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In vitro antidiabetic potential of *Euphorbia hirta* Linn.: A nutritionally significant plant

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Abstract
Euphorbia hirta is an ethno medicinally important plant of the family Euphorbiaceae. The leaves of the plant are consumed as a vegetable by tribal people. Traditionally, various parts of the plant are used to treat against worm infestations in babies, dysentery, gonorrhea, jaundice, digestive disorders, cancer, and diabetes. The root extract is used against vomiting, diarrhea, and also as snake anti-venom. In the present study, the nutritional quality of the leaves of the plant was assessed by the estimation of various nutritional and antinutritional components. The *in vitro* antidiabetic potential of the plant was determined by alpha-amylase inhibition assay. The results revealed that the leaves of this plant are nutritionally significant, and also the plant has substantial inhibitory activity on alpha-amylase.

Keywords: *Euphorbia hirta*, nutritional, antinutritional, antidiabetic, alpha-amylase

Introduction
The world plant biodiversity is the largest source of herbal medicine, and about 80% of the world population depends on plant-based medicines, which are being used since ancient times as traditional health care systems. The bioactive compounds of these herbal plants have many physiological effects on the human body, which may be successfully utilized for the treatment of various diseases. These plant-derived natural compounds form the base of many of the modern drugs that we use today [1]. Tribal populations provide vital information about the use of many plants and plant parts. These traditional systems of medicine play an essential role in their primary health care.

Diabetes mellitus is a chronic metabolic disorder that occurs either due to insufficient insulin secretion or due to the insensitivity of insulin receptors. It includes a group of metabolic symptoms characterized by hyperglycemia, in which blood sugar levels are elevated. The enzyme alpha-glucosidase is responsible for the breakdown of oligo and disaccharides to monosaccharides. Hence the inhibition of this enzyme leads to a decrease in the blood glucose level because the monosaccharides are the form of carbohydrates that can be absorbed through the mucosal border in the small intestine. Another effective method to control diabetes is to inhibit the activity of the alpha-amylase enzyme, which converts starch into simple sugars (dextrin, malt triose, maltose, and glucose). This can be done by using alpha-amylase inhibitors, which delay the glucose absorption rate and thereby reducing the blood glucose level in hyperglycemic individuals.

Recently herbal medicines are getting more importance in the treatment of diabetes as they are free from side effects and less expensive when compared to synthetic hypoglycaemic agents. Many medicinal plants are being promoted as sources of antidiabetic drugs in the drug market, and there is intense research on the identification and characterization of the active principles present in them.

Euphorbia hirta Linn. Is a medicinally important plant belonging to the family Euphorbiaceae. Tribal people consume the leaves of this plant as vegetables [2]. The various parts of this plant are traditionally used to treat against worm infestations in babies, dysentery, gonorrhea, jaundice, digestive disorders, cancer, and diabetes. The root extract is used against vomiting, diarrhea, and also as snake anti-venom. In Africa and Australia, the leaf extracts are used to treat hypertension and inflammation [3]. The present study was intended to evaluate the nutritional as well as antidiabetic efficacy of *E. hirta*, which is an ethnomedicinally significant plant.

Materials and Methods
Nutritional analysis
Various nutritional and antinutritional factors present in the fresh leaves of *Euphorbia hirta* were estimated using standard procedures.

Moisture content
Moisture content was estimated as per ISTA [4]. One gm of fresh leaf sample was weighed and taken in a pre-weighed Petri plate. The sample was dried at 110 ℃ in a hot air oven. After 24 hours, the sample was weighed again, and the difference in weight was determined. The percentage of moisture was calculated by the following formula:

% of moisture = (fresh weight-dry weight) x100/weight of sample taken

Determination of chlorophyll and carotenoids
Chlorophylls and carotenoids were estimated by using Arnon's formula [5]. One gm of fresh leaf tissue was homogenized in 80% of 10 ml acetone, filtered and centrifuged at 5000 rpm for five minutes. Collected the supernatant and made up to known volume. One ml of aliquot was taken and made up to 5 ml by adding acetone. Absorbance was measured by UV-Visible spectrophotometer at 645, 663, and 490 nm against 80% acetone as blank.

Total proteins
Estimation of protein was done by Lowry's method [6]. One gm of fresh leaf tissue was homogenized in phosphate buffer (pH 7). The homogenate was filtered and centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and made up to known volume by phosphate buffer. Five ml of the solution was taken, added with 5 ml of 10% trichloroacetic acid, and kept in the refrigerator for 15 minutes after thorough shaking. The mixture was then centrifuged at 10000 rpm for 10 minutes. The pellet was dissolved in a known volume of 0.1 N NaOH. Then an aliquot (0.1 ml) was pipetted out and made up to 1 ml using 0.1 N NaOH followed by the addition of 5 ml of copper sulfate solution (reagent C). After 10 minutes, added 0.5 ml Folinciocateau reagent (reagent D). Mixed well and kept for 30 minutes in the dark at room temperature. The blue color developed was measured at 670 nm against the blank.

Total carbohydrates
The amount of total carbohydrates was estimated as per the Anthrone method [7]. One gm of fresh leaf tissue was homogenized in 10 ml distilled water, filtered and centrifuged at 10000 rpm for 10 minutes. The supernatant was collected and made up to known volume by using distilled water. Four ml of anthrone reagent was added and kept in a boiling water bath for 10 minutes. Absorbance was read at 620 nm against the blank.

Estimation of lipids
The lipid content in the sample was estimated by the method of Bligh and Dyer [8]. One gm of fresh leaf tissue was homogenized in 10 ml chloroform: methanol (2:1). The homogenate was filtered. Transferred the filtrate to a separating funnel by adding few drops of chloroform, 1 ml of saturated NaCl, and water. The mixture was shaken well and allowed to settle. The lower layer was collected in a pre-weighed petri dish. The solvent was evaporated in a hot air oven at 60 ℃ overnight. The percentage of lipid was estimated gravimetrically.

Thiamine
Thiamine content was determined according to the method of Deepak [9]. One gm of fresh sample was homogenized in 10 ml ethanolic NaOH. The homogenate was filtered and centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and made up to known volume with ethanolic NaOH. One ml of the sample was added with 10 ml of potassium dichromate. The absorbance was read at 630 nm against the blank.

Riboflavin
Riboflavin content was determined according to the method of Indian Pharmacopeia [10]. To one gm of sample powder, 30 ml of water and 1 ml of glacial acetic acid were added in a conical flask. The solution was boiled for 5 minutes and then cooled. After that, 6 ml of 0.1 M NaOH was added and diluted to 500 ml with distilled water. The solution was filtered, and absorbance was measured at 444 nm in Shimadzu UV-1201-spectrophotometer.

Estimation of ascorbic acid
Estimation of ascorbic acid was done by the method of Sadasivam and Manickam [11]. One gm of fresh leaf tissue was homogenized in 10 ml of 4% oxalic acid. The homogenate was centrifuged at 5000 rpm for 15 minutes. The supernatant was made up to known volume with 4% oxalic acid. Pipetted out 5 ml of the sample into a conical flask and added 10 ml of 4% oxalic acid and titrated against the dye solution. The endpoint is the appearance of pink color. About 5 ml of the working standard was pipetted out, added 10 ml of 4% oxalic acid and titrated against the dye solution until pink color appears. The amount of ascorbic acid was calculated by the standard formula.

Niacin
Weighed accurately 2 gm of sample and homogenized with about 10 ml of distilled water and centrifuged at 3000 rpm for 10 minutes. The supernatant was transferred into the conical flask for titration and added few drops of either phenol red or phenolphthalein and titrated against 0.1 N NaOH until the endpoint is reached. The amount of niacin was calculated by using standard formula.

Estimation of reducing sugars
The estimation of reducing sugars was done by the dinitrosalicylic acid method [12]. One gm tissue was homogenized in 10 ml distilled water. The homogenate was centrifuged at 10000 rpm for 10 minutes. The supernatant was made up to known volume by distilled water. An aliquot was taken, made up to 3 ml, and 2 ml of DNS reagent was added. The mixture was kept in a boiling water bath for 10 minutes and then cooled. The absorbance was measured at 540 nm against the appropriate blank.

Antidiabetic activity assay
The methanolic extract of the whole plant was subjected to alpha-amylase inhibition assay.

Alpha-amylase inhibition assay [13]
Appropriate methanolic extracts of dilution 0-200 µl and 500 µl of 0.02 M Sodium phosphate buffer (P^H 6.9 with 0.006 M NaCl) containing porcine pancreatic alpha-amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25 ℃ for 10 minutes. Then 500 ml of 1 % starch solution in 0.02 M Sodium phosphate buffer (p^H 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixture was incubated at 25 ℃ for 10 minutes and stopped with 1 ml of dinitrosalicylic acid color reagent; after that, the mixture was incubated in a boiling water bath for 5 minutes and cooled to room temperature. The

reaction mixture was then diluted by adding 10 ml of distilled water and absorbance measured at 540 nm. The alpha-amylase inhibitory activity was expressed as percentage inhibition.

Inhibition % = (Absorbance of control-Absorbance of extract) / Absorbance of control x 100

Results and Discussion

Nutritional analysis

The present study involved the analysis of nutritional and antinutritional constituents present in the fresh leaves of *Euphorbia hirta* Linn. The analysis of nutritional factors showed that carbohydrates, proteins, ascorbic acid, niacin, lipids, and thiamine are found in higher amounts compared to others (Table. 1) and are shown in (Fig.1).

The moisture content of fresh leaves of *Euphorbia hirta* was 10.7%. The general requirement for the moisture content in the crude drugs was found to be not more than 14%, the low moisture content of the leaves and stem would hinder the growth of micro-organisms, and the storage life would be high. The quantification of nutritional factors revealed that *Euphorbia hirta* contains good amount of nutritional constituents in its fresh leaves.

Carbohydrates are the most common source of energy for most organisms, including humans. The carbohydrate content was found to be 96.7 mg/g in the leaves of *E. hirta*, which is comparable to that of *Moringa oleifera* leaves (76 - 125 mg/g) [14]. Other nutritional factors, such as proteins, lipids, and vitamins, are also present in sufficient quantities.

Table 1: Nutritional constituents of fresh leaves of *Euphorbia hirta*

Sl. No.	Parameters	Concentration (mg/g)
1	Chlorophyll a	0.189
2	Chlorophyll b	0.00603
3	Total chlorophyll	0.0249
4	Carotenoids	0.1062
5	Proteins	1.1644
6	Carbohydrates	96.7
7	Reducing sugar	0.00422
8	Ascorbic acid	25.15
9	Niacin	6.0133
10	Riboflavin	0.03912
11	Thiamine	2.387
12	Lipids	41.6
13	Moisture content	10.7%

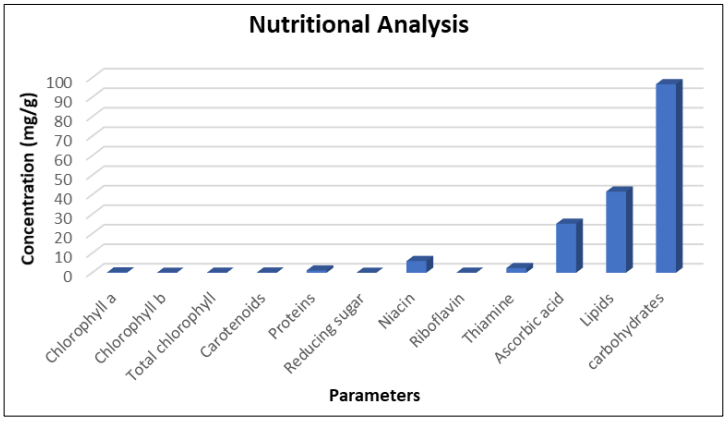


Fig 1: Nutritional analysis of fresh leaves of *Euphorbia hirta* L.

Antinutritional analysis

Antinutritional factors are also as important as the nutritional content of any edible plant part. It is generated in natural food substances by the normal metabolism of species which exert effects contrary to the optimum nutrition. The antinutritional analysis of fresh leaves of *Euphorbia hirta* showed the various amount of antinutrients such as oxalates, phytate, and phenols (Table. 2).

In plants, antinutrients are compounds designed to protect from bacterial infections and attack by insects. Oxalates in green leafy vegetables can bind to calcium and prevent it from being absorbed. Phytate mainly reduces the absorption of several minerals such as iron, zinc, magnesium, etc. Phenolics may interfere with protein digestion and also reduce the absorption of metals such as iron, zinc, etc.

Among the three antinutritional components, phenols are found in minute quantities, whereas oxalates and phytates are present in comparatively higher amounts. But the oxalate content is lesser than that of Amaranthus spinosus leaves (10.9 mg/g). The phytate content in the leaves was found to be higher than that of *Sesbania grandiflora* (1.35 mg/g), which is another commonly used leafy vegetable [15]. But the phytate content can be reduced by various conventional cooking procedures. Though antinutritional factors were found to be present in the leaves, the levels were such that consumption would not result in any deleterious effect on the consumer.

Table 2: Antinutritional constituents of fresh leaves of *Euphorbia hirta*

Sl. No.	Parameters	Concentration (mg/g)
1	Phenols	0.005
2	Phytate	13.33
3	Oxalates	6.9

Antidiabetic activity assay

The antidiabetic activity of *Euphorbia hirta* was studied by *in vitro* alpha-amylase inhibition assay. In this study, the methanolic extract of *Euphorbia hirta* showed concentration-dependent inhibitory activity against α-amylase enzyme (Table. 4) with an IC_{50} value of 0.748 mg/ml. The *in vitro* antidiabetic activity of the extract was compared with that of the standard drug Metformin with an IC_{50}value of 0.58 mg/ml (Table. 3), and the result is depicted in (Fig.2). Since the methanolic extract of *E. hirta* exhibited an IC_{50} value comparable to that of the standard drug, it possesses significant inhibition on alpha-amylase, and hence it may have antidiabetic potential. Certain other plants of the family Euphorbiaceae such as *Phyllanthus amarus, Acalypha indica, and Euphorbia thymifolia* were also reported to have significant antidiabetic potential by their inhibitory effect on the alpha-amylase enzyme.

Table 3: Alpha-amylase inhibition assay- Metformin

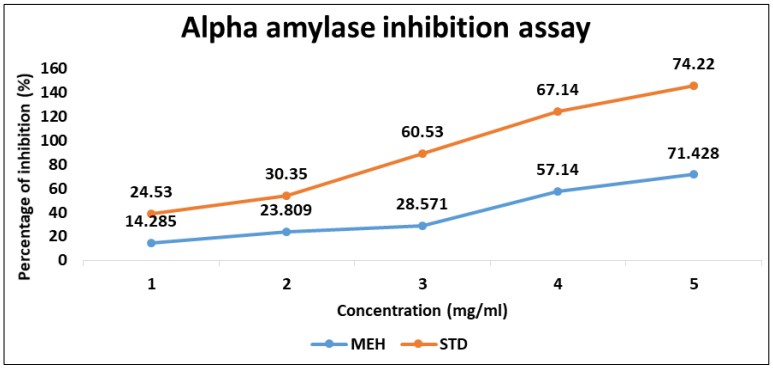
Concentration (mg/ml)	Percentage of inhibition
0.2	24.53
0.4	30.35
0.6	60.53
0.8	67.14
1	74.22

IC_{50} Value = 0.580 mg/ml

Table 4: Alpha-amylase inhibition assay – Methanolic extract of *Euphorbia hirta*

Concentration (mg/ml)	Percentage of inhibition
0.2	14.285
0.4	23.809
0.6	28.571
0.8	57.14
1	71.428

IC_{50} Value = 0.748 mg/ml



MEH – Methanolic extract of *Euphorbia hirta*; STD - Standard drug Metformin

Fig 2: Antidiabetic activity assay of methanolic extract of *E. hirta*

Conclusion

The nutritional analysis of fresh leaves of *E. hirta* revealed that the leaves contain sufficient amounts of nutritional components like carbohydrates, proteins, lipids, vitamins, etc. whereas the antinutritional factors are found in minimum quantities. Hence the leaves of this plant may be used as a leafy vegetable. Also, the plant showed significant inhibition on the enzyme alpha-amylase in a concentration-dependent manner with an IC_{50} value comparable to that of the drug metformin. So the plant may provide significant antidiabetic compounds that can be used in the treatment of diabetes.

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