Anti-hyperglycaemic and antioxidant effects of *Bidens tripartita* and quantitative analysis on its active principles

Nilüfer Orhan 1*, Ülkü Gökçen İçöz 2, Levent Altun 3, Mustafa Aslan 1

1 Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330, Etiller, Ankara, Turkey
2 Republic of Turkey Social Security Institution, 06520, Balgat, Ankara, Turkey
3 Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06500, Tandogan, Ankara, Turkey

**ABSTRACT**

**Objective(s):** *Bidens* species are used for their antidiabetic properties traditionally in many countries. Aim of this study is to evaluate hypoglycaemic and antidiabetic activity of *Bidens tripartita* extract and to identify its active compounds through bioactivity guided isolation technique.

**Materials and Methods:** Hypoglycaemic effects of *B. tripartita* extract and its sub-extracts were investigated in normal and glucose-hyperglycaemic rats. Streptozotocin induced diabetic rats were used to examine antidiabetic activity of the extract and its sub-extracts after acute and sub-acute administration. Additionally, *in vitro* enzyme inhibitory and antioxidant activities were evaluated. HPLC analyses were carried out to determine the active constituents of the extract and its sub-extracts.

**Results:** Through *in vivo* bioactivity-guided fractionation process, ethyl acetate and n-butanol sub-extracts were found to have potent antidiabetic activity. *In vitro* enzyme inhibitory activities of the same sub-extracts were found to be potent. The highest total phenol, flavonoid contents and radical scavenging activity was determined in ethyl acetate sub-extract. According to LC-MS analyses, chlorogenic acid, luteolin and 7-O-glucoside of luteolin (cynaroside) were determined as the main components of the active sub-extracts.

**Conclusion:** According to our results, *B. tripartita* has potent antidiabetic activity and its active constituents might be beneficial for diabetes and its complications.

**Introduction**

*Bidens tripartita*, commonly known as “three-lobed beggarticks, three-part beggarticks, leafy-bracted beggarticks or trifid bur-marigold, is a flowering plant in the Asteraceae family (1). It is a worldwide weed native to Northern hemisphere growing near the river banks, marshes, ditches and wet sites (2).

Infusion of the aerial parts of *B. tripartita* L. is widely used in the treatment of catarrhal rhinitis, angina, acute respiratory infections, and as an anti-inflammatory in colitis, gout, and infantile rickets in Russia as traditional medicine (3). It is also used as a diaphoretic and a diuretic in nephrolithiasis (4), as an antiseptic and as a bath for children to treat allergy symptoms (5). Decoctions of *Bidens* species are used to treat diabetes in different regions of the world (6-11). Additionally, young leaves of *Bidens* species are added to salads, soups or stews and young shoot tips are used to make tea (12). Hence, they have been used as food and medicine traditionally without noticeable adverse effects for centuries (13).

Many pharmacological studies have been conducted on *B. tripartita* and its anticancer, anti-inflammatory, antimicrobial, antioxidant, antithrombin, antiiucer, hepatoprotective, and hypotensive effects are reported in the literature. The Herba Bidentis Monograph is included both in the World Health Organization Monographs on Medicinal Plants Commonly Used in the Newly Independent States (NIS) in 2010 and in Russian Pharmacopoeia. By revealing more information about *Bidens*, broad studies of this plant can lead to an improved appreciation of the extent of the applications of this herb in medicine (14).

Diabetes is one of the most common and important metabolic disorders in the growing world. As of 2014, an estimated 387 million people have diabetes worldwide. Diabetes can affect many parts of the body and is associated with serious complications, such as heart disease, stroke, blindness, kidney failure, and lower-limb amputation, among other conditions (15). Oxidative stress is known to have the major role in the progress of these conditions. Therefore, it is important to evaluate both the antioxidant potential and the

---

*Corresponding author: Nilüfer Orhan, Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330, Etiller, Ankara, Turkey. Tel: +90-312-2023174; Fax: +90-312-2235018; email: nympis@gmail.com*
hypoglycaemic activity of antidiabetic drugs. On the other hand, one of the therapeutic approaches to treat diabetes is to decrease postprandial hyper-glycaemia and this can be achieved by the inhibition of carbohydrate hydrolyzing enzymes like α-amylase and α-glucosidase. They are the major enzymes in the digestion of carbohydrates and they are the potential targets in the development of lead compounds for the treatment of diabetes (16). As well as insulin and oral antidiabetics, many traditional plant remedies or herbal formulations exist from ancient times and are still widely used (17).

In the present study, we aimed to: 1. evaluate the potential antidiabetic effect of B. tripartita extract and its sub-extracts by in vivo models, 2. elucidate the probable antidiabetic mechanism by in vitro models, 3. determine the active principles by using bioactivity guided isolation technique, 4. investigate antioxidant capacity and 5. determine the chemical profile of the active extracts.

Materials and Methods

Plant materials

Aerial parts of B. tripartita L. (Asteraceae) were collected at the end of August in 2011 from the lakeside of Yeniçağa, Bolu (Turkey). The voucher specimen (AEF 25996) is stored in the Herbarium of Ankara University, Faculty of Pharmacy.

Preparation of the extract and sub-extracts

Plant materials were dried under shade and coarsely powdered for extraction. A portion of the material (100 g) was extracted with 80% ethanol (EtOH) (1.5 l) on shaker for 24 hr and filtered. 1 l of 80% ethanol was added to the pulp and extraction was completed on a shaker after 24 hr. Combined ethanol extracts were evaporated to dryness under reduced pressure and then lyophilized (EtOH extract, yield 15.63%).

The EtOH extract (40 g) was dissolved in 500 ml of distilled water and fractionated by successive solvent extraction with chloroform (9 × 500 ml), ethyl acetate (11 × 500 ml) and n-butanol saturated with H₂O (7 × 500 ml). Each sub-extract as well as the remaining aqueous sub-extract was evaporated to dryness under reduced pressure to obtain “Chloroform sub-extract” (Yield %:14.60), “Ethyl acetate sub-extract” (Yield %:19.05), “n-Butanol sub-extract” (Yield %:15.54) and “Remaining water sub-extract” (Yield %:44.22). All the obtained sub-extracts were used in animal experiments at the doses calculated according to their yields.

In-vivo studies

Animals

Wistar Albino male rats (150-200 g) were used in this study with the approval of Animal Experiments Local Ethical Committee (GUET-06.087). Animals were purchased from the animal breeding laboratories of Experimental Animal Research Center of Gazi University (GUDAM) (Ankara, Turkey) and the experiments were carried on at the same research center. The animals were maintained on standard pellet diet and water ad libitum throughout the experiment.

Blood Collection and determination of blood glucose levels

Blood samples were collected from the tip of tail at the defined time patterns and blood glucose concentrations (mg/dl) were determined using an Ascensia-Elite commercial test (Serial No. 9123232, Bayer), based on the glucose oxidase method.

Effect on normoglycaemic animals

Fasting blood glucose level of each animal was determined at initial time, after overnight fasting (12 hr) with free access to water. Tolbutamide (100 mg/kg of body weight [BW]) was used as the reference drug. The extract, sub-extracts and the reference were suspended in 0.5% aqueous carboxymethylcellulose (CMC) suspension in distilled water prior to oral administration to animals (10 ml/kg of BW). Control group was received 0.5% CMC (10 ml/kg BW). Blood samples were collected at 1/2, 1, 2 and 4 hr after the oral administration of test samples.

Effect on glucose-hyperglycaemic animals [OGTT: Oral glucose tolerance test]

After overnight fasting (12 hr), the blood glucose levels of animals were determined and immediately test samples were administered orally. Two g/kg glucose was loaded to the rats orally at the 30th minute and the blood glucose concentrations were determined at the 1st, 2nd and the 4th hour of the experiment.

Effect on diabetic animals

Streptozotocin (STZ, 60 mg/kg, intraperitoneal) dissolved in citrate buffer was used to induce experimental diabetes. Seven days after the injection of STZ, the blood glucose levels were measured and the animals with blood glucose levels higher than 250 mg/dl were considered to be diabetic. Diabetic rats were fasted 8 hr before the experiment.

For determination of acute antidiabetic effect, the blood glucose levels were measured at the beginning of the experiment before the administration of CMC/tolbutamide/extract/sub-extracts. Then, in the blood glucose levels were monitored at the 30th, 60th, 120th, 240th minute of the experiment.

The ethanol extract, its sub-extracts (chloroform, ethyl acetate, n-butanol, remaining water), CMC and tolbutamide were administered to the diabetic animals seven days consecutively for determination of antidiabetic effect after sub-acute administration. During the experiment, blood glucose levels were
measured at the 1st, 4th and 8th days at 10.00 a.m. just before the administration of test samples. The effect of all test samples on body weight was also monitored in the same days.

**In-vitro studies**

*Assay for α-amylase inhibitory activity*

The α-amylase inhibitory activities of *B. tripartita* ethanol extract and its sub-extracts were determined by the method of Ali et al. (2006) (18). Porcine pancreatic α-amylase type VI (EC 3.2.1.1, Sigma) was dissolved in distilled water. As substrate solution, potato starch (0.5%, w/v) in phosphate buffer (pH 6.9) was used. Experiments were carried out with three replicates. Acarbose was used as the positive control. At the end of the experiment absorbances of the mixtures were read at 540 nm. The absorbance (A) due to maltose generated was calculated according to following formula:

\[ A_{\text{Control or Sample}} = A_{\text{Test}} - A_{\text{Blank}} \]

The amount of maltose generated was calculated by using the maltose standard calibration curve (0.01% w/v) and the obtained net absorbance. Percent of inhibition was calculated as:

\[ \text{Inhibition} \% = \left(\frac{\text{Maltose Control} - \text{Maltose Sample}}{\text{Maltose Control}}\right) \times 100 \]

*Assay for α-glucosidase inhibitory activity*

The method of Lam et al. (2008) was used to evaluate α-glucosidase inhibitory activity (19). α-Glucosidase type IV enzyme (Sigma Co, St. Louis, USA) from *Bacillus stea rothermophilus* was dissolved in phosphate buffer (0.5 M, pH 6.5). The enzyme solution and extracts dissolved in MeOH-H2O were preincubated in a 96-well microtiter plate for 15 min at 37 °C. After that, the substrate solution [20 mM p-nitrophenyl-α-d-glucopyranoside (NPG), Sigma] was added. The mixture was incubated for 35 min at 37 °C. The increase in the absorption at 405 nm due to the hydrolysis of NPG by α-glucosidase was measured by an ELISA microtiter plate reader. Acarbose (Bayer Group, Turkey) was used as positive control. The inhibition percentage (%) was calculated by the equation:

\[ \text{Inhibition} \% = \left(\frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}}\right) \times 100 \]

*Total antioxidant activity by phosphomolybdenum assay*

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphorus/Mo (V) complex at acidic pH. *B. tripartita* ethanol extract and its sub-extracts were added to test tubes containing molybdate reagent solution (Molybdate reagent:1 ml 0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). Vortexed tubes were incubated at 90 °C for 90 min. Then, tubes were cooled to room temperature and the absorbances of the samples were measured at 695 nm. Results were expressed as mg ascorbic acid equivalent/g extract (20). Trolox was used as positive control.

**Radical scavenging activity by DPPH assay**

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities of the extract/sub-extracts were determined according to the method reported by Jung et al. (2011) (21). Extract/sub-extracts were mixed with DPPH solution and incubated in darkness for 30 min. Then the absorbance was measured at 520 nm utilizing a 96-well ELISA microplate reader (VersaMax, Molecular Devices, USA). Ascorbic acid was used as a positive control at 0.5, 1 and 2 mg/ml concentrations.

**Phytochemical analysis**

*Determination of total phenol content*

The method of Zongo et al. (2010) was used to determine total phenol contents. The extract and sub-extracts were mixed with Folin-Ciocalteu reagent and samples were incubated for 5 min. at room temperature (22). Then, sodium carbonate solution was added. The absorbance of mixture was measured at 735 nm after 30 min. The mean of three readings was used and the total phenol content was expressed in mg of gallic acid equivalents/g extracts. Calibration curve equation was:

\[ y(\text{Abs.}) = 5.306x(\text{Conc.}) + 0.0587 \] and the coefficient of determination was \( r^2 = 0.9986 \).

*Determination of total flavonoid content*

Total flavonoid contents of the *B. tripartita* extract and its sub-extracts were measured by the method of Kosalec et al. (2004) (23). Extract and sub-extracts were dissolved in ethanol. Ethanol, sodium acetate and aluminium chloride solution were added to the samples and the mixture was diluted by distilled water to 5 ml. After 30 min, the absorbance of yellow mixtures was measured at 415 nm. Results were expressed in mg of quercetin equivalents/g extracts. Calibration curve equation was:

\[ y(\text{Abs.}) = 2.4214x(\text{Conc.}) - 0.051 \] and the coefficient of determination was \( r^2 = 0.9998 \).

**LC-MS and HPLC analysis**

**Solvents and chemicals**

HPLC grade methanol and acetonitrile, analytical grade trifluoroacetic acid, and chromatographic grade double-distilled water were used for LC-MS and HPLC analysis. Presence of selected compounds was investigated. Ascorbic acid (57803), caffeic acid (C0625), chlороic acid (06957), chlorogenic acid (C3878), rosmarinic acid (R4033), catechin (43412), hyperoside (00180595), kaempferol (K0133), luteolin (L9283), luteolin-7-O-glucoside (cyanaroside)
Table 1. Acute hypoglycaemic and antidiabetic effects of Bidens tripartita ethanol extract

| Test Model | Group | Dose (mg/kg) | Mean blood glucose concentration (mg/dl) ±SEM (Inhibition %) |
|------------|-------|-------------|-----------------------------------------------------------|
|            |       | Initial     | ½ hr | 1 hr | 2 hr | 4 hr |
| NG         | Control | 88.0 ± 4.98 | 110.2 ± 5.57 | 103.7 ± 5.60 | 99.8 ± 4.77 | 109.9 ± 5.36 |
| Tolbutamide | 100   | 88.4 ± 4.93 | 81.4 ± 1.69*** | 68.2 ± 4.06*** | 57.5 ± 3.48*** | 60.3 ± 4.33*** |
| B. tripartita | 250   | 88.7 ± 3.56 | 95.2 ± 3.51 (13.6) | 88.7 ± 1.52 (14.5) | 83.2 ± 1.05* (16.6) | 97.2 ± 2.18* (11.6) |
| 500        | 82.2 ± 1.30 | 81.8 ± 3.28*** | 25.8 | 85.5 ± 4.17 (17.3) | 79.3 ± 1.98** (20.5) | 93.0 ± 4.13* (15.4) |
| OGTT       | Control | 85.0 ± 4.75 | 88.0 ± 3.94 | 134.2 ± 3.11 | 118.2 ± 5.31 | 95.6 ± 5.08 |
| Tolbutamide | 100   | 86.5 ± 4.30 | 64.6 ± 4.33*** | 80.9 ± 7.51*** | 52.8 ± 4.76*** (55.3) | 44.2 ± 6.90*** (53.8) |
| B. tripartita | 250   | 83.2 ± 2.93 | 90.0 ± 3.90 | 118.8 ± 5.17* (11.5) | 104.2 ± 7.52 (11.8) | 81.0 ± 3.31* (15.3) |
| 500        | 76.7 ± 1.98 | 81.5 ± 1.73 (7.4) | 117.7 ± 3.57* (12.3) | 104.5 ± 2.05 (11.6) | 75.5 ± 2.22* (21.0) |
| Diabetic   | Control | 335.4 ± 19.53 | 303.4 ± 13.01 | 294.6 ± 16.60 | 265.8 ± 18.60 | 204.4 ± 15.42 |
| Tolbutamide | 100   | 300.8 ± 17.68 | 253.7 ± 11.18* (16.4) | 235.6 ± 15.40* (20.0) | 220.0 ± 7.40* (16.5) | 210.0 ± 9.78 |
| B. tripartita | 250   | 308.8 ± 16.68 | 260.8 ± 4.64* (14.0) | 249.2 ± 13.09* (15.4) | 206.0 ± 12.00* (22.5) | 158.8 ± 1.62** (22.3) |
| 500        | 307.6 ± 16.97 | 229.4 ± 8.69** (24.3) | 214.0 ± 19.40** (27.4) | 178.8 ± 12.43*** (32.7) | 152.6 ± 8.37* (25.3) |

* P<0.05, ** P<0.01, *** P<0.001; SEM: standard error of the mean; NG: normoglycaemic; OGTT: oral glucose tolerance test

Experiment

Major chemical constituents of active extract and its sub-extracts were determined by using LC-MS. HPLC was used in the quantitative analysis. The analysis was first carried out with an LC-MS system (Agilent Technologies, ACE column (5 µ, C18 (150 mm x 4.6)) was used for the analysis. Injection volume was 20 µl. Mobile phase was acetonitrile and trifluoroacetic acid (0.03%) in water with a flow rate of 1 ml/min. A mass detector (G1956) was used to investigate the presence of selected compounds given above.

HPLC analysis were performed by using HP-1200 (Agilent Technologies Inc., California, USA) HPLC with an ACE 5 µ, C18 (150 x 4.6 mm) column. Water (0.03% trifluoroacetic acid) and acetonitrile were used in gradient elution as the mobile phase with a flow rate 1 ml/min. The composition of gradient was 10:90 (Acetonitrile: TFA Solution) at 0 min and changed to 30:70 (Acetonitrile: TFA Solution) in 30 min. Injection volume was 20 µl. The duration between runs was 10 min. Different wavelengths (210, 254, 280 and 360 nm) was scanned by a G1315D diode array detector (DAD). Then, DAD was set at 210 nm and peak areas were integrated automatically by computer using Agilent Software. Spectrums of selected compounds, B. tripartita ethanol extract and its sub-extracts were compared and their UV spectrums were superimposed. Presence of chlorogenic acid, cyanoside and luteolin were proved. Thus, quantitative analysis of these 3 compounds in B. tripartita ethanol extract, ethyl acetate sub-extract and n-butanol sub-extract were done.

Quantification and validation procedures

Ethanol extract of B. tripartita and its n-butanol sub-extract (10 mg) were dissolved in 10 ml of water: methanol (1:1) mixture. Ethyl acetate sub-extract (10 mg) was also dissolved in 10 ml methanol. All solutions were filtered from 0.45 µm filters and directly injected. The stock solutions of the pure compounds were prepared by dissolving 10 mg of chlorogenic acid and luteolin in 10 ml methanol and 10 mg of cyanoside in 10 ml water:methanol (1:1) mixture. For calibration, six concentrations of the standards (0.01-0.5 mg/ml) were prepared by diluting stock solutions. Triplicate 10 µl injections were made for each concentration and the calibration equations were obtained by using peak areas of standard solutions.

Limit of detection (LOD) and limit of quantification (LOQ) were established at a signal to noise ratio (S/N) of 3 and 10, respectively. LOD and LOQ concentrations were experimentally verified by 9 injections of standard compounds. Precision tests were performed by the evaluation of intra-day variations of the same standard solutions of all compounds at the LOQ level. The area values were recorded and RSD% was calculated.

Statistical analysis

Instat2 software was used to evaluate the data statistically. Values are presented as means±SEM Statistical differences between the treatments and the controls were tested by one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. A difference in the mean values of P<0.05 was considered to be statistically significant.

Results

In-vivo activity studies

Hypoglycaemic and antidiabetic effects of B. tripartita after acute administration

Effects of B. tripartita ethanol extract on normoglycaemic, glucose loaded and streptozotocin induced diabetic rats are shown in Table 1. Reference drug tolbutamide (100 mg/kg BW) was found to have a potent hypoglycaemic activity (26.1-55.3%) on normoglycaemic and glucose loaded rats. B. tripartita extracts reduced blood glucose levels significantly (11.5-25.8%) on normoglycaemic and glucose loaded rats compared to healthy control group.

(49968), quercetin (4951), rutin (R5143) were purchased from Sigma-Aldrich (Germany) and Fluka.

Iran J Basic Med Sci, Vol. 19, No. 10, Oct 2016

1117
Hypoglycaemic and antidiabetic effects of Bidens tripartita sub-extracts after acute administration

Chloroform, ethyl acetate, n-butanol, and remaining water sub-extracts of *B. tripartita* were obtained by successive solvent extraction of the ethanol extract. Doses of the extracts were calculated according to the percentage of their yields. The sub-extracts were administered at two different doses to rats to determine the hypoglycaemic and antidiabetic effects. None of the sub-extracts reduced blood glucose levels significantly in normoglycaemic animals (Table 3). In oral glucose tolerance test, reference drug tolbutamide lowered blood glucose levels significantly between 28.5-47.7%. Sub-extracts demonstrated significant and promising Hypoglycaemic effect in different ratios (10.3-27.9%). Among all tested extracts, only n-butanol sub-extract showed dose dependent effect on glucose loaded animals.

### Table 2. Sub-acute hypoglycaemic effect of *Bidens tripartita* extract on STZ-induced diabetic rats

| Group               | Dose (mg/kg) | Mean blood glucose conc. (mg/dl) ± SEM (Inh. %) | Mean body weight±SEM (Change % to 1st day) |
|---------------------|--------------|------------------------------------------------|------------------------------------------|
|                     |              | 1st Day | 4th Day | 8th Day | 1st Day | 4th Day | 8th Day |
| Control             | 335.4±19.53  | 391.5±17.44 | 328.7±12.63 | 180.2±11.64 | 193.0±11.35 | 185.7±13.61 | +7.1 | +3.1 |
| Tolbutamide         | 100          | 300.8±17.68 | 336.5±15.64* | 266.3±8.73* | 194.2±10.09 | 200.8±9.50 | 186.5±10.84 | +3.4 | -4.0 |
| *B. tripartita*     | 250          | 308.8±16.68 | 342.4±10.45* | 282.6±8.25* | 183.0±11.99 | 189.8±13.10 | 196.2±13.91 | +3.7 | +7.2 |
|                     | 500          | 307.6±16.97 | 252.3±18.44*** | 195.3±24.93*** | 169.6±17.76 | 176.2±30.20 | 194.3±30.25 | +3.9 | +14.6 |

*P <0.05**, **P <0.01, ***P <0.001, SEM: standard error of the mean; STZ: streptozotocin

### Antidiabetic effect of *B. tripartita* after sub-acute administration

Ethanol extract of *B. tripartita* was given to STZ-induced diabetic rats for 8 days at two different doses and the effects on blood glucose levels and body weights were monitored (Table 2). Antidiabetic effect of the extract at 500 mg/kg (24.3-32.7%) was significant and this effect was higher than the effect of the reference drug tolbutamide (0.0-20.0%) at all measurements.

### Table 3. Hypoglycaemic effect of *Bidens tripartita* sub-extracts on normoglycaemic and glucose loaded rats

| Test Model | Group               | Dose (mg/kg) | Mean blood glucose conc. (mg/dl) ± SEM (Inhibition %) |
|------------|---------------------|--------------|------------------------------------------------------|
|            |                     | Initial | % | 1 hr | 2 hr | 4 hr |
| NG         | Control             | 70.7±5.24 | 80.0±1.40 | 73.5±1.65 | 63.5±2.56 | 58.3±4.67 |
|            | Tolbutamide         | 100     | 68.0±1.81 | 55.2±4.74** (31.0) | 45.2±3.54** (38.5) | 42.7±4.60** (32.8) | 53.0±7.49 (9.09) |
|            | Chloroform SE       | 78      | 67.3±6.62 | 74.7±2.70 (6.6) | 69.8±3.10 (5.0) | 64.7±2.23 | 80.7±22 |
|            | Ethyl acetate SE    | 102     | 71.7±3.11 | 76.2±2.99 (4.8) | 72.5±2.51 (1.6) | 69.3±3.05 | 76.2±23.28 |
|            | Butanol SE          | 204     | 69.3±3.99 | 73.5±2.88 (8.1) | 74.3±3.41 | 64.0±3.24 | 70.2±3.38 |
|            | Remaining water SE  | 83      | 74.8±1.49 | 88.2±2.35 | 81.7±2.69 | 71.2±3.13 | 80.5±2.66 |
|            |                     | 166     | 66.5±1.98 | 76.3±4.25 (4.6) | 70.0±1.39 (4.8) | 63.8±3.28 | 71.5±4.50 |
| OGTT       | Control             | 236     | 72.5±1.84 | 80.7±1.31 | 77.0±1.57 | 69.2±1.78 | 72.2±3.77 |
|            | Tolbutamide         | 473     | 71.3±1.84 | 87.8±2.48 | 83.2±2.20 | 71.2±4.14 | 77.0±3.37 |

*NG: normoglycaemic, OGTT: oral glucose tolerance test, SE: sub-extract, *P <0.05, **P <0.01, ***P <0.001, SEM: standard error of the mean*
Table 4. Acute antidiabetic effects of *Bidens tripartita* sub-extracts on STZ-induced diabetic rats

| Group                | Dose (mg/kg) | Mean blood glucose conc. (mg/dl) ± SEM (Inhibition %) | Mean body weight ± SEM (Change % to 1st day) |
|----------------------|--------------|------------------------------------------------------|-------------------------------------------|
|                      |              | Initial | 1/2 hr | 1 hr | 2 hr | 4 hr | 1st Day | 4th Day | 8th Day | 1st Day | 4th Day | 8th Day |
| Control              | 388.0±5.40   | 377.7±6.75 | 35.1±2.50 | 327.0±15.00 | 300.0±6.50 |
| Tolbutamide          | 100          | 381.0±4.25 | 369.0±14.65 | 341.0±10.50 | 331.0±14.00 | 265.0±13.00* |
| Chloroform SE        | 78           | 376.0±8.60 | 375.5±7.20 | 363.2±6.40 | 355.0±11.50 | 336.8±13.40 |
|                      | 156          | 391.0±13.90 | 328.0±13.60 | 312.6±12.90* | 294.6±16.70 | 235.4±11.21** |
| Ethyl acetate SE     | 102          | 388.0±3.90 | 395.0±15.80 | 363.0±9.50 | 345.0±10.40 | 314.3±11.20 |
|                      | 204          | 371.8±15.59 | 337.8±16.00 | 338.6±15.00 | 272.4±18.00* | 212.6±9.07** |
| n-Butanol SE         | 83           | 380.0±10.50 | 412.0±6.80 | 374.0±6.50 | 395.0±7.80 | 268.0±7.60 |
|                      | 166          | 375.0±15.00 | 338.4±19.00 | 293.2±5.00** | 255.2±9.40** | 158.2±5.80*** |
| Remaining water SE   | 236          | 380.0±7.20 | 400.0±7.30 | 342.0±8.50 | 334.0±8.90 | 290.0±10.50 |
|                      | 473          | 378.0±14.00 | 415.5±15.70 | 354.0±18.00 | 325.5±12.00 | 273.5±9.50* |

SE: sub-extract, *P<0.05, **P<0.01, ***P<0.001, SEM: standard error of the mean; STZ: streptozotocin

Results of acute antidiabetic activities of *B. tripartita* sub-extracts are given in Table 4. Except remaining water sub-extract, high doses of all tested sub-extracts showed promising antidiabetic effect on STZ-induced diabetic rats (11.1-47.3%). n-butanol sub-extract at 166 mg/kg dose was the most active one, its effect was significant and continuous (16.6-47.3%). Ethyl acetate sub-extract at 204 mg/kg dose lowered blood glucose levels significantly at 2nd and 4th hour measurements (16.7, 29.1%) and chloroform sub-extract showed a similar antidiabetic effect at 156 mg/kg (9.4-21.5%) after acute administration.

Antidiabetic effects of *B. tripartita* sub-extracts after sub-acute administration

Antidiabetic activity of high doses of the *B. tripartita* sub-extracts after sub-acute administration was investigated and the results are given in Table 5. According to the 4th and 8th days measurements, chloroform and remaining water sub-extracts were found to be inactive. On the other hand, antidiabetic effect of the ethyl acetate and n-butanol sub-extracts were higher than the other sub-extracts and reference drug tolbutamide on severe diabetic animals. Their antidiabetic properties were found to be similar.

Therefore, all other analysis were conducted on these sub-extracts. BW of the diabetic animals were also recorded during the sub-acute administration. However the changes in the body weights of the groups were not significant compared to control group due to short experiment time.

**In-vitro activity studies**

Inhibition of carbohydrate digestive enzymes

α-Glucosidase and α-amylase inhibitory activities of *B. tripartita* ethanol extract and its sub-extracts were tested at three different concentrations (2, 1, and 0.5 mg/ml) (Table 6). α-Glucosidase inhibitory activity of ethanol extract of *B. tripartita* was found to be moderate (49.87-25.02%) at tested

Table 5. Sub-acute hypoglycemic effect of *Bidens tripartita* sub-extracts on STZ-induced diabetic rats

| Group                | Dose (mg/kg) | Mean blood glucose conc. (mg/dl)±SEM (Inh.) | Mean body weight ± SEM (Change % to 1st day) |
|----------------------|--------------|--------------------------------------------|-------------------------------------------|
|                      |              | 1st Day | 4th Day | 8th Day | 1st Day | 4th Day | 8th Day |
| Control              | 388.5±5.40   | 424.5±20.00 | 372.0±10.09 | 173.5±11.00 | 182.6±6.80 | 190.3±4.20 | (+1.76) |
| Tolbutamide          | 100          | 381.0±4.25 | 456.0±16.00 | 447.0±19.00 | 179.2±5.40 | 186.0±4.40 | (+9.5) |
| Chloroform SE        | 156          | 391.0±13.90 | 385.0±18.00 | 379.6±15.00 | 181.0±3.60 | 185.0±5.20 | (+5.1) |
| Ethyl acetate SE     | 204          | 371.8±15.59 | 396.0±17.50 | 320.2±17.00* | 170.3±4.20 | 176.1±3.40 | (+1.8) |
| n-Butanol SE         | 166          | 375.0±15.00 | 371.5±15.40* | 333.0±8.50* | 172.5±3.20 | 167.0±4.10 | (+3.2) |
| Remaining water SE   | 473          | 378.0±14.00 | 425.0±16.00 | 392.5±14.90 | 170.0±2.40 | 173.0±5.20 | (-0.5) |

SE: sub-extract, *P<0.05, **P<0.01, ***P<0.001, SEM: standard error of the mean; STZ: streptozotocin
concentrations compared to the reference drug Acarbose (71.11-47.99 at 1-0.25 μg/mL). All subextracts showed inhibitory activity on α-glucosidase enzyme at 2 mg/ml. Among these, ethyl acetate subextract showed the highest activity (65.56-27.38%).

B. tripartita ethanol extract was inactive on α-amylase enzyme and its sub-extracts (chloroform, ethyl acetate, and n-butanol) have a peddling inhibitory effect only at 2 mg/ml concentration. Reference drug Acarbose showed a dose dependent inhibitory activity against α-amylase enzyme at tested concentrations (47.68% at 1 mg/ml, 23.75 at 0.3 mg/ml and 5.34 at 0.1 mg/ml respectively).

Antioxidant activities

Total antioxidant capacities of extract and subextracts were determined by phosphomolibdenum assay. Antioxidant capacity of the ethanol extract was promising (352.51) and close to antioxidant capacity of the reference compound Trolox (382.5). The highest total antioxidant capacity was found in chloroform sub-extract (1532.45). On the contrary, no activity was observed by the same sub-extract on DPPH radical scavenging activity test. All other tested extract and sub-extracts were active in different percentages (62.86-19.35%) at tested concentrations (2-0.5 mg/ml). Ethyl acetate extract was the most active sub-extract (62.86-59.60%) among all and its effect was similar to reference compound ascorbic acid (70.88-65.62%) in DPPH radical scavenging assay.

Phytochemical analysis

Total phenol and flavonoid contents of the ethanol extract and its sub-extracts were measured (Table 7). Total phenol content of the ethanol extract was found to be 120.99 mg gallic acid equivalent in 1 g extract. Total flavonoid content was determined as 127.47 mg quercetin equivalent in 1 g extract. Both total phenol and flavonoid contents were determined very high in ethyl acetate extract (280.93 mg GAE/g extract and 297.62 mg QE/g extract respectively). Total flavonoid content of chloroform sub-extracts (213.79 mg QE/g extract) was higher than the flavonoid content of ethanol extract. Also phenol and flavonoid contents of n-butanol sub-extract were found close to the results of ethanol extract as expected.

After determination of phenol and flavonoid contents, active extract and sub-extracts (ethyl acetate and n-butanol) were examined for their chemical profile. Among all investigated flavonoids and phenolics; chlorogenic acid, luteolin-7-O-glycoside (cyanoside) and luteolin were found to be the major compounds according to LC-MS and UV analysis. Quantities of these three compounds were measured by using HPLC in ethanol extract of B. tripartita and its active sub-extracts. Amount and retention time of the compounds, wavelengths, calibration and validation data are given in Table 8 and 9. These three compounds were found to be in the highest level in ethyl acetate sub-extract (chlorogenic acid; 10.63%, cyanoside 10.87% and luteolin 7.58%). On the other hand, a small amount of chlorogenic acid (4.51%) and cyanoside (0.33%) were determined in the n-butanol sub-extract. It was interesting that luteolin is not detected in n-butanol sub-extract.

Table 6. α-Glucosidase and α-amylase inhibitory activity of Bidens tripartita ethanol extract and its sub-extracts

| Extract             | α-glucosidase inhibitory activity (Inhibition % ± SD) | α-Amylase inhibitory activity (Inhibition % ± SD) |
|---------------------|------------------------------------------------------|--------------------------------------------------|
|                     | 2 mg/ml                                              | 0.5 mg/ml                                        |
|                     | 1 mg/ml                                              | 2 mg/ml                                          |
|                     |                                                       | 1 mg/ml                                          |
|                     |                                                       | 0.5 mg/ml                                        |
| B. tripartita EtOH Ext. | 49.87 ± 1.16                                        | 25.02 ± 1.64                                     |
| Chloroform SE.       | 35.77 ± 4.50                                        | 21.05 ± 2.30                                     |
| Ethyl acetate SE.    | 64.56 ± 0.75                                        | 22.12 ± 3.19                                     |
| n-Butanol SE.        | 11.51 ± 1.74                                        | 5.31 ± 0.40                                     |
| Remaining water SE.  | 7.55 ± 1.30                                         | 7.11 ± 1.40                                     |
| Reference            | 1 μg/ml                                              | 0.25 μg/ml                                      |
| Acarbose             | 71.11 ± 2.08                                        | 47.99 ± 1.76                                    |

Table 7. Total phenol, total flavonoid contents and antioxidant activities of Bidens tripartita ethanol extract and its sub-extracts

| Extract             | Total phenol content (mg GAE/g ± SD) | Total flavonoid content (mg QE/g ± SD) | Total antioxidant capacity (AAE ± SD) | DPPH radical scavenging activity (Inhibition % ± SD) |
|---------------------|-------------------------------------|---------------------------------------|---------------------------------------|--------------------------------------------------------|
|                     | 2 mg/ml                             | 1 mg/ml                               | 0.5 mg/ml                             |
| B. tripartita EtOH Ext. | 12.099 ± 6.02                        | 352.51 ± 67.59                       | 28.65 ± 1.18                         | 25.57 ± 1.38                                           |
| Chloroform sub-ext.   | 58.04 ± 3.93                        | 1532.45 ± 39.02                      | 25.65 ± 1.18                         | 21.35 ± 3.00                                           |
| Ethyl acetate sub-ext. | 280.93 ± 11.05                      | 136.18 ± 30.70                       | 62.86 ± 0.80                         | 59.91 ± 0.46                                           |
| n-Butanol sub-ext.    | 10.43 ± 5.95                         | 91.94 ± 22.53                        | 27.96 ± 7.25                         | 25.19 ± 5.63                                           |
| Remaining water sub-ext. | 26.44 ± 0.94                      | 23.11 ± 17.03                        | 46.70 ± 6.22                         | 40.40 ± 3.48                                           |
| Reference             | 3 mg/ml                              | 2 mg/ml                               | 1 mg/ml                               | 0.5 mg/ml                                              |
| Acarbose              | NT                                  | 70.88 ± 4.7                           | 68.27 ± 1.36                         | 65.62 ± 4.67                                           |
| Trolox                | NT                                  | 382.50 ± 17.03                       | NT                                    | NT                                                     |

Total flavonoid contents of the extracts are expressed as mg quercetin equivalent (QE)/g extract and total phenol contents are expressed as mg gallic acid equivalent (GAE)/g extract.
Bioactivity & photochemistry of *Bidens tripartita*  

*Orhan et al.*

**Figure 1.** LC-MS chromatograms of *Bidens tripartita* extract, ethyl acetate and *n*-butanol subextracts

**Table 8.** Chlorogenic acid, cyanoside and luteolin contents of *Bidens tripartita* plant, its ethanol extract and its active sub-extracts

| Extract             | Chlorogenic acid | Cynaroside | Luteolin |
|---------------------|------------------|------------|----------|
| *B. tripartita*     | 0.8066           | 0.6363     | 0.3267   |
| *B. tripartita* EtOH ext. | 5.160           | 4.07       | 2.09     |
| Ethyl acetate sub-ext. | 10.63           | 10.87      | 7.58     |
| *n*-Butanol sub-ext. | 4.51            | 0.33       | -        |

Results are given as w/w%

**Discussion**

For centuries, plants are used to cure many ailments and several species of medicinal plants are used in the treatment of diabetes mellitus. Among them, *Bidens* species have attracted the attention of many scientists for a long time. Many studies have been conducted on the antidiabetic activity and antidiabetic constituents of *Bidens pilosa* extracts so far. According to the results of these elaborate animal experiments, it has been proved that extracts prepared from leaves and aerial parts of *B. pilosa* have antidiabetic effect. These studies have also revealed that *B. pilosa* extracts stimulate insulin secretion from pancreatic islets and increase insulin levels, decrease HbA1c and blood glucose levels and improve insulin sensitivity. Additionally, they can prevent autoimmune diabetes by modulating the differentiation of helper T cells. (24-28).

In this study, we aimed to evaluate the antidiabetic activity potential of *B. tripartita* ethanol extract. Results of our experiments revealed that the extract has potent hypoglycaemic effect on normoglycaemic and glucose loaded rats and also promising antidiabetic effect on streptozotocin induced diabetic rats by acute and sub-acute administration. Sub-extracts lowered blood glucose levels in oral glucose tolerance test remarkably. Additionally, ethyl acetate and *n*-butanol sub-extracts have shown outstanding antidiabetic activity after acute and sub-acute administration to diabetic animals.
In enzyme inhibition assays, ethyl acetate was the most effective sub-extract on α-glucosidase and α-amylase enzymes. Also its antioxidant activity was found to be remarkable by phosphomolibdenum and DPPH radical scavenging assays. It is estimated that high phenol and flavonoid contents may be responsible for the antioxidant activity of this sub-extract. According to LC-MS and HPLC analysis, major compounds in the ethyl acetate and n-butanol sub-extracts were determined as cyanaroside and chlorogenic acid, also luteolin was detected in the ethyl acetate sub-extract (Figure 1).

It is well-known that the genus Bidens is rich in flavonoid and polyacetylene derivatives. Thus, these compounds are commonly considered as chemotaxonomic markers for the species in this genus. Lv and Zhang have studied the chemical constituents of B. tripartita and they have isolated 14 flavonoids and 2 polyacetylenes from the aerial parts of the samples collected from China (29). Additionally, phytochemical studies on B. tripartita herb have shown the presence of flavones, flavanones, chalcones, aurones, coumarins, carotenoids and volatile compounds (30).

In a recent study (30), luteolin, cyanaroside and flavanomarein have been detected and quantified in B. tripartita herb and flower samples collected from Poland. The amount of flavanomarein, cyanaroside and luteolin is 0.229, 0.116 and 0.047 (in % of dry weight) respectively in flower extract and 0.157, 0.179 and 0.031 (in % of dry weight) respectively in herb extract. Cyanaroside and luteolin content of Turkish B. tripartita samples (Table 8) have been found higher than Poland samples. Cyanaroside amount in the ethanol extract is 4.07% and luteolin amount is 2.09% in our study. Moreover, DPPH radical scavenging activity of ethyl acetate extracts of B. tripartita flower and herbs have been investigated in the same study. Ethyl acetate extracts have exhibited a significant radical scavenging activity (flower extract: 98%, herb extract: 89%). These results are also compatible with our antioxidant activity results.

In recent years, many in-vivo and in-vitro studies have been conducted on the antidiabetic activities of phenolic acids and flavonoids. Chlorogenic acid is a major polyphenolic compound found in many plants and is an ester of caffeic acid and quinic acid. It has been isolated as one of the main active compounds in Cecropia obtusifolia. Its antidiabetic effect has been investigated on STZ induced diabetic rats at 10 and 15 mg/kg doses. Blood glucose levels of diabetic animals in chlorogenic acid administered groups have been decreased significantly compared to diabetic control (31). In in-vitro studies, α-glucosidase and α-amylase inhibitory activity of chlorogenic acid has been found to be significant and dose dependent (32, 33).

On the other hand, luteolin has shown a high inhibitory activity against yeast α-glucosidase (92% at 200µM) and against porcine pancreatic α-amylase (61% at 500 µM) (34). It has also inhibited rabbit muscle glycogen phosphorylase (IC₅₀ = 31.7 µM), aldose reductase enzymes (IC₅₀ = 5.1 µM) and inhibited sorbitol accumulation on erythrocytes (79.2% at 200 µM) (35). In addition, luteolin and cyanaroside have been found to have strong inhibitory activity on yeast α-glucosidase and porcine pancreatic α-amylase (36). Rauter et al have evaluated antihyperglycaemic and protective effects of many flavonoids on STZ induced diabetic rats (37). Blood glucose contenttrations of diabetic animals after 7 day administrations and on glucose tolerance test have been significantly reduced by cyanaroside (4 mg/kg BW).

**Conclusion**

In the light of literature survey and according to our results, it can be concluded that Bidens tripartita extracts containing chlorogenic acid, cyanaroside and luteolin have antidiabetic activity and its active components reveal this activity via many pathways. Further studies are necessary to obtain toxicological data and to observe long-term effects of the extracts and their active components.

**Acknowledgment**

This study is a part of Ülkü Gökçen İçöz’s PhD thesis called “Pharmacognosic studies on some Bidens L. taxaes growing in Turkey”. This study was financially supported by the Scientific and Technological Research Council of Turkey (TUBITAK-112S015). We are grateful to Ayşe Mine

---

**Table 9. Linear regression data, precision of the method at the LOQ level (n=9) and other method validation data**

| Parameters of analysis and validation | Chlorogenic acid | Cyanaroside | Luteolin |
|--------------------------------------|-----------------|-------------|----------|
| Wavelength (nm)                      | 210             | 210         | 210      |
| Retention Time (min)                 | 6.77            | 16.77       | 29.586   |
| Calibration Curve, r²                | y=6892.2x-31.614, r²=0.9991 | y=20236.62x-15.2951 r²=0.9999 | y=28020x-46.072, r²=0.9998 |
| Slope RSD %                          | 0.358           | 0.057       | 0.218    |
| Intersection RSD %                   | 2.822           | 1.249       | 6.177    |
| Peak Area (Mean), RSD %              | 35.02857, 3.421 | 40.69286, 0.585 | 39.77314, 0.850 |
| LOD (µg/ml), RSD %                   | 1.0386, 3.047   | 0.924, 3.003 | 1.024, 1.858 |
| LOQ (µg/ml), RSD %                   | 3.1158, 3.421   | 2.7725, 0.585 | 3.072, 0.849 |

RSD % = (SD / Mean) X 100, SD: Standart Deviation
Gencler Ozkan for her help in species determination.
We thank Burcin Ergene Oz for her help in LC-MS and HPLC analysis. We are also thankful to Bayer Group Turkey for providing us with Acardbose.

References

1. Sandu R, Miron A, Zaghat M, Ghicuic C, Lupusoru C. Experimental researches on acute toxicity of a Bidens tripartita extract in mice-preliminary investigation. Rev Med Chir Soc Med Nat Iasi 2012; 116: 1230-1234.
2. Kupicha K, Bidens L. In: Davis PH, editor. Flora of Turkey and the East Aegean Islands. 2nd ed. Edinburgh; Edinburgh University Press; Vol. 5. 1997. p. 46-47.
3. Sokolov SV. Phytotherapy and Phytopharmacology: The Manual for Doctors. Moscow: Medical News Agency; 2000.
4. Sezik E, Yesilada E, Shadidoyatov H, Kulvey Z, Nigmatullayev AM, Aripov HN, et al. Folk medicine in Uzbekistan I. Toshkent, Djuizzax, and Samargand provinces. J Ethnopharmacol 2004; 92: 197-207.
5. Blinova KY, Yakovlev GP. Botanical Pharmacognostic Dictionary. Moscow: Vyshyashkhiola; 1990.
6. Andreade-Cetto A, Heinrich M, Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 2005; 99: 325-348.
7. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine 1995; 2: 137-199.
8. Mootosamy A, Mahomoodally MF. Ethnomedical application of native remedies used against diabetes and related complications in Mauritius. J Ethnopharmacol 2014; 151: 413-444.
9. Pereira RL, Ihimarin T, Lucchetti L, da Silva AJ, Goncalves de Moraes VL. Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from Bidens pilosa L. Immunopharmacol 1999; 43: 31-37.
10. Tag H, Kalita P, Dwivedi P, Das AK, Namda ND. Herbal medicines used in the treatment of diabetes mellitus in Arunachal Himalaya, northeast, India. J Ethnopharmacol 2012; 141: 787-795.
11. Ubillas RP, Mendez CD, Jolad SD, Luo J, King SR, Carlson TJ, et al. Anti-phytogenic acetylenic glucosides from Bidens pilosa. Planta Med 2000; 66: 82-83.
12. Morton JF. Spanish needles (Bidens pilosa L) as a wild food resource. Econ Bot 1962; 16: 173-179.
13. Yang W-C. Botanical, pharmacological, phytochemical, and toxicological aspects of the antidiabetic plant Bidens pilosa L.vid Based Complement Altern Med 2014; 2014:698617.
14. Shikov AN, Pozharitskaya ON, Makarov VG, Wagner H, Verpoorte R, Heinrich M. Medicinal plants of the Russian Pharmacopoeia; their history and applications. J Ethnopharmacol 2014; 154: 481-536.
15. International diabetes federation. key findings 2014. Available from: http://www.idf.org/diabetesatlas/update-2014 [Last access date: 20.11.2015]
16. Subramanian R, Asmawi MZ, Sadikun A. In vitro α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochim Pol 2008; 55: 391-398.
17. Alexiou P, Demopoulos VJ. Medicinal plants used for the treatment of diabetes and its long-term complications Plants, in: Kokkalou, E. (Ed.) Traditional and Modern Medicine: Chemistry and Activity. Kerala: Transworld Research Network; 2010. p. 69-175.
18. Ali H, Houghton PJ, Soumyanath A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. J Ethnopharmacol 2006; 107: 449-455.
19. Lam SH, Chen JM, Kang CJ, Chen CH, Lee SS. α-Glucosidase inhibitors from the seeds of Syagrus romanzoffiana. Phytochemistry 2008; 69: 1173-1178.
20. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex, specific application to the determination of vitamin E. Anal Biochem 1999; 269: 337-341.
21. Jung HA, Jin SE, Choi RJ, Manh HT, Kim YS, Min BS, et al. Anti-tumorigenic activity of sophoraflavescenol against Lewis lung carcinoma in vitro and in vivo, Arch Pharm Res 2011; 34: 2087-2099.
22. Zongo C, Savadogo A, Ouattara L, Bassole IHN, Ouattara CAT, Ouattara AS, et al. Polyphenols content, antioxidant and antimicrobial activities of Ampelocissus grantii (Baker) Planch. (Vitaceae): A medicinal plant from Burkina Faso. Int J Pharmacol 2010; 6: 880-887.
23. Kosalec I, Bakmaz M, Pepeljnikaj S, Vladimir-Knezevic S. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acta Pharm 2004; 54: 65-72.
24. Alarcon-Aguilar FJ, Roman-Ramos R, Flores-Saenz JL, Aguirre-Garcia F. Investigation on the hypoglycaemic effects of extracts of four Mexican medicinal plants in normal and alloxan-diabetic mice. Phytother Res 2002; 16: 383-386.
25. Chang S-L, Chang CL-T, Chang Y-M, Hsieh R-H, Tzeng C-R, Wu T-K, et al. Polyacetylenic compounds and butanol fraction from Bidens pilosa can modulate the differentiation of helper t cells and prevent autoimmune diabetes in non-obese diabetic mice. Planta Med 2004; 70: 1045-1051.
26. Chien S-C, Young PH, Hsu Y-J, Chen C-H, Tien Y-J, Shiu S-Y, et al. Anti-diabetic properties of three common Bidens pilosa variants in Taiwan. J Ethnopharmacol 2009; 70: 1246-1254.
27. Dimo T, Rakotominina SV, Tan PV, Azay J, Dongo E, Cros G. Leaf methanol extract of Bidens pilosa prevents and attenuates the hypertension induced by high-fructose diet in Wistar rats. J Ethnopharmacol 2012; 83: 183-191.
28. Hsu Y-J, Lee T-H, Chang CL-T, Huang Y-T, Yang W-C. Anti-hyperglycemic effects and mechanism of Bidens pilosa water extract. J Ethnopharmacol 2009; 122: 379-383.
29. Lv J-L, Zhang L-B. Flavonoids and polyacetylenes from the aerial parts of Bidens tripartita. Biochem Syst Ecol 2013; 48: 42-44.
30. Wolniak M, Tomczykowa M, Tomczyk M, Gudej J, Wawer L. Antioxidant activity of extracts and
flavonoids from *Bidens tripartita*. Acta Pol Pharm 2007; 63: 441-447.
31. Andrade-Cetto A, Wiedenfeld H. Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. J. Ethnopharmacol 2001; 78: 145-149.
32. Narita Y, Inouye K. Kinetic analysis and mechanism on the inhibition of chlorogenic acid and its components against porcine pancreas α-amylase isoenzymes I and II. J Agric Food Biochem 2009; 57: 9218-9225.
33. Oboh G, Agunloye OM, Adeyemi SA, Ademiluyi AO. Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes *(in vitro)*: a comparative study. J Basic and Clin Physiol Pharmacol 2015; 26: 165-170.
34. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of α-glucosidase and α-amylase by flavonoids. J Nutr Sci Vitaminol 2006; 52: 149-153.
35. Kato A, Minoshima Y, Yamamoto J, Adachi I, Watson AA, Nash RJ. Protective effects of dietary chamomile tea on diabetic complications. J Agric Food Chem 2008; 56: 8206-8211.
36. Kim J-S, Kwon C-S, Son KH. Inhibition of alpha-glucosidase and amylose by luteolin, a flavonoid. Biosci Biotechnol Biochem 2000; 64; 2450-2461.
37. Rauter AP, Martins A, Borges C, Mota-Filipe H, Pinto R, Sepodes B, et al. Antihyperglycaemic and protective effects of flavonoids on streptozotocin-induced diabetic rats. Phytother Res 2010; 24: 133-138.