# *In vitro* α-glucosidase and α-amylase inhibition by aqueous, hydroalcoholic, and alcoholic extract of *Euphorbia hirta* L.


## Abstract

**Background:** *Euphorbia hirta* L. (Euphorbiaceae), commonly known as Dudhani, is distributed in the warm region of India and China. Traditionally, it is used in respiratory and gastrointestinal disorders. In addition, the antidiabetic property of the plant was also reported in the literature. This study was designed to evaluate the effect of aqueous, hydroalcoholic, and methanolic extracts (MEHs) of *E. hirta* on α-glucosidase and α-amylase *in vitro*. **Materials and Methods:** Aqueous, hydroalcoholic, and MEHs of *E. hirta* were prepared as per application program interface. In α-glucosidase activity, α-glucosidase (0.1 μ/mL) and substrate, 2.5 mM p-nitrophenyl-α-D-glu copyranoside was used; absorbance was recorded at 405 nm. In α-amylase activity, α-amylase solution (1.0 μ/mL) and substrate, 0.25% starch were used, and absorbance was measured at 540 nm. The $IC_{50}$ values were calculated by linear regression. **Results:** All the extracts showed α-glucosidase inhibitory activity comparable to acarbose with MEH having highest inhibitory activity among tested extracts. The observed $IC_{50}$ values were 213.63, 146.9, 78.88, and 8.07 μg/mL for aqueous, hydroalcoholic, MEH, and acarbose, respectively. All the extracts have shown mild α-amylase inhibitory activity compared to acarbose. Lineweaver–Burk plot has shown that the MEH is a mixed noncompetitive inhibitor for α-glucosidase enzyme. **Conclusion:** The results from this *in vitro* study clearly indicated that MEH of *E. hirta* had strong inhibitory activity against α-glucosidase and mild inhibitory activity against α-amylase. It can be used for management of postprandial hyperglycemia with lesser side effects, and provide a strong rationale for further animal and clinical studies.



**Key words:**
*Euphorbia hirta, Euphorbiaceae, α-amylase, α-glucosidase*


## Introduction

Diabetes mellitus (DM), a global public health problem, is now emerging as an epidemic worldwide.[1] The world prevalence of diabetes among adults was 6.4%, affecting 285 million adults, in 2010, and will be increased to 7.7%, affecting 439 million adults by 2030.[2] Among all cases of diabetes, approximately 90% are type II diabetes.[3] Postprandial hyperglycemia (PPHG) is a more important risk factor in onset and the development of Type II diabetes.[4] Dietary carbohydrates such as starch on hydrolysis yield glucose which is the prime source of glucose in PPHG. Dietary polysaccharides are hydrolyzed by α-amylase to oligosaccharides and disaccharides, which are further hydrolyzed to monosaccharides by α-glucosidases.[5]

Inhibition of α-glucosidase and α-amylase can significantly decrease PPHG and thus reduce the diabetes progression.[6] In the past few decades, some synthetic α-glucosidase inhibitors,


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such as acarbose, miglitol, and voglibose, have been developed and have received considerable attention for the management of type II diabetes.[7] Adverse effects such as abdominal distention, flatulence, meteorism, diarrhea, and pneumatosis cystoides intestinalis are common for these drugs.[8,9] Such adverse effects might be caused by the excessive inhibition of pancreatic α-amylase resulting in the abnormal bacterial fermentation of undigested carbohydrates in the colon.[10,11] The α-glucosidase inhibitors from plant sources are potential alternative to control PPHG. Lower inhibitory effect against α-amylase activity and stronger inhibition activity against α-glucosidase can be an ideal approach for the management of PPHG with minimal side effects.[10,11] *Euphorbia hirta* (Euphorbiaceae), commonly known as asthma weed, is often used in traditional medicine in many parts of Africa, America, Asia, Australia, and Pacific countries for the treatment of several ailments such as respiratory diseases, gastrointestinal disorders, skin diseases, diabetes, and kidney stones.[12] Pharmacological activities such as antioxidant[13] and antidiabetic[14,15] have also been reported. Phytochemical investigation of this medicinal plant revealed the presence of several flavonoids[16,17] and polyphenols.[18]

In the literature, the α-glucosidase inhibitory activity of *E. hirta* has been reported. However, the correlation between the presence of flavonoids and polyphenols to its activity has not been explored. Further in this study, type of inhibition is reported for the first time. Therefore, the objective of this research was to correlate *in vitro* α-glucosidase and α-amylase inhibitory activity of different extracts of *E. hirta* to their composition and to dwell the type of inhibition.

## Materials and Methods

### Chemicals and enzymes

α-glucosidase (EC 3.2.1.20), porcine pancreatic amylase (EC 3.2.1.1, type VI), quercetin, and gallic acid were purchased from Sigma–Aldrich (St. Louis, USA). Acarbose was obtained as a gift sample from Medley Pharmaceuticals Ltd., India. All chemicals and reagents used were of analytical grade.

### Plant material

*E. hirta* (whole plant) was collected from the garden of Maktabah Jafariyah Knowledge and Research Academy, India, geographically located on 23°58'12.5"N, 72°19.02'17"E in the month of May 2011. The plant material was authenticated by Dr. H. B. Singh, Scientist G and Head, Raw Materials, Herbarium and Museum, NISCAIR, New Delhi, India. The specimen was deposited there with the reference number Ref. NISCAIR/RHMD/Consult/2011-12/1785/85.

### Preparation of extracts

The air dried and powdered sample (200 g) were separately extracted with water, methanol-water (1:1, v/v), and methanol by maceration for 24 h with occasional stirring. Extracts were filtered and concentrated under reduced pressure. The dry extracts were stored at 4°C until use.

### Phytochemical analysis

Preliminary, the phytochemical analysis was carried out qualitatively.[19] The quantitative estimation of total phenolic and total flavonoid contents (TFCs) was performed by Folin–Ciocalteu assay[20] and aluminum chloride assay,[21] respectively.

### *In vitro* α-glucosidase inhibition

The α-glucosidase inhibitory activity was measured by standard method.[4] Briefly, 50 μL of sample and 50 μL of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.1 μ/ml) was incubated at 37°C for 10 min. After, 50 μL of 2.5 mM p-nitrophenyl-α-D-glucopyranosi de (PNPG) solution in the same buffer was added to each well and incubated for 20 min. Then, the reaction was stopped by adding 100 μL of 0.2 M $Na_2CO_3$ and absorbance (A) was recorded at 405 nm by microplate reader (BioTek, USA) and compared to control, which had 50 μL of buffer solution in place of the extract.

### *In vitro* α-amylase inhibition

The α-amylase inhibitory activity was measured by a standard method.[4] The 50 μl of sample (0.020 M phosphate buffer, pH 6.9) was premixed with 100 μL of α-amylase solution (1.0 μ/mL in the pH 6.9 buffer), and incubated at 25°C for 10 min. After, 200 μL of a 0.25% starch solution was added to start the reaction for 5 min and terminated by addition of 1.0 mL of the DNS reagent. The test tubes were then kept on a boiling water bath for 5 min and cooled to room temperature, and absorbance was measured at 540 nm using a ultraviolet-visible spectrophotometer (Shimadzu, Japan). The control had 200 μL of buffer solution in place of the α-amylase solution.

The enzyme inhibitory activity was expressed as %inhibition and was calculated as follows:

$$\text{Enzyme inhibitory activity (\%)} = (A_{control} - [A_{test} - A_{background}])/A_{control} \times 100$$

Where $A_{control}$, $A_{test}$, and $A_{background}$ are defined as the absorbance of 100% enzyme activity (only the solvent with the enzyme), test sample with the enzyme and test sample without the enzyme, respectively.

The concentration of inhibitors required for inhibiting 50% of the enzyme activity under the assay conditions was presented as the $IC_{50}$ value.

### Kinetic of α-glucosidase inhibition

Methanolic extract (MEH) was further analyzed for the type of inhibition. In this method, initial rates at

different MEH concentration (0, 12.5, 25, 50, 100, and 200 µg/mL) were measured at different concentration of PNPG, substrate (0.075, 0.156, 0.313, 0.625, 1.25, 2.5, and 5.0 mM) in 0.1 M phosphate buffer containing α-glucosidase solution (0.1 µ/mL), pH 6.8 at 37°C, and release of p-nitrophenol was measured as described above. The inhibition pattern was evaluated by Lineweaver–Burk plot.

## Results

### Phytochemical analysis

Qualitative chemical tests have shown the presence of flavonoids, tannins, alkaloids, glycosides, steroids, terpenoids, and absence of proteins, saponins, sugars, and amino acid in MEH. Aqueous and hydroalcoholic extracts had shown similar chemical constituents. Quantitative estimation of total phenolic and TFCs was higher in MEH followed by hydroalcoholic and aqueous extract as showed in Table 1.

### *In vitro* α-glucosidase inhibition

All the extracts showed concentration-dependent inhibition of α-glucosidase [Figure 1]. The MEH showed highest α-glucosidase inhibition (75.18 ± 2.2) followed by acarbose (69.40 ± 3.8) and aqueous extract of *E. hirta* (AEH) (42.18 ± 2.9), respectively at their highest concentration tested. However, based on $IC_{50}$ values, α-glucosidase inhibitory activity of MEH (78.88 µg/mL) was higher than that of acarbose (8.07 µg/mL), and lower than that of hydroalcoholic extract of *E. hirta* (HEH) (146.9 µg/mL) and AEH (213.63 µg/mL) [Table 2].

### *In vitro* α-amylase inhibition

All the extracts and acarbose showed dose-dependent inhibition of α-amylase [Figure 2]. Interestingly, MEH showed weakest α-amylase enzyme inhibition (33.49 ± 3.6) followed by HEH (43.79 ± 2.5), AEH (46.89 ± 2.6), and acarbose (73.48 ± 2.7), respectively at their highest concentration tested [Figure 2]. Similarly, $IC_{50}$ values of α-amylase inhibitory activity of MEH (280.71 µg/mL) was higher than that of HEH (202.24 µg/mL) and AEH (180.09 µg/mL), and acarbose (7.46 µg/mL) as summarized in Table 2.

### Relationship between the α-glucosidase and α-amylase inhibition versus total phenolic content and total flavonoid content

The correlation coefficients ($R^2$) of the highest α-glucosidase and α-amylase inhibition versus total phenolic content (TPC) and TFC in the aqueous, hydroalcoholic, and MEH of *E. hirta* were shown in Figure 3a and b, respectively. The $R^2$ value of the highest α-glucosidase inhibition versus TPC and TFC were 0.9872 and 0.956, respectively. Results

**Table 1: Total phenolic and flavonoid content of *Euphorbia hirta* extracts**

| Extract | TPC[a] | TFC[b] |
|---|---|---|
| AEH | 45.29 ± 1.78 | 21.08 ± 1.69 |
| HEH | 64.21 ± 1.55 | 38.70 ± 1.01 |
| MEH | 118.04 ± 1.94 | 53.35 ± 2.02 |

[a]Total phenolic content expressed as gallic acid equivalent in mg/g of extract; [b]Total flavonoid content expressed as quercetin equivalent in mg/g of extract. Values were expressed as the mean±SD of three replicated samples. AEH – Aqueous extract of *Euphorbia hirta*; HEH – Hydro-alcoholic extract of *Euphorbia hirta*; MEH – Methanolic extract of *Euphorbia hirta*; SD – Standard deviation; GAE – Gallic acid equivalent; QE – Quercetin equivalent

**Table 2: Maximum inhibition of enzymes by *Euphorbia hirta* extracts and acarbose**

| Inhibitors | Maximum inhibition (%) | | $IC_{50}$ (µg/mL)* | |
|---|---|---|---|---|
| | α-glucosidase | α-amylase | α-glucosidase | α-amylase |
| Acarbose | 69.40 ± 3.8 | 73.48 ± 2.7 | 8.07 | 7.46 |
| AEH | 42.18 ± 2.9 | 46.89 ± 2.6 | 213.63 | 180.09 |
| HEH | 54.16 ± 1.7 | 43.79 ± 2.5 | 146.9 | 202.24 |
| MEH | 75.18 ± 2.2 | 33.49 ± 3.6 | 78.88 | 280.71 |

*$IC_{50}$ values were calculated by linear regression. At least, five serially diluted solutions of each analyte were taken for calculation of the $IC_{50}$ values. Values are the means±SD of three replicated samples. AEH – Aqueous extract of *Euphorbia hirta*; HEH – Hydro-alcoholic extract of *Euphorbia hirta*; MEH –Methanolic extract of *Euphorbia hirta*; SD –Standard deviation

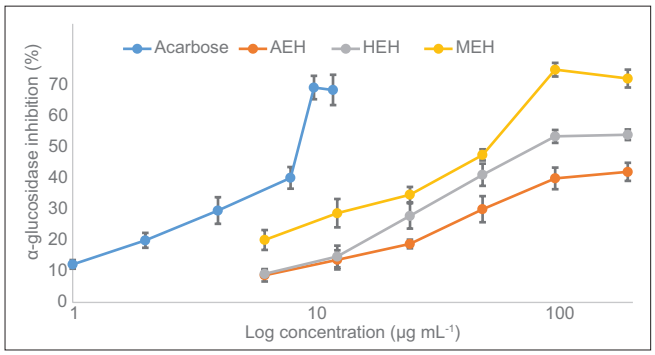


**Figure 1:** α-glucosidase inhibitory activity of *Euphorbia hirta* and acarbose against α-glucosidase (0.1 µ/mL)

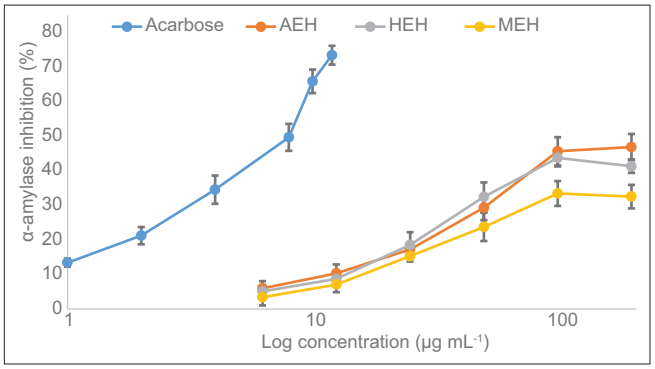


**Figure 2:** α-amylase inhibitory activity of *Euphorbia hirta* and acarbose against α-amylase (1.0 µ/mL)

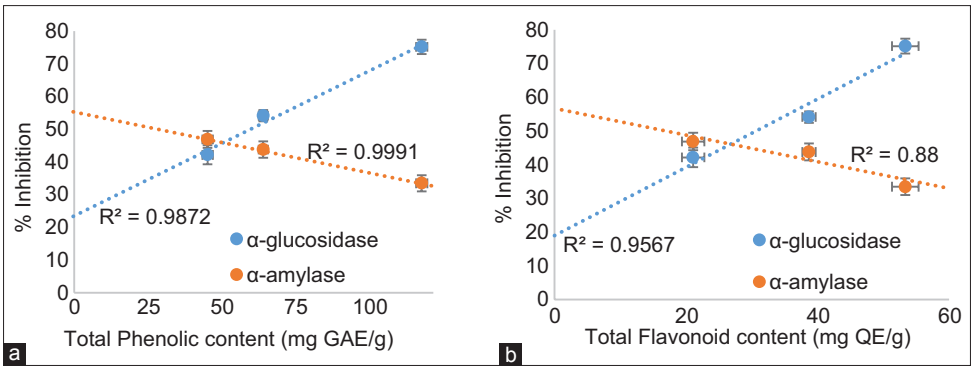


**Figure 3:** Correlation coefficients ($R^2$) of highest α-glucosidase and α-amylase inhibition versus total phenolic content (a) and total flavonoid content (b) in the aqueous, hydroalcoholic, and methanolic extracts of *Euphorbia hirta*

suggested that a positive correlation. Similarly, the $R^2$ value of the highest α-amylase inhibition versus TPC and TFC were 0.999 and 0.88, respectively, suggested that a negative correlation [Figure 3].

### Kinetics of α-glucosidase inhibition of methanolic extract

Lineweaver–Burk plot analysis of α-glucosidase inhibition by MEH showed that with increasing MEH concentration, the vertical axis intercept ($1/V_M$) increased and the horizontal axis intercept ($-1/K_M$) varied. These results indicate that the velocity of the reaction catalyzed by α-glucosidase was slowed down with increasing MEH concentration, and the $K_M$ value of α-glucosidase was also affected, which was correlated with the classical pattern of mixed noncompetitive inhibition [Figure 4].

Therefore, weak inhibitory effect against α-amylase activity and a stronger inhibition activity against α-glucosidase can be an ideal approach for the managing of PPHG. Flavonoids and phenolic compounds enriched extracts have shown high α-glucosidase inhibitory activity combined with low α-amylase inhibitory activity.[11,22,23]

In this study, the inhibitory activity of aqueous, hydroalcoholic, and MEH of *E. hirta* upon α-glucosidase and α-amylase was elucidated and compared with acarbose. Further, the correlation between total phenolic and flavonoid content with α-glucosidase and α-amylase inhibition, and the type of α-glucosidase inhibition were also evaluated.

TPC and TFC estimation showed a high amount of polyphenols and flavonoids in MEH of *E. hirta*. The MEH exhibited strong α-glucosidase inhibition followed by acarbose, HEH, and AEH, respectively [Figure 1 and Table 2]. In contrast, the α-amylase inhibitory activity was lowest in MEH compared to acarbose, HEH, and AEH [Figure 2 and Table 2]. It might be due to the presence of high phenolic and flavonoid content as these inhibitions were perfectly correlated with total flavonoid and phenolic contents from

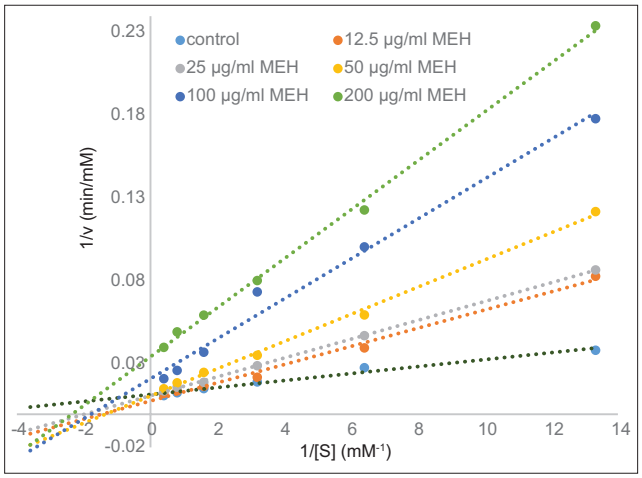


**Figure 4:** Lineweaver–Burk plot analysis of the inhibition kinetics of α-glucosidase inhibitory effects by methanolic extract of *Euphorbia hirta*

the results [Figure 3]. It is evident that MEH was a strong inhibitor for α-glucosidase with mild α-amylase inhibitory activity. Moreover, MEH has showed the classical pattern of mixed noncompetitive inhibition for α-glucosidase further suggesting that MEH may bind close to the active site, or by binding elsewhere on enzyme site but has an influence on the active site.[24]

## Discussion

The results from this study clearly demonstrated that MEH of *E. hirta* had strong inhibitory activity against α-glucosidase and mild α-amylase inhibitory activity. It can be potentially useful to manage the glucose-induced hyperglycemia and provide the rationale for further phytochemical and preclinical studies. DM, a metabolic disorder with multiple etiologies, is characterized by chronic hyperglycemia with metabolic disturbances.[25] Initial management of PPHG is of prime importance in the early prevention of diabetic complications, especially in type II diabetes.[26] Acarbose is a competitive inhibitor of α-glucosidase as well as a mixed noncompetitive inhibitor of α-amylase.[27] In this study, the

inhibitory activity of aqueous, hydroalcoholic, and MEH of *E. hirta* upon α-glucosidase and α-amylase was elucidated and compared with acarbose. Further, the correlation between total phenolic and flavonoid content with α-glucosidase and α-amylase inhibition, and the type of α-glucosidase inhibition were also evaluated.

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**Conflicts of interest**

There are no conflicts of interest.

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