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Research Article

Evaluation of possible mechanisms of three plants for blood glucose control in diabetes
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Abstract

This study was conducted to provide the evidence for the mechanism of anti-diabetic activity of Cocculus orbiculatus, Leea indica and Venilago maderaspatana. This was accomplished by employing methods like uptake of glucose, glycogen synthesis and inhibition of α-glucosidase. For uptake of glucose, diaphragms were dissected out in Tyrode solution with 2% glucose and assayed for glucose content. In glycogen synthesis methodology liver, skeletal muscle and cardiac muscles were isolated, homogenized and glycogen content was analyzed. In α-glucosidase enzyme inhibition procedure involved estimation of α-glucosidase enzyme inhibition. All the three plant extracts exhibited significant (p<0.05 - p<0.01) anti-diabetic activity by increasing glucose uptake, glycogen content and α-glucosidase enzyme. Among the three plants, V. maderaspatana (500 mg/kg) exhibited higher glucose uptake, glycogen content and α-glucosidase inhibition activity (IC₅₀ 145 µg/mL). The present experimental results evidenced the anti-diabetic activity of three plants by all the three mechanisms.

Introduction

Diabetes mellitus is characterized by impaired production of insulin and/or diminished stimulation of insulin sensitive peripheral tissues associated with a marked decrease in glucose uptake and metabolism in response to insulin. The defective glucose transport system plays a significant role in the pathogenesis of peripheral insulin resistance. Glucose uptake in target tissues is a crucial step in maintaining glucose homeostasis and in lowering the postprandial glucose load (Shulman, 2000). Direct stimulation of glucose transport and metabolism in muscle and fat cells lead to enhanced glucose utilization. To attenuate the glucose uptake by peripheral cells, biguanides are employed. Cellular assays are utilized to investigate the mechanism of action of natural compounds using isolated rat diaphragms. It is highly preferred to explore modern anti-diabetic agents from natural sources that stimulate glucose uptake/disposal by peripheral tissues such as adipose tissue or muscle cells.

The pivotal enzyme for carbohydrate digestion is α-glucosidase. This is a therapeutic target for the modulation of postprandial hyperglycemia, the earliest abnormality that occurs in NIDDM (Kim et al., 2005). Dietary carbohydrates are the major source for blood glucose. These carbohydrates are hydrolyzed by α-glucosidase, so as to be absorbed by small intestine. Therefore, the most effective treatment is to inhibit the activity of α-glucosidase (Krentz and Bailey, 2005). α-Glucosidase inhibitors such as acarbose, miglitol and voglibose reduce postprandial hyperglycemia by inhibiting the activity of carbohydrate digesting enzymes and delaying glucose absorption.

Previously, we have reported anti-diabetic, anti-hyperlipidemic and anti-oxidant activity of three medicinal
plants Cocculus orbiculatus, Lea indica and Ventilago maderaspatana in the treatment of diabetes (Damayanthi and Satyavati, 2015); Damayanthi et al., 2014; Dama- 
yanthi and Satyavati, 2015). But, till date there are no 
scientific evaluation reports available to support the 
mechanisms responsible for anti-diabetic activity. 
Therefore, the present investigation was aimed to 
ascertain in vivo anti-diabetic activity by methods such 
as glucose uptake activity using isolated rat diaphragm 
and glycogen synthesis in liver, skeletal muscle and 
cardiac muscle and in vitro anti-diabetic activity by 
inhibiting α-glucosidase enzyme.

Materials and Methods

Plant materials

Aerial parts of C. orbiculatus were collected from 
Tirumala forest area, Tirupathi. L. indica leaves were 
procured from Karthikavanam forest area, Dhulapally, 
Hyderabad. V. maderaspatana roots were obtained from 
Tirumala forest area, Tirupathi. The plants were 
authenticated by Prof. Madhava Chetty, Department of 
Botany, Sri Venkateshwara University, Tirupathi, India.

Chemicals

Streptozotocin was procured from Sigma-Aldrich. Glu-
cose estimation kit was obtained from Erba Diagnostics, 
Mannheim. Gibenclamide (Oglucon) was purchased from 
Alpha pharmaceuticals, Apollo pharmacy, Bathalapalli, 
Ananthapur. Insulin (Novo Nordisk) was obtained 
from Alpha pharmaceuticals, Bathalapalli, Anantha-
pur. Glucose was purchased from Sd Fine Chemicals, 
India.

Preparation of plant extract

Aerial parts of C. orbiculatus were finely powdered, 
packed in soxhlet apparatus and then extracted with 
hydroalcohol (60:40). Leaves of L. indica were air dried 
at room temperature, coarsely powdered extracted by 
maceration with hydroalcohol (3:1). V. maderaspatana 
roots were finely powered and extracted by using 
soxhlet apparatus with hydroalcohol solvent (60:40). 
Percentage yield of the plants C. orbiculatus, L. indica, V. 
maderaspatana was found to be 16.7, 25.6 and 15.8% 
respectively.

Preliminary phytochemical analysis

All the three plant extracts were subjected to prelim-
inary phytochemical analysis to determine the phyto-
constituents employing standard tests (Harbone, 1998).

Animals

Wistar albino rats weighing about 200-250 g were 
procured from Raghavendra enterprises, Banglore. The 
animals were acclimatized (2 weeks); housed under 
standard laboratory conditions (temperature 23 ± 2°C), 
humidity 55-70% and fed with commercial diet Durga 
feeds, Bangalore.

α-glucosidase inhibitory assay

This assay was assigned to investigate the in vitro inhibi-
tory activity of three plant extracts on α-glucosidase 
enzyme. α-Glucosidase (100 µL of 1 U/mL) was mixed 
with phosphate buffer (100 µL, pH 7.0) containing 100 
µL of three plant extracts (25-1600 µL) or standard drug 
acarbose (0.1-3.2 µg/mL). This mixture is incubated at 
37°C for 60 min in maltose solution. Later the mixture 
is kept in boiling water for 2 min and cooled. The boiling 
stops the α-glucosidase action on maltose. Glucose 
reagent (2 mL) was added and absorbance is measured 
at 540 nm to estimate the amount of liberated glucose 
by the action of α-glucosidase (Kuppusamy et al., 2011)

Glucose uptake in normal and streptozotocin-induced 
diabetic rats

The glucose uptake using rat hemidiaphragm was 
estimated according to the method reported elsewhere 
(Walass and Walass, 1952; Chattopadhyay et al., 1992), 
but with some modifications. After 18 hours fasting; 
rats were killed by decapitation. Diaphragms were 
dissected out quickly with minimal trauma and divided 
into two halves. Hemidiaphragms were then rinsed in 
cold Tyrode solution (without glucose) to remove blood 
clots. Then these were weighed and placed in test tubes. 
The volumes in all test tubes were made equal by 
adding distilled water. The test tubes were incubated 
for 30 min at 37°C in an atmosphere of 100% oxygen 
and were shaken at 140 cycles/min. Hemidiaphragms 
were taken out. Glucose content of the incubated 
medium before and after incubation was measured. 
This was carried out by employing Erba Diagnostic 
Mannheim kit using GOD-POD method (Barham and 
Trinder, 1972) and Erba Mannheim Chem-7 
semiautoanalyzer. Glucose uptake was calculated as the 
difference between initial and final glucose content. 
Glucose uptake was expressed as mg/g of tissue per 30 
min of incubation. Rats were divided into 12 groups; six 
in normal group and six in diabetic control group of 
five rats each. In normal group: Group I (normal rats; 
received 2 mL of 0.1%); Group II (received Tyrode solution 
and 0.6 mL of 0.4 IU/mL insulin); Group III (administered Tyrode solution and standard drug 
metermin- 2 mL of 0.1%); Group IV (received Tyrode solution and 300 mg/kg of C. 
orbiculatus); Group V (received Tyrode solution- 
400 mg/kg of L. indica); Group VI (administer-
ed Tyrode solution and 500 mg/kg V. maderaspatana).

In diabetic control group: Group VII (diabetic control 
group, untreated); Group VIII (received Tyrode solu-
tion and 0.6 mL of 0.4 IU/mL insulin); Group IX 
(administered Tyrode solution and standard drug, 
metermin- 2 mL of 0.1%); Group X (received Tyrode 
solution and 300 mg/kg C. orbiculatus);
Group XI (received Tyrode solution and 400 mg/kg L. indica); Group XII (administered Tyrode solution and 500 mg/kg V. maderaspatana)

**Glycogen estimation in normal and streptozotocin-induced diabetic rats**

The glycogen content in liver, skeletal muscle and cardiac muscle was estimated by Carroll et al., (1956). Rats were divided into ten groups: Five in normal group and five in diabetic control group of five rats each.

Normal group: Group I (normal rats; received 1% sodium carboxymethyl cellulose); Group II (received standard drug, glibenclamide 10 mg/kg); Group III (administered 300 mg/kg of C. orbiculatus); Group IV (administered 400 mg/kg of L. indica) Group V (administered 500 mg/kg V. maderaspatana)

Diabetic control group: Group VI (diabetic control group, untreated); Group VII (diabetic control received standard drug, glibenclamide 10 mg/kg); Group III (diabetic control administered 300 mg/kg of C. orbiculatus); Group IV (diabetic control administered 400 mg/kg of L. indica); Group V (diabetic control administered 500 mg/kg of V. maderaspatana)

After 18 hours of fasting, plant extracts were administered to different groups. Two hours later they were sacrificed by decapitation. The liver, skeletal muscle and cardiac muscle were isolated, weighed and homogenized using 10 mL of 4% trichloroacetic acid and centrifuged for 10 min. Supernatant was decanted and precipitate is discarded. To 2 mL of supernatant 4 mL of anthrone reagent was added. Later test tubes were allowed to cool for 30 min. Absorbance was measured at 620 nm using spectrophotometer. Glycogen content was expressed as milligram for 100 g of tissue.

Glycogen content = DU x 0.2 x volume of the extract x 1000 DS x weight of the tissue

DU= Absorbance of the sample; DS= Absorbance of the standard

**Statistical analysis**

The experimental results were presented as mean ± standard error mean (SEM). Statistical analysis was performed by graphpad instat version 3.2. Probability value of analysis p<0.01 and p<0.05 was considered to be statistically significant.

**Results**

**Preliminary phytochemical analysis**

Phytochemical analysis of C. orbiculatus exhibited positive results for alkaloids, glycosides, carbohydrates, flavonoids, saponins, tannins, terpenoids, polyphenols and starches (Table I). Phytochemical analysis of L. indica exhibited the presence of alkaloids, terpenoids, carbohydrates, flavonoids, tannins and saponins. Preliminary phytochemical analysis of V. maderaspatana revealed the presence of alkaloids, glycosides, emodin, cardiac glycosides, carbohydrates, flavonoids, tannins and saponins. The constituents like aporphine

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>C. orbiculatus</th>
<th>L. indica</th>
<th>V. maderaspatana</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
<td>-</td>
<td>++</td>
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<tr>
<td>Carbohydrates</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
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<td>++</td>
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<tr>
<td>Tannins</td>
<td>++</td>
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<td>Starches</td>
<td>++</td>
<td>-</td>
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</tr>
<tr>
<td>Polyphenols</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>--</td>
<td>++</td>
<td>--</td>
</tr>
<tr>
<td>Gallic acid</td>
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<td>++</td>
<td>--</td>
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<tr>
<td>β-Sitosterol</td>
<td>--</td>
<td>++</td>
<td>--</td>
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<tr>
<td>Cardiac glycosides</td>
<td>--</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Emodin</td>
<td>--</td>
<td>--</td>
<td>++</td>
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<tr>
<td>Dam-karrer test</td>
<td>--</td>
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<td>++</td>
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<tr>
<td>Juglone test</td>
<td>--</td>
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<td>++</td>
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</tbody>
</table>

Note: Present (++); Absent (--)
and berberine, ursolic acid, gallic acid, β-sitosterol, emodin and physcion were found by the analysis of *C. orbiculatus*, *L. indica* and *V. maderaspatana*.

**α-Glucosidase inhibitory activity**

*C. orbiculatus*, *L. indica* and *V. maderaspatana* hydroalcoholic extracts exhibited 8.3, 10.3 and 15.1% inhibition of α-glucosidase activity at 25 µg/mL and 85.7, 90.3 and 95.8% inhibition at 1600 µg/mL respectively (Table II). The IC₅₀ values were 325, 265 and 145 µg/mL. IC₅₀ value of acarbose was found to be 0.2 µg/mL.

**Effect on peripheral glucose uptake**

Table III shows glucose uptake in an isolated rat hemidiaphragm muscle of normal and diabetic animals. Addition of *C. orbiculatus*, *L. indica* and *V. maderaspatana* hydroalcoholic extracts elicited significant increase in glucose uptake by the rat hemidiaphragm in normal animals. *V. maderaspatana* seemed to be more effective in enhancing peripheral glucose uptake than *L. indica*, *C. orbiculatus* and metformin. The glucose uptake by rat hemidiaphragm was significantly higher in all the groups when compared to control group. Diabetic control animals exhibited significant increase in glucose uptake of *C. orbiculatus*, *L. indica* and *V. maderaspatana* (37.7, 40.8 and 48.0% respectively). Glucose uptake of *V. maderaspatana* was significantly higher when compared to *L. indica* and *C. orbiculatus*.

**Effect on glycogen content in liver, skeletal muscle and cardiac muscle in normal animals**

Hydroalcoholic extracts of *C. orbiculatus*, *L. indica* and *V. maderaspatana* showed significantly increased glycogen content in liver (Table IV). Increase in glycogen content was more for *V. maderaspatana* than *L. indica*, *C. orbiculatus* and glibenclamide. Skeletal muscle glycogen content was increased more for all the three plant extracts. Increase was greater for *V. maderaspatana* than *L. indica*, *C. orbiculatus* and glibenclamide. Significant

### Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (µg/mL)</th>
<th>% Inhibition</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. orbiculatus</em></td>
<td>25</td>
<td>8.3 ± 0.5</td>
<td>325.1</td>
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<tr>
<td></td>
<td>50</td>
<td>17.1 ± 0.5</td>
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<td></td>
<td>100</td>
<td>23.2 ± 0.4</td>
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<td></td>
<td>200</td>
<td>37.1 ± 0.5</td>
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<td></td>
<td>400</td>
<td>55.3 ± 1.7</td>
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<tr>
<td></td>
<td>800</td>
<td>67.3 ± 1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>85.7 ± 1.4</td>
<td></td>
</tr>
<tr>
<td><em>L. indica</em></td>
<td>25</td>
<td>10.3 ± 0.4</td>
<td>265.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>20.5 ± 0.7</td>
<td></td>
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<tr>
<td></td>
<td>100</td>
<td>33.7 ± 0.5</td>
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<tr>
<td></td>
<td>200</td>
<td>45.3 ± 0.8</td>
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<tr>
<td></td>
<td>400</td>
<td>62.2 ± 0.9</td>
<td></td>
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<tr>
<td></td>
<td>800</td>
<td>79.5 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>90.3 ± 1.8</td>
<td></td>
</tr>
<tr>
<td><em>V. maderaspatana</em></td>
<td>25</td>
<td>15.1 ± 0.3</td>
<td>145.0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>29.3 ± 0.4</td>
<td></td>
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<tr>
<td></td>
<td>100</td>
<td>43.7 ± 0.7</td>
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<td></td>
<td>200</td>
<td>56.3 ± 0.5</td>
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<tr>
<td></td>
<td>400</td>
<td>75.3 ± 1.0</td>
<td></td>
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<tr>
<td></td>
<td>800</td>
<td>88.9 ± 1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>95.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Acarbose</td>
<td>0.1</td>
<td>30.7 ± 0.4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>47.3 ± 0.7</td>
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<tr>
<td></td>
<td>0.4</td>
<td>59.2 ± 1.1</td>
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<tr>
<td></td>
<td>0.8</td>
<td>73.1 ± 2.1</td>
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<td></td>
<td>1.6</td>
<td>81.2 ± 1.9</td>
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<tr>
<td></td>
<td>3.2</td>
<td>96.2 ± 1.7</td>
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</table>
In current study, we investigated the mechanism for anti-diabetic activity of three plants (C. orbiculatus, L. indica, and V. maderaspatana) by employing glucose uptake, glycogen synthesis, and α-glucosidase enzyme inhibition methods. Three plants showed significant uptake of glucose and are more or less effective than insulin. Glibenclamide has evidenced greater uptake of glucose than L. indica, C. orbiculatus, metformin and comparatively equal to insulin. Glibenclamide showed increased glycogen content in muscle cells. Therapy with the three plants evidenced increased glucose uptake and thereby glycogen storage in liver, skeletal muscle and cardiac muscle. V. maderaspatana increased glycogen content greater than L. indica and C. orbiculatus. The three plants also evidenced α-glucosidase inhibitory activity among which V. maderaspatana exhibited greater activity.

The impaired glucose uptake is linked with decrease in the translocation of glut 4 and is the major cause of insulin resistance. Metformin and insulin stimulate glucose uptake in muscle cells by increasing GLUT 4 (Klip and Leiter, 1990). Berberine has been investigated to activate AMPK in skeletal muscle and adipose tissue that lead to AMPK phosphorylation and subsequently activate glycogen synthase. The mechanism was observed for V. maderaspatana than L. indica and C. orbiculatus. V. maderaspatana exhibited increased cardiac muscle glycogen content than L. indica, C. orbiculatus and glibenclamide.

**Effect on glycogen content in liver, skeletal muscle and cardiac muscle in diabetic animals**

Three plants have elicited significantly increased liver glycogen content. V. maderaspatana manifested higher liver glycogen content than L. indica and C. orbiculatus. Significantly increased skeletal muscle glycogen content was observed for V. maderaspatana L. indica and C. orbiculatus. Glycogen content was more in V. maderaspatana than L. indica and C. orbiculatus. Cardiac muscle glycogen content increase was observed for all the three plants. Among these V. maderaspatana exhibited increased glycogen content than L. indica and C. orbiculatus.

**Discussion**

In current study, we investigated the mechanism for anti-diabetic activity of three plants (C. orbiculatus, L. indica and V. maderaspatana) by employing glucose uptake, glycogen synthesis and α-glucosidase enzyme inhibition methods. Three plants showed significant uptake of glucose and are more or less effective than insulin. V. maderaspatana has evidenced greater uptake of glucose than L. indica, C. orbiculatus, metformin and comparatively equal to insulin. Glibenclamide showed increased glycogen content in muscle cells. Therapy with the three plants evidenced increased glucose uptake and thereby glycogen storage in liver, skeletal muscle and cardiac muscle. V. maderaspatana increased glycogen content greater than L. indica and C. orbiculatus. The three plants also evidenced α-glucosidase inhibitory activity among which V. maderaspatana exhibited greater activity.

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increased glucose uptake (Yin et al., 2008; Cheng et al., 2006; Kim et al., 2007; Lee et al., 2006; Zhou et al., 2007; Wang et al., 2004; Ko et al., 2005). Similarly gallic acid the constituent of L. indica has been implicated to mediate insulin stimulated glucose transport in muscle and adipocytes (Vishnu Prasad et al., 2010). Finally VMHAЕ possess emodin that mediated Glut 1 and Glut 4 expression to enhance glucose uptake in skeletal muscles and adipocytes (Ying Yang et al., 2007). Glibenclamide increased glycogen content in muscle but adverse effects like hypoglycemia and weight gain limits the use. Hence drugs that attenuate glycogen content without adverse effects were desirable for long term anti-diabetic therapy. The three plants evidenced enhanced glucose uptake and glycogen storage in liver, skeletal muscle and cardiac muscle. The increased glycogen levels by the three plants might be due to the berberine of C. orbiculatus that evidenced glucose utilization in Hep G2 cells and 3T3-L1 adipocytes increasing glycogen content (Yin J et al., 2002; Zhou et al., 2003; Zhou et al., 2003). Gallic acid, the constituent of L. indica by insulin secretagogue action produced increased glycogen levels (Punithavathi et al., 2011). Emodin of V. maderaspatana, the natural PPAR activator, up-regulated PPARy and increased glycogen content (Yonemitsu et al., 2001). Acarbose, the α-glucosidase inhibitor has common side effects (flatulence and abdominal bloating). Herbal drugs devoid of side effects are desired to improve compliance for diabetic patients. The constituents like berberine, β-sitosterol and emodin of the plants have reported α-glucosidase inhibitory activity (Pan et al., 2003; Sunil Kumar et al., 2013; Yang et al., 2014). Thus active constituents of the experimental study might be responsible for the elicited α-glucosidase inhibitory activity of C. orbiculatus, L. indica and V. maderaspatana.

Conclusion

The experimental reports evidenced C. orbiculatus, L. indica and V. maderaspatana plants act through multiple mechanisms like increased glucose uptake, glycogen synthesis in muscle cells and α-glucosidase inhibition to control blood glucose in diabetes. V. maderaspatana elicited higher antidiabetic activity compared to L. indica and C. orbiculatus.

Financial Support

Self-funded

Ethical Issue

Experiments were conducted in accordance with the guidelines of CPCSEA REGD No. 878/ac/05/CPCSEA/21/2015. The study protocol was approved by Institutional Animal ethics committee.

Conflict of Interest

Authors declare no competing interest

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