International Journal of Web of Multidisciplinary Studies
( Peer-Reviewed, Open Access, Fully Refereed International Journal )
website: *http://ijwos.com*
Vol.02 No.12.
OPEN ACCESS
E-ISSN : 3049-2424
DOI: https://doi.org/10.71366/ijwos

# IN VITRO ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF ETHANOLIC WHOLE PLANT EXTRACT OF BIDENS PILOSA THROUGH CARBOHYDRATE DIGESTIVE ENZYME INHIBITION AND GLUCOSE UPTAKE ENHANCEMENT

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***Article Info***

***Article History:***

*Published:31 Dec 2025*

***Publication Issue:***
*Volume 2, Issue 12*
*December-2025*

***Page Number:***
*917-927*

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***Abstract:***

The ethanolic extract of Bidens pilosa (EEBP) was evaluated for its inhibitory effect on carbohydrate digestive enzymes, including α-glucosidase and α-amylase, alongside antioxidant activity and glucose uptake in vitro. The phytochemical screening revealed the presence of flavonoids, phenols, steroids, and terpenoids. Antioxidant studies showed notable DPPH, superoxide, and nitric oxide radical scavenging activities. EEBP significantly inhibited α-glucosidase and α-amylase, with IC50 values of 248.26 µg/ml and 71.35 µg/ml respectively. Glucose uptake studies in rat hemi-diaphragm confirmed the insulin-like potential of EEBP. These results suggest that Bidens pilosa may be a promising natural therapeutic agent for diabetes management.

***Keywords:*** Bidens pilosa, α-glucosidase, α-amylase, glucose uptake, antioxidant, diabetes mellitus.

## 1. INTRODUCTION

Diabetes mellitus is a serious chronic metabolic ailment originated by innate and /or pancreatic deficit in insulin making, or by a decrease in insulin production[1]. It is represented by the impairment of carbohydrate, protein and fat metabolism that has developed from the interaction of various hereditary and ecological factors. It had a major crash on the health and happiness and life span of diseased patients, along with the economies of health care system[2].

Diabetes mellitus is symbolized by hyperglycemia, diabetic specific micro vascular complications in the eye, kidney and peripheral neurons and macro vascular complications affecting arteries that supply the heart, brain and other organs. Chronic hyperglycemia is an important manifestation for diabetic vascular complications causing blindness, renal failure, neuropathy, atherosclerosis and cardiovascular disease[3].

## 2. MATERIALS

### 2.1 Plant material

The whole plant of *Bidens Pilosa* was collected from Seshachalam forest, Tirumala, Chitoor Dt and identified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Chitoor Dt, A.P.

The collected whole plant was immediately dried at room temperature for one month, powdered mechanically sieved (10/44) and stored in air tight containers.

**2.2 Chemicals**

1, 1-diphenyl-2-picryl-hydrazyl (DPPH), Nicotinamide adenine di nucleotide (NADH) and Phenazine methosulphate (PMT) were obtained from Sigma Chemicals Co., St. Louis, MO. Sodium nitroprusside, methanol and dimethyl sulphoxide were obtained from Ranbaxy Fine Chemicals Ltd., Mohali, India. Sulphanilic acid was obtained from Himedia Laboratories Ltd., Mumbai, India. Naphthyl ethylene diamine dihydrochloride was obtained from Loba Chemie, Mumbai, India. Nitro blue tetrazolium (NBT) was obtained from Merk, Germany. Streptozotocin was purchased from SRL private Ltd., Mumbai, India. Ascorbic acid and rutin were obtained from S.D Fine Chem., Biosar, India. All the other chemicals used were of analytical grade.

**2.3 Experimental animals**

Male and female Wistar albino rats (130-160gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22±2° C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized at least 7days to the laboratory conditions prior to experimentation. Tap water and food pellets were provided ad libitum. Food pellet was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of "Guide for the care and Use of Laboratory animals [4,5].

**3. METHODOLOGY**

**3.1 Preparation of extract**

After collection of the plant, shade drying the whole plant of *Bidens Pilosa* were then blended in to fine powder with a blender and used for the aqueous and ethanol extracts. Ethanol extract was extracted by using soxhlet extractor for 18-20 h. The extract obtained, was concentrated under reduced pressure at controlled temperature (40-50° C) and finally made powdered[6,7].

**3.2 Test for Alkaloids[8,9]:**

**a. Mayer's test:**

To the 1 ml of the extract, a drop or two drop of Mayer's reagent was added by the side of test tube. A white or creamy precipitate indicates the test as positive.

**b. Wagner's test:**

To 1 ml of the extract, few drop of Wagner's reagent was added. A reddish brown colour indicates the test as positive.

**Test for Carbohydrates:**

**a. Benedict's test:**

To the 5 ml of Benedict's reagent, 1 ml of the extract solution was added and boiled for 2 minute and cooled. Formation of red precipitate shows the presence of carbohydrate.

**b. Molisch's test:**

To the 2 ml of extract, two drops of alcoholic solution of ά-nephthol was added and shaken well. 1 ml of concentrated sulphuric acid was added slowly along the side of the test tubes and allowed to stand. A violet ring indicates the presence of carbohydrates.

**3.3 Test for Protein and Amino acid:**

**a. Million's test:**

To the 2 ml of extract, few drops of Million's reagent were added. A white precipitate indicates the presence of proteins.

**b. Ninhydrin test:**

To the 2 ml of extract, two drops of Ninhydrin solution was added. A characteristic purple colour indicates the presence of amino acids, proteins and peptides.

**3.4 Test for Glycosides:**

**a. Legal's test:**

To the 2 ml of extract was dissolve in the solution of pyridine and to it sodium nitropruside was added, to make it alkaline. The formation of pink to red colour shows the presence of glycosides.

**b. Baljet's test:**

To the 1 ml of extract, 1 ml of sodium picrate solution was added. The changing of colour from yellow to orange reveals the presence of glycosides.

**3.5 Test for Flavonoids:**

**a. Shinoda test:**

To the1 ml of extract, add magnesium turnings was added and 1-2 drops of concentrated hydrochloric acid was added drop wise. Formation of pink to crimson colour indicates the presence of flavonoids.

**b. Alkaline reagent test:**

The aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicates the presence of flavonoids.

### 3.6 Test for Fixed Oil[10,11]:

**a. Spot test:**

A small quantity was pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

**b. Saponification test:**

A few drops of 0.5 N alcoholic potassium hydroxide solution was added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on water bath for 2 hrs. Formation of the soap or partial neutralization of alkali indicates the presence of fixed oil.

### 3.7 Antioxidant activity

**In vitro antioxidant activity[12]**

EEBP was evaluated for its anti oxidant activity by selecting the following activities. 1, 1-Diphenyl-2-pycrylhydrazy (DPPH) free radical scavenging activity, Superoxide anion radical scavenging activity and Nitric oxide radical inhibition assay

**In vitro studies**

**Inhibition of carbohydrate digesting enzymes**

**α-glucosidase inhibitory assay**

The purpose of this experiment was to examine the in vitro inhibitory effect on α-glucosidases such as maltase and sucrase. Although α-glucosidase isolated from yeast is widely used as an evaluation material for α-glucosidase inhibitors, the results may not always agree with those obtained in animals. In order to better replicate the in vivo state, we used a rat small intestine homogenate as α-glucosidase solution[13].

The method used by "Dahlqvist" has been somewhat modified to calculate the inhibitory effect. A portion of the animal's small intestine below the duodenum and immediately above the caecum was cut, cleaned with ice-cold saline, and homogenized with 12 milliliters of maleate buffer (100 millimoles, pH 6) following a 20-hour fast. The homogenate was utilized as a solution for α-glucosidase. The sample extract (50–500 µg/ml), 2% (w/v) of each glucose substrate solution (100 µl), and 100 mM maleate buffer (pH 6) make up the test mixture. After the mixture was pre-incubated for five minutes at $37^0$C, α-glucosidase (50 µl) solution was added, and the mixture was then incubated again for ten minutes at 370C to start the reaction[14,15].

### 3.8 Glucose uptake

**Procedure**

At the end of 28 days of therapy rats were killed by decapitation and hemi diaphragm from selected rats were isolated. In a cultured tubes containing 2ml tyrode solution with 2g percent glucose, hemi diaphragms were put and incubated for 30 min at 37˚C in an environment of 95% $O_2$ 5% CO with shaking. Six models of similar experiments had been done, in which,

Group-I - diabetic control

Group-II - reference standard insulin (0.25 IU/ml)

Group-III - EEBP (200 mg/ml)

Group-IV - EEBP (400 mg/ml)

Group-V - Insulin (0.25 IU/ml) + EEBP (200mg/ml)

Group-VI - insulin (0.25 IU/ml) + EEBP (400 mg/ml) respectively.

The hemi diaphragms had been taken out and weighed following incubation. The glucose content of the incubated medium had been calculated. Glucose uptake had been determined as the distinction between the initial and final glucose content in the incubation medium[16].

## 4. RESULTS

### 4.1 Preliminary phytochemical screening:

Preliminary phytochemical screening of the ethanolic extract revealed the presence and absence of various secondary metabolites. The results indicated that the extract tested positive for flavonoids, tannins, steroids, terpenoids, phenols, and proteins. Conversely, alkaloids, glycosides, saponin glycosides, and carbohydrates were found to be absent. These findings demonstrate that the ethanolic extract is rich in several bioactive phytoconstituents, particularly phenolic and terpenoid compounds.

| S No | Constituent | Ethanolic extract |
|---|---|---|
| 1 | Alkaloids | -ve |
| 2 | Glycosides | -ve |
| 3 | Saponin glycosides | -ve |
| 4 | Flavonoids | +ve |
| 5 | Tannins | +ve |
| 6 | Steroids | +ve |
| 7 | Terpenoids | +ve |
| 9 | Phenols | +ve |
| 10 | Proteins | +ve |

| | | |
|---|---|---|
| 11 | Carbohydrates | -ve |

+ve sign indicates presence; - ve sign indicates absence;

**4.2 In vitro antioxidant activity**

The in vitro antioxidant activity of the ethanolic extract of *EEBP* was evaluated using DPPH, superoxide, and nitric oxide radical scavenging assays, and the results are expressed as $IC_{50}$ values (µg/ml) with corresponding SEM (Table 1).

The ethanolic extract exhibited strong DPPH radical scavenging activity with an $IC_{50}$ value of 8.97 ± 0.73 µg/ml. This activity, although lower than the standard antioxidant ascorbic acid ($IC_{50}$ = 3.45 ± 0.16 µg/ml) and rutin ($IC_{50}$ = 4.03 ± 0.74 µg/ml), indicates significant free radical scavenging potential. In the superoxide radical scavenging assay, EEBP showed moderate antioxidant activity with an $IC_{50}$ value of 29.23 ± 3.19 µg/ml. Standard compounds were not evaluated for this assay. The nitric oxide scavenging activity of EEBP was comparatively lower, with an $IC_{50}$ value of 149.28 ± 2.16 µg/ml. Rutin, used as a reference standard for nitric oxide scavenging, demonstrated higher activity with an $IC_{50}$ value of 83.41 ± 3.07 µg/ml.

Overall, the results demonstrate that EEBP possesses appreciable in vitro antioxidant activity, particularly against DPPH and superoxide radicals, indicating its potential as a natural source of antioxidant compounds.

**Table 1: In vitro antioxidant activity of EEBP**

| Test material | IC 50 (µg/ml) ± SEM[a] | | |
|---|---|---|---|
| | DPPH | Super oxide | Nitric oxide |
| EEBP | 8.97±0.73 | 29.23±3.19 | 149.28±2.16 |
| Ascorbic acid | 3.45±0.16 | - - | - - |
| Rutin | 4.03±0.74 | - - | 83.41±3.07 |

**4.3 In vitro studies on EEBP for its antidiabetic activity**

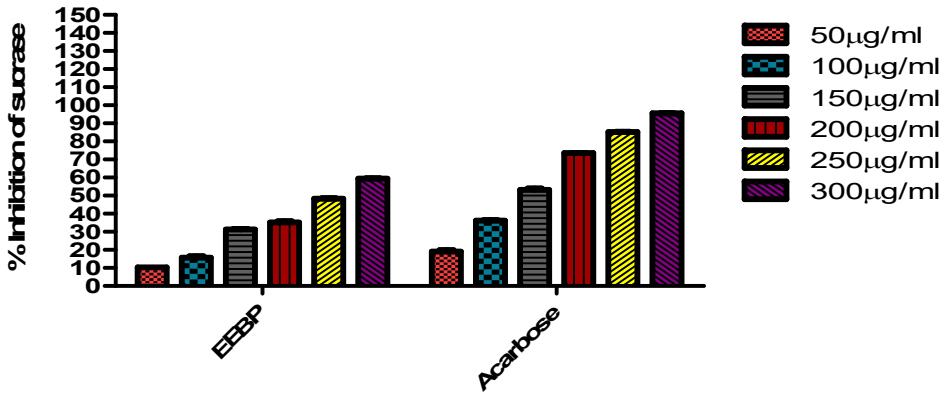
**α- glucosidase and α-amylase inhibitory activities**

The in vitro antidiabetic activity of the ethanolic extract of EEBP was evaluated by assessing its inhibitory effect on carbohydrate-hydrolyzing enzymes, specifically α-glucosidase (sucrase) and α-amylase, and the results were compared with the standard drug acarbose. EEBP exhibited a concentration-dependent inhibition of sucrase activity across the tested concentration range of 50–300 µg/ml. At the lowest concentration (50 µg/ml), EEBP showed 10.32 ± 0.06% inhibition, which gradually increased to 60.02 ± 0.20% at 300 µg/ml. In comparison, acarbose demonstrated significantly higher inhibitory activity, with percentage inhibition increasing from 18.97 ± 0.59% at 50 µg/ml to 94.83 ± 0.05% at 300 µg/ml.

The $IC_{50}$ value for sucrase inhibition by EEBP was found to be 248.26 ± 0.31 µg/ml, indicating moderate inhibitory potential. The standard drug acarbose showed a lower $IC_{50}$ value of 141.02 ± 0.06 µg/ml, reflecting its stronger enzyme inhibitory activity. The results indicate that EEBP possesses measurable α-glucosidase and α-amylase inhibitory activities in vitro, with enzyme inhibition increasing in a dose-dependent manner, although its potency is lower than that of the standard antidiabetic drug acarbose.

| **Concentration (µg/ml)** | **Percentage inhibition (%) of sucrase by** | | **IC $_{50}$ (µg/ml)** | |
|---|---|---|---|---|
| | **EEBP** | **Acarbose** | **EEBP** | **Acarbose** |
| 50 | 10.32 ± 0.06 | 18.97 ± 0.59 | 248.26 ± 0.31 | 141.02± 0.06 |
| 100 | 15.53 ± 0.69 | 36.42 ± 0.26 | | |
| 150 | 32.05 ± 0.48 | 53.52 ± 0.35 | | |
| 200 | 35.74 ± 0.57 | 72.94 ± 0.10 | | |
| 250 | 49.12 ± 0.43 | 84.75 ± 0.04 | | |
| 300 | 60.02 ± 0.20 | 94.83 ± 0.05 | | |

**Fig 1 α-glucosidase (sucrase) inhibitory activity of EEBP**




**4.4 Glucose uptake**

The effect of EEBP on glucose uptake was evaluated using isolated tissue incubated in Tyrode solution containing 2 g% glucose, and the results are presented in Table 2. Glucose uptake is expressed as mg/g tissue/30 min.

The diabetic control group (Group I) showed a baseline glucose uptake of 4.13 ± 0.29 mg/g/30 min. Treatment with insulin alone (Group II) significantly increased glucose uptake to 6.29 ± 0.91 mg/g/30 min (***$p < 0.001$) when compared to the diabetic control.

Administration of EEBP alone produced a dose-dependent increase in glucose uptake. At a concentration of 200 mg/ml (Group III), EEBP increased glucose uptake to 5.41 ± 0.52 mg/g/30 min (*$p < 0.05$), while a higher concentration of 400 mg/ml (Group IV) resulted in a more pronounced and significant increase to 6.84 ± 0.12 mg/g/30 min (***$p < 0.001$), compared to the diabetic control.

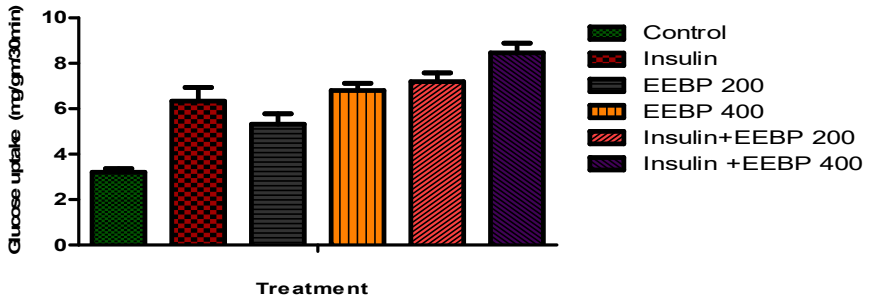
Combined treatment with insulin and EEBP further enhanced glucose uptake. Group V, treated with insulin and EEBP at 200 mg/ml, showed glucose uptake of 7.19 ± 0.42 mg/g/30 min (***$p < 0.001$). The highest glucose uptake was observed in Group VI, which received insulin along with EEBP at 400 mg/ml, recording 8.53 ± 0.75 mg/g/30 min (***$p < 0.001$).

**Table 2 Effect of EEBP on glucose uptake**

| Groups & Treatment | Glucose uptake (mg/gm/30 min) |
|---|---|
| I. Tyrode solution in glucose(2g%)+Diabetic control | 4.13±0.29 |
| II. Tyrode solution in glucose(2g%)+Insulin (0.25 IU/ml) | 6.29±0.91*** |
| III. Tyrode solution in glucose (2g%)+ EEBP(200mg/ml) | 5.41±0.52* |
| IV. Tyrode solution in glucose(2g%)+ EEBP(400mg/ml) | 6.84±0.12*** |

| | |
|---|---|
| V. Tyrode solution in glucose(2g%)+Insulin (0.25 IU/ml)+EEBP(200mg/ml) | 7.19±0.42*** |
| VI. Tyrode solution in glucose(2g%)+Insulin(0.25 IU/ml)+EEBP(400mg/ml) | 8.53±0.75*** |

**Figure 5.3 Effect of EEBP on glucose uptake**




## 5. CONCLUSION

The present work was undertaken with a positive approach to put down standards which could be useful to detect the potency and authenticity of *Bidens Pilosa* for their invitro anti diabetic activity. *Bidens Pilosa* claims to treat various disease ailments like neurological disorders and inflammation etc. In Indian indigenous system of medicine was as anti diabetic, however the scientific studies have not been done. Therefore, the invitro anti diabetic activity of ethanolic extract of *Bidens Pilosa* (EEBP) was evaluated.

Both the doses of ethanolic extract registered significant activity in increasing the peripheral glucose uptake by the isolated rat hemi diaphragm. The test doses additionally found to increase the glycogen degree that might be because of the increased transformation of glucose to glycogen that would be attributed as a result of reactivation of this glycogen synthetase system. The property for the test doses to recuperate the body weight of animals suggesting increased usage and decreased retention of protein.

Hence, *Bidens Pilosa* may have great potential as an alternative to the therapeutic agents currently available for treatment of diabetes.

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