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# EVALUATION OF IN VITRO ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY POTENTIAL OF ROOTS OF EUPHORBIA HIRTA LINN

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***Keywords:*** *antidiabetic, alpha amylase, alpha glucosidase, in-vitro, Euphorbia hirta*

## *Abstract*

**Aim:** The present study was designed to investigate the in vitro inhibitory potential of Euphorbia hirta root extract on alpha-amylase and alpha glucosidase enzymes.

**Materials and Methods:** Alcoholic extract of Euphorbia hirta was subjected to inhibitory effect of alpha-amylase and alpha-glucosidase using specific standard in vitro procedure.

**Results:** The results revealed that extract successfully inhibited the activity of both enzymes in an in vitro model. The alcoholic root extract of Euphorbia hirta inhibited the alpha amylase and alpha glucosidase enzymes as 79.73 ± 0.18% and 81.35 ± 0.12% respectively.

**Conclusion:** The present study showed that, the alcoholic extract showed a significant inhibitory effect on alpha amylase and alpha glucosidase enzymes, thus validating the traditional use of the plant.

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## Introduction

Diabetes mellitus is a metabolic disorder characterized by deficiency in insulin production by pancreas, or by improper action of the insulin produced, or both. Diabetes mellitus is one of the most common metabolic disorders which is associated with damage of vital organs like eyes, nerves, heart and blood vessels.[1] According to WHO, it is estimated that 3.5% of the world's population have suffered from diabetes and the prevalence is expected to be double by the year 2025 to 7.2%. Various Synthetic antidiabetic agents like sulphonylureas, biguanides etc, are available, but management of diabetes without any side effect is still a challeng.[2] In comparison with synthetic agents, herbal drugs are safer, cheaper and much effective, so traditional antidiabetic herbal drugs can be used as alternative drugs.[3] Thus aim of the present study is to investigate the in vitro antidiabetic potential of root extract of *Euphorbia hirta*. *Euphorbia hirta* belongs to family Euphorbiaceae commonly known as *Dudhi*. The roots are fibrous and dark brown in colour.[4] It is found abundantly along the roadsides and in open grasslands areas. [5,6] It has been reported to contain alkaloids, tannins, saponins and flavonoids. Traditionally, it is used in the treatment of bronchial and respiratory diseases, gastrointestinal disorders and diabetes. [7] It also shows antipyretic, anxiolytic, antifertility, antimalarial, analgesic and anti-inflammatory activities. [8,9] Euphorbia hirta is reported to contain Quercitrin, Myricitrin, Afzelin, euphorbin-A, euphorbin-B, euphorbin-C, euphorbin-D, shikmic acid, choline kaempferol, gallic acid derivatives and protocatechuic acid.[10,11] Our literature survey revealed that there is no evidence of antidiabetic effect of roots of the plant. Therefore, the present study was carried out to investigate the in vitro antidiabetic potential of root extract of *Euphorbia hirta*.

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## Materials and methods

**Collection of Plant material**
The roots of Euphorbia hirta were collected in the month of June 2019 from Hodal, India. A voucher specimen has been retained at the School of Medical and Allied Sciences, K.R. Mangalam University , Sohna Road, Gurgaon. The roots were cleaned thoroughly with distilled water to remove any type of contamination. Washed roots were air dried in shade.

**Preparation of the plant extract**
To prepare root extract of *Euphorbia hirta* Linn, the dried roots were powdered by using dry grinder and passed through sieve. This powder was packed into soxhelet apparatus and extracted successively with alcohol. The process was continued until the solvent in the thimble becomes transparent. The extract was solidified under reduced pressure in a rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below $10^0$ C. [12]

**In vitro methods for antidiabetic studies**

***Alpha-amylase inhibition assay***
0.5% w/v starch solution was prepared by mixing 0.125gm of potato starch in 100 ml of 0.02 M of sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The enzyme solution was prepared by mixing 1U/ml of alpha-amylase in the same buffer. The reagent was prepared by adding sodium potassium tartarate solution to 3, 5-di nitro salicylic acid solution. The starch solution was added to each tube and incubated at 25°C for 10 minutes. 1 ml of dinitrosalicylic acid color reagent was added and the contents were heated for 15 minutes on a boiling water bath and cooled to room temperature. The final volume was made up with distilled water, and the absorbance was measured at 540 nm using spectrophotometer. The alpha amylase inhibitory activity was expressed as percentage inhibition. [13]
Inhibition % = (Absorbance of control-Absorbance of extract) / Absorbance of control x 100

***Alpha- glucosidase inhibition assay***
The alpha -glucosidase enzyme inhibitory activity was determined by incubating 1 ml of starch substrate solution (2% w/v maltose) with 0.2 M Tris buffer and various concentrations of alcoholic root extract at 37°C for 10 minutes. The reaction was started by adding 1 ml of alpha-glucosidase enzyme and incubated for 60 minutes at 37°C. To stop the alpha -glucosidase reaction, the above reaction mixture was kept in boiling water for 2 minutes and cooled to room temperature. To this, 2ml of glucose reagent was added and absorbance was measured at 540 nm. [14]
Inhibition % = (Absorbance of control-Absorbance of extract) / Absorbance of control x 100
Calculation of 50% Inhibitory Concentration ($IC_{50}$)

The concentration of the plant extract required to inhibit 50% of the enzyme activity ($IC_{50}$) was calculated by using the percentage inhibition potential of alcoholic root extract of *Euphorbia hirta* at five different concentrations.

**Statistical Analysis**
The data was expressed as mean ± standard deviation (SD).The Student's t-test was used for statistical comparisons between results obtained from different samples and between results from samples and controls. A probability value of less than 0.05 was considered as significant.

## Results and Discussion
The inhibition of alpha-amylase and alpha-glucosidase activities is one of the main practical strategies for evaluation of antidiabetic effect. The antidiabetic activity of *Euphorbia hirta* was studied by in vitro alpha-amylase and alpha-glucosidase inhibition assay. In this study, the alcoholic extract of Euphorbia hirta showed concentration dependent inhibitory activity against alpha-amylase and alpha-glucosidase enzyme. The in vitro antidiabetic activity of the extract was compared with that of the standard drug Metformin. Table 1 (Fig. 1) shows the results of in vitro Antidiabetic activity of alcoholic extract of *Euphorbia hirta* roots and standard drug Metformin by the use of alpha amylase enzyme. At a concentration of 0.2 mg/ml Metformin and alcoholic extracts of *Euphorbia hirta* have the

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inhibitory activity of 27.32 ± 0.02% and 26.30 ± 0.06% respectively. The maximum concentration of 1.0 mg/ml of the alcoholic extract showed 79.73 ± 0.18% of inhibitory effect against the alpha amylase enzyme. Table 2 (Fig. 2) shows the results of in vitro antidiabetic activity of the alcoholic root extract of *Euphorbia hirta* and standard drug Metformin in alpha glucosidase method. The extract showed the significant inhibitory action of alpha glucosidase enzyme in a dose dependent manner. In alcoholic extract, the inhibitory percentage varied from 28.21% - 81.35%, and the inhibitoty effect of Metformin varied from 36.13% - 92.35%. Alcoholic extract of *Euphorbia hirta* shows inhibitory effect against the alpha amylase and alpha glucosidase enzyme with an $IC_{50}$ value 0.537 mg/ml and 0.544 mg/ml respectively whereas standard drug Metformin shows inhibitory effect against the alpha amylase and alpha glucosidase enzyme with an $IC_{50}$ value 0.488 mg/ml and 0.425 mg/ml respectively (Table 3, Fig. 3). These results indicated that the extract has a marked antidiabetic effect.

## Conclusion

Traditional herbal medicines are used in the world for treatment of various diseases. Worldwide, over 2500 species of plants have been reported to be used as traditional medicine for treatment of diabetes. Antidiabetic effect of plants was due to presence of the plant metabolites which have ability to increase glucose transport and to stimulate insulin secretion. [15] Herbal drugs are used widely because of their effectiveness, lesser side effects and relatively low cost. [16] Therefore, research on such metabolites from traditional herbal plants has become more important. Based on reported traditional uses, the alcoholic root extracts of *Euphorbia hirta* was investigated for its antidiabetic activity and the results showed that alcoholic extract possess noticeable antidiabetic effect. The plant showed significant inhibitory effect against the alpha amylase and alpha glucosidase enzyme in a dose-dependent manner with an $IC_{50}$ value comparable to that of the drug metformin. The antidiabetic activity of roots may be due to its active constituents like flavanoids, tannins and alkaloids etc. The present study demonstrates that alcoholic extract represents potential inhibiton of alpha-amylase and alpha glucosidase enzyme and also encourages for further in vivo studies, isolation and characterization of active compounds.

## Acknowledgements

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**Tables:**

***Alpha-amylase inhibition assay***

**Table 1.** ***In Vitro Antidiabetic Activity of Alcoholic Extract Of Euphorbia Hirta Roots And Metformin By Alpha Amylase Method***

| Concentration mg/ml | % Inhibition | |
|---|---|---|
| | Metformin | Alcoholic Extract |
| 0.2 | 27.32 ± 0.02 | 26.30 ± 0.06 |
| 0.4 | 34.67 ± 0.16 | 31.50 ± 0.11 |
| 0.6 | 63.14 ± 0.05 | 61.31 ± 0.13 |
| 0.8 | 82.50 ± 0.1 | 74.80 ± 0.01 |
| 1.0 | 91.80 ± 0.07 | 79.73 ± 0.18 |

Values represent mean ± SD. (n=6)

***Alpha- glucosidase inhibition assay***

**Table 2.** ***In Vitro Antidiabetic Activity of Alcoholic Extract Of Euphorbia Hirta Roots And Metformin By Alpha glucosidase Method***

| Concentration mg/ml | % Inhibition | |
|---|---|---|
| | Metformin | Alcoholic Extract |
| 0.2 | 36.13 ± 0.02 | 28.21 ± 0.03 |
| 0.4 | 39.46 ± 0.05 | 31.27 ± 0.1 |
| 0.6 | 66.12 ± 0.06 | 57.12 ± 0.16 |
| 0.8 | 84.75 ± 0.1 | 72.48 ± 0.01 |
| 1.0 | 92.35 ± 0.07 | 81.35 ± 0.18 |

Values are expressed as mean ± S.D (n=6)

**50% Inhibitory Concentration ($IC_{50}$)**

**Table 3.** ***Antidiabetic Potential ($IC_{50}$) Of Alcoholic Extract of Euphorbia Hirta And Metformin***

| STANDARD/EXTRACTS | $IC_{50}$ VALUE (mg/ml) | |
|---|---|---|
| | Alpha amylase assay | Alpha glucosidase assay |
| Metformin | 0.488 | 0.425 |
| Alcoholic root extract | 0.537 | 0.544 |

Values are expressed as mean ± S.D (n=6)

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**FIGURES:**

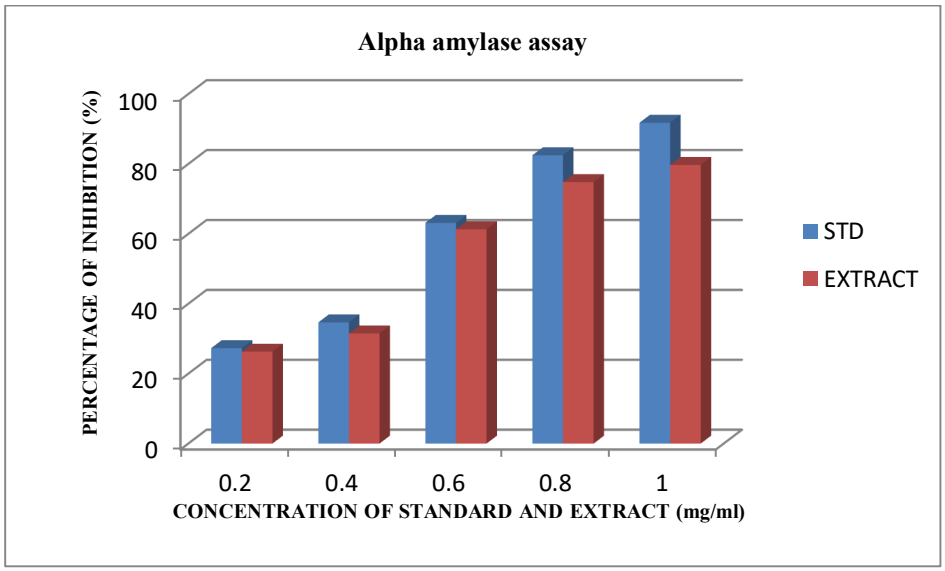


*Fig. 1 In vitro Antidiabetic activity of alcoholic extract of Euphorbia hirta roots and Standard by alpha amylase method*

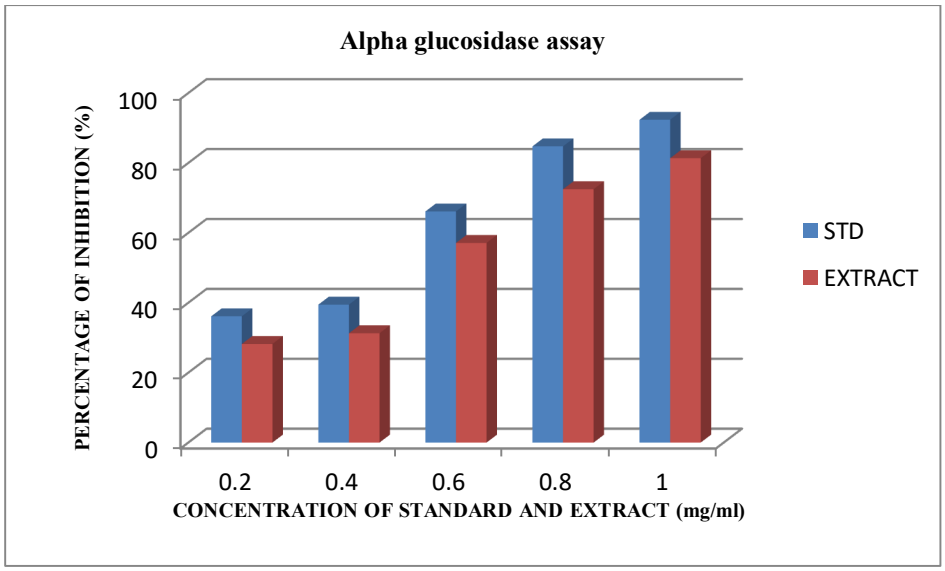


*Fig. 2 In vitro Antidiabetic activity of alcoholic extract of Euphorbia hirta roots and Standard by alpha glucosidase method*

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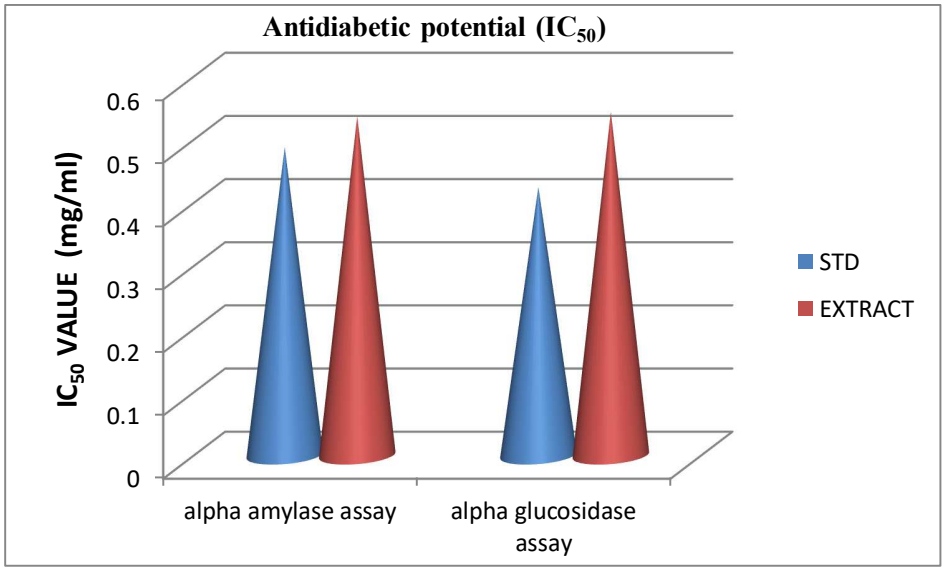


*Fig. 3 Antidiabetic potential ($IC_{50}$) of alcoholic extract of Euphorbia hirta and Standard*