phytochemicals. They also play an important role in protecting plants against microbial infestations or infections caused by pests.\textsuperscript{[1]} Meanwhile, phytochemistry is known as the study conducted on natural products. Phytochemicals have been categorized and characterized from spices such as turmeric, beverages such as green tea and red wine, vegetables such as broccoli and onion, fruits such as grapes and apples, and many other sources.\textsuperscript{[2]} Ethnopharmacology is the scientific study that emphasizes the application of these indigenous or local medicinal remedies which include the use of plants to treat diseases. However, this practice has been around since the ancient times. Moreover, besides ethnopharmacology being the traditional medicine of high importance in the entire world, it is incorporated into mainstream medicine recently.
In fact, the plants used in phytomedicine consist of complex chemical composition with many elements. However, the quality of element and active constituent’s content of herbal medicine are depending on numerous factors. These include choosing the highest yielding age, geographical origin (climatic variation), growth conditions, genetic composition of the plant, variety, plant species, and particular parts of the plants which are harvested before getting processed. There are several major groups of compounds which play a role in the phytomedicine activity from plants. These include alkaloids, terpenoids, coumarins, tannins, flavonoids, saponins, quinones, phenolic acids, and phenolics. Of all these compounds, alkaloids are the largest group of secondary chemical constituents with a high content of ammonia compounds. Basically, there are contents of nitrogen bases in these compounds, which are synthesized from amino-acid building blocks. This is where one or more hydrogen atoms are replaced with various radicals in the peptide ring. As a result, oxygen occupied most of the peptide ring. The function of these nitrogenous compounds is to guard plants against pathogens and herbivores. Besides, due to their strong biological activities, there is a wide use of them as poisons, narcotics, stimulants, and pharmaceuticals. The use of alkaloids is also applied pharmacologically, which can be seen from the creation of anaesthetics and CNS stimulants. Although it is known that there are more than 12,000 alkaloids residing in approximately 20% of plant species, the exploitation of them for medicinal purposes is low. On the other hand, glycosides are categorized based on the pharmacological action, chemical nature of aglycone, and types of sugar component. Glycosides is a colourless, crystalline carbon and a compound with the presence of oxygen and hydrogen (some have sulphur and nitrogen). Additionally, flavonoids are a primary group of polyphenols, which can be seen from their high availability in floras. They are separated into various categories such as leucoanthocyanidins, chalcones and catechin, proanthocyanidins, anthocyanidins, flavonols, flavans, dihydroflavonols, and flavones. Most of the time, phenolics compounds can be produce in plants from phenylalanine through the process of phenylalanine ammonia lyase (PAL). Aside from being multifunctional, they hold high importance for plants. They are also broken into several classifications: (i) phenolic acids, (ii) flavonoid polyphenolics (catechins, xanthones, flavones, and flavanones), and (iii) non-flavonoid polyphenolics. Apart from that, caffeic acid is acknowledged as the most common group under the phenolic compounds. They are available in floras.

Following that, chlorogenic acid is a group known to be the source of allergic dermatitis in humans. The origin of saponin is Saponaria vaccaria (Quillaja saponaria), a plant with abundance of saponins, and it was once utilized as soap. This is the reason for its ‘soaplike’ behaviour, where the foam is formed when placed in water. Sapogenin consists of two types: steroidal and triterpenoidal. Therapeutically, they hold high importance due to their anticancer and hypolipidemic activities. Furthermore, saponins are important for cardiac glycosides activity. Conversely, tannins are phenolic compounds with high molecular weight. Complexes are formed from these compounds and proteins, alkaloids, gelatin, and carbohydrates. They are also specified into hydrolysable tannins and condensed tannins. Furthermore, a number of diseases are cured with medicinal plants. In Ayurveda, in order to treat diseases such as leucorrhoea, rhinorrhoea, and diarrhoea, medicines created from tannin-rich plants have been formulated. Terpenes are one of the chemically various groups of natural products.

Terpenoids are categorized in accordance with the amount of isoprene units included for the formation of these compounds. Plant steroids (or steroid glycosides), also known as ‘cardiac glycosides’, are one of the plant phytoconstituents with the most natural formation. Through this, therapeutic applications such as cardiac drugs or arrow poisons are discovered. It has been shown that cinnamaldehyde, as a phytoconstituent extract, shows significant antihyperglycaemic effects, which lead to the decrease of the levels of both total cholesterol and triglyceride. At the same time, they are capable of increasing HDL-cholesterol level in Streptozotocin (STZ)-induced diabetic rats. This investigation unveiled the potential of cinnamaldehyde as a natural oral agent which exhibits both hypolipidemic and hypoglycaemic effects.
Based on the recent reports, it has been indicated that *Cinnamon* extract and polyphenols with procyanidin type-A polymers have the potential to increase the amount of glucose transporter-4 (GLUT4), insulin resistance (IR), and thrombotic thrombocytopenic purpura) and in 3T3-L1 adipocytes. It has also been stated that the possible factor of the mechanism of *Cinnamon*’s insulin-like activity is the increase in the amount of GLUT4, IRβ, and TTP. In addition, it is possible that the roles of *Cinnamon* polyphenols are numerous as anti-inflammatory and/or anti-angiogenesis agents.[7]

**Materials and methods**

*Preparation of the Ocimum tenuiflorum Lnn. extracts and fraction*

Chemical and equipment: Solvent used: n-hexane (QREC (Asia) SDN BHD); chloroform (Tri Chloroform): CHCL3 (QREC (Asia) SDN BHD); methanol: CH3OH (QREC Product); ethyl acetate (QREC Product); n-butanol (R&M Marketing, Essex, UK); ethanol (QREC Product), separating funnel, rotary evaporation (Buchi, Switzerland, EYELA N1200B 1101348), and freeze dryer (Millrok Technology, LD53, Kingston, USA).

Preparation of the extracts (serial extraction): The freeze-dried (Millrok Technology) leaves were powdered using a milling machine. An amount of 200 g of the powder was sequentially extracted by maceration (40–60ºC) with five solvents, i.e., n-hexane, chloroform, ethyl acetate, methanol, and water, for 7 consecutive days that yielded extracts of n-hexane (HE), chloroform (CE), ethyl acetate (EE), methanol (ME), and water (WE), respectively. The extracts obtained were filtered with Whatman No. 1 filter paper and concentrated in vacuum by rotary evaporation (Buchi) at reduced pressure and 30ºC. The concentrated extracts (HE, CE, EE, ME, and WE) were dried in an oven (40ºC) until the organic solvent evaporated, whereas the concentrated WE was dried in a freeze dryer. The dried extracts were kept in the freezer (−25ºC) until further analysis. All extracts were dissolved using 5% Tween 80 in normal saline prior treatment.

Preparation of the fractions: The most active extract (ME) of 25 g was sequentially fractionated by liquid-liquid extraction with five solvents, i.e., chloroform, ethyl acetate, butanol, ethanol, and water, to yield fractions of chloroform (CF), ethyl acetate (EAF), n-butanol (nBF), ethanol (EF), and water (WF), respectively. The fractions procedure was repeated three times, respectively, using separating funnels. The fractions obtained were then concentrated in vacuum by rotary evaporation (Buchi) at reduced pressure and 30–40ºC. The concentrated fractions (CF, EAF, EF, nBF, and WF) were dried in an oven (40ºC) until the organic solvent evaporated, whereas the concentrated WF was dried in the freeze dryer. The yield complete dried fractions were kept in the freezer (−25ºC) until further analysis. All fractions were dissolved in 5% tween 80 in normal saline prior treatment.

**Phytochemical screening of crude extracts of Ocimum tenuiflorum Lnn. leaves and methanol fractions.**[8]

Identification by chemical test: 0.5 g of the sample (extract) was dissolved in distilled water and filtered. The filtrate was heated with Fehling solutions A (1 ml) and B (1 ml). The red precipitate of cuprous oxide indicates the presence of reducing sugars. Fehling A: 7 g of CuSO₄.7H₂O in 100 ml distilled water. Fehling B: 24 g of KOH+ 34.6 g Na-K-tart rate in 100 ml distilled water.

Identification of tannins: 0.5 g of the extract was stirred with 10 ml of distilled water and filtered. Four drops of 1% ferric chloride solution was added to 2 ml of the filtrate. Blue-black, green, or blue-green precipitate indicates the presence of tannins.

Identification of steroid (LiebermannBurchard test): 0.2 g of the extract was mixed with 2 ml of acetic acid and cooled well on ice followed by the addition of concentrated H₂SO₄ carefully in the fume chamber. The formation of violet, blue, or bluish-green steroid ring indicates the presence of steroids.

Identification of terpenoids: 0.1 g of the extract was dissolved in 10 ml ethanol. Then, 1 ml of acetic anhydride was added and followed by the addition of 3 drops of H₂SO₄. Pink or violet colour indicates the presence of terpenoids.

Identification of flavonoids (Ferric chloride test): 0.5 g of the extract was boiled with 3 ml of distilled water and filtered; 2 ml of the filtrate and 5 drops of 10% ferric chloride solution were
added. The colour changes to green-blue or violet, which indicates the presence of the phenolic hydroxyl group.

Identification of soluble starch: 0.1 g of the extract was boiled with 1 ml of 5% KOH cooled and acidified with concentrated H\textsubscript{2}SO\textsubscript{4}. A yellow colouration was taken as the presence of soluble starch.

Identification of saponin: 0.2 g of the extract was dissolved in 5 ml of distilled water bath until boiled. The solution was then cooled and filtered, and 3 ml of distilled water was added to the filtrate and shaken.

Identification of glycoside: 0.5 g of the extract was dissolved in 1 ml of distilled water. Aqueous NaOH of 2 M was added to the solution, and yellow colouration indicates the presence of glycoside.

Identification by liquid chromatography–mass spectrometry (LC-MS): In Pharmaceutical industry, LC-MS has become the method of choice in many stages of drug development. Recent advances include electrospray, thermospray, and ion spray ionization techniques which offer unique advantages of high detection sensitivity and specificity, and liquid secondary ion mass spectroscopy; later, laser mass spectroscopy with 600 MHz offers accurate determination of molecular weight proteins, peptides. Isotopes pattern can be detected by this technique.\textsuperscript{[9]}

**Chromatographic conditions**

The HPLC analysis was performed at room temperature (25ºC) using the following: Zorbax SB-C18, column (2.1 mm x 150 mm 3.5 µm particle size). The mobile phase A, 20 mM ammonium acetate + 0.25% formic acid in ultrapure water at the flow rate of 0.2 ml/min at room temperature (25ºC). The mobile phase A, 20mM ammonium acetate + 0.25% formic acid in ultrapure water at the flow rate of 0.2ml/min at room temperature (25ºC) and the mobile phase B, 20 mM ammonium acetate + 0.25% formic acid in methanol at the flow rate of 0.2 ml/min at room temperature (25ºC) was prepared. The sample injection volume was 5 µl. Gradients were followed at different times and conditions: time 0, 50, 55, 55.1, 60 min); mobile phase % A:%B (95:5, 0:100,0:100, 95:5, 95:5).

**Mass spectrometric parameters**

Mass spectrometric parameters are as follows: capillary voltage (4 kV), nebulizer gas pressure 3.5 bar, dry gas flow rate 6.0 L/min, and dry gas temperature 300ºC. An amount of 2.01 mg of methanol crude extract was dissolved in 201 µl methanol to get solutions with the concentration of 10 mg/ml and then centrifuged at 13,000 rpm and 5 µl of supernatant was injected into the LCMS system.

**Statistical analysis**

Values were represented as a standard error of the mean (SEM). A one-way ANOVA, followed by Dunnet’s HSD test, was employed for the statistical analysis. GraphPad Prism (version 4) software was used for all statistical analyses. p-Values <0.05 were considered to be significant.

**Results and discussion**

**Phytochemical identification of crude extracts and fractions by the chemical test**

Table 1 shows the results of phytochemical screening of hexane, chloroform, ethyl acetate, methanol, and aqueous crude extract. Qualitative assay of hexane, chloroform, ethyl acetate, methanol, and aqueous extract showed the presence of tannin, flavonoid, and glycoside. However, except aqueous steroid, terpenoid was present in all other crude extracts. On the other hand, saponin was detected in all crude extracts except hexane.
Table 1. Phytochemical screening of HE, CF, EA, ME, and AQ of Ocimum tenuiflorum leaves.

| No. | Test            | HE   | CF  | EA  | ME  | AQ  |
|-----|-----------------|------|-----|-----|-----|-----|
| 1   | Reducing sugar  | (+)  | (+) | (+) | (+) | (+) |
| 2   | Tannin          | (+)  | (+) | (+) | (+) | (+) |
| 3   | Steroid         | (+)  | (+) | (+) | (+) | (+) |
| 4   | Terpenoid       | (+)  | (+) | (+) | (+) | (+) |
| 5   | Flavonoid       | (+)  | (+) | (+) | (+) | (+) |
| 6   | Soluble starch  | (-)  | (-) | (-) | (-) | (-) |
| 7   | Saponin         | (-)  | (+) | (+) | (+) | (+) |
| 8   | Glycoside       | (+)  | (+) | (+) | (+) | (+) |

(+): detected.  
(-): not detected.

Hexane (HE), chloroform (CF), ethyl acetate (EA), methanol (ME), aqueous (AQ) crude extracts.

Table 2. Phytochemical screening of CF, BU, EA, ETH, and AQ.

| No. | Test            | CF  | BU  | EA  | ETH | AQ  |
|-----|-----------------|-----|-----|-----|-----|-----|
| 1   | Reducing sugar  | (+) | (+) | (+) | (+) | (-) |
| 2   | Tannin          | (+) | (+) | (+) | (+) | (+) |
| 3   | Steroid         | (+) | (-) | (+) | (-) | (-) |
| 4   | Terpenoid       | (+) | (+) | (+) | (+) | (+) |
| 5   | Flavonoid       | (+) | (+) | (+) | (+) | (+) |
| 6   | Soluble starch  | (-) | (-) | (-) | (-) | (-) |
| 7   | Saponin         | (+) | (+) | (+) | (+) | (+) |
| 8   | Glycoside       | (+) | (+) | (+) | (+) | (+) |

(+): detected.  
(-): not detected.

Chloroform (CF), butanol (BU), ethyl acetate (EA), ethanol (ETH), aqueous (AQ) fractions.

Table 2 and Table 4 displayed the results of chloroform, butanol, ethyl acetate, ethanol, and aqueous fractions consequently. Qualitative assay of chloroform, butanol, ethyl acetate, ethanol, and aqueous fractions showed the presence of tannins, flavonoid, saponin, and glycoside, whereas butanol, ethanol, and aqueous fractions did not show steroid. Furthermore, all the fractions showed the presence of terpenoid except the aqueous fraction.

Identification by liquid chromatography mass spectrometry (LCMS)

Table 3 shows compounds of the methanol crude extract, butanol, and ethyl acetate fractions of Ocimum tenuiflorum leaves. The compounds were found to consist of many constituents that may have contributed to its medicinal activity. The analysis of LC-MS identification of compounds was based on the quality of compounds matching with the library software by more than 90% similarity(>90%). The compounds identified in methanol crude extract and its fractions of Ocimum tenuiflorum leaves were phenolic, flavonoid, flavon, flavones, and isoflavone that are reported with high medicinal aspects. The phenolic compounds present in the crude extract and

| Sample | Retention Time (min) | Identified Compounds                        |
|--------|----------------------|---------------------------------------------|
| ME     | 29.4                 | 3,4-Dimethoxycinnamic acid (phenolic)       |
|        | 31.6                 | Kaempferol, luteolin (flavonoid)            |
|        | 32.0                 | Caffeic acid (phenolic)                     |
|        | 34.6                 | Peinidin (anthocyanidins), diosmetin (flavone glycoside), kaempferide (flavonol), chrysoeriol (flavan) |
|        | 36.4                 | Nevadensin (flavones, glycosides), pedunculin, xanthomicrol (flavonoid) |
|        | 37.2                 | 3.4,5-Trimethoxycinnamic acid               |
|        | 45.0                 | Di-n-butyl phthalate, dibutyl phthalate     |
|        | 54.7                 | Di (ethylhexyl) phthalate                   |
|        | 57.3                 | Glycolipid                                  |

(+): detected.  
(-): not detected.
its fractions of *Ocimum tenuiflorum* leaves were 3,4-dimethoxycinnamic acid, caffeic acid, permethrin, and rosmarinic acid; other compounds such as kaempferol, luteolin (flavonoid), kaempferide (flavonol), chrysoeriol (flavonoid), xanthomicrol (flavonoid), isosakuranetin (Flavanone) and robinetin trimethyl ether (flavonoids) were reported. Other compounds identified from *Ocimum tenuiflorum* leaves crude extract and its fractions such as peonidin, diosmetin and nevadensin were anthocyanidins, flavone glycoside, flavones, and glycosides consequently.

The novel compounds identified in *Ocimum tenuiflorum* leaves crude extract and its fractions for the first time were pedunculin, 3,4,5-trimethoxycinnamic acid, di(ethylhexyl) phthalate and 4,4′-methylene-bis(2-methyl aniline), isosakuranetin, and robinetin trimethyl ether frequently.

However, based on this study, it was found that chloroform, ethyl acetate, methanol, and aqueous extract of *Ocimum tenuiflorum* L. leaves had tannin, flavonoid, and glycoside content within. Meanwhile, the presence of steroid and terpenoid was not seen in aqueous extract. However, the presence of saponin was discovered in all crude extracts, with the exception of hexane. Apart from that, there was consistency between the results of this study and the previous study by Joshi et al.\[10\] The presence of tannins, flavonoid, saponin, and glycoside was shown through the qualitative essay regarding methanol fractions. However, ethanol and aqueous fractions of steroid were discovered in other fractions, with the exception of butanol. Additionally, the presence of terpenoid was not seen in the aqueous fraction. The results of *Ocimum sanctum*, which were reported in the previous study, covered the results from phytochemical identification of crude extracts and fractions of *Ocimum tenuiflorum* L. leaves.\[11\] Similarly, a few similar compounds such as flavonoid, tannin, glycoside, and terpenoids were reported from the other plant under the same group.\[12\]

The medicinal activity of the hydroalcoholic extracts of *Ocimum tenuiflorum* is the result of phytochemical constituents. Besides, although saponins are normally taken as antinutrients, they are also regarded to offer benefits in the human diet, specifically in the control of cholesterol. Therefore, it could be suggested that it poses a medicinal value. Additionally, the presence of saponins in traditional medicine preparation is evident.\[13,14\] Traditionally, tannins have been considered as antihemorrhoidal compounds. It is suggested from their presence in plants that they pose medicinal value due to their potential of antiviral,\[8\] antibacterial, and antiparasitic effects.\[11\] Based on other reports regarding rich flavonoid and ethanolic extract of *Ocimum sanctum*, its hypoglycaemic and hypolipidemic effects and its ability to increase insulin and glucose tolerance and peptide levels in diabetic rats have been shown.\[15\]

Meanwhile, flavonoids and other phenolics, such as hydroxycinnamic and hydroxybenzoic acid compounds, are secondary plant metabolites which fall under the most abundant groups of compounds. Furthermore, an important component of both human and animal diets is formed through these compounds.\[16\] Recently, these compounds have obtained much attention and they have been studied by many researchers due to their physiological functionality, such as biological functions.

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**Table 4. Phytochemical components identified in butanol (BU) and ethyl acetate (EA) fractions.**

| Sample | Retention Time (min) | Identified Compounds |
|--------|----------------------|----------------------|
| BU     | 9.0                  | Adenosine (purine nucleoside) |
|        | 23.4                 | Kaempferol-3-glucuronide |
|        | 23.7                 | Luteolin, kaempferol, isosakuranetin (lavanone) |
|        | 25.6                 | Baicalin (lavonoids) |
|        | 29.4                 | 3,4-Dimethoxycinnamic acid |
|        | 34.3                 | Apigenin, genistein (isoflavone) |
|        | 54.7                 | Permethrin |
| EA     | 24.7                 | Rosmarinic acid (phenolic) |
|        | 28.1                 | 4,4′-Methylene-bis(2-methyl aniline) |
|        | 31.7                 | Kaempferol, luteolin, isosakuranetin (flavanone) |
|        | 34.1                 | Apigenin, genistein |
|        | 34.7                 | Peonidin-3-O-alpha-arabinopyranoside, peonidin-3-O-beta-d-arabinopyranoside, peonidin-3-O-beta-galactopyranoside |
|        | 36.6                 | Robinetin trimethyl ether, (flavonoids), nevadensin, xanthomicrol, pedunculin |
Moreover, phenolic phytochemicals have the potential for the prevention of many chronic-oxidation-related diseases, such as diabetes and micro- and macro-cardiovascular diseases.

Based on what has been illustrated, identification of several phytochemicals that control blood glucose in diabetic rats was performed through the results of HPLC chromatograms and mass spectrum results for the methanol crude extract. The same case went for the low level of toxicity in the vital body organs of the STZ-induced rats. For example, some compounds such as 3,4-dimethoxycinnamic acid, caffeic acid, and kaempferol/luteolin have been reported for their impact on the reduction of blood glucose level in diabetic rats.\(^{17}\) Based on the evidence shown in other findings, it is possible that kaempferol is a naturally occurring anti-diabetic compound which offers protection for pancreatic beta-cell survival. Besides, it functions in a hostile environment which would otherwise result in Type 2 diabetes.\(^ {18}\) It has also been proposed from previous findings that the flavonoid fraction (MAF) and luteolin (0.5% w/w) are possibly beneficial for the acceleration of wound healing process amongst diabetic individuals. The possible reason is the free-radical scavenging activity of plants, in which antioxidant, anti-inflammatory, and neuron-protective activities take place in luteolin, a flavonoid separated from Cirsium japonicum.\(^ {19}\) It has also been suggested by researchers that besides the possibility of promoting insulin receptor tyrosyl phosphorylation, two phenolic acids could upregulate the expression of insulin signal-associated proteins. This includes glucose transporter-2, glycogen synthase, phosphatidylinositol-3 kinase, and insulin receptors. As an outcome of the effects of phenolic compounds, they lead to the increase in the consumption of glucose and the reinforcement of insulin resistance in cells. Examples of such compounds are cinnamic and caffeic acids, which are two phenolic acids commonly available in coffee, vegetables, and fruits.\(^ {20}\)

Our result was supported by the result of Collier et al.\(^ {21}\) Formerly, flavonoids, including flavones, flavonols, flavanones, isoflavones, and anthocyanidins, have been proposed to be effective supplements for the management and prevention of diabetes and cardiovascular disease. Recently, the results reported by Farrell et al.\(^ {22}\) indicated that 3,4-dimethoxycinnamic acid (dimethoxy cinnamic acid) in Robusta coffee beans showed a beneficial association between coffee intake and a reduced risk of Type 2 diabetes as well as colorectal cancer.\(^ {22}\) Research on the flavonol kaempferol reported by Zhang and Liu\(^ {18}\) found that kaempferol, which is a flavonol compound in some Chinese medicinal herbs, has cytoprotective effects on cultured clonal beta-cells and pancreatic human islets. These findings provide evidence that kaempferol may be a naturally occurring anti-diabetic compound by protecting pancreatic beta-cell survival that would lead to control Type 2 diabetes.\(^ {18}\)

Flavonoid compounds such as luteolin and apigenin were isolated from the ethanol extract of Martynia annua Linn. leaves, and the extract showed a wound-healing effect in streptozotocin-induced rats.\(^ {19}\) Among the other phenolic compounds, caffeic acid and cinnamic acid were commonly present in many fruits, vegetables, and coffee. The reported pharmacological properties of the caffeic acid include antioxidative, anticancer, and antimutagenic activities.\(^ {20}\) A previous study reported that the caffeic and cinnamic acids have anti-hyperglycaemic effects.\(^ {23,24}\) Among the most important anthocyanidins are pelargonidin, cyanidin, peonidin, delphinidin, and Maldivian. Previous studies of anthocyanidins have shown their effect in the prevention of cardiovascular and neurological diseases\(^ {25}\) as well as cancer and diabetes.\(^ {26,27}\) Several of these biological effects are related to the radical scavenging (antioxidant) properties of these compounds. In addition, multiple studies have evaluated the antioxidant properties of anthocyanidins (ArOH); however, the relationship of these properties and the chemical structure of anthocyanidins are not yet fully understood. Some reports suggested that an increase in the number of hydroxyl groups has a consequent increase in the antioxidant capacity of the flavonoids, but this is not always true. Interesting experimental data obtained by using oxygen radical capacity activity method (ORAC) indicated that delphinidin, which has three OH on the B ring, showed a lower antioxidant than pelargonidin, malvidin, cyanidin, and peonidin compounds with one or two hydroxyl groups on the same ring.

Diosmetin contributed to excellent α-amylase inhibitory activity than the standard because of its structural parameters. These molecular docking analyses of the selected compounds could lead to further development to find the potent α-amylase inhibitors for the treatment of diabetes.\(^ {28}\) Based on
the previous studies, the researcher found the synthesized form of compound from the group of alkylated flavonoids such as chrysin, diosmetin, apigenin, and luteolin, showed that their α-glucosidase inhibitory activity in diabetic rats.\cite{29} Recent research indicated that the chrysoeriol as a flavon, one of the major flavonoid classes, exhibited many activities such as anti-inflammation and antihistamine and anti-diabetic activities.\cite{30} Biological activities of nevadensin, which is identified from the seeds of Zanthoxylum alatum with antibacterial, antifungal, anthelmintic, anti-diabetic, antiproliferative, and insecticidal, have been reported.\cite{31} The seeds of Ocimum americanum Linn. extract show that nevadensin as a phytochemical compound indicated anti-diabetic activity, improved glucose tolerance was observed in diabetic patients who were given 30 g seed/day for 1 month, and lowering of fasting plasma glucose level up to 30% was also reported.\cite{32} The α-amylase inhibition property and antioxidant capacity of the major detected compounds such as caffeic acid, luteolin glucoside, xanthomicrol, and carvacrol from Origanum glandulosum were reported as a pharmacological effect.\cite{33}

In previous reports, researchers were reported that 3,4,5-trimethoxycinnamic acid as flavones showed antioxidant, anti-proliferative, antitumour, antimicrobial, oestrogenic, acetylcholinesterase, and anti-inflammatory activities, and was also used in treating cancer, cardiovascular disease, neurodegenerative disorders, etc., but we could not find any report on antidiabetic activity.\cite{34} Phthalates (di(ethylhexyl) phthalate) are widely used as a plasticizer and are considered as a typical endocrine-disrupting chemical.\cite{35} Epidemiological studies have associated serum or urinary phthalate metabolites with the prevalence of Type 2 diabetes or related phenotypes.\cite{36} However, the influence of phthalates on glucose homeostasis and atherosclerosis in hyperlipidemic mice was reported.\cite{37} Previous studies indicate that the synthetic glycolipid OCH can prevent insulin and diabetes in non-obese diabetic mice.\cite{38} However, till now, researchers have not reported anti-diabetic effect of di-n-butyl phthalate and pedunculin, 4,4′-methylene-bis(2-methylaniline), isosakuranetin (flavone), and robinetin trimethyl ether (flavonoids).

Regarding previous study, anti-inflammatory activity and improvement of kidney function of adenosine identified from butanol fraction of Ocimum tenuiflorum leaves were reported.\cite{39,40} Previous studies showed that the compounds such as baicalin (flavonoids) showed antihyperglycaemic effects on streptozotocin-nicotinamide-induced diabetic rats\cite{41} and α-glucosidase inhibitors\cite{42} and apigenin (isoflavone) α-glucosidase and amylase inhibitory activities and antioxidant effects.\cite{43,44} Genistein (isoflavone) was proposed as a promising compound for the treatment of metabolic disorders, obesity and Type 2 diabetes, oxidative stress, and the protective effects on pancreatic B-cells\cite{45} and α-glucosidase inhibitors.\cite{42} Effect ofpermethrin in the treatment of patients suffering from diabetes mellitus was reported.\cite{46} Diabetic nephropathy,\cite{47} blood glucose lowerin,\cite{48} and hypoglycaemia due to the high α-glucosidase inhibitory\cite{49} from rosmarinic acid (phenolic) were reported.

**Conclusion**

In the present study, kaempferol, luteolin, caffeic acid, 3,4-dimethoxycinnamic acid, and rosmarinic acid (phenolic) were considered as main compounds extracted from Ocimum tenuiflorum L. leaves by methanol crude extract, butanol, and ethyl acetate fractions as an antihyperglycaemic activity in streptozotocin-induced rats with single and different dose administrations in lowering blood glucose and controlling iabetes.

**Acknowledgments**

This work was funded by a grant of Universiti Sains Malaysia RU grants (1001/PTEKIND/6711526), which contributed to the funding of this research. The financial support of PhD Fellowship from University Sains Malaysia for author Leila Mousavi is gratefully acknowledged.
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