Food Research 2 (4) : 368 - 377 (August 2018)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Evaluation of *in vitro* sucrase Inhibitory and non-enzymatic glycation properties of *Ocimum tenuiflorum* L. leaves

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Article history:
Received: 11 March 2018
Received in revised form: 18 April 2018
Accepted: 20 April 2018
Available Online: 2 May 2018

Keywords:
Sucrase,
Advanced glycation end products,
Non-enzymatic glycation,
Diabetes mellitus,
Hyperglycemia

DOI:
https://doi.org/10.26656/fr.2017.2(4).076

Abstract

Different concentrations of aqueous and ethanolic extracts of *Ocimum tenuiflorum* L. leaves were evaluated for their sucrase inhibition and non-enzymatic glycation inhibitory effects. The results revealed that aqueous extracts, particularly the one from oven-dried leaves had significant inhibition potential for all three assays. The IC_{50} values for sucrase inhibition assay, BSA-glucose assay, and BSA-fructose assay were, 370.48±2.14 µg/ml, 188.19±2.52 µg/ml, and 166.16±1.99 µg/ml, respectively and the highest inhibition found in1250 µg/ml were, 91.88±0.16%, 118.46±0.27%, and 104.15±0.21%, respectively. Other than that, the aqueous extract of fresh leaves and 50% ethanol extract of oven dried leaves also showed good inhibition activity. Though, the results presented the highest inhibition activity for BSA-glucose assay. The leaves also attained 50% inhibition with the lowest concentration for BSA-fructose assay. The overall result of *O. tenuiflorum* leaves suggested that it can be used for treating diabetes mellitus and the complications associated with this.

1. Introduction

Diabetes mellitus (DM) and its complications are increasing with time in every part of the world (Tupe *et al.,* 2017). It is a metabolic disorder characterized by increased blood glucose level because of insulin production deficient or defects in insulin action, sometimes both (Singh *et al.,* 2016). With the onset of type IIDM it links with hyperglycemia, an unusual elevation of postprandial blood glucose level (Li *et al.,* 2014). It is crucial to control in the postprandial hyperglycemia in the early treatment of DM as it can cause different health complications (Ahmed *et al.,* 2009; Hu *et al.,* 2013). Different carbohydrate hydrolyzing enzymes such as α-amylase, α-glucosidase, and sucrase in the small intestine can reduce the absorption of glucose level and thus, postprandial hyperglycemia (Rhabasa-Lhoret and Chiasson, 2004). The postprandial hyperglycemia can be reduced by inhibiting the activity of carbohydrate hydrolyzing enzymes in the brush border of the small intestine. This will slow down the process of carbohydrate digestion and absorption. Hence, the rate of glucose absorption will be delayed. As a result, postprandial plasma glucose rise will be hindered (Mohamed *et al.,* 2012).

Prolonged hyperglycemia leads to failure and damage to multiple organs and develops secondary complications in diabetic patients (Kaewnarin and Rakariyatham, 2017). Acute complications can cause lipid and protein metabolic mutation and more persistent complications such as retinopathy, cataracts, atherosclerosis, neuropathy, aging and many more (Pashikanti *et al.,* 2010). The major reason for these complications is Advanced Glycation End Products (AGEs). The non-enzymatic glycation of protein and is an elementary cause of these secondary complications (Brownlee, 2001). Non-enzymatic protein glycation is occurred by the reaction between the carbonyl group of reducing sugars (glucose, fructose, and ribose) and the amino group of proteins. This is also known as Maillard reaction (Kaewnarin and Rakariyatham, 2017). A Schiff base is formed during the early stage which afterward rearranged through oxidative pathways to make more stable Amadori products (Brownlee, 2001). These amadori products go through different complicated reactions (dehydration, oxidation, cyclisation) and several highly Reactive Carbonyl Species (RCS) are formed. These reactive carbonyl compounds such as glyoxal, methylglyoxal (MGO) and 3-deoxy-glucosone react with amino groups of both extra-cellular and intra-cellular protein that forms fluorescent and non-fluorescent AGEs (Cervantes-Laurean *et al.,* 2006; Deve

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eISSN: 2550-2166

et al., 2014). The AGEs develop cross-links with long-lived proteins like collagens and alters their function (Hsieh *et al.,* 2007).

Inhibition of both the carbohydrate hydrolyzing enzyme and AGEs are greatly needed to avoid the early and the secondary complications induced by hyperglycemia (Peng, Zheng, Cheng *et al.,* 2008; Hu *et al.,* 2013). There are some popular inhibitors like acarbose, miglitol, and voglibose to inhibit the carbohydrate hydrolyzing enzymes like sucrase (Bailey, 2003). Acarbose has been used in treating the diabetic patients in recent times. It can also reduce complications like retinopathy and neuropathy but synthetic acarbose are often reported to cause gastrointestinal side effects as it can induce abdominal pain, flatulence or diarrhoea because of the bacterial fermentation of undigested carbohydrate in the colon (Derosa and Maffiloli, 2012; He *et al.,* 2014). Similarly, many synthetic drugs, for example, aminoguanidine, aspirin, taurine, vitamin B6 and quercetin have been reported in many research to reduce non-enzymatic glycation. Among all these drugs, aminoguanidine was reported as a specific inhibitor of AGE formation, cross-linking of protein and it can also inhibit accelerated diabetic retinopathy (Matsuura *et al.,* 2002). The disadvantage of using these drugs is, a very high dose required for the inhibition of which lead to various side effects including toxicity (Pripdeevech *et al.,* 2010).

The side effects of these synthetic drugs have lead researchers to look for natural alternatives. To treat the non-communicable chronic diseases, traditional and complementary medicines like herbal therapies are becoming popular with time (Tupe *et al.,* 2015). A recent concern has been arising is the use the bioactive compounds from plants to inhibit the formation of AGEs as well as reduce the activity of carbohydrate hydrolyzing enzymes (Peng, Cheng, Ma *et al.,* 2008). Different species of the genus *Ocimum* have shown strong effects such as antioxidant, anti-stress, antimicrobial, anticancer, antidiabetic and so many (Bano *et al.,* 2017). The species *O. tenuiflorum*, as well as the other species of *Ocimum* (*Ocimum basillicum, Ocimum americanum, Ocimum gratissimum*), have been known for reducing the blood glucose level. It has been used as a remedy for DM and high cholesterol since ancient times (Kaewnarin and Rakariyatham, 2017). The reports of significant antioxidant property of this plant have made it a potential natural source to inhibit the AGEs because oxidation stress in hyperglycemia induces free radicals by glucose auto-oxidation and interruption of electron transport chain with also assists in the formation of AGEs (Naowaboot *et al.,* 2009; Nahak *et al.,* 2011; Deo *et al.,* 2013). As for the inhibitory property of *O. tenuiflorum* against the non-enzymatic glycation, this area still has a lot to explore. There are only a few works that have been done on this property of the plant as well as its sucrase inhibitory effect.

This research mainly focuses on the carbohydrate hydrolyzing enzyme inhibitory and non-enzymatic glycation inhibitory properties of different extracts of *O. tenuiflorum* leaves by surase inhibition assay and BSA-glucose and BSA-fructose assay.

2. Materials and methods

2.1 Plant materials

Fresh *O. tenuiflorum* plants were collected from Kuala Kurau, Perak. The authentication of the leaves was done at Universiti Sains Malaysia (voucher specimen number 11400). Leaves with no physical damages and proper maturity were chosen as sample.

2.2 Sample preparation and extraction

The fresh leaves from the *O. tenuiflorum* plants (2.6 kg) were separated and washed thoroughly with water to remove any dust particles or other foreign materials. Then, the extra water on the leaf surface was removed by drying in open air at room temperature of 28°C. The leaves were divided into two equal batches. One batch of the leaves was dried in the oven overnight at 55^{o}C and then pulverized and prepared for solvent extraction (Hannan *et al.,* 2006; Basu *et al.,* 2013). Both the raw and oven-dried leaves were extracted with water and different strengths of ethanol (50%, 75%, and 100%) were used. For water extraction, the oven-dried and raw leaves were soaked in water overnight at room temperature (28°C). Then, the supernatant was collected and centrifuged at 3000 rpm to remove any remaining solid particle. The final supernatant was collected and freeze-dried to make lyophilized powder. For the ethanol extraction, both raw and oven-dried leaves were soaked in different strengths of ethanol for 48 hours. Then, the extracts were filtered, and the filtrates were kept in a water bath at 60^{o}C for three days to remove the excess amount of solvent and obtained the desired samples. All the samples were stored in the refrigerator for future use.

2.3 Analysis of antihyperglycemic activity

2.3.1 Sucrase inhibition assay

Inhibitory effect of sucrase was determined with slight modifications of the method of a previous study (Ahmed *et al.,* 2009). Enzyme (sucrase), sucrose and different concentration of sample solutions (250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml, 1250 µg/ml), respectively were prepared using Phosphate Buffer

Saline (PBS). The enzyme solution of 10µl and varying concentration of samples were placed in glass test tubes followed by incubation at 37°C for 10 min. After that, the volume was made up to 200 µl by malate buffer (pH 6.0) and 100 µl of 60 mM sucrose was added to the tube. The tubes were again left for incubation at 37°C for another 30 min. After completing the incubation 200 µl of DNS reagent was added to each tube. The reaction was terminated by putting the tubes in boiling water bath for 10 min. The absorbance of the samples was read at UV-Vis spectrophotometer at a wavelength of 540 nm. Inhibition of sucrase was calculated according to the following formula:

$$\%\text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.3.2 Non-enzymatic glycation assay by BSA-glucose model

Antiglycation assay had been done to measure the percent of inhibition of Advanced Glycation End (AGE) products. In all experiments, the final reaction volume was 1 ml and the reactions were performed in 1.5 ml micro-centrifuge tubes. A 500 µl of BSA (1 mg/ml) was incubated with 400 µl of glucose (400 mM) in the presence of 100 µl of different concentration of extracts (250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml, 1250 µg/ml), arbutin (positive control), or PBS as the control buffer at the specified concentration. The reaction could proceed at 60°C for 24 h. The reaction mixture was allowed to cool at room temperature, and the reaction was stopped by adding 10 µl of 100% (w/v) trichloroacetic acid (TCA). The TCA-added mixture was kept at 4°C for 10 min before centrifugation at 13000 rpm. The precipitate was redissolved with 4 ml PBS (pH 7.2), and the relative amount of glycated bovine serum albumin (BSA) was immediately determined based on fluorescence intensity by use of a cary-eclipse spectrofluorometer. The excitation and emission wavelengths used were at 370 and 440 nm, respectively (Chen *et al.,* 2011). Results were expressed as percentage inhibition of formation of the glycated protein. Inhibition of AGE's was analysed according to the formula:

$$\%\text{ Inhibition} = \left[1 - \left(\frac{\text{Fluoroscence of sample} - \text{Fthe luoroscence of sample negative}}{\text{fluoroscenceofcontrol} - \text{fluoroscenceofcontrol blank}}\right)\right] \times 100$$

2.3.3 Non-enzymatic glycation assay by BSA-fructose model

The assay was done with slight modifications of a previous study (Meenatchi *et al.,* 2017) which was also like non-enzymatic glycation by BSA-glucose model. Just for the incubation of BSA, approximately 400 µl of fructose (400 mM) was used instead of glucose. The relative amount of glycated bovine serum albumin (BSA) was determined based on fluorescence intensity by use of a cary-eclipse spectrofluorometer. The excitation and emission wavelengths used were at 370 and 440 nm, respectively. Results were expressed as percentage inhibition of formation of the glycated protein. Inhibition of AGE's was analysed according to the formula:

$$\%\text{ Inhibition} = \left[1 - \left(\frac{\text{Fluoroscence of sample} - \text{Fluoroscence of sample negative}}{\text{fluoroscenceofcontrol} - \text{fluoroscenceofcontrol blank}}\right)\right] \times 100$$

2.3.4 Statistical analysis

Each experiment was performed in triplicate for every concentration of all the samples. The data were expressed as mean±SD (Standard Deviation of the mean). Statistical analysis was performed for ANOVA (analysis of variance) followed by Duncan's test with SPSS version 20.0. Values of $p \leq 0.05$ were considered as significant.

3. Results

Eight different extracts of *Ocimum tenuiflorum* L. leaves were evaluated for its inhibitory properties by sucrase inhibition assay and non-enzymatic glycation (BSA-glucose and BSA-fructose model) assay. The aqueous and the three different ethanol extracts of both fresh and oven-dried leaves Ethanol:water (50:50), Ethanol:water (75:25) and Ethanol:water (100:0) were prepared in five different concentration: 250 µg/ml; 500 µg/ml; 750 µg/ml; 1000 µg/ml; and 1250 µg/ml. The best sample had been selected by their ability of inhibition in all three assays.

3.1 Sucrase inhibition assay

The inhibition of sucrase by different concentrations of sample extracts is presented in Figure 1. According to the result, the oven-dried aqueous leaf extract of 1250 µg/ml showed the strongest inhibitory effect against sucrase which is 91.88±0.16%. Followed by, fresh aqueous leaf extract of same concentration and oven-dried aqueous leaf extract of 1000 µg/ml, which were respectively 84.44±0.03% and 81.30±0.04%. The percentages were higher than that of the reference standard acarbose which was 82.58±0.07 and 75.06±0.00, respectively. The oven-dried aqueous leaf extract had demonstrated better result than the other extracts for all concentrations. Among all extracts, the 100% ethanol extract of oven-dried leaves had shown a minimum percentage of inhibition compared to the other extracts. The highest percentage of inhibition was 65.64±0.00% for 1250 µg/ml, and the lowest was 19.23±0.64% for 250 µg/ml, which were much lower than both acarbose and oven-dried aqueous leaves. All the sample extracts had shown a gradual increase in the inhibition with their concentration except for the concentration of 750 µg/ml and 1000 µg/ml which had

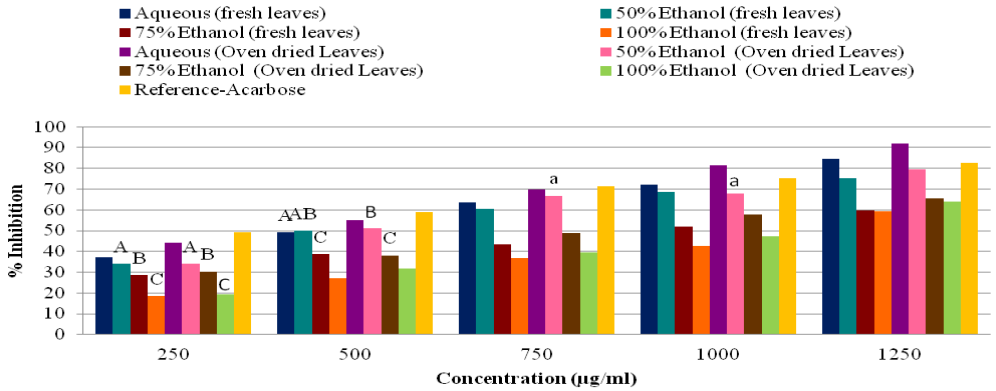

Figure 1. Sucrase inhibition assay. Values are expressed as mean±SD. Values followed by same capital letters in same concentration had no significant difference and values followed by same small letters in different concentration had no significant difference.

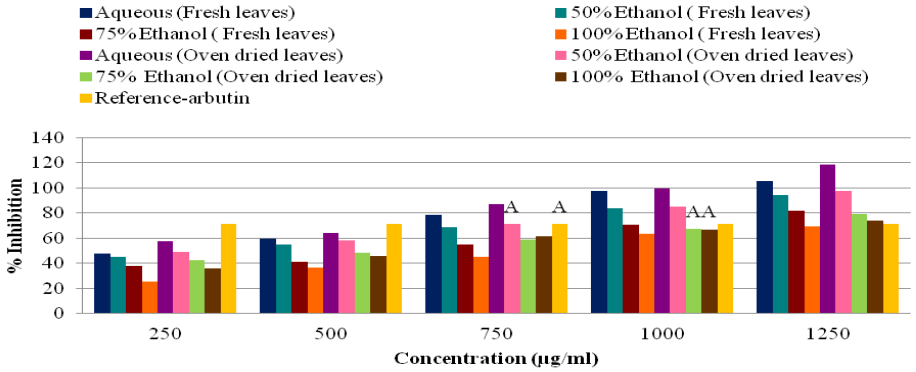

Figure 2. Non-enzymatic glycation by BSA-glucose model. Values are expressed as mean±SD. Values followed by same letters in same concentration have no significant difference.

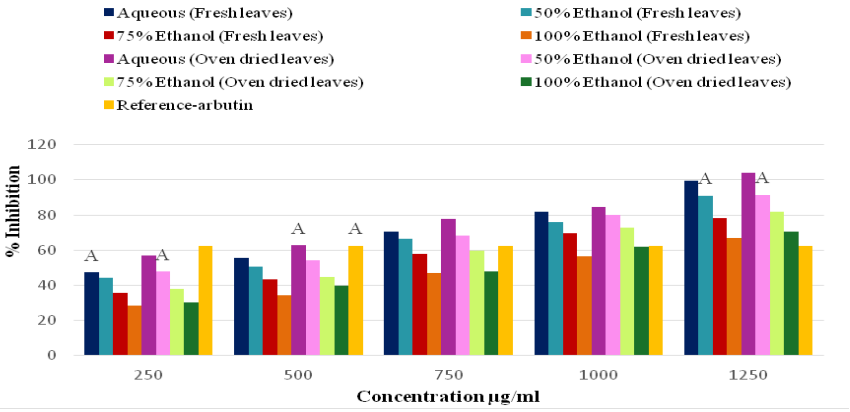

Figure 3. Non-enzymatic glycation by BSA-fructose model. Values are expressed as mean±SD. Values followed by same letters in same concentration have no significant difference.

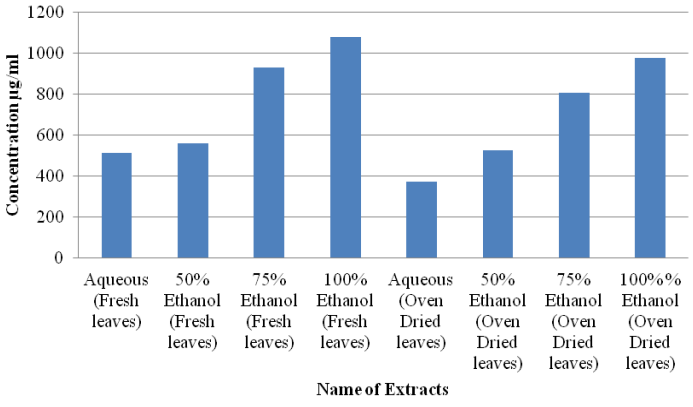

Figure 4. IC_{50} values for sucrase inhibition assay. Values are expressed as mean±SD.

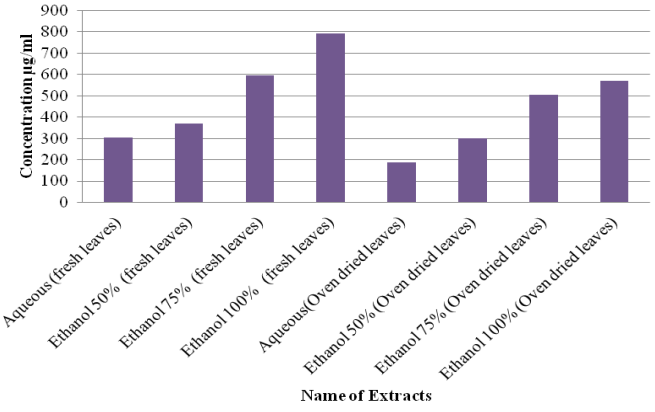

Figure 5. IC_{50} values for Non- enzymatic glycation assay by BSA-glucose model. Values are expressed as mean±SD.

no significant differences ($p>0.05$). The highest inhibition percentage for 50%, 75% and 100% ethanolic oven-dried leaf extracts were 79.67±0.00%, 65.64±0.00% and 63.91±0.06%respectively, in 1250 µg/ml. For fresh leaves, the results were 75.06±0.00%, 59.70±0.13%, and 59.38±0.04%, respectively for the same concentration. All the samples exhibited low percentage of inhibition for the concentration of 250 µg/ml for both oven-dried and fresh leaves, as 50% and 75% ethanolic extracts had inhibition of 33.97±1.2%, 30.34±0.37%, 33.97±0.64% and 28.63±0.98%, respectively. Though there are significant differences in all the samples, some samples had shown similar inhibitory potentials. For 250 µg/ml concentration, all the ethanol extracts for both fresh and oven-dried leaves had no significant differences ($p>0.05$) in their inhibitory potential. The IC_{50} value for oven-dried aqueous leaves was 370.48±2.14 µg/ml (Figure 4). Furthermore, for fresh aqueous leaf extracts, it was 511.50±0.50 µg/ml. For fresh leaves with 50%, 75% and 100% ethanolic extracts the IC_{50} values were 561.48±7.22 µg/ml, 928.33±9.82 µg/ml, 1080.95±6.45 µg/ml, respectively and for oven-dried 50%, 75% and 100% ethanolic leaf extracts, 524.95±12.69 µg/ml, 808.57±13.21 µg/ml and 927.96±8.45 µg/ml respectively.

3.2 Non-enzymatic glycation assay by BSA-glucose model

Figure 2 represents the results for inhibition of AGEs by non-enzymatic glycation. BSA-glucose model was used for this assay and the results showed the inhibition percentages of the glycated protein of different *O. tenuiflorum* leaf extracts. The aqueous extracts of oven-dried leaves were found to have the strongest inhibitory effect against AGEs with 118.46±0.27% for 1250 µg/ml. For the aqueous extract of fresh leaves, the inhibition was 105.45±0.40% for the same concentration. All sample extracts had a stronger inhibitory effect on AGEs in 1250 µg/ml concentration than the reference standard arbutin, which was found to be 71.29±0.00% for all concentrations of sample extracts. Arbutin is a well-known drug with good antiglycation properties and used by other researchers in previous studies (Perera *et al.,* 2014). For 1000 µg/ml, only the aqueous and 50% ethanolic extract of both fresh and oven-dried leaves had better inhibition than arbutin which were 97.71±0.15%, 99.20±0.26%, 83.55±0.40% and 84.96±0.26%, respectively. Other than that, aqueous leaf extracts of 750 µg/ml of both fresh and oven-dried leaves had demonstrated better inhibitory properties than arbutin which were 78.18±0.40% and 87.07±0.26%, respectively. The 250 µg/ml and 500 µg/ml concentrations for all the sample extracts had lower inhibition percentages than the reference standard. All the samples had a continuous increase in the inhibition with the increase in concentration. Among all the samples, the oven-dried aqueous leaves had the best effect on inhibiting AGEs with an IC_{50} value (Figure 5) of 188.19±2.52 µg/ml which was lower than all the other samples. It could mean that it can obtain 50% inhibition at the lowest concentration. The aqueous extract with fresh leaves had an IC_{50} value of 302.66±1.25 µg/ml. All the ethanolic extracts had higher IC_{50} values than both the aqueous extract except 50% ethanol extract of oven-dried leaves which is lower than fresh aqueous leaf extract with 300.65±3.16 µg/ml. Though, all the results clearly showed that 50% inhibition can be obtained with much lower concentration than 1250 µg/ml.

3.3 Non-enzymatic glycation assay by BSA-Fructose

The results for non-enzymatic glycation by BSA-fructose model is presented in Figure 3. The figure shows the inhibition properties of all the sample extracts. The reference standard had also been evaluated with arbutin**.** In accordance with the other two assays, oven-dried aqueous leaves extract had exhibited the strongest potential for inhibiting glycated protein. The highest inhibition of oven-dried aqueous leaves was 104.15±0.21% for 1250 µg/ml and it was 57.10±0.32% for 250 µg/ml. The results were better than all other sample extracts for the same concentration. Other than this, the fresh leaves aqueous extract and 50% ethanolic extracts for both fresh and oven-dried leaves showed fair percentages of inhibition. All the samples, except 100% ethanol extract of fresh leaves for 1250 µg/ml, showed better inhibitory potential than arbutin which was 69.53±0.00%. For both the 100% ethanol extracts, the results were comparatively poor than the other samples. As well as the BSA-glucose model, a decrease in the inhibitory property had been observed with increasing amount of ethanol. There also had been an increase in inhibition with increasing concentrations in the entire sample. But, for some sample concentrations, no significant difference ($p>0.05$) was observed in the inhibition. For example, aqueous extract of fresh leaves and 50% ethanol extract of oven-dried leaves showed a similar percentage of inhibition at 250 µg/ml. The 75% ethanolic extract of fresh leaves had similar inhibition potential as arbutin with 69.78±0.32 % of inhibition. For 250 µg/ml and 500 µg/ml, all the samples had lower inhibition percentage than arbutin. The IC_{50} values for this assay have been also presented in Figure 6. In Figure 6, the IC_{50} value for oven-dried aqueous leaves extract is 163.16±1.99 µg/ml which is much lower than the lowest selected concentration 250 µg/ml. The IC_{50} value for aqueous extracts of fresh leaves was 350.23±3.66 µg/ml which is nearly similar to 50% ethanol extract of oven-dried leaves value for IC_{50} of 345.90±11. The highest

IC_{50} value is for 100% ethanolic extract of fresh leaves which is 833.34±4.83 µg/ml which also had the lowest inhibition percentage. Observing the IC_{50} values it can be said that, all the samples attained 50% inhibition level with much lower concentration than the highest one used for this assay especially, oven-dried aqueous leaves extract.

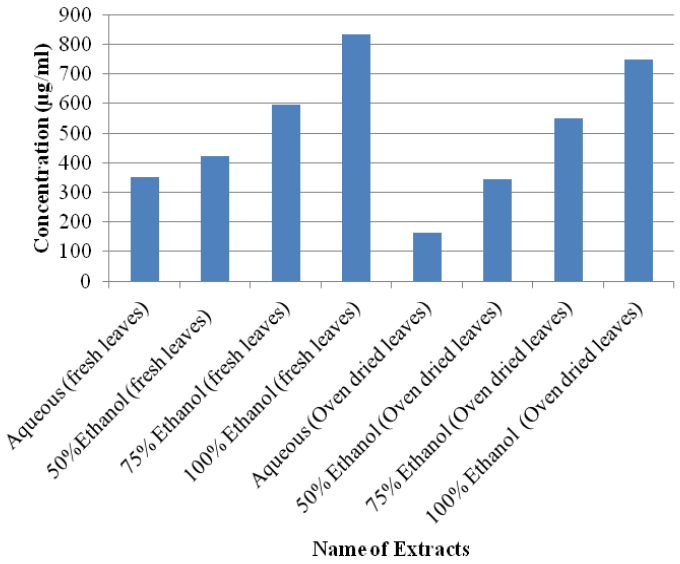

Figure 6. IC_{50} values for Non- enzymatic glycation assay by BSA-fructose model. Values are expressed as mean±SD.

All these three assays which were performed to evaluate the different extracts of *O. tenuiflorum* leaves had reported that aqueous extract of both oven-dried and fresh leaves had strong potential to contribute in reducing both early and secondary complications in type two DM followed by 50% ethanolic extract of oven-dried leaves and fresh leaves respectively. The other extracts had moderate inhibition percentages compared with these.

4. Discussion

The antioxidant potential of *O. tenuiflorum* plants had played the key role in its antihyperglycemic activities. The antioxidant properties of a plant depend on the amount of different phenolic components present in it (Inampudi *et al.,* 2010). There are several reports that suggested that phenolic compounds have antidiabetic activities (Gandhi *et al.,* 2011; Abdallah *et al.,* 2011; You *et al.,* 2012; Hu *et al.,* 2013). *O. tenuiflorum* plants had been recognised for their strong antioxidant effects as they had proved their potential in both in-vitro and in-vivo studies (Kath and Gupta, 2006). Therefore, it can be assumed that this property had likely been contributed towards its sucrase and AGE inhibition in this study.

Sucrase is one of the key enzymes in brush borders of the small intestine which involves in starch breakdown and intestinal glucose absorption. These enzymes catalyze the hydrolysis of sucrose to glucose and fructose. Inhibition of sucrase can reduce the rate of glucose absorption in the blood (Li *et al.,* 2014). Both aqueous extracts of leaves showed stronger inhibitory properties than other against sucrase. This inhibitory property could be related to their total phenolic components. Aqueous extract of *O. tenuiflorum* leaves had been reported to have a high amount of phenolic components than hexane, chloroform, ethyl acetate and methanol extracts (Shetty *et al.,* 2008; Mahomoodally *et al.,* 2012; Mousavi *et al.,* 2016).The presence of these phenolic compounds such as eugenols, rosmarinic acids, luteolin, apigenin, glucosides, saponins etc. could be thought as the possible reason for their sucrase inhibitory property (Singab *et al.,* 2014; Verma, 2016; Kaewnarin and Rakariyatham, 2017). Among these, eugenol had been used for reducing glucose metabolism (Srinivasan *et al.,* 2014). A report had been found for *Ocimum basillicum*, another species from *Ocimum* genus had shown significant inhibiting effects with aqueous extracts against sucrase and malatase due to the presence of phenolic compounds and flavonoid (El-Beshbishy and Bahashwan, 2012). This can further suggest that the aqueous extract of *O. tenuiflorum* had already been reported to have α-amylase and α-glucosidase inhibitory effects which are also carbohydrate hydrolyzing enzymes (Sudha *et al.,* 2011; Rege and Chowdhary, 2014). So, it can be said that for sucrase, it had followed a similar pathway. Researchers also found that both the aqueous and ethanol extracts inhibited the enzyme more potently than acarbose (Rege and Chowdhary, 2014) which also partially relates with our study as both the aqueous extracts were able to show inhibition more than acarbose in the higher concentrations. There were a few studies that reported a significant amount of polyphenols present in water-alcohol fractions of *O. tenuiflorum* leaves (Wangcharoen and Morasuk, 2007) which is similar to our study but the inhibition percentages are less than the aqueous extracts. The possible reason for this phenomenon could be the presence of a lower amount of polyphenols in those extracts.

Our study showed the inhibition of AGEs by measuring the inhibition percentages of glycated BSA with both BSA-Glucose and fructose model. Most of the studies were done for non-enzymatic glycation followed an incubation period of 3-4 weeks with 37°C before addition of TCA (Adisakwattana *et al.,* 2012; Ramkissoon *et al.,* 2013; Adisakwattana *et al.,* 2014) which made this assay a relatively time-consuming one. The increase of temperature can reduce this time such as increasing the incubation temperature to 60°C the incubation time reduced to 48 hours (Matsuura *et al.,* 2002). In our experiments with both glucose and

fructose, we followed an incubation period of 24 h at 60° C which was able to form glycated BSA. Both two assays showed a higher amount of inhibition for glycated BSA for the aqueous extracts of oven-dried and fresh leaves, and the 50% ethanol extract of oven-dried leaves than the other samples. When it comes to the formation of AGEs, oxygen free radicals such as ROS plays an important part in the glycation of protein by lipid peroxidation. When these free radicals join the glycation process, they help in the formation of AGEs (Sabu and Kuttan, 2002). Thus, reducing the formation of these free radicals could possibly contribute to the inhibition of AGEs. Antioxidants such as polyphenols, flavonoids, saponins etc. had been known to reduce the number of free radicals as reported in several studies (Nikolova, 2011; Gupta *et al.,* 2012). There are several plants with high antioxidant potential which has delivered a significant inhibition against the non-enzymatic glycation (Naowaboot *et al.,* 2009; Ramkissoon *et al.,* 2013). As reported earlier, aqueous extracts were found to be high in polyphenols and flavonoids, we can assume this to be the primary reason for its inhibition of glycated BSA. Some reports have suggested that sesquiterpenes and eugenol methyl ether had demonstrated antiglycation activities and these two are found in *O. tenuiflorum.* Thus, we can say they contribute to the leaves inhibition activity against glycated BSA (Singh *et al.,* 2016; Hafizur *et al.,* 2017). Another contributing factor could be the presence of saponin in hydro-alcoholic extracts of leaves of *O. tenuiflorum* (Devendran and Balasubramanian, 2011). Saponins had been reported to have antiglycation activity previously (Xi *et al.,* 2008, Chen *et al.,* 2011). A significant amount of saponin, eugenol and other phytochemical constituents which are responsible for antiglycation activity could have been present in the aqueous extracts also which justified our obtained results. There are not many studies conducted on the antiglycation potential of *O. tenuiflorum* plants. However, there were a few reports that suggested the potentiality of *O. tenuiflorum* having the antiglycation property. This indicates that plant phytochemical constituents are responsible for such activity (Mahomoodally *et al.,* 2012; Tube *et al.,* 2017).

5. Conclusion

Our present work evaluated the aqueous and ethanolic extracts of *O. tenuiflorum* leaves for its potential role to reduce diabetic complications by inhibiting sucrase and glycation of protein. The leaves had exerted a significant effect in its aqueous extracts and 50% ethanolic extracts. Some moderate inhibition activities were also observed for other extracts. As diabetic patients are increasing with time in the world, preliminary and secondary diabetic complication are causing chronic health issues among the patients. Various medicines are being used for treating these problems but that comes with some side effects which are unwanted. If the leaves of this plant could be used successfully and effectively, there are possibilities that reduction of diabetic complications can be achieved and possibly without side-effects.

Conflicts of Interest

We declare that we have no conflict of interest with this presented work.

Acknowledgment

The research has been financially supported by Research University (RU) with grant 1001/ PTEKIND/812176. We are thankful for their financial support towards the research.

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eISSN: 2550-2166