Evaluation of Antidiabetic Potential of Ethanolic Extracts of Cissus quadrangularis and Cinnamomum tamala - An in vitro Study

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ABSTRACT

Background: Diabetes is a rapidly growing metabolic disorder of the present generation. Uncontrolled diabetes leads to various complications which in turn affects the vascular system, neuropathy, and more. Medicinal plants are always a good source of drug equivalent currently present. Antidiabetic drugs lead to a large amount of side effects. A comparative study was made between Cissus quadrangularis and Cinnamomum tamala for its antidiabetic potential.

Aim: To evaluate the antioxidant and antidiabetic potential of ethanolic extract of Cissus quadrangularis and Cinnamomum tamala and to compare its efficacy.

Materials and Methods: Ethanolic extracts of Cissus quadrangularis and Cinnamomum tamala were tested for its phytoconstituents, antioxidant and antidiabetic potential. The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test and it was used to see the statistical significance among the groups. The results with the p<0.05 level were considered to be statistically significant.

Results: Phytochemical screening showed a strong presence of flavonoids and terpenoids in both
the plant extract. Results showed that plant extract had antidiabetic and antioxidant activity. Among them Cissus quadrangularis exhibited significantly more antidiabetic and antioxidant activity.

**Conclusion:** The ethanolic extract of Cissus and Cinnamomum tamala exhibits potent antidiabetic and antioxidant properties. With further invivo and invitro studies the plant extract can be formulated into a potent antidiabetic drug.

**Keywords:** Phytochemicals; antidiabetic potential; antioxidants; innovative technology; novel method.

### 1. INTRODUCTION

Diabetes is a growing problem worldwide causing enormous financial burden and maintaining care policy issues [1]. According to the international diabetes federation (IDF) the number of individuals with diabetes in 2011 crossed 3.66 million with an estimation of 4.6million death each year [1,2]. Indian subcontinent emerged as the capital of the diabetes epidemic. Indians show a significantly higher age related prevalence of diabetes when compared to other folks. Diabetes is characterized by metabolic dysregulation primarily of carbohydrate manifested by hyperglycemia leading to defects in insulin secretion, impaired insulin action or in many cases both the above mentioned stages. Untreated or uncontrolled diabetes leads to a plethora of complications which inturn affect the vascular disease, neuropathy, nephropathy. Diabetes is referred to as a chronic metabolic disorder that affects around 40.9million population in India. Antidiabetic drugs such as biguanides and sulfonylureas can be used but they have prolonged side effects.

Medicinal plants have always been a good source of drugs and currently there are many drugs which are directly or indirectly extracted from them [3]. The ethnobotanical information says that about 800 plant species posses antidiabetic potential among which Momordica charantia, Pierocarpus marsupium, Cissus quadrangularis, Trigonella foenum greacum are reported beneficial for treatment of diabetees.

Several herbs have shown antidiabetic activity when evaluated using different experimental techniques [4]. Wide arrays of plant derived active principles representing different kinds of biological activity has been explored. Among which alkaloids, glycosides, galactomannan, peptidoglycan, amino acids,terpenoids, glycoprotein, carbohydrates and other inorganic ions are demonstrated activity including treatment for diabetes.

Several classes of oral hypoglycemic drugs exerts antidiabetic effect through different mechanisms like sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones and non-sulfonylureas secretagogues. Although synthetic oral hypoglycemic drugs alongside insulin are the main cause for controlling diabetes they fail to reverse the course of its complication completely and further worsen it by producing side effects [5,6,7,8]. This forms the main alternative source discoveries for antidiabetic agents. Despite the significant progress made in treatment of diabetes using oral antidiabetic agents in the past 3 decades, the results of treatment of diabetic patients are still far from perfect. There are several disadvantages reported related to the use of oral hypoglycemic agents, including drug resistance which reduces the efficiency, adverse effect and even the toxicity [5,6,7,8]. Due to several limitations the search for newer antidiabetic drugs from natural sources continues.

One of the popular plants which helps in antidiabetic treatment is Cissus quadrangularis which belongs to the family Vitaceae and are distributed in hotter parts of india and sri lanka. The stem of the plant has been reputed in ayurveda as an alternative, anthelmintic, dyspeptic, digestive, analgesics in eye and ear disease, irregular menstruation, asthmatic and mainly as antidiabetic. It also proves to reduce swelling and proves to promote the relevance of pain and healing of simple fractures as well as in curing the allied disorders associated with fracture [9]. Some phyto-constituents like flavonoids, phytosterol, tannins and triterpene present in the plant extract are responsible for antidiabetic activity, Cinnamomum tamala, Indian bay leaves which is also known as tejpatt, is a tree which belongs to the Lauraceae family native to India, Nepal, Bhutan and china. They are commercially cultivated in certain parts of the country for leaf production and essential oils [5]. Previous studies suggest that about 3g/d for 30 days decreases the risk factor of diabetes,
cardiovascular disease and is beneficial for people with type-1 diabetes [10]. Cinnamomum tamala also possesses antidiabetic, gastroprotective, hypolipidemic, immunomodulatory, cytoprotective and antigenotoxic effects. The antidiabetic activity of Cinnamomum tamala is due to the presence of steroids, terpenoids, tannins and polysaccharides [11-31]. This research is carried out to evaluate the antioxidant and antidiabetic potential of ethanolic extract of Cissus quadrangularis and Cinnamomum tamala and to compare its efficacy [5,6].

2. MATERIALS AND METHODS

2.1 Phytochemical Screening Test

2.1.1 Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

2.1.2 Test for carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The Development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

2.1.3 Test for flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

2.1.4 Test for alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

2.1.5 Test for terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H₂SO₄ was added. Red color ppt obtained indicates the presence of terpenoids.

2.1.6 Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

2.2 Detection of Saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

2.3 Test for Steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2.3.1 DPPH free radical scavenging activity of Cissus quadrangularis and Cinnamomum Tamala

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

DPPH radical scavenging (%) = (Control OD - Sample OD X100) / Control OD

2.4 In vitro Antidiabetic Activity

2.4.1 In vitro alpha amylase inhibitory activity of Cissus quadrangularis and Cinnamomum tamala

α-amylase inhibitory activity of the extract was carried out according to the standard method of Ademiluyi et al [14]. In a test tube, reaction mixture containing 500 microliters of phosphate buffer (100 Mm, pH = 6.8), 100 μl of α-amylase (2 U/ml) and varying concentrations of T. email (0.1 to 0.5 mg/ml) was pre incubated at 3 degree Celsius for 20 min. Then, 200 μl of 1% soluble starch (100mM phosphate buffer of pH = 6.8)
was added as a substrate and incubated further at 37 degree Celsius for 30 min; 1000 microliters of 3.5- dinitrosalicylic acid (DNS) color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using multiple readers. Acarbose at various concentrations (0.1-0.5 mg/ml) was used as standard. The results were expressed as percentage inhibition, which was then calculated using the formula,

\[
\text{Inhibitory activity (\%)} = (1 - \frac{As}{Ac}) \times 100 \text{ Where,}
\]

As – absorbance in presence of test substance, Ac – absorbance of control

### 2.4.2 α-glucosidase inhibitory activity of *Cissus quadrangularis* and *Cinnamomum Tamala*

α-glucosidase inhibitory activity of extract was carried out according to the standard method of Ademiluyi et al [14]. In a test tube, reaction mixture containing 500 μl of phosphate buffer (100 Mm, pH = 6.8), 100 microliters of α-glucosidase (1 U/ml) and varying concentrations of *T. ammi* oil (0.1 to 0.5 mg/ml) was preincubated at 37⁰C for 20 min. The reaction was stopped by adding 50 μl of Na2CO3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using multiple readers. Acarbose at various concentrations (0.1-0.5mg/ml) was used as a standard. The result was expressed as percentage inhibition which was calculated using the formula,

\[
\text{Inhibitory activity (\%)} = (1 - \frac{As}{Ac}) \times 100 \text{ Where,}
\]

As – absorbance in presence of test substance, Ac – absorbance of control

### 2.5 Statistical Analysis

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan’s multiple range test to assess the significance of individual variations between the groups. In Duncan’s test, significance was considered at the level of p<0.05.

### 3. RESULTS AND DISCUSSION

Fig. 1 Bar graph depicts the In vitro antioxidant activity of ethanolic extract of *Cissus quadrangularis* and *Cinnamomum tamala*. The X axis represents the different concentrations of *Cissus quadrangularis* and *Cinnamomum tamala* and the Y axis represents the percentage of inhibition. The blue colour denotes Vitamin C, the orange colour denotes *Cissus quadrangularis* ethanolic extracts and purple denotes ethanolic extracts of *Cinnamomum tamala*. The difference was statistically significant. Each line represents Mean ± SEM of 3 independent observations. Significance at p ≤ 0.05.

Fig. 2 Bar graph depicts the In vitro alpha amylase inhibitory activity of ethanolic extracts of *Cissus quadrangularis* and *Cinnamomum tamala*. The X axis represents the different concentrations of ethanolic extracts of *Cissus quadrangularis* and *Cinnamomum tamala* and the Y axis represents the percentage of inhibition. Blue colour denotes the concentration of the standard drug Metformin, red colour denotes the ethanolic extracts of *Cissus quadrangularis* and purple denotes the ethanolic extracts of *Cinnamomum tamala*. The difference was statistically significant. Each line represents Mean ± SEM of 3 independent observations. Significance at p ≤ 0.05.

### Table 1. Phytochemical analysis of Ethanolic extract of *Cissus quadrangularis* and *Cinnamomum tamala*

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th><em>Cissus quadrangularis</em></th>
<th><em>Cinnamomum tamala</em></th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Alkaloids</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Terpenoids</td>
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<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Fig. 1. *In vitro* antioxidant activity of ethanolic extracts of *Cissus quadrangularis* and *Cinnamomum tamala*

Fig. 2. *In vitro* alpha amylase inhibitory activity of ethanolic extracts of *Cissus quadrangularis* and *Cinnamomum tamala*

Fig. 3. *In vitro* alpha glucosidase inhibitory activity of ethanolic extracts of *Cissus quadrangularis* and *Cinnamomum tamala*
The qualitative phytochemical analysis of Cissus quadrangularis ethanolic extract showed the presence of proteins, flavonoids, alkaloids, terpenoids, and saponins whereas ethanolic extracts of Cinnamomum tamala showed the presence of proteins, alkaloids, terpenoids, steroids and saponins (Table 1). Phytochemical screening is generally done for identification of medically active substances. The ethanolic extracts of Cissus quadrangularis and Cinnamomum tamala showed antioxidant activity (Fig. 1), alpha amylase inhibitory activity (Fig. 2) and alpha glucosidase inhibitory activity (Fig. 3).

Low levels of insulin achieves adequate response and insulin resistance in target tissues. Some of the patients with type 2 diabetes are asymptomatic during their early phase of diabetes, but symptoms include polyuria, polydipsia, weight loss, blurred vision and polyphagia [32]. Where uncontrolled diabetes leads to complications like stupor, coma, if not treated may even lead to death, due to the ketoacidosis or from nonketotic hyperosmolar syndrome [32,33]. Diabetes is generally associated with patients with endocrine disease such as acromegaly, cushing syndrome, glucagonoma and pheochromocytoma. It can also be seen in patients with genetic syndrome like down syndrome, klinefelter syndrome, turners syndrome and wolfram syndrome. As in discussion with prediabetes it correlates with increased cardiovascular mortality and cancer [34].

The phytochemical analysis of the plant extract Cissus quadrangularis and Cinnamomum tamal were studied. Where the extracts exhibited a strong presence of saponins, terpenoids, flavonoids and alkaloids. Phytochemical screening refers to the identification, extraction and screening of medicinally active substances which are found in plants. They are widely used in order to enhance the values of particular plants for particular disease and disorders. Previous studies on the plant Ephedra intermedia for the presence of phytochemicals reveals the presence of tannins, terpenoids, glycosides and saponins [35].

Oxidants are free radical compounds which cause harm if their levels are high in our body. They are linked to multiple illnesses like diabetes, heart disease and cancer. Antioxidants protect the cells from free radicals and play an important role in heart disease, cancer and other diseases. Free radicals are produced when the human body encounters breakdown of food and when they are exposed to toxins [36]. The most commonly known antioxidants are Vit C and Vit B. Ethanolic extract of the plants showed in vitro antioxidant activity in a concentration dependent manner where vit c is used as a standard drug. Antioxidant activity with IC 50= 320ug/ml and 380ug/ml was exhibited by Cissus quadrangularis and Cinnamomum tamal respectively (Fig. 1).

One of the antidiabetic therapeutic strategies is the inhibition of carbohydrate digesting enzymes like alpha amylase and alpha glucosidase. Where glucosidase enzymes lead to catalysis of starch to simple sugar and in humans they increase the blood glucose levels. In vitro antidiabetic activity was analysed for both the plant extracts and the result prove that ethanolic extract of Cissus quadrangularis showed a significantly increased activity compared to Cinnamomum tamala (Fig. 2 and Fig. 3).

4. CONCLUSION

From the study it can be concluded that ethanolic extracts of Cissus quadrangularis and Cinnamomum tamala showed a potent antioxidant and antidiabetic activity which was evident from DPPH scavenging activity, alpha amylase inhibitory activity and alpha glucosidase inhibitory activity respectively. A dose-dependent antidiabetic activity was observed from extracts and the standard drug Metformin. In the present study, the standard drug Metformin showed greater activity compared to extracts in all concentrations though the natural drugs tested showed potential, significantly so. Validation needs to be done on natural herbal extracts to make it into a potential alternative for synthetic drugs which possess a lot of side effects.
5. SOURCE OF FUNDING

The present study was supported by the following agencies.

- Saveetha Institute of Medical and Technical Sciences (SIMATS)
- Saveetha Dental College
- Saveetha University
- Ultracare sanitary napkins, chengalpattu

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of medical and technical Sciences, Saveetha University for providing research laboratory facilities to carry out the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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