Original Article

Exploring the potential of Mangifera indica leaves extract versus mangiferin for therapeutic application

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A B S T R A C T

There has been an alarming increase in the prevalence of diabetes in India and worldwide. As an alternative medicine to treat diabetes mellitus, many herbal extracts and bioactives are being assessed all over the world. One of the strategic approaches for diabetes management is to control gastrointestinal glucose production and its absorption into the blood stream. The purpose of the study was to evaluate the anti-diabetic potential of aqueous extract of Mangifera indica leaves (mango leaves) and to compare it with its main biomolecule (mangiferin). An in vitro method using carbohydrate metabolism (α-amylase and α-glucosidase inhibition) was performed. The concentrations required for 90% inhibition of enzymes activity were determined to investigate the relative potency of the extract and mangiferin. The results showed that mangiferin strongly inhibited both α-amylase and α-glucosidase activity, with much higher potency than Acarbose and mango leaves extract, while mango leaves extract showed higher anti-oxidant activity compared to mangiferin. The results also showed that mango leaves extract and mangiferin have a bright future in the therapy of diabetes mellitus. Such a comparison helps to evaluate whether the active bio-molecules are playing a role or if the whole extract is required for the management of a disease. This would be beneficial in designing a proper process for utilization of natural medicines.

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Introduction

Diabetes Type 2 (diabetes mellitus) is a chronic endocrine disorder which occurs when the pancreas is not able to produce insulin efficiently, thereby leading to failure in monitoring the glucose uptake by body cells and in the long run leading to hyperglycemia. It is usually associated with disturbances in carbohydrate, fat and protein metabolisms resulting from a lack of insulin secretion (American Diabetes Association, 2009). It has become a concern these days as it is evident in many people in India and reports state that it is expected to affect 300 million by 2025 with India likely to have the largest number of diabetic cases (Sutha et al., 2011; Obied et al., 2005). There are several drugs prevalent in the market for the treatment of Type 2 diabetes. Some of the drugs used belong to the classes of biguanides, sulfonylureas and glinides, which in the long term, have led to side effects such as nausea, abdominal pain, abnormal weight gain, allergic reaction, low blood glucose, dark urine, fluid retention and complications in pregnancy (Anbu et al., 2012). Considering these severe side effects of the synthetic drugs, the use of natural products for treating diabetes and other diseases is becoming popular, since drugs with herbal origin have no side effects, there are subject to increasing interest, with various bioactives derived from medicinal plants being screened in developing many of the traditional and modern medicines (Tyler, 1999; Taylor, 2000; Donald, 2000). Despite the growing interest in the use of natural medicine, there are many plants that are still unexploited properly. The present study deals with one the least-exploited plants, Mangifera indica leaves with mangiferin being its major constituent. Mangifera indica leaves were chosen as India is one of the largest mango producing countries. Moreover, mango leaves are used for holy and cultural purposes (Pariona, 2018). Hence, it was considered useful to identify a means of using these leaves in a proper and beneficial way. There are reports on the use of medicinal plants for the management of Diabetes Type 2 (Jayaprasad et al., 2012; Rao et al., 2010; Nair et al., 2013; Girón et al., 2009). However, to date, little is known about the applicability of mango leaves in treating various diseases (Yoshikawa et al., 2001; Bhuvaneswari, 2013; Sellamuthu et al., 2013; Mujawdia and Suman, 2015). There are reports on anti-diabetic, anti-oxidant, anti-tumor and anti-viral properties of mangiferin (Guha et al., 1996; Miura et al., 2001). Bhuvaneshwari

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et al. (2014) reported similar studies focusing on the activities of tender and mature leaves. Similarly, Malherbe et al. (2014) reported on the anti-oxidant activities of mangiferin from *Cyclopia genis-toides*. There are many such reports published on the anti-oxidant capacity of mangiferin or mango leaves but these researchers did not compare the anti-oxidant capabilities of mangiferin and mango leaves. Furthermore, the researchers extracted the mangiferin in organic solvent and then used it as an anti-diabetic agent. Solvent-extracted mangiferin not only increases the cost but makes it less acceptable in the more stringent markets such as pharmaceuticals, food or cosmetics. Hence, this work deals with the water-based extraction of mangiferin and its use as anti-diabetic agent in pharmaceuticals. Moreover, there was no evidence in the literature of any report that provides information about the comparison and efficacy of mangiferin with mango leaves in fighting diabetes. This comparison is much needed as it would provide knowledge about the basis of the treatment and whether the active ingredient (mangiferin) is responsible for the desired activity or if the crude extract is playing a major role. This would also help in designing a suitable downstream process along with proper documentation. Hence, the anti-diabetic potential of *Mangifera indica* and mangiferin was assessed for their efficacy in the treatment of Diabetes Type 2. This was achieved by doing in vitro tests for inhibition of α-amylase and glucosidase enzymes. Pancreatic α-amylase plays an active role in catalyzing the initial reaction in the hydrolysis of carbohydrates to smaller oligo or disaccharides. These are then acted on by α-glucosidases and further converted to glucose, which on absorption enters the blood-stream. (Eichler et al., 1984). Therefore, it is important to inhibit or restrict these key enzymes in order to keep the glucose levels under control. Apart from these enzymes, oxidative stress is also responsible in the development of diabetes complications: due to glucose oxidation and oxidative degradation of glycated proteins, large numbers of free radicals are formed during diabetes (Mehta et al., 2006). Therefore, the present study also considered an evaluation of the anti-oxidant capacity of the mangiferin and mango leaves extract.

**Materials and methods**

**Plant collection and crystallization method**

*Mangifera indica* leaves were collected from the Institute of Chemical Technology, Mumbai, India. The leaves were washed, sun-dried for 48 h and then powdered to a particle size of approximately 1–2 mm. A moisture content of the powder was around 9%. Different concentrations ranging from 100 to 500 μg/mL of crude aqueous extract were prepared. Mangiferin (10–500 μg/mL concentrations) was prepared and purified using crystallization according to Bhuvaneswari (2013), with modifications. For crystallization, the powdered plant materials were defatted with petroleum ether (60–80 °C) and then extraction was performed. The extraction was carried out in a microwave glass vessel placed in a microwave oven. Powder of *Mangifera indica* leaves was added in the glass vessel along with water as a solvent for 7 min as per the method described by Kulkarni and Rathod (2016). Samples were withdrawn at specific time intervals, filtered and analyzed using high performance liquid chromatography. Other extraction conditions were: extraction time 5 min, solid-to-solvent ratio 1:20 and microwave power 272 W. Subsequently, the extract was concentrated by nanofiltration using a 150 Da membrane until the desired volume was achieved. Later, the concentrated extract was partitioned with dichloromethane at the ratio of 1:4 to remove the chlorophylls and other pigments. Then the aqueous phase was hydrolyzed by reflux with 2N sulfuric acid at pH 3 for 1 h with stirring at 100 revolutions per minute. After cooling the former mass to room temperature (30±2 °C), it was partitioned with ethyl acetate (1:3 ratio). Then, the ethyl acetate layer was dried using a vacuum rotary evaporator. The dried ethyl acetate fraction was dissolved in ethanol and stored at 4–8 °C overnight. After that, the precipitate was obtained, which was dissolved in 70% aqueous ethanolic solution and left in a refrigerator (4–8 °C) overnight. Finally, the pale-yellow, needle-shaped crystals of mangiferin were obtained. All experiments were performed in triplicate and the mean value was reported.

**Evaluation of anti-diabetic potential**

### a-amylose inhibitory activity

The α-amylase inhibitory activity of mango leaves extract and mangiferin was performed according to the standard method with certain modifications (Telagari and Hullatti, 2015). In a 96-well plate, a reaction mixture containing 50 μL phosphate buffer of 100 mM, pH = 6.8, 10 μL α-amylase (2 U/mL), and 20 μL of varying known concentrations of mango leaves extract and mangiferin were pre-incubated at 37 °C for 20 min. Then, the 20 μL of 1% soluble starch (100 mM phosphate buffer, pH 6.8) were added as a substrate and incubated further at 37 °C for 30 min; 100 μL of the DNS (2-hydroxy-3,5-dinitrobenzoic acid) color reagent was added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using a multiplate reader. Acarbose at various concentrations was used as a standard for comparison. Samples without the test (extract and fractions) substance were set up in parallel as a control and each experiment was performed in triplicate. The results were expressed as the percentage inhibition, which was calculated using Equation (1):

\[
\text{Inhibitory activity(%) = } (1 - \frac{A_{s}}{A_{c}}) \times 100
\]

where, As is the absorbance in the presence of the test substance and Ac is the absorbance of the control. The IC_{50} value, i.e. a measure of the concentration of the substance needed to inhibit 90%, of enzyme activity, was calculated using logarithmic regression and goal seek in the Excel 2013 software package, Microsoft Inc., Redlands, WA, USA.

### a-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of mango leaves extract and mangiferin was carried out according to the standard method with slight modifications (Telagari and Hullatti, 2015). In a 96-well plate, reaction mixture samples containing 50 μL phosphate buffer (100 mM, pH = 6.8), 10 μL α-glucosidase (1 U/mL), and 20 μL of varying known concentrations of mango leaves extract and mangiferin were pre-incubated at 37 °C for 15 min. Then, 20 μL P-NPG (5 mM) was added as a substrate and incubated at 37 °C for 20 min. The reaction was terminated by adding 50 μL Na₂CO₃ (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using the multiplate reader. Acarbose at various concentrations was used as a standard. Samples without the test substance were set up in parallel as a control and each experiment was performed in triplicate. The results were expressed as the percentage inhibition, which was calculated using Equation (2):

\[
\text{Inhibitory activity(%) = } (1 - \frac{A_{s}}{A_{c}}) \times 100
\]

where, As is the absorbance in the presence of the test substance and Ac is the absorbance of the control. Logarithmic regression was carried out on the values and the IC_{50} value was obtained using goal seek in the Excel 2013 software package, Microsoft Inc., Redlands, WA, USA.
Anti-oxidant activity

2,2-Diphenyl-1-picrylhydrazyl radical assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay was carried out according to the procedure described by Kulic et al. (2004) modified by Obied et al. (2005). The DPPH solution was prepared by dissolving 32 mg in 1 L of 80% methanol. Various concentrations of aqueous mango leaves extract and mangiferin along with standard gallic acid were prepared. Each test involved adding 3 mL of DPPH solution to a glass cuvette followed by the addition of 200 μL of sample. The mixture was shaken well and kept in the dark at room temperature for 1 h. Absorbance was measured at 517 nm using a spectrophotometer. The absorbance of 80% methanol was considered as the blank while the negative control (DPPH solution) was also run simultaneously.

The percentage inhibition was measured according to Equation (3):

\[
\text{Percentage inhibition(\%)} = \left( \frac{A_c - A_s}{A_c} \right) \times 100
\]

where \(A_c\) is the absorbance of the control and \(A_s\) is the absorbance of the sample. Logarithmic regression was undertaken on the values and the IC\(_{90}\) value was obtained using goal seek in the Excel 2013 software package, Microsoft Inc., Redlands, WA, USA.

Ferrous reducing anti-oxidant capacity assay

The ferrous reducing anti-oxidant capacity (FRAC) of samples was evaluated using the method of Oyaizu (1986). Different known concentrations of 0.25 mL samples per standard solution were added to 0.625 mL potassium buffer (0.2 M) and 0.625 mL 1% potassium ferricyanide, \([K_3Fe(CN)_{6}]\) solution. The reaction mixture was then incubated for 20 min at 50 °C to complete the reaction. Then, 0.625 mL of 10% trichloroacetic acid (TCA) solution were added to terminate the reaction. The total mixture was centrifuged at 3000 rpm for 10 min after which, 1.8 mL supernatant was withdrawn from the test tube and was mixed with 1.8 mL distilled water and 0.36 mL 0.1% ferric chloride (FeCl\(_3\)) solution. The absorbance of the solution was measured at 700 nm using a spectrophotometer against the blank. The blank solution contained the same solution without plant extracts/standard and it was incubated under the same conditions and the absorbance of the blank solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing capacity.

Total phenolic content and total flavonoid content assay

The total phenolic content (TPC) of aqueous mango leaves extract was determined using the Folin-Ciocalteu colorimetric method according to Li et al. (2008) and Wong et al. (2006) with slight modification. A sample of 1 mL of 50-times-diluted (volume per volume; v/v) aqueous mango leaves extract was mixed with 1 mL of 1:10 (v/v, in deionized water) diluted Folin-Ciocalteu reagent (FCR). After 4 min, 800 μL of sodium carbonate solution (7.5%, w/v) was added into the reaction mixture. Then, the mixture was again mixed for 5 s and stored in the dark for 2 h. A blank was also prepared by replacing the 1 mL of diluted aqueous mango leaves extract with 1 mL of deionized water. The absorbance of the mixture was measured at 765 nm against the blank using an ultraviolet light spectrometer. The total flavonoid content (TFC) of crude extract was estimated using the procedures described by Karadeniz et al. (2005) and Ozsoy et al. (2007). A sample of 1.25 mL of deionized water was added into 0.25 mL of aqueous mango leaves extract followed by the addition of 75 μL of 5% (weight per volume; w/v) sodium nitrite solution. The mixture was allowed to stand for 6 min and then 150 μL of 10% (w/v) aluminum chloride solution was added and further kept for another 5 min followed by addition of 0.5 mL of 1 M sodium hydroxide solution and 275 μL of deionized water. Subsequently, the mixture was mixed for 5 s and its absorbance was determined at 510 nm against the blank using the ultraviolet light spectrometer. A blank was prepared by replacing the 0.25 mL of undiluted crude extract with 0.25 mL of deionized water.

Results and discussion

Anti-diabetic activity

α-amylose inhibition assay

In this study, α-amylose inhibition assay of aqueous mango leaves extract and mangiferin was performed. The results obtained from α-amylose inhibition assay of aqueous mango leaves extract and mangiferin were compared with the Acarbose which was considered as a standard for α-amylose inhibition assay. The results of the study are shown in Fig. 1 which shows that mangiferin induced significant inhibition of α-amylose enzyme at concentrations 10 μg/mL, 25 μg/mL, 50 μg/mL with 91.3%, 95.1%, and 100%, respectively, α-amylose enzyme inhibition. Acarbose, used as a reference standard at the same concentrations of 10, 25, 50 μg/mL showed 24.6%, 28.9% and 44.7% inhibition of α-amylose activity.

Fig. 2 illustrates the comparison of aqueous mango leaves extract and Acarbose for α-amylose inhibition assay. It was observed that aqueous mango extract showed comparable inhibition of amylase enzyme compared to Acarbose. At 100 μg/mL, 250 μg/mL, and 500 μg/mL, mango leaves extract was able to inhibit amylase enzyme up to 64.5%, 86.5% and 96.2%, respectively. Furthermore, from Table 1, the IC\(_{90}\) values for α-amylose inhibition assay of aqueous mango leaves extract, mangiferin and Acarbose were 132.27 μg/mL, 8.64 μg/mL, and 363.21 μg/mL, respectively. Thus, the IC\(_{90}\) value for mangiferin was considerably lower than that in aqueous mango leaves extract and Acarbose. The lower IC\(_{90}\) value indicated that the drug is highly potent as it will require a lower concentration to achieve 90% inhibitory activity.

Dineshkumar et al. (2010) carried out α-amylose inhibition assay for Mangifera indica wherein they made use of the stem and bark of Mangifera indica and not the leaves. In addition, their preparation method of the crude extract was different from the current study as they used a maceration process and unlike the microwave assisted extraction in the current study. The current study indicated that aqueous mango leaves extract and mangiferin could significantly inhibit α-amylose enzyme. One of the important findings was that the standard drug Acarbose and aqueous mango leaves extract showed comparative inhibition of amylase enzyme compared to Acarbose. At 100 μg/mL, 250 μg/mL, and 500 μg/mL, mango leaves extract was able to inhibit amylase enzyme up to 64.5%, 86.5% and 96.2%, respectively. Furthermore, from Table 1, the IC\(_{90}\) values for α-amylose inhibition assay of aqueous mango leaves extract, mangiferin and Acarbose were 132.27 μg/mL, 8.64 μg/mL, and 363.21 μg/mL, respectively. Thus, the IC\(_{90}\) value for mangiferin was considerably lower than that in aqueous mango leaves extract and Acarbose. The lower IC\(_{90}\) value indicated that the drug is highly potent as it will require a lower concentration to achieve 90% inhibitory activity.

Fig. 1. α-Amylase inhibition assay of mangiferin and Acarbose.
required higher concentrations (nearly double) of mangiferin. Thus, it can be concluded that mangiferin is a potent inhibitor of α-amylase. However, the mechanism of mangiferin is not very clear regarding how and why it inhibits α-amylase and the α-glucosidase enzyme. It might be that mangiferin by acting as a competitive inhibitor of α-amylase and the α-glucosidase enzyme is able to control the carbohydrate breakdown. Furthermore, it has been reported that mangiferin stimulates the enzymes involved in glycolysis while slowing the rate of gluconeogenesis, thus helping to control the blood glucose level (Sellamuthu et al., 2013).

α-glucosidase inhibition assay

The α-glucosidase is present in the brush border of the small intestine. This enzyme is useful in catalyzing the breakdown of oligo or disaccharides into simple sugars and therefore, inhibitors of this enzyme can hold back the uptake of dietary carbohydrates and thus can control the blood glucose level (Hossain et al., 2008). Therefore, the efficiency of aqueous mango leaves extract and mangiferin in controlling this enzyme was evaluated. The results are shown in Fig. 3. At concentrations of 100 μg/mL, 250 μg/mL and 500 μg/mL, mango leaves extract was able to inhibit α-glucosidase up to 77.8%, 83.4% and 95.7%, respectively. However, as observed from Fig. 4, a lower concentration of mangiferin (10 μg/mL, 25 μg/mL or 50 μg/mL) was sufficient to inhibit α-glucosidase (up to 86.85%, 92.35% and 99.11%, respectively).

From Table 1, the IC₉₀ values for α-glucosidase inhibition assay of aqueous mango leaves extract, mangiferin and Acarbose were 345.79 μg/mL, 16.05 μg/mL, and 447.44 μg/mL, respectively, with the IC₉₀ value for mangiferin being significantly lower than that of aqueous mango leaves extract and Acarbose. The IC₉₀ values for both α-amylase inhibition assay and α-glucosidase inhibition assay show that mangiferin can be highly efficacious in the treatment of diabetes.

Table 1

<table>
<thead>
<tr>
<th>IC₉₀ Values (μg/mL)</th>
<th>Aqueous mango leaves extract</th>
<th>Mangiferin</th>
<th>Acarbose</th>
<th>Gallic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-amylase inhibition assay</td>
<td>132.27 ± 4.23</td>
<td>8.64 ± 0.12</td>
<td>363.21 ± 7.62</td>
<td>Not applicable</td>
</tr>
<tr>
<td>α-glucosidase inhibition assay</td>
<td>345.79 ± 6.22</td>
<td>16.05 ± 0.67</td>
<td>447.44 ± 3.13</td>
<td>Not applicable</td>
</tr>
<tr>
<td>DPPH assay</td>
<td>156.08 ± 2.03</td>
<td>Not applicable</td>
<td>Not applicable</td>
<td>189.29 ± 5.49</td>
</tr>
</tbody>
</table>

a The anti-oxidant activity was low at the selected concentration.

b IC₉₀ value is a measure of the concentration of the substance needed to inhibit 90%, of enzyme activity.

DPPH is 2,2-diphenyl-1-picrylhydrazyl radical assay

DPPH assay is based on the anti-oxidant ability of a compound of interest to combat with DPPH radical action generated in the assay system. DPPH is a protonated radical widely used as a scavenger for other radicals. DPPH has distinguished absorption...
maxima at 517 nm that decreases with the addition of the proton radical present in the plant extracts. These rate reductions of a chemical reaction form the basis for studying the anti-oxidant nature of a compound. Anti-oxidants are important as they inhibit free radical reactions and thus protect from cellular damage (Young and Woodside, 2001; Bagchi and Puri, 1998). A number of activities such as several biochemical reactions, generation of reactive oxygen species and reactive nitrogen species in the human body lead to oxidative stress that is particularly responsible for various disease states including Diabetes Type 2 (Kim and Byzova, 2014). The oxidative stress can be managed effectively by improving cellular defenses with the use of anti-oxidants (Pal and Nimse, 2006). Oxidative stress is one of the factors that lead to diabetes. Thus, ideally, an anti-diabetic drug should also have some anti-oxidant properties. Consequently, the anti-oxidant properties were inspected for the similar concentrations of mango leaves extract and mangiferin, which were used to test anti-diabetic activity. From Fig. 5, it is observed that at 100 μg/mL, 250 μg/mL and 500 μg/mL mango leaves extract inhibited around 89.4%, 90.49%, and 92.02%, respectively, while gallic acid used as a standard in DPPH at similar concentrations showed 87.94%, 91.51% and 93.20% inhibition, respectively. Fig. 6 illustrates that at 10 μg/mL, 25 μg/mL, and 50 μg/mL, mangiferin inhibited 4.92%, 19.86%, and 24.95%, respectively while gallic acid inhibited 49.40%, 78.26%, and 85.22%, respectively. Considering that the primary objective was to evaluate mangiferin for its anti-diabetic properties, it was an added advantage that mangiferin also shows some anti-oxidant property. In addition, as the anti-oxidant property of mango leaves extract was comparable to the standard, it can be concluded that there are constituents in mango leaves other than mangiferin which also provide anti-oxidant activity, perhaps because the compounds in mango extract act as electron donors and also react with free radicals and terminate the radical chain reaction. It has been proposed that the higher anti-oxidant activity is also due to the presence of numerous hydroxyl groups (Cao et al., 1997). As can be seen from Table 1, the IC₉₀ values for DPPH inhibition assay of aqueous mango leaves extract and gallic acid were 156.08 μg/mL and 189.29 μg/mL, respectively. The IC₉₀ value for aqueous mango leaves extract was lower than that in gallic acid.

Ferric reducing anti-oxidant power

The FRAP assay is used to determine the reduction of ferric (Fe³⁺) to ferrous (Fe²⁺) ions in the presence of anti-oxidants (Sonawane and Arya, 2013). Therefore, measuring the formation of Perl’s Prussian blue at 700 nm can monitor the Fe²⁺ concentration. In Figs. 7 and 8, the readings are plotted as absorbance versus concentration, where a higher absorbance indicates a higher reducing ability of a compound. The reducing ability in mangiferin at 10 μg/mL, 25 μg/mL and 50 μg/mL was 0.34, 0.41 and 0.53, respectively, while for gallic acid it was 0.14, 0.37 and 0.64, respectively, while in Fig. 8, the reducing ability in mango leaves extract at 100 μg/mL, 250 μg/mL and 500 μg/mL was 2.40 and 5.44, respectively and at the same concentration of gallic acid it was 1.20, 2.94 and 6.91, respectively. Thus, the reducing ability of mangiferin and mango leaves was comparable with the standard, giving them an added advantage in effectiveness as an anti-diabetic drug.
Total phenolic content and total flavanoid content

Flavonoids and phenols present in the plants also contribute to anti-oxidant activity; it has been reported that the ability of phenolic compounds to donate hydrogen helps in the termination of radical chain reactions and thus gives them anti-oxidant activity (Baumann et al., 1980). It is also known that the free radical scavenging activity of phenolic compounds is governed by the number and position of phenolic hydrogen in their molecules (Rice-Evans et al., 1996). In the current study, the total phenolic content was expressed in terms of the gallic acid equivalent (GAE). The mechanism of anti-oxidant activity of flavonoids involves stabilization of the reactive oxygen species; due to the high reactivity of the hydroxyl group present in flavonoids, the radical reactions are terminated (Korkina and Afanas Ev, 1996). The highest total phenolic content was 64 mg GAE/100 g at 500 mg/mL of crude extract. The flavonoid content was also determined to be 127 mg QE (quercetin)/100 gm at 500 mg/mL of crude extract.

Characterization of mangiferin crystal

Fourier-Transform InfraRed (FTIR) spectra of isolated and pure mangiferin are shown in Figs. 9 and 10, respectively. It can be seen from the spectra that both have mostly identical peaks, confirming the structure of mangiferin. The broad peak obtained in the pure mangiferin crystal is sharply isolated due to vacuum drying of the isolated materials leading to the loss of intact moisture.

The anti-diabetic and anti-oxidant potentials of aqueous Mangifera indica leaves along with its active components were studied and compared. This is the first reported time such a comparison between the mangiferin (an active biomolecule) and a crude water extract of mango leaves has been performed. The in vitro evaluation used several methods (α-amylase inhibition, α-glucosidase inhibition and other anti-oxidant tests) which showed that mangiferin even at a lower concentration of 50 μg/mL produced good results as an anti-diabetic agent when compared to the standard drug (Acarbose) and aqueous mango leaves extract at 500 μg/mL. This study suggests that the mangiferin in crude extract has less potential as an anti-diabetic drug compared to mangiferin in pure form. However, the process of isolation and purification of mangiferin would add to the cost of formulation. On the other hand, there is no requirement for additional isolation and purification steps with the crude extract, as that extract can be used directly. Thus, the decision on whether to use the extract or mangiferin can be made depending on the application and complexity of the disease. The comparison undertaken in the current study was necessary as the findings have demonstrated that in cases where mangiferin or drugs are not an option, the extract can be used and vice versa. For example, when there is a need to apply the anti-oxidant benefits of mangiferin in the cosmetic industry, then crude mango leaves extract is applicable, whereas in cases where stringent purity conditions need to be maintained such as in the pharmaceutical industry, isolation and application of mangiferin may be more suitable. Thus, depending on the application, the proper use of mango leaves extract or mangiferin should be carried out.

Conflict of interest

There is no conflict of interest for this manuscript.

Acknowledgement

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References


Ozsoy, N., Can, A., Yanardag, R., Akev, N., 2007. Total flavonoid content and anti-
Pal, D., Nimse, S.B., 2006. Screening of the anti-oxidant activity of Hydrilla verti-

Yakugaku Zasshi 121, 371
