Evaluation of Antioxidant Potential and α-Amylase and α-Glucosidase Inhibitory Action of Indian Under-Utilized Fruits

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Abstract: Different parts of Tinospora cordifolia (Menispermace) and Persea americana (Lauraceae) have been used in traditional medicine for a wide range of illness and some of these uses have been proven scientifically. However, the medicinal property of fruit sample of these plants was not yet investigated. Hence the present work was carried out to evaluate the phytochemical compounds, antioxidant activity and anti-hyperglycemic potential (inhibition of α-amylase and α-glucosidase enzymes) of ethanol and aqueous extracts of fruit samples of T. cordifolia and P. americana with a view to apply them as herbal anti-diabetic drug. Ethanol and aqueous extracts were prepared from powdered fruit samples and preliminary phytochemical screening was carried out and total phenolic and flavonoid contents were quantified by spectrometric methods. Antioxidant activity of crude extracts was determined by in vitro methods (DPPH radical scavenging and reducing power assays) and α-amylase & α-glucosidase enzyme inhibitory activities of fruit extracts were analyzed using standard methodologies. The ethanolic extract of both the plants has flavonoids and terpenoids while only the presence of flavonoids was noted in aqueous extracts. Ethanolic extract of P. americana fruits exhibited the highest level of antioxidant activity but possess lower levels of total phenolics and flavonoids when compared to T. cordifolia fruit extract. The aqueous and ethanolic extracts of T. cordifolia recorded remarkable inhibition activities against α-amylase and α-glucosidase enzymes, respectively. Hence, consumption of such fruits will prevent the oxidative stress and hyperglycemic complications in human beings. However, further detailed scientific investigations are necessary to prove the nutraceuticals properties of these Indian under-utilized fruits.

Key words: Tinospora cordifolia, Persea americana, Phytochemicals, Antioxidant, α-Amylase, α-Glucosidase.

1. INTRODUCTION

Ethnobotanical studies on traditional herbal remedies used for diabetes have identified more than 1,200 species of plants with hyperglycemic activity [1, 2]. A number of medicinal plants and their formulations are used for treating diabetes in the traditional Indian systems of medicine as well as in ethno-medicinal practices. In tropical countries like India, rural people traditionally harvest wide range of leafy vegetables, roots, tubers and fruits from wild because of its nutritional effect, taste, and cultural uses, as supplements or to tide over food storage. Wild plants have been recognized to have potential to meet the house hold food and income security [3, 4]. Many wild fruits notably, Amla, Harida, Bel, Elephant apple have been exploited from wild for centuries across Indian subcontinent on account of its food and medicinal properties. In India, the indigenous fruits collected from wild habitats play a significant role in the food and nutrional security of rural poor and tribal communities. Some wild fruits have been identified to possess better nutritional value than cultivated fruits [5, 6]. In this connection, the present study focused on two such wild fruits namely Tinospora cordifolia and Persea americana.

Tinospora cordifolia (Wild.) Miers ex Hook. F. and Thomas (Tc), commonly known as Guduchi, is an herbaceous vine of the family Menispermaceae, indigenous to the tropical areas of India, Myanmar and Sri Lanka. Guduchi is widely used in veterinary folk / ayurvedic system of medicine as tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arithmetic, anti-allergic and anti-diabetic drug [7 - 9]. This plant is used in Ayurvedic Rasayanas to improve the immune system and the body resistance against infections. The root of this plant is known for its anti-stress, anti-leptotic and anti-malarial activities [10]. The active adaptogenic constituents are diterpene compounds including tinosporone, tinosporic acid, cordifolisides A to E, syringe [11, 12]. The active principles of T. cordifolia were found to possess anti-complementary and immune-modulatory activities. Recent research has demonstrated that a combination of guduchi and turmeric extract is effective in preventing the hepatotoxicity caused by the pharmaceutical treatment for tuberculosis with rifampicin [13].

Persea americana Mill belongs to the laurel family (Lauraceae), which includes many aromatic trees and shrubs. It is commonly called as Avocado in English, which refers to the testicle shape of the fruits. Avocados have long been cultivated for their edible fruits, the flesh is highly nutritious and has a composition different from that of other fruits. It is high in calories
(220 kcal per 100 g), contains vitamins A, B and E and is a good source of folic acid, riboflavin, niacin, thiamin, iron and potassium [14]. It is eaten with salads and desserts and used as a sandwich filling and an ingredient in dips and spreads (such as guacamole), ice-creams and milkshakes. It is a multipurpose plant which has diverse applications in ethno-medicines ranging from treatment of diarrhea, dysentery, tooth-ache, kidney and liver troubles, diabetes and intestinal parasites to the area of skin treatment and cosmetics [15].

Even though different plants of *T. cordifolia* and *P. americana* have been used in Indian traditional systems of medicine for a long time and their therapeutic properties were proved scientifically in earlier studies, the information regarding the antioxidant and anti-diabetic related properties of fruit samples of these plants are found to be meager. Hence, the present study was carried out to analyze the phytochemical compounds of solvent extracts of fruit samples of *T. cordifolia* and *P. americana* and also to evaluate their antioxidant and anti-hyperglycemic properties.

2. MATERIALS AND METHODS

2.1. Sample Collection

The fruit samples of *T. cordifolia* and *P. americana* were collected from natural stands in Coimbatore and Ooty, Tamil Nadu. The fruit samples were shade-dried and powdered in a lab mill to a particle size of 1 mm and used for further experiments.

2.2. Preparation Extracts

Fifty grams of air-dried powder were taken in 200 ml of each of ethanol and water in separate conical flasks, plugged with cotton wool and shaken at room temperature for 2 days. Then the contents were filtered and the filtrate was collected and the solvent was evaporated and the dry extract was re-suspended in respective solvents at 1 mg/ml ratio.

2.3. Phytochemical Analysis

Qualitative phytochemical screening was carried out in aqueous and alcoholic extracts of *T. cordifolia* and *P. americana* fruits using standard procedures to identify the phytochemical constituents [16, 17]. The total phenolic content was determined by Folin-Ciocalteu reagent method [18]. Suitably diluted extracts of each sample or gallic acid (standard phenolic compound) was mixed with 5 ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 4 ml of 1 M aqueous sodium carbonate. The mixtures were allowed to stand for 15 min at 37°C and the total phenols were determined by measuring the absorbency at 765 nm in a Spectrophotometer (Make: Perkin Elmer, USA). Similarly, total flavonoid content was analyzed in the extracts [19]. Each plant extract (0.5 ml) was mixed with 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. This mixture was kept at room temperature for 30 min and the absorbance was measured at 415 nm using a Spectrophotometer.

2.4. DPPH Radical Scavenging Assay

DPPH scavenging activity was carried out using different concentrations (1000, 500, 250, 125, and 62.5 mg/ml) of extract was taken in test tubes in triplicates [20]. Then 5 ml of 0.1 mM methanol solution of DPPH (2,2- Diphenyl-1- Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37°C for 20 min. The control was prepared without any extracts. Ethanol was used for base line corrections and the absorbance of sample was measured at 517 nm and the radical scavenging activity was expressed in percentage.

2.5. Reducing Power Assay

Reducing activity was carried out by using different concentrations (1000, 500, 250, 125, and 62.5 mg/ml) of the extract was taken in the test tubes in triplicates [21]. To the test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50°C for 20 min. After incubation, 2.5 ml of 10% TCA was added and were kept for centrifugation at 3000 rpm for 10 min. After centrifugation 5.0 ml of supernatant were taken and to this 5.0 ml of distilled water was added. To this about 1.0 ml of 1% ferric chloride was added and was incubated at 35°C for 20 min and then the absorbance was taken at 700 nm. The blank was prepared by adding all the reagents without extract and ferric chloride (0.1 %) and the control was prepared by adding all other solution without the extract.

2.6. α-Amylase Inhibition Assay

Fruit extracts of different concentrations was mixed with 100 μl of 0.02 M sodium phosphate buffer (pH 6.9) and 100 μl of α-amylase solution (4.5 Units/ml/min) and pre-incubated at 25°C for 10 min [22]. Starch solution (100 μl, 1%) was then added to the solution and
incubated at 25°C for 30 min; the reaction was stopped by the addition of 1.0 ml of dinitrosalicylic acid reagent. Afterwards, the test tubes were incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was then diluted (10-fold) with distilled water and the absorbance was measured at 540 nm. The readings were related with the control, which contained buffer instead of extract, and the per cent of α-amylase enzyme inhibition was calculated.

2.7. α-Glucosidase Inhibition Assay

Fruit extracts of different concentrations was mixed with 100 μl of 0.1 M phosphate buffer (pH 6.9) and 100 μl of α-glucosidase solution (1 Unit/ml/min) and pre-incubated at 25°C for 5 min [22]. Substrate (100 μl of 5 mM p-nitrophenyl-α-D-glucopyranoside) was then added and the reaction mixture was incubated at 25°C for 10 min. After the incubation period, the absorbance readings were recorded at 405 nm and allegorized to a control that had 100 μl of buffer in place of the extract. The results were calculated and expressed on a percent basis.

2.8. Statistical Analysis

All the results were expressed as mean ± standard error of triplicate analysis. The significant difference between experimental groups were analyzed through ANOVA using Graph-Pad Prism software (Version 5).

3. RESULTS AND DISCUSSION

3.1. PHYTOCHEMICAL SCREENING

In nature all the plants synthesize phytochemicals to perform defense activities. In present study, the investigated plants have exhibited different kinds of secondary metabolites. Ethanol and aqueous extracts of fruit samples were subjected to qualitative analysis of phytochemical compounds. Ethanolic extract of *T. cordifolia* fruit has positively answered for alkaloids, terpenoids and flavonoids while presence of flavonoids and terpenoids were detected in the ethanolic extract of *P. americana* fruits (Table 1). In both the samples, aqueous extract showed the presence of flavonoids. Phytochemical constituents isolated from the aqueous extract of *T. cordifolia* stem [23] showed similar phytochemicals to that of current study. The medicinal value of the presently studied fruit samples are mainly attributed by the presence of phytochemical substances that exhibit definite medicinal effect in the human body.

3.2. Quantification of Phytochemicals

The concentration of total phenols and flavonoids were quantified in the ethanol and aqueous extracts of two different fruit samples. The concentration of total phenols and flavonoids in the examined fruits was expressed in terms of gallic acid and quercetin equivalents, respectively. The concentration of total phenols in the examined fruit extract was ranged from 325 to 445 mg GAE/100 g while the total flavonoids ranged from 112 to 217 mg QE/100 g (Table 2). Significantly highest concentration of total phenols and flavonoids were noted in the ethanol extract of *T. cordifolia* when compared to *P. americana*. The concentrations of phytochemicals in the extracts depend on the polarity of solvents and type of plant material used for the extractions.

A large number of wild edible fruits and their purified constituents have shown beneficial therapeutic potentials. Various wild edible fruits have been reported to exhibit antioxidant activity, including *T. cordifolia* and *P. americana* fruits. The majority of the antioxidant activity is due to the presence of flavonoids, flavones,
isoﬂavones, anthocyanins, coumarins, lignans [24]. Phenolic and other phytochemical antioxidants found in fruits are bioactive compounds capable of neutralizing free radicals and may play a role in the prevention of certain diseases. Phenolic compounds are widely found in food products derived from plant sources, and they have been shown to possess signiﬁcant antioxidant activities. In presently investigated samples, the T. cordifolia fruit shows high levels of total phenols and ﬂavonoids content, which might be responsible for its high antioxidant activity. This is in agreement with that of previous report on Malus domestica [25].

3.3. Antioxidant Activity

The antioxidant potential of presently analyzed fruit samples were determined by performing radical scavenging assay against 2,2-diphenyl-1-picryl hydrazine (DPPH). DPPH assay is known to give reliable information concerning the antioxidant ability of the tested extracts. In both the samples, a maximum scavenging activity was offered by ethanol extract of P. americana fruits (84.43%) and T. cordifolia fruit (76.83%) followed by aqueous extract of the respective plants (Table 3). The ethanol and aqueous extracts of the two fruit samples of the present study showed signiﬁcantly higher DPPH scavenging activity when compared with standard ascorbic acid at 1000 mg/ml. The presently studied ethanolic extract of both the samples was found to exhibit more effective free radical inhibition activity against DPPH. The free radical inhibition activity of ethanolic extract of the presently analyzed two different fruit samples was ranged between 76.83 and 84.43 %, which is in agreement with that of previous reports on Hylocereus undatus [26]; Malus domestica [25]; Minusa elengi, Terminalia citrina, Toddalia asiatica, Solanum torvum and Ziziphus rugosa [27]. Among the two different samples, P. americana fruits showed the highest free radical scavenging activity.

The reducing power of the presently studied extracts of two different fruit samples were given in Table 4. The reducing power was found to be ranged from 0.082 to 0.876 for the investigated extracts. The reducing power of the fruit extract increased in concentration dependent manner. Among the two different fruit samples, P. americana has exhibited signiﬁcantly highest level of reducing power (0.876%).

### Table 2. Total Phenols and Flavonoid Content of Ethanol and Aqueous Extracts of T. cordifolia and P. americana Fruits

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compounds</th>
<th>T. cordifolia Fruit Extracts</th>
<th>P. americana Fruit Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
<td>Aqueous</td>
</tr>
<tr>
<td>1</td>
<td>Total phenols</td>
<td>$445^a \pm 0.05$</td>
<td>$325^c \pm 1.43$</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>$217^a \pm 0.02$</td>
<td>$184^c \pm 0.05$</td>
</tr>
</tbody>
</table>

The data are mean of triplicate determinations ± Standard error. Values in the same row with different superscripts are statistically different ($p < 0.05$).

### Table 3. DPPH Radical Scavenging Activity of Ethanol and Aqueous Extracts of T. cordifolia and P. americana Fruits

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract Concentration (mg/ml)</th>
<th>DPPH Radical Scavenging Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T. cordifolia Fruit Extracts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td>1</td>
<td>1000</td>
<td>$76.83^a \pm 2.02$</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>$47.13^a \pm 2.20$</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>$29.73^a \pm 1.55$</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>$16.50^a \pm 2.50$</td>
</tr>
<tr>
<td>5</td>
<td>62.5</td>
<td>$9.60^a \pm 1.21$</td>
</tr>
<tr>
<td>6</td>
<td>Ascorbic acid (1000 mg/ml)</td>
<td></td>
</tr>
</tbody>
</table>

The data are mean of triplicate determinations ± Standard error. Values in the same row with different superscripts are statistically different ($p < 0.05$).
The ethanol and aqueous extracts of both the fruit samples of the present study showed significantly higher reducing power when compared with the aqueous extract and standard ascorbic acid (1000 mg/ml). The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow colour of the ferric ion solution change to dark bluish green depending on the reducing power of the extract. The reducing power of the extract might be due to their hydrogen donating ability. Possibly, *P. americana* fruit extract contains high amounts of reducing phytochemicals, which could react and reduce the ferric ions. Present investigated fruit extracts showed high reducing power due to their better ability to donate electrons. Both the fruit extracts were found to exhibit high level of reducing power in a dose- dependent manner. The results are comparable to earlier report on *Hylocereus undatus* [26] and *Malus domestica* [25].

### 3.4. Anti Hyperglycemic Activity

The medicinal plants which are used in Ayurvedic traditional system to treat diabetes are a valuable source of novel anti-diabetic agents in India. *T. cordifolia* stem is widely used in Ayurveda for treating diabetes mellitus by regulating the blood glucose [28]. Pancreatic α-amylase and α-glucosidase inhibitory action of phytochemicals from plant sources offer an effective strategy to lower the levels of post-prandial hyperglycemia via control of starch breakdown [29].

In the present study two different fruit samples were analyzed for hypoglycemic properties in terms of their enzyme inhibition potential against α-amylase and α-glucosidase enzymes. Different solvent extracts of fruit samples revealed α-amylase and α-glucosidase inhibition activity. α-Amylase inhibition activities of fruit samples of *T. cordifolia* and *P. americana* were ranged from 20.67 to 35.14 % (Figure 1). Aqueous extract of *T. cordifolia* exhibited significantly strong inhibitory activity (35.14 %) at 100 mg/ml concentration. α-Glucosidase inhibition activity of fruit samples of *T. cordifolia* and *P. americana* was found to be ranged between 27.20 and 42.01 % (Figure 2). Ethanol extract of *T. cordifolia* strongly inhibited the α-glucosidase activity. Among the two different fruit samples studied, the aqueous and ethanolic extracts of *T. cordifolia* fruit showed significant inhibitory activity against α-amylase and α-glucosidase enzymes, respectively. This is comparable with that of earlier report on seeds of *Linum usitatissimum*, leaves of *Morus alba* and *Ocimum tenuiflorum* [29]; *Aloe vera*, *Adansonia digitata*, *Allium sativum*, *Casia fistula*, *Catharanthus roseus*, *T. cordifolia*, *Gymnema sylvestre*, *T. cordifolia*, *Eugenia jambolana* and *Aegle marmelos* [30-34].

#### Table 4. Reducing Power of Ethanol and Aqueous Extracts of *T. cordifolia* and *P. americana* Fruits

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extract Concentration (mg/ml)</th>
<th>Reducing power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>T. cordifolia</em> Fruit Extracts</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>Aqueous</td>
</tr>
<tr>
<td>1</td>
<td>1000</td>
<td>0.769 ± 1.52</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>0.352 ± 2.08</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>0.191 ± 2.00</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>0.122 ± 2.30</td>
</tr>
<tr>
<td>5</td>
<td>62.5</td>
<td>0.096 ± 1.00</td>
</tr>
<tr>
<td>6</td>
<td>Ascorbic acid (1000 mg/ml)</td>
<td>0.685 ± 0.23</td>
</tr>
</tbody>
</table>

The data are mean of triplicate determinations ± Standard error. Values in the same row with different superscripts are statistically different (*p* < 0.05).


4. CONCLUSION

Ethanolic extract from the fruits of *Tinospora cordifolia* and *Persea americana* exhibited remarkable levels of phytochemicals, antioxidant activity and anti-hyperglycemic properties. On the basis of the results of this present study, the *P. americana* fruits was found to exhibit highest antioxidant activity when compared to *T. cordifolia*. The aqueous and ethanolic extracts of *T. cordifolia* strongly inhibited the activity of hyperglycemia-linked enzymes such as α-amylase and α-glucosidase. Hence, such wild fruit samples could be considered as potential candidates for the management of hyperglycemia and oxidative stress-related diseases. However, further scientific investigations in suitable animal model would be required to evaluate their medicinal properties and mechanism of action.

REFERENCES


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