Diabetes is an emerging endocrine disorder, caused by progressive insulin resistance, impaired glucose utilization in adipose tissues and impairment of functioning beta cells. Drugs currently used in the treatment of diabetes focus on preventing the complications arising from diabetic neuropathy and nephropathy. However, prolonged use of these drugs aggravates side effects like nausea, diarrhea, hypoglycemia, increased body weight and sometimes can affect the functioning of the liver. α-amylase enzyme catalyzes the hydrolysis of α (1-4) glycosidic linkage of polysaccharides and supports in the regulation of blood sugar level. The inhibitors of this enzyme would lower the glucose absorption and control the elevation of glucose levels. The practice of traditional medicine for the treatment of various diseases has gained a global reputation and search of molecules with therapeutic activity. The methanol fraction of the leaves was reported for its activity against bacterial diabetic foot ulcer. In the present study, extracts of Tragia involucrata Linn. were screened for qualitative phytochemical analysis and investigated for the α-amylase enzyme inhibitory potential.

**MATERIALS AND METHODS**

**Chemicals and Reagents**

α-Amylase enzyme (EC 3.2.1.1) and acarbose were obtained from Sigma Aldrich. All other solvents and chemicals were purchased from SD Fine chemicals and used as such for the experiment.

**Plant Material Collection and Processing**

The fresh leaves of Tragia involucrata Linn. were collected from various regions of Vellore, Tamil Nadu, India and further authentication was carried out at the Plant Anatomy Research Centre in 2013 and voucher specimen was deposited (PARC/2013/2170).

**Preparation of Extracts**

The fresh leaves of Tragia involucrata Linn. were washed with distilled water and shade dried at room temperature. The dried leaves were milled using a mixer and sieved. The powdered sample of 50 g was successively extracted with solvents of increasing polarity: Petroleum ether (TI-P), Ethyl Acetate (TI-EA), Chloroform (TI-C) and Water (TI-A) by soxhlet method. The extracts are then...
concentrated under reduced pressure using a rotary evaporator and stored in a desiccator for further analysis.

**Phytochemical Screening**

The preliminary phytochemical screening of TI-P, TI-EA, TI-CH and TI-A extracts of *Tragia involucrata* Linn. were carried out by standard methods as described.

**α- Amylase Inhibitory Assay**

The extracts were screened for α-amylase inhibitory activity by following the method reported by Ashok Kumar *et al.* with simple modification. Briefly 50 µl of α-amylase (5 U/ml) was taken in three test tubes and extracts of different concentrations (50, 100, 200 µg/ml) were added and pre-incubated at room temperature for 20 minutes. The catalytic reaction was initiated by the addition of 0.5 % starch substrate dissolved in 20 mM phosphate buffer (pH 6.9) followed by incubation for 20 minutes at 37°C. The catalytic activity was terminated by addition of 2 ml of DNS reagent (1 % 3, 5-dinitrosalicylic acid and 12 % sodium potassium tartrate in 0.4 M NaOH) to the reaction mixture and refluxed at 100°C for 15 minutes. The percentage inhibition of amylase activity was determined by measuring the absorbance at 540 nm. Each concentration was performed in triplicates and the mean absorbance was used to calculate the percentage of amylase inhibition.

\[
\text{Ac-As} = \frac{A_c - A_s}{A_c} \times 100
\]

Where “% A” is the percentage of α-amylase inhibition, Ac is the absorbance of control and As is the absorbance of the sample. Acarbose, a known α-amylase inhibitor was used as the standard.

**Statistical Analysis**

All the experiments were performed in triplicates and the data were expressed as mean± standard deviations by using SPSS software version 16.0.

### Table 1: The phytochemical screening of leaf extracts of *Tragia involucrata* Linn.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Tragia involucrata Linn. Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TI-A</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>Protein and Amino Acids</td>
<td>-</td>
</tr>
<tr>
<td>Phenols and Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) Indicates absence and (+) indicates presence
RESULTS AND DISCUSSION

The preliminary phytochemical screening reveals the presence of various phytoconstituents mainly steroids, terpenoids, phenols and carbohydrates. The results are tabulated in Table 1. The amylase enzyme inhibitory role of Tragia involucrata Linn. extracts were studied by Dinitrosalicylic acid method. The TI-P extract showed lowest inhibitory activity compared to standard acarbose (Figure 1). The TI-A, TI-EA and TI-CH extracts exhibited potent inhibitory activity with an increase in concentration in a dose dependent manner. The increasing incidences of diabetes mellitus studies focus on identification of potential compound with greater efficacy. Recent studies showed novel steroids 28 Nor-22 (R) with 2, 6, 23-trienolide, β-sitosterol and terpenoids to possess potent antidiabetic activity. The phytochemical studies also reveal steroids and terpenoids to be the major components, which might be responsible for the α-amylase inhibitory potential of the extracts.

CONCLUSION

It is shown that the extracts of Tragia involucrata Linn. possess potent α-amylase enzyme inhibitory activity. In earlier studies, steroids and terpenoids have been reported to possess anti-diabetic activity individually. The presence of bioactive secondary metabolites like terpenoids and steroids might play an important role in controlling enzymatic activity. The combined presence of terpenoids and steroids in aqueous, ethyl acetate and chloroform extracts might contribute to the evident anti-diabetic activity of the extracts. Thus, further investigation on individual phytoconstituents would lead to the identification and development of potent lead molecules.

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