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# IN-VITRO EVALUATION OF ANTI-DIABETIC ACTIVITY OF ETHANOLIC EXTRACT TRICHOLEPIS GLABERRIMA

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**Abstract**

*Tricholepis glaberrima* commonly known as "Brahmadandi" belongs to the family Asteraceae. It is prominently used by traditional healers as an aphrodisiac. The plant contains several phytochemical constituents such as carbohydrates, flavonoids, tannins, steroids and triterpenoids and glycosides. Even though so many allopathy are available but can act certain extent only, hence there is a need for the traditional formulation development. With this aim we have started the research. In this research we aimed to evaluate the anti-diabetic activity of ethanolic extract *Trichloepis glaberrima* using *In-Vitro* process. The dried root of *Tricholepis glaberrima* was extracted with ethanol & was prepared by soxhlation using soxhlet apparatus with ethanol as a solvent extract evaluated for anti- diabetic activity by *In-vitro* (i.e., by **α** -amylase inhibition assay, **α**-glucosidase inhibition assay). From the results ethanolic extract *Tricholepis glaberrima* has exhibited significant anti-diabetic activity.

**Key words:** *Trichloepis glaberrima*, Albino rats, Anti-diabetic activity, *In vitro*, Alpha amylase inhibition assay, Alpha glucosidase inhibition assay.

**1. Introduction:** *Tricholepsis glaberrima* commonly known as "Brahmadandi" belongs to the family Asteraceae. It is prominently used by traditional healers as an aphrodisiac[1]. In this research we aimed to evaluate the anti-diabetic activity of ethanolic extract Trichloepsis glaberrima on albino rats[2]. The plant contains several phytochemical constituents such as carbohydrates, flavonoids, tannins, steroids and triterpenoids and glycosides. It is prominently used by traditional healers as an aphrodisiac. It also used in cough, leucoderma, skin diseases & also nourishes skin, swellings, heals wounds, oedema. It has anticancer activity, anti-septic properties[3]. In addition, ash is mixed with oil for treating chronic non-healing wound. It is also claimed to be beneficial in haematuria such as condition, blurring micturition, and maintaining the natural colour of urine. According to International Diabetes Federation (IDF), worldwide 382 million people were affected by diabetes in 2013 and it is expected to raise to 592 million by 2035. IDF estimates 65 million diabetic patients in India in 2013and it is expected to cross 109 million by 2030. In India diabetic patients are increasing day by day may be because of the change in food pattern, i.e. fast food diet intake and change in lifestyle[4]. Management of diabetes is a tough task. The medicines used in diabetic treatment are either too costlier or have adverse effects like hypoglycaemic coma, insulin resistance, hypersensitivity and metallic taste etc[5]. Hence, in the recent years, herbal compounds are gaining popularity in both developed and developing countries because of their natural origin, low adverse effects. Even though so many allopathy formulations are available but can act certain extent only, hence there is a need for the traditional formulation development. With this aim we have extracted the *Tricholepis glaberrima* with ethanol as a solvent extract was evaluated for anti-diabetic activity by *In-vitro* methods (i.e., by **α** -amylase inhibition assay, **α**-glucosidase inhibition assay).

## 2. MATERIALS AND METHODS:

### 2.1 Plant material

The bark of the *Tricholepis glaberrima* plant was collected from the Tirupati, Chittoor district, Andhra Pradesh, India and was authenticated by Botanist Dr K Madhavashetty at Sri Venkateswara University.

**2.2 Extraction and phytochemical investigation :** The plant material was grounded in a mixer. The crude drug powder passed through sieve no. 20 and retained part, was used for extraction by using ethanol. The ethanolic extract of the drug was packed in Soxhlet extractor and extracted using chloroform as a solvent.

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**3. Experimental process:**

**3.1 Alpha-Amylase Inhibition Assay:** The alpha-amylase assay was performed according to the method described by Odeyemi[7] . Briefy, 15 µl of the plant extract at diferent concentrations (50 µg/ml – 200 µg/ml) (diluted in a phosphate bufer) was added to 5 µl of enzyme porcine pancreatic solution into 96-well plate. Afer 10 min of incubation at 37∘ C, the reaction was initiated by adding 20 µl of starch solution and further incubated for 30 min at 37∘C. The reaction was then stopped by adding 10 µl 1M of HCl to each well followed by 75 µl of iodine reagent. A blank containing phosphate buffer (pH 6.9) instead of the extract and a positive control (acarbose, 64 µg/ml) were prepared. No enzyme control and no starch control were included for each test sample. The absorbance was measured at 580 nm and the percentage inhibitory activity was calculated by using the following equation:

$$\% \text{ Inhibition} = \frac{(1 - \text{Absorbance of the untreated (Control)}}{\text{(Absorbance of the test well)}} \times 100$$

**3.2 Evaluation of *In vitro* α-glucosidase inhibitory activity using EE of TG extract :**

The alpha-glucosidase inhibition assay was determined using a method described by Sancheti et al[8]. with slight modifcation. Briefly, 5 µl of the plant extract (prepared at concentration of 50 µg/ml, 100 µg/ml, and 200 µg/ml) was added to 20 µl of 50 µg/ml alpha-glucosidase solution into a well of a 96-well plate. Thereafter, 60 µl of 67 mM potassium phosphate buffer (pH 6.8) was added. After 5 min of incubation, 10 µl of 10 mM p-nitrophenyl- α-D-glucoside solution was then added and further incubated for 20 min at $37^0$C. After incubation, 25 µl of 100 mM $Na_2CO_3$ (sodium carbonate) solution was added and the absorbance was measured at 405 nm. A blank sample was also prepared by adding 5 µl of deionised water instead of plant extract and 20 µl of deionised water instead of enzyme respectively.

Epigallocatechin gallate (10 µg/ml) was used as a positive control. The percentage inhibition

was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{(1 - \text{Absorbance of the test well})}{\text{Absorbance of the untreated (control)}} \times 100$$

**4. Results :**

**Table:1 Effect on *In-vitro* α-amylase inhibitory activity using Ethanolic bark extract of *Tricholepis glaberrima***

| Sample | Concentration in µg/ml | % of inhibition | $Ic_{50}$ |
|---|---|---|---|
| Ethanolic extract of T.G | 100 | 26.88 ±2.11 | 72.44 ± 3.20 |
| | 200 | 34.76±1.09 | |
| | 400 | 36.39±2.43 | |
| | 800 | 48.20±0.32 | |
| | 1000 | 55.66 ± 1.32 | |
| Acarbose | 100 | 32.73 ± 1.84 | 41.40±1.09 |
| | 200 | 46.14 ± 3.21 | |
| | 400 | 55.86 ± 4.22 | |
| | 800 | 61.45 ± 3.11 | |
| | 1000 | 78.21 ± 0.44 | |

**Graph:1 Graphical representation of effect on *In-vitro* α-amylase inhibitory activity using Ethanolic bark extract of *Tricholepis glaberrima***

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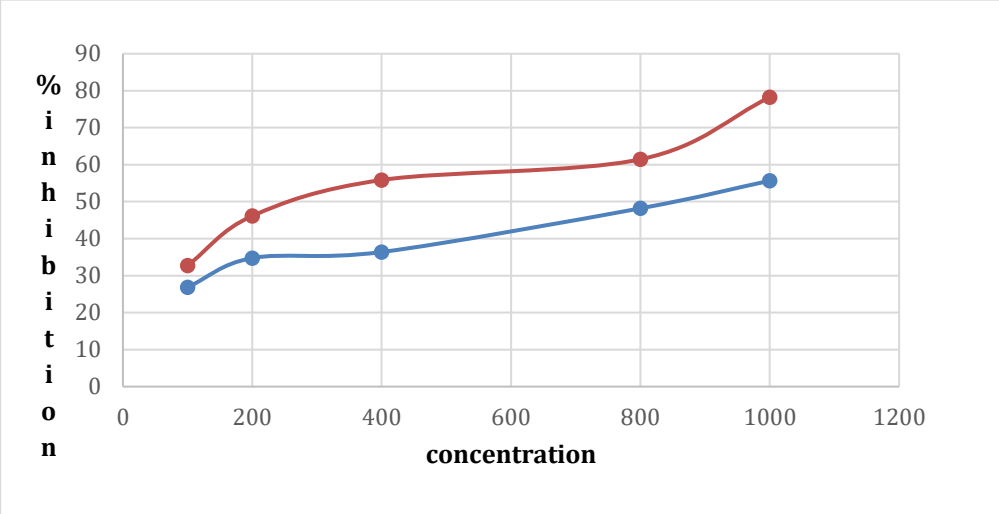


**Table:2 Effect on *In vitro* α-glucosidase inhibitory activity using Ethanolic bark extract of *Tricholepis glaberrima***

| Sample | Concentration in µg/ml | % of inhibition of alpha glucosidase | $Ic_{50}$ |
|---|---|---|---|
| Ethanolic extract of T.G | 100 | 31.10± 3.21 | 49.98 ±1.20 |
| | 200 | 36.82 ± 3.29 | |
| | 400 | 47.09± 1.03 | |
| | 800 | 55.31 ± 0.32 | |
| | 1000 | 71.39 ± 3.40 | |
| Acarbose | 100 | 42.43 ± 2.32 | 32.84±0.87 |
| | 200 | 47.14 ± 1.76 | |
| | 400 | 58.09 ± 2.09 | |
| | 800 | 76.31 ± 2.07 | |
| | 1000 | 88.31 ±2.86 | |

**Graph:2 Effect on in vitro α-glucosidase inhibitory activity using Ethanolic bark extract of *Tricholepis glaberrima***

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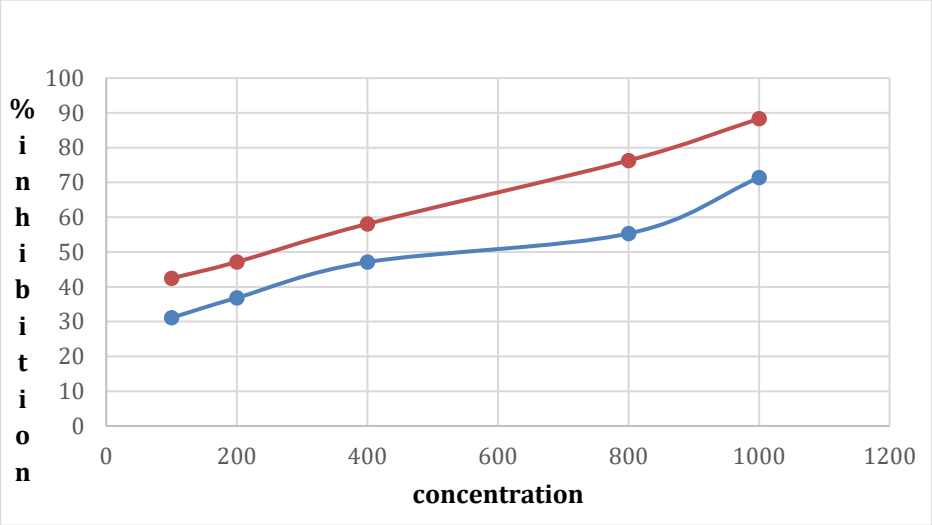


## 5. Discussion

Lack of insulin affects the metabolism of carbohydrates, proteins, fat and causes significance disturbance of water and electrolyte homeostasis . Recent advances in understanding the activity of intestinal enzymes (α-amylase and α-glucosidase both are important in carbohydrate digestion and glucose absorption) have lead to the development of newer pharmacological agents. A high postprandial blood glucose response is associated with micro- and macrovascular complications in diabetes and is more strongly associated with the risk for cardiovascular diseases than are fasting blood glucose. α-Glucosidase enzymes in the intestinal lumen and in the brush border membrane play main roles in carbohydrate digestion to degrade starch and oligosaccharides to monosaccharides before they can be absorbed. It was proposed that suppression of the activity of such digestive enzymes would delay the degradation of starch and oligosaccharides, which would in turn cause a decrease in the absorption of glucose and consequently the reduction of postprandial blood glucose level elevation. Alpha-glucosidase inhibitor retards the digestion of carbohydrates and slows down the absorption. Acarbose and miglitol are competitive inhibitor of α-glucosidases and reduces absorption of starch and disaccharides. Hence one of the therapeutic approaches for reducing postprandial (PP)blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Inhibition of these enzymes (α-amylase and α-glucosidases) reduced the high postprandial ( PP) blood glucose peaks in diabetes. Acarbose and miglitol are competitive inhibitor of α glucosidases and reduces absorption of starch and disaccharides . The α-amylase from the Table:1 and Graph:1 reports inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates. Acarbose is complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effect such as abdominal pain, diarrhoea and soft faeces in the colon. Our finding reveals that *Tricholepis glaberrima* efficiently inhibits α-amylase enzyme *In vitro*. The results suggest that ethanol extract of *Tricholepis glaberrima* bark efficiently inhibits α-glucosidase enzymes *In-Vitro*. The antidiabetic action of *Tricholepis glaberrima* bark can also be attributed to the intestinal α-glucosidases inhibitory activity. The CD methanol extract revealed a significant inhibitory action on α-glucosidase enzyme (Table 2 and Graph:2 reports). The percentage inhibition at 100-1000 µg/ml concentrations of TG extract showed a concentration dependent increase in percentage inhibition. The percentage inhibition varied from 71.39 ± 3.40 to 31.10± 3.21for highest concentration to the lowest concentration of 100 µg/ml. The concentration required for 50% inhibition (IC50) was found to be 49.98 ±1.20 µg/ml where as the α -glucosidase inhibitory activity of positive control acarbose produced percentage of 42.43 ± 2.32 for 100 µg/ml and 88.31 ±2.86 for 1000 µg/ml. The IC50 value of standard drug acarbose against α-glucosidase was found to be 32.84±0.87 µg/ml.

## 7.Conclusion :

Conclusion Data accrued from the present study clearly indicate that the ethanolic extract of the bark and leaves exhibited significant antihyperglycemic by inhibiting alpha amylase and glucosidase by *In-Vitro* studies. Further investigation is necessary to determine the exact phytoconstituent(s) responsible for anti-DM effect.

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**8. References:**

1. Padashetty S.A. & S.H. Mishra (2007); Aphrodisiac Studies of *Tricholepis glaberrima*. with Supportive Action from Antioxidant Enzymes, Pharmaceutical Biology, 45:7,580-586.

2. AvinashPatil A, SwapneelKoli A, Darshana A, Patil B, Vinod Narayane C, Anita V. Phatak (2013), A. Evaluation of effect of aqueous slurry of Curculigoorchioidesaertn. rhizome in streptozotocin induced diabetic rats. Journal of pharmacy research; 7: 747-53 .

3. Kim JS, Kwon CS, Son KH. 1944, Bioscience Biotechnology and Biochemistry 2000; 64(11): 2458- 2461. 22.

4. IDF Diabetes Atlas. 6th Ed. Belgium: International Diabetes Federation: 2013.

5. Devi Manickam, LathaPeriyasamy (2013). Antidiabetic effect of methanolic extract of Decalepishamiltonii root in normal and alloxan induced diabetic rats. Journal of pharmacy research; 16(6): 166-72.

6. U.K. Prospective Diabetes Study Group, "U.K. Prospective Diabetes Study 27: plasma lipids and lipoproteins at diagnosis of NIDDM by age and sex," Diabetes Care, vol. 20, pp. 1683– 1687, 1997.

7. A. Nirmala, J. Eliza, M. Rajalakshmi, E. Priya, and P. Daisy, "Effect of hexane extract of TG barks on blood glucose and lipid profile in Streptozotocin diabetic rats," International Journal of Pharmacology, vol. 4, no. 4, pp. 292–296, 2008.

8. Madhav SN, Upadhyaya K, Bisht A. Phytochemical screening and standardization of polyherbal formulation for dyslipidemia. Int J Pharm PharmSci2011;13:235-8.