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IN VITRO ANTIDIABETIC ACTIVITY OF *TRAGIA INVOLUCRATA* LINN. LEAF EXTRACTS

V. Vinodhini[1], M. Himaja[2]*, V. Sai Saraswathi[3], Das Poppy[1]

[1]Research Scholar, Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore, Tamil Nadu, India

[2]Professor, Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore, Tamil Nadu, India

[3]Assistant Professor (Sr), Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore, Tamil Nadu, India

*Corresponding author

Prof. Dr(Mrs) M Himaja, Program Chair & Division Chair, Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore-632014 Tamil Nadu, India E-mail: drmhimaja@gmail.com

ABSTRACT

Tragia involucrata Linn. a perennial herb, is a potential source of phytoconstituents like terpenoids, sterols, phenols and carbohydrates. The plant is used as traditional medicine for treatment of skin eruption, diabetes, and cephelagia in many regions. Rationale of the present study was to carry out phytochemical screening and examine the in-vitro α-amylase inhibitory role of *Tragia involucrata* Linn. extracts. The α-amylase inhibitory potential of extracts was studied by dinitrosalicylic acid (DNS) method and it was found that aqueous extract (TI-A), ethyl acetate extract (TI-EA) and chloroform extract (TI-C) with increasing concentration restrain effective α-amylase enzyme inhibitory activity.

Keywords: *Tragia involucrata* Linn., Phytochemical screening, α-amylase, anti diabetes.

INTRODUCTION

Diabetes is an emerging endocrine disorder, caused by progressive insulin resistance, impaired glucose utilization in adipose tissues and impairment of functioning beta cells[1,2]. Drugs currently used in the treatment of diabetes focus on preventing the complications arising from diabetic neuropathy and nephropathy[3]. 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[4]. α-Amylase enzyme catalyzes the hydrolysis of α (1-4) glycosidic linkage of polysaccharides and supports in the regulation of blood sugar level[5]. 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 efficiency provides opportunities for identification of novel leads[6]. It is still noteworthy that there is a lack of knowledge about active plant compounds of desired effects. *Tragia involucrata* Linn. is a traditional folk medicine belonging to Euphorbiaceae family. *Tragia* species have been reported to contain wide pharmacological properties in the treatment of bronchitis[6], liver disorders, eczema, viral infections[7] and inflammations[8]. *Tragia involucrata* Linn. extracts are potential source of phytoconstituents like phenols, terpenoids, steroids and carbohydrates. Previous studies reported the in-vivo anti-diabetic potential of whole plant extract[9] and experiments revealed that the leaf extracts and its isolated compounds possess potent antimicrobial activity[10]. The methanol fraction of the leaves was reported for its activity against bacterial diabetic foot ulcer[11]. 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[12].

α- Amylase Inhibitory Assay

The extracts were screened for α-amylase inhibitory activity by following the method reported by Ashok Kumar *et al*.[13] 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 37^0C. 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^oC 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.

$$\% \ A = \frac{Ac-As}{Ac} \times 100$$

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.

Phytoconstituents	*Tragia involucrata* Linn. Extracts			
	TI-A	TI-E	TI-C	TI-P
Alkaloids	-	-	-	-
Carbohydrates	+	+	+	+
Glycosides	-	-	-	-
Phytosterols	+	+	+	+
Protein and Amino Acids	-	-	-	-
Phenols and Tannins	-	-	-	+
Terpenoids	-	+	+	+
Saponins	+	-	-	+
Flavonoids	-	-	-	-

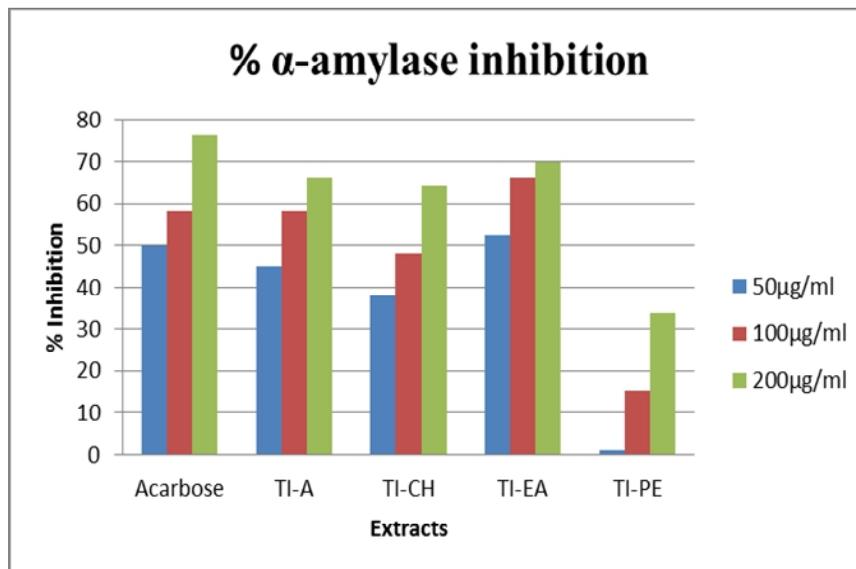(-) Indicates absence and (+) indicates presence

Figure 1: % inhibition of α-Amylase activity

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) witha 2, 6, 23-trienolide[14], β-sitosterol[15] and terpenoids[16] 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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