Effect of ethyl acetate fraction of *Lespedeza cuneate* in the treatment of streptozotocin-induced type-2 diabetes mellitus in ICR mice

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Abstract

In the recent past, phytomolecules are exponentially applied in the discovery of anti-diabetic drug. The natural products derivatives associated anti-diabetic drugs are gaining an appeal in both developing and developed countries due to less adverse effects. This work screened the active solvent fraction of Lespedeza cuneate based on the phytochemical, enzyme inhibition, antioxidant properties. The antioxidant efficacy of the different fractions of the L. cuneate were appraised by DPPH, ABTS, ferric reducing power, hydrogen peroxide and hydroxyl radical scavenging assays. The enzyme (α-amylase and α-glucosidase) inhibitory activity were measured. Phytochemical composition of Ethyl acetate fractions of L. cuneate (Lc-EAF) were studied by UHPLC-Q-TOF-MS/MS. The effect of Lc-EAF treatments in glucose uptake were studied in insulin resistance HepG2 cells (IR-HepG2). Further, the anti-diabetic effect of Lc-EAF in streptozotocin (STZ) induced diabetes mice were demonstrated. Ethyl acetate, hexane and methanol fractions of the L. cuneate showed a notable antioxidant, α-amylase and α-glucosidase inhibitory properties. Among the fractions, Lc-EAF found to be potent than the other fractions tested. The Lc-EAF exhibited the IC50 of 205.32 ± 23.47 µg/mL and 105.32 ± 13.93 µg/mL for α-amylase and α-glucosidase inhibition respectively. Also, 75 µg/mL of Lc-EAF exposure were enhanced the 68.23% of glucose uptake in IR-HepG2 cells. In vivo study indicated that treatment Lc-EAF (100 mg/kg b.wt) maintained the blood glucose level through reduction of insulin level, while improved the lipid profile, hepatic, and renal markers. The present findings suggested that Lc-EAF could be considered a prominent source of isolation of molecules with antidiabetic, anti-hyperlipidemic and ROS-associated diseases control.

Introduction

In the present world scenario, around 422 million people suffering from obesity-related type II diabetes mellitus. According to World Health Organization statistics, by 2030, this number will be almost double (Ali et al. 2021). Diabetes mellitus (DM) is one the most severe widespread carbohydrate metabolic disorders and it is one of the third leading causes of death worldwide, predominantly in developed and developing countries, including South Korea (Magliano et al. 2021). The sudden increase of DM in South Asia is more than 150% between 2000 and 2035. The drastic incidence of DM was significantly increased in the last three-decade in the South Korean population (13.7%) (Oh et al. 2021). According to the Korean National Health and Nutrition Examination Surveys, the diabetic's prevalence was increased from 8.9% to 11.1 in the young Korean population. Ageing, high, obesity, urbanization, food habits, and socioeconomic status are the common risk factor for DM. Due to globalization, the Increasing prevalence of diabetes was recorded worldwide during the last three decades not only from the increasing age but also from the age-specific prevalence (Mirzaei et al. 2020, Oyewande et al. 2020, Wang et al. 2020).

The current therapeutical system was failed to cure metabolic diseases, including DM, completely, due to the poor target specificity and less availability of the diabetic drug. While medical researchers have made advances in diabetic prevention and treatment, they continue to look for new antidiabetic drugs (Mohan et al. 2020). However, the remarkable accomplishment of synthetic drugs from "bench to bedside" for human use has met a restricted success because many of the drugs have a significant proportion of unfavourable effects. The use of medicinal plants and their phytoconstituents for DM is not just a search for safer alternatives to pharmaceuticals, which effectively lowers blood glucose levels and insulin resistance, decreases diabetic associated metabolic complications, and also improves the insulin secretion and antioxidant system (Vinayagam et al. 2016, Vinayagam et al. 2017). Several research studies were proven that phytoconstituents have always guided the search for a clinical trial. Hence, there is a emergence of novel dietary phytoconstituents with natural antioxidants that can be used as an antidiabetic compound (Sun et al. 2020, Wang et al. 2013).

Based on this scientific knowledge, the present study is designed to evaluate the antidiabetic effect of the active fraction of Lespedeza cuneate through the deactivation of the streptozotocin induced diabetic model. *Lespedeza cuneate*, is a perennial herbaceous shrub, belongs to the family of Fabaceae. It is widely distributed in the Korean peninsula, Japan, India, China, Taiwan, Nepal, Vietnam, and Bhutan, also cultivated in Australia, North America, South America and the Caribbean (Lee et al. 2011). The root and leaves part of the *L. cuneate* contains vitamin, minerals and the phytochemical analysis explored that; it has a rich source of flavonoids, pinitols, phenylpropanoids, sterols, tannins, triterpenoids, lignin's, etc. Preliminary reports confer anti-inflammatory, anticancer, antioxidants, antimicrobial, antiaging and hepatoprotective activities (Cho et al. 2009, Kim &Kim 2010, Kim &Kim 2007, Lee et al. 2013, Zhang et al. 2016). Besides, the extract of the plant material has a long history in folk medicine to treat severe chronic cough, abscess, asthma, eye diseases (Lee et al. 2019). Flavonoid compounds isolated from *L.cuneata* has a considerable free radical scavenging activity (Kim et al. 2011). Aqueous extracts of *L. cuneate* has considerably inhibited the diabetic-related enzyme of DPP-IV and α-glucosidase activity (Sharma et al. 2014). Moreover, few studies have focused on the antidiabetic efficacy of the bioactive fraction of the *L. cuneate*. Hence our present study intended to explore the detailed study on the phytochemical profile, antioxidants and STZ induced type II diabetic studies in ICR mice.

Materials And Methods

Chemicals

Streptozotocin, Insulin, citrate buffer, Sodium carbonate, sodium nitrate, sodium hydroxide, Ascorbic acid, aluminium chloride, potassium ferricyanide, trichloroacetic acid, EDTA, potassium acetate, ferric chloride, quercetin, gallic acid, ABTS reagent, DPPH reagent, Folin-Ciocalteau reagent, ascorbic acid and ethanol were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, ethyl acetate and n-hexane were procured from Daejung Chemicals & Metals Co. Ltd (South Korea). The cell culture media, fetal bovine serum, antibiotic solution, phosphate buffer saline and trypsin EDTA were procured from Gibaco chemicals and Croning (USA). The WST solution (Cellomax^{TM}) was purchased from Mediflab, South Korea.

Preparation of plant extracts/ active fraction

Dried leaves of *L. cuneate* were purchased from the local market of Chuncheon, South Korea. The taxonomic authentication was done by Professor. MH Wang at Department of Bio-Health Convergences and the voucher specimen (KNUH-BMC-2020-006) was deposited in the Herbarium of Department of Biomedical convergence, Kangwon National University, South Korea. The leaves part of *L. cuneate* were crushed in a grinder. Then, 100 g powdery samples of *L. cuneate* were percolated with 500 methanol three times at room temperature. After the two days of incubation, the resulting mixture was filtered with Whatman No 1 filter paper and then it was concentrated with reduced pressure. Then, 10 g of concentrated methanol crude extracts were used to partition the active fractionation using hexane, ethyl acetate, and methanol as a solvent. All the solvents generate the best extraction yield. Under the reduced pressure atmosphere in a rotary vacuum evaporator at 40ºC, the collected fraction was concentrated. Then the dried sample was stored at -20 ºC for further analysis.

Phytochemicals, in vitro antioxidant, and antidiabetic assay

The total phenolic content (TPC) was determined by using the Folin-Ciocalteau reagent, and the results were expressed in mg of Gallic acid equivalents (GAE)(Singleton et al. 1999). Total flavonoid content was measured by the method described by Zhishen et al. (1999), and the results were expressed in mg of Quercetin equivalents (GAE)(Zhishen et al. 1999). The antioxidant activity of the extracts and fractions of *L. cuneate* was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS, hydroxy radical and reducing power assay as described previously (Halliwell et al. 1987, Leong &Shui 2002, Oraiza 1986, Re et al. 1999). Sudha et al. (Sudha et al. 2011) method was used to document the α-amylase activity, and the α-glucosidase inhibitory activity was assessed by Li et al. (Liu et al. 2016). The assay was repeated three times.

Biocompatible nature of *L. cuneate*

The biocompatibility of *L. cuneate* extracts and active fractions was tested using the NIH3T3 mouse fibroblast cell line. 100 µL of trypsinised, well-matured NIH3T3cells were cultivated in a 96-well plate and incubated overnight. Then, 10 µL of varying concentrations (10-100µg/mL) of Lc-EAF were dissolved and placed in culture plates. After that, the plates were placed in a humidified CO2 chamber for overnight incubation. After that, 10 µL of WST solution was added to each well and reared for another 4 hours to determine the cytotoxicity. A multi-functional microplate reader was used to record (OD at 450 nm) the biocompatibility of the tested sample. Three parallel samples measured this assay and the findings were statistically represented by mean and standard deviation(Mariadoss et al. 2020).

Screening of in vitro Antidiabetic assay in HePG2 cells

Glucose uptake assay was tested in Insulin resistance HEpG2 (IR-HepG2) cells to establish the antidiabetic activity of the Lc-EAF. IR-HepG2 cells were firstly generated according to the protocol reported elsewhere(Saravanakumar et al. 2021). The well-established IR-HepG2 cells ($1x10^4$ cells/well) were cultured in high glucose DMEM included with FBS (10%) and antibiotic solution (1%) in a 5% CO_2 incubator for 24 h. For the treatment, various concentrations of Lc-EAF (4.68–300 g/mL) were added to cells and reared 24 h in the conditions mentioned above. Besides, the positive control (HepG2) cells were maintained. After the incubation, the cells, including the culture media, were harvested and centrifuged at 440 g for 5 min, and the supernatant was used for glucose assay by DNS method. Glucose uptake (%) was estimated using the method described elsewhere.

UHPLC-Q-TOF–MS/MS analysis

UHPLCQ-TOF-MS/MS was used to identify metabolites present in the ethyl acetate fractions of the *L.cuneate*. The test sample was characterized using a UHPLC quadrupole-TOF-MS/MS (Sciex 5600, included with the Eksigent UHPLC system) with a 50–1200 m/z scanning range negative mode of ionization. The Hypersil GOLD UHPLC Column was employed, which is 100 mm 2.1 mm 3 m. The mobile gradient phase was made up of H2O and CH3CN (each containing 0.1 percent HCOOH and 5 mM $HCOONH_4$). With a 0.25 mL/min flow rate and a 20 L injection volume, gradient elution from 10% to 90% CH_3CN was performed. Sciex Peak View 2.1 software, ACD/MS Fragmenter (ACD/Lab), and Chemispider Database were used to analyze the data. With the use of reported values from the literature, resolved peaks were further found.

Computational study

Lipinski's rule of five was employed to predict the drug-likeness of the identified compound from UHPLCQ-TOF-MS/MS analysis. The parameters of no more than five hydrogen bond donors, below ten hydrogen bond acceptors, less than 500 Da of molecular weight and an octanol-water partition coefficient log P less than 5(Lipinski 2004, Lipinski et al. 1997). Based on Lipinski analysis, the selected compounds (Vanillic acid 4-O-b-D-glucoside, Glucosyringic acid, trans-o-Coumaric acid 2-glucoside, Ferulic acid glucoside, Roseoside and Isovitexin) toxicity profile was analyzed by a web server based ADMET-SAR online tool. The parameters of AMES toxicity, carcinogen, acute oral toxicity, and acute rat toxicity were considered for this analysis(Guan et al. 2019). A computer-based PASS programme (Prediction of spectra for substances) was used to explore the diabetic associated pharmacological activity of the selected compounds from *L.cuneate.* The predication scale was based on the probability to be active (pa) and probability to be inactive (pi) (Khurana et al. 2011). In addition, *In Silico* molecular docking study was implied to validate the binding efficiency of selected compounds against the diabetic-related target of α-Amylase and α-Glucosidase. The target molecule of α-amylase (IOSE) and α-glucosidase (3A4A) was retrieved from RCSB-PDB protein structure. The non-protein, other ligand and water molecule were removed for before the docking analysis. The selected phytocompounds were retrieved from NIH PubChem and its energy minimalization was performed by USCF chimera software (Ver 1.14). The docking analysis was performed by Argus lab 4.0.1 and the results were visualized by using BIOVIA discovery studio visualizer V20 and the parameters like binding energy (Kcal/mol) and intermolecular energy (Kcal/ mol) were contemplated to select the best docked compounds(Anand Mariadoss et al. 2018).

In vivo antidiabetic study

Induction of experimental diabetes

Male ICR mice weighing 19-21 g will be used for this study. The mice were randomly separated to cages at 18-22°C under normal lighting conditions and allowed access with ad libitum and water. All procedures were approved by the Kangwon National University Animal Experimental Ethics Committee (Ethical approval No: 200813; Dt:23.12.2020). Experimental diabetes was induced in 12 h fasted rats by successive i.p. injection of STZ (50 mg/kg b.wt) dissolved in cold citrate buffer (0.1 M, pH 4.5) once a day, five days in a row(Talchai et al. 2012). STZ-injected animals were given 20% glucose solution to prevent the initial drug-induced hypoglycemia. The presence of diabetes in rats was established 72 hours after injection with STZ by measuring increased plasma glucose (using the glucose oxidase assay). The fasting glucose level > 250 mg/dl were selected as diabetic control for the experiment.

Study design

The experimental animals were separated into five groups, with each group consisting of at least six animals, as indicated below. **Lc-EAF** and Metformin were orally given for 28 days.

Group I : Normal rats

Group II : Diabetic rats

Group III : Diabetic + **Lc-EAF** (100 mg/kg b. wt)

Group IV : Diabetic + Metformin (50 µg/kg b.wt)

Group V : Normal + **Lc-EAF** (100 mg/kg b. wt)

Detection of Biochemical Indexes in Serum

After the treatment period, the rats were fasted overnight by sacrificed cervical decapitation. Fasting blood glucose will be estimated by a commercially available glucose kit based on the glucose oxidase method. Serum samples will be collected and used for biochemical analysis. The levels of glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (ALT), creatinine and blood urea nitrogen (BUN) content were measured by colorimetric method. The liver, pancreas and kidney sections were carefully dissected and fixed with 10% buffer formaldehyde solution for 24 h before paraffin embedding. Hematoxylin and eosin (H&E) staining was performed on the paraffin-embedded tissue sample (5 µm), and the stained sections were examined using optical microscopy.

Statistical analysis

One-way analysis of variance was used to evaluate group comparisons, followed by Duncan multiple comparison. IBM SPSS 20 was used for the statistical analysis, and all data were represented as mean, standard deviation. Statistical significance is defined as a value of $p < 0.05$.

Results And Discussion

Extraction yield, total phenolic and flavonoid content

The yield of methanol, hexane, and ethyl acetate fractions of the *Lespedeza cuneata* was shown in Table 1. Herein, a different solvent system was selected for the partition of *L.cuneata*. This different solvent system has the prime role in influencing the extraction yield and the quantity of the phytoconstituents, and it is also reflecting the upcoming findings. In particular, the ethyl acetate fractions have the maximum extraction yield of 5.59±2.01 mg followed by methanol (2.67 ±0.98 mg) and hexane (1.81±0.53 mg). It shows the ethyl acetate fractions were considered a suitable choice for the optimum extract yields from the leaves part of the *L.cuneata*. In addition, total phenolic and flavonoid content was measured by the standard spectroscopic method (Table 1). It revealed that the total phenolic and flavonoid content were significantly (p<0.05) higher in Lc-EAF than Lc-HF and Lc-MF. Comparatively, the Lc-EAF has a higher amount of total phenolic (395.54±5.04 mg/g extracts) and flavonoid content (209.63±0.63 mg/g extracts). Previous studies also revealed the *L.cuneata* has a higher amount of flavonoid content. Especially the ethyl acetate fraction has a higher amount of phenolic and flavonoid content due to its polarity nature. Our results endorsed these findings (Yoo et al. 2015, Zhang et al. 2016). In line with previous studies suggested that a higher yield of polyhydroxy compounds, glycones, and other organic compounds were extracted from the ethyl acetate fractions due to their polarity nature (Kifayatullah et al. 2015). These substances are responsible for various pharmacological properties, including antioxidants, antidiabetic, antiviral, anticancer, anti-inflammatory activities (Cho et al. 2009, Kim &Kim 2010, Lee et al. 2013, Yoo et al. 2015).

Antioxidants and free radical scavenging activity

A variety of *in vitro* studies assessed the antioxidant activity of the plant material. DPPH, ABTS, hydrogen peroxide scavenging, hydroxyl radical scavenging, and ferric reducing power are the most regularly used methods. Based on this, the free radical and antioxidants scavenging abilities of the active fraction of the *L.cuneata* associated DPPH, ABTS, hydroxyl radical and reducing power assay was studied (Table 2). We found that the Lc-EAF fraction was effectively inhibited the ABTS with the IC50 value of 58.32±4.21 µg/mL, whereas Lc-HF and Lc-MF showed the IC50 value of 149.86±10.73 µg/mL and 237.23 ±19.35 µg/mL, respectively. Likewise, Lc-EAF fraction (IC50 value of 99.54±4.43 µg/mL) were affording the dose-dependent inhibiting DPPH radical scavenging activity. On the contrary, the DPPH radical scavenging activity was comparatively higher than the ABTS assay. As a result, the scavenging activity of *L.cuneata* fraction in decreasing order was Lc-EAF > Lc-HE > Lc-MF. It showed that ethyl acetate fractions are more active than other fractions (p<0.05). These observations suggest a close linkage between the phytochemical content and antioxidant activity, such as the radical-scavenging effect on DPPH, ABTS assay (Fernandes de Oliveira et al. 2012). Similarly, dose-dependent hydroxyl radical scavenging activity was observed in our study. IC50 value the hydroxy radical scavenging properties of Lc-EAF (103.16±7.34 µg/mL), Lc-HF (198.44±6.11 µg/mL) and Lc-MF (452.95± 19.84 µg/mL). On the contrary, the IC50 value of the reducing power activity of Lc-EAF (254.37±35.52 µg/mL), Lc-HF (505.81±24.91µg/mL), and Lc-MF(952.62±15.67 µg/mL) showed to substantial inhibition activity. At the highest concentration of 1000 µg/mL, Lc-EAF, Lc-HF, and Lc-MF showed considerable antioxidant activity. These results revealed that the antioxidants and free radical scavenging potential of *L.cuneata* were increased with the increasing concentrations.

α -amylase and α -glucosidase inhibition assay

To explore the inhibitory effect of *L.cuneata*, an *in vitro* α-amylase and α-glucosidase inhibition assay was performed, and the results were presented in Table 2. The inhibitory action of the α-amylase against the active fraction of *L.cuneata* was found to be dose-dependent from 10 to 1000 µg/ml concentrations. Lc-EAF showed the lowest value, which indicates the increasing inhibitory activity of the enzyme. The IC50 value of the α-amylase inhibitory activity of Lc-EAF (205.32±23.47 µg/mL), Lc-HF (407.85±25.54 µg/mL), and Lc-MF (682.23±30.86 µg/mL) showed the considerable enzyme inhibitory activity. IC50 value of the α-glucosidase inhibitory activity of Lc-EAF (105.32±13.93 µg/mL), Lc-HF (286.80±18.86 µg/mL), and Lc-MF (403.52±20.17 µg/mL). From the results, it was clear that the phenolic enriched ethyl acetate fraction of *L.cuneata* was much more effective in inhibiting the activity of α-amylase and α-glucosidase. Consequently, it might be envisaged to be an effective strategy to control or treat DM (Honda &Hara 1993). Other results were broadly in line with the phytocompound can inhibit the alpha-amylase and alpha-glucosidase by neither interacting nor inhibiting certain positions of the enzyme (Rohn et al. 2002, Unuofin et al. 2017).

Biocompatibility analysis

To verify the biocompatibility nature of the Lc-EAF, we examined the WST based cytotoxicity assay in a non-cancerous cell line of NIH3T3 cells. NIH3T3 cells were subjected to increasing concentration of Lc-EAF, the cell viability was monitored for 24 h incubation. The results revealed that Lc-EAF has fewer cytotoxic effects on NIH3T3 cells. i.e., 1.97±0.87, 3.19±0.46, 6.60±0.18, 8.22±0.47, 9.49±0.69, 10.17±0.56 and 14.85±1.23 µg/mL at a concentration of 4.68, 9.37, 18.75, 37.5, 75, 150 and 300 µg/mL. From these findings, it could be suggested that Lc-EAF does not have any toxic nature compounds (Fig. 1a). Many previous studies revealed that most plant-based phytocompounds have biocompatible, non-toxic effects and immensely enhance cell viability. For instance, our previous studies reported that the active fraction of

Helianthus tuberous considerably enhances the cell viability of non-cancerous cells (Mariadoss et al. 2021). The fermented and non-fermented extracts of *L.cuneata* exposure surmised that the Hs68 (human dermal fibroblast cells) viability does not significantly differ from the untreated cells. A similar finding was also documented by Park et al. (2020), who revealed that the aqueous extracts of *A.manihot* increase cell proliferation (Park et al. 2020).

Glucose uptake in IR-HePG2 cells

The liver is a vital metabolic organ of the body accountable for the normal metabolic pathway. The imbalance in liver metabolism, including glucose and lipid homeostasis, leads to diabetes mellitus through insulin resistance (IR). Hence, developing a stable and reliable IR hepatocyte model for researching the molecular mechanism of IR in diabetic treatment (Röder et al. 2016). Based on this, the liver cancer cell line of HepG2 was ideally used to examine IR because the hepatic cells have similar morphological and biochemical features. The hepatic embryonal cancer cells of HepG2 were ideally used cell lines to examine the IR because the hepatic cells have similar morphological and biochemical features (Donato et al. 2015). Several studies also endorsed this model. We were developed IR- HepG2 using a culture media containing high glucose medium and $5x10^7$ M of Insulin, and these established cells were used for this study. Our results explored that the glucose uptake in the IR cells was much lower than in the control cells ($p<0.001$). In comparison to the IR cell, the glucose absorption was significantly boosted after treatment with Lc-EAf in a dose dependent manner ($p<0.05$). Among the tested concentration, 75 µg/mL of Lc-EAf showing about 68.23% of glucose uptake. On the contrary, the uptake levels were considerably lower for the concentrations 150 and 300µg/mL (Fig. 1b). However, in line with the findings of Nomura et al. (2008), it can be suggested that the bioactive compounds including quercetin, kaempferal, luteolin, and apigenin can suppress the IR signaling pathway through the activation of the AKT pathway and inhibition insulin phosphorylation (Nomura et al. 2008).

UPLC-QTOF-MS/MS analysis

Of note, the Lc-EA fraction was shown to have the most potent radical scavenging ability and intriguing antidiabetic properties. It could be owing to the enrichment of bioactive compounds. As a result, for the UPLC-QTOF-MS/MS analysis, Lc-EA fractions were used. The findings were presented with tentatively identified phytocompound along with formula, RT (min), (M-H)-, m/z, Response, Mass Error (ppm), and Fragmentation (m/z) (Fig. 2 and Table 3). The identified phytocompounds were characterized into five groups: Flavonoid, Flavonoid glycosides, Phenolic glycosides, Lignan glycosides, and saponin compounds. From the Lc-EA fractions, 28 compounds were identified by UPLC-QTOF-MS/MS assessment, including nine Phenolic glycosides (vanillic acid 4-O-b-D-glucoside, glucosyringic acid, trans-o-coumaric acid, ferulic acid glucoside, Isolariciresinol 9'-O-beta-D-glucoside, cuneataside A, cuneataside D and triterpene glycoside), nine flavonoid glucosides (Luteolin di-C-hexose, Taxifolin O-glucopyranside, Isorhamnetin-3-O-β-rutinoside, Apigenin C-pentosyl-C-hexoside, apigenin di-C-pentose, Apigenin C-hexoside-O-pentoside, Apigenin di-C-hexose, Apigenin O-hexose) and seven flavonoids ([Iso]Orientin, Quercetin-O-rhamnose-O-glucoside, [Iso]vitexin, Kaempferol-3-glucuronide, Nicotiflorin, Quercetin-3-O-β-D-glucopyranoside, Luteolin O-rutinoside, Roseoside). The bioactive organic fraction of Lc-EAF also contains lignan glycosides of Secoisolariciresinol-4-O-β-D-glucopyranoside and the saponin nature of the triterpene glycoside. Some of the identified phytocompound of Glucosyringic acid, Vanillic acid 4-O-b-D-glucoside, Ferulic acid glucoside trans-o-Coumaric acid, 2-glucoside along with the other phenolic compound has a significant therapeutic activity including antidiabetic activity (Shahidi and Yeo, 2018). The next category of flavonoids including Luteolin di-C-hexose, Taxifolin, Isorhamnetin-3-O-β-rutinoside, [Iso]Orientin, Quercetin-O-rhamnose-O-glucoside, [Iso]vitexin, Nicotiflorin, Kaempferol-3-glucuronide, Quercetin-3-O-β-D-glucopyranoside, and Luteolin O-rutinoside, which are to have a remarkable antidiabetic, antimicrobial, antimutagenic, and anticancer activity (Kumar &Pandey 2013, Middleton et al. 2000). In addition, there are four apigenin derivates abundantly present in the Lc-EA fractions. It is well known that flavonoid based phytocompounds has significant antidiabetic activity in several types of cell line and experimental animals (Malik et al. 2017, Qin et al. 2016).

Computational study

Lipinski's rule was adopted to explore the drug-likeness properties of the isolated compounds from Lc-EAF using a web tool of SwissADME. Also, ADMET-SAR online server predicted the toxicological properties of the selected compounds (Sup. Table 1 & 2). The analysis revealed the selected compounds (Vanillic acid 4-O-b-D-glucoside, Glucosyringic acid, trans-o-Coumaric acid 2-glucoside, Ferulic acid glucoside, Roseoside and Isovitexin) are non-carcinogenic and had low rat toxicity and acute oral toxicity values. However, the phytocompound of trans-o-Coumaric acid 2-glucoside, Ferulic acid glucoside showed an AMES toxicity. Besides, the selected compounds also underwent the PASS online tool to screen the diabetic-related activities, and the potential compounds displayed a higher Pa value than Pi (Sup. Table 3). *In silico* docking analysis showed that selected phytocompounds were acts as a potential inhibitor of α-Amylase and α-Glucosidase. The interaction poses of Vanillic acid 4-O-b-D-glucoside, Glucosyringic acid, trans-o-Coumaric acid 2-glucoside, Ferulic acid glucoside, Roseoside and Isovitexin with the target protein of α-Amylase (Fig. 3) and α-Glucosidase (Fig. 4 and Table 4). Our studies revealed that among the tested compounds, trans-o-Coumaric acid 2-glucoside binds with the α-amylase with higher affinity with -9.99503 kcal/mol of docking score. Trans-o-Coumaric acid 2-glucoside were directly bound to the amino acid residue of Gly304, Arg 346, Thr 314, Asp 317, Arg 267 in IOSE. The other

residual of Phe 348, Gly 309, Asp 353, Arg 303, Gln 302, Trp 316, Leu 313, Ile 312, Trp 269, Ala 310 and Gly 351 also showed a hydrophobic and other interaction with trans-o-Coumaric acid 2-glucoside. Besides, Glucosyringic acid has a docking score of -8.59kcal/mol with 3A4A (α-glucosidase). It was directly bound with the amino acid residues of Leu 434, Trp 402, Lys 400, Tyr 407, Asn 401 in 3A4A through the hydrogen bonding. The other residual of Val 404, Pro403, His 444, Phe 399, AlA 438, AsN398, Thr 358, Leu 439, Ile 437, Glu 435, Trp 402 also showed a hydrophobic and other interaction with Glucosyringic acid. The docking results of other tested phytocompounds docking results were shown in Fig. 3 & Table 4.

In vitro antidiabetic study

Diabetes is characterized by high blood glucose levels, excessive urination, excessive thirst, and weight loss despite increased appetite. Table 5 shows the blood glucose level, body weight, kidney, and liver weight of the experimental animals in each group. In the end, the STZ alone treated mice showed an increased blood glucose level (387.21±9.34 mg/dL). It indicates the ICR mice in diabetic status, whereas these levels were significantly reduced in Lc-EAF treatment. Besides, the body weight and organ weight were significantly decreased in diabetic animals compared to non-treated control mice. The STZ alone treated mice lost the bodyweight of -3.12±0.93, and the relative liver weight was found to be 4.17±0.81. These levels were significantly lower in the rest of the other experimental groups. Lc-EAF treatment has significantly balanced the body weight and organ weight in diabetic mice. We also monitored the animals' caloric intake and water intake daily while participating in the study. A significant reduction in body weight was seen in STZ-induced diabetic mice instead of their control counterparts(Saadane et al. 2020). Lack of Insulin may account for this, as it causes glucose to be unable to enter the cell, thereby increasing the percentage of sugar in the blood. To eliminate excess sugar, the body attempts to rid itself of the sugar through excretion in the urine (Cantley &Ashcroft 2015). An increase in urine production will lead to dehydration and weight loss. While hyperglycaemic STZ-induced ICR mice were found to have significantly increased food and water intake, they also found that the augmented food and water intake of these mice are likely due to a reduction in glucose utilization and significant loss of glucose in the urine, resulting in a stimulus to eat and drink (Data are not shown). The improvement in polyphagia, polydipsia, and preventing weight loss seen in STZ-induced ICR mice was strongly correlated with improved metabolic status and intestinal absorption in Lc-EAF-supplemented mice.

The low dose of STZ (50 mg/kg b.wt) causes pancreatic β-cells to be destroyed in rats, which results in insufficient insulin secretion. This model mimics the clinical condition of type 2 diabetes. The level of plasma glucose increased while the level of Insulin decreased (Table 5). Lc-EAF's ability to stimulate insulin secretion from the remnant β-cells and increase glucose utilization by the tissues is responsible for reducing fasting plasma glucose levels in diabetic rats. These findings were supported by evidence that insulin secretion increased in rats with diabetes due to Lc-EAF treatment and our histopathological analysis revealed a rise in the number of insulin-secreting cells in pancreatic islets. We also discovered that Lc-EAF could protect the pancreatic islets from free radical-induced damage by STZ by acting as a scavenger.

The current scenario has shown that elevated levels of hepatic lipids are commonly found in people with diabetes, which can serve as a valuable risk factor for cardiovascular problems. Additionally, increased concentrations of fatty acids also promote the oxidation of fatty acids, resulting in more acetyl CoA and cholesterol, which causes hypercholesterolemia (Martín-Timón et al. 2014). As indicated by increased plasma cholesterol, TGs, LDL, and diminished HDL, dyslipidemia was detected in STZ-induced ICR mice in the current study. Because of this, we discovered that Lc-EAF could treat hyperlipidemia by reducing cholesterol, TGs, LDL, and increasing HDL levels in diabetic mice (Table 6). Because of an increase in insulin secretion, there was a reduction in cholesterol synthesis, which accounts for the anti-hyperlipidemic effect. In addition, Lc-EAF is thought to affect lowering cholesterol levels by inhibiting the uptake of cholesterol from the intestines by binding to bile acids, which subsequently increases bile acid excretion and decreases cholesterol absorption from the intestines. These findings concur with Kim et al. [44], who reported that hesperetin reduced the hepatic lipid profile in hypercholesterolemic hamsters, resulting in reduced blood lipid levels (Kim et al. 2010).

We assessed microscopic histological observations of the endocrine pancreas to learn whether biochemical modifications led to structural changes. After a treatment period with menthol, histological assessment of the pancreas revealed significant improvement in both the changes in the islets and the numbers of pancreatic β-cells. The number of insulin-producing β-cells in STZ-injected ICR mice were diminished, which lowered the amount of insulin in the blood. Interestingly, the application of Lc-EAF to STZ-induced ICR mice resulted in improvements in islet cell rejuvenation and increased insulin secretion, suggesting that Lc-EAF can defend and repair pancreatic β-cells from free radical exploitation (Fig. 5). Because of this, Lc-EAF could be of great help in helping to repair pancreatic β-cell damage and assist in the production of Insulin. Increased glucose utilization in diabetic rats mediated by the promotion of b-cell regeneration and insulin secretion in the pancreas explains the antihyperglycemic effect of Lc-EAF.

Although in the current assessment, diabetic rats have demonstrated hepatic damage as well, current research also points to liver dysfunction and changes in circulating enzymes as additional contributors to hepatic injury. Decreased blood insulin, primarily due to leakage of these enzymes from the liver cytosol into the bloodstream, led to elevated ALT, AST, and ALP levels in the serum (Ollerton et al. 1999). Experimental ICR mice have significantly higher levels of ALT, AST, and ALP compared to normal mice. Our results showed that administration of Lc-EAF

prevented the rise in hepatic injury enzymes beyond normal levels, which may be due to the hepatoprotective effects of Lc-EAF (Table 7). Histological studies revealed that Lc-EAF improves cellular liver damage, and so it successfully handles diabetic complications (Fig. 6).

Next, we found out that BUN and creatinine are excreted in the urine along with urea nitrogen. The presence of this waste product may indicate enhanced protein breakdown in both the liver and plasma in experimental diabetes (Ozcan et al. 2012). The current study discovered that elevated BUN levels are indicators of renal dysfunction in hyperglycemic mice. In diabetes, increased serum creatinine levels indicate a decreased GFR (Table 7). This study's finding suggests that extracts possess the potential to attenuate renal injury caused by a hyperglycemic state, which is linked directly to the antioxidant capacity of this extract. Our study's findings on Lc-EAF, as well as cutting-edge research on this extract and its significant benefits for treating diabetic nephropathy, have been proven to be accurate. The administration of Lc-EAF showed significantly improved STZ-induced histopathological changes in the kidneys of the ICR mice and minimal tubular damage and less necrotic damage (Fig. 7). The biochemical findings corroborate histopathological findings, and the biochemical results indicate the potential nephroprotective properties of Lc-EAF.

Conclusion

The ethyl acetate fraction of the *L. cuneata* has a higher amount of polyphenols and flavonoids, favoring the potent antioxidant and antidiabetic activities. The mechanism of action of the Lc-EAF might be increasing the insulin secretion and sensitivity in extract-treated diabetic mice. The findings will be hopeful to provide new insights into the plant-derived antidiabetic drug. It might pave the way for next-generation treatment for diabetes which might be less expensive and with minimum side effects. However, more research will be conducted to ascertain the active hypoglycemic components in *L.cuneata*.

Declarations

Data availability statement The data available from the corresponding authors upon reasonable request and with permission of the study sponsor.

Author contribution Arokia Vijaya Anand Mariadoss: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Roles/Writing-original draft; Writing review & editing. SeonJu Park: Formal analysis; Investigation. Kandasamy Saravanakumar: Data curation; Formal analysis; Validation; Review & Editing. Anbazhagan Sathiyaseelan: Software; Formal analysis; Data curation; Validation. Myeong-Hyeon Wang: Funding acquisition; Project administration; Resources; Software; Supervision; Validation.

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Ethics approval All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All animal experiments were performed under protocol approved by the Local Institutional Animal Ethics Committee of Kangwon National University, Republic of Korea.

Consent for publication All authors have approved the manuscript for submission.

Competing interest The authors declare there is no conflict of interest in this study.

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Tables

Table 1 Extraction yield and Total phenolic content, and total flavonoid content fractions from *Lespedeza cuneata*.

Particulars	LC-MF	LC-HF	LC-EAF
Extraction yield (mg)	2.67 ±0.98^{b}	1.81±0.53^{a}	5.59±2.06^{c}
Total phenol content (Gallic acid equivalent (GAE) mg/g of extract)	46.33 ± 4.97^{b}	39.62±0.32^{a}	359.54±5.04^{c}
Total flavonoid content (Quercetin equivalent (QE) mg/g of extract)	32.73 ± 0.31^{a}	67.97±1.85^{b}	209.63±0.63^{c}

The different superscript letters in the same column of the different fractions shows the significant difference by Duncan's multiple range test ($p < 0.05$)

Table 2 IC50 value of antioxidants (ABTS, DPPH radical), α -amylase and α -glucosidase inhibitory activity of different fractions of *Lespedeza cuneata*.

Particulars	LC-MF	LC-HF	LC-EAF
ABTS	237.23±19.35^{c}	149.86±10.73^{b}	58.32±4.21^{a}
DPPH	395.73±42.97^{c}	263.62±18.42^{b}	99.54±4.43^{a}
Hydroxy radical	452.95±19.84^{c}	198.44±6.11^{b}	103.16±7.34^{a}
Reducing power	952.62±15.67^{c}	505.81±24.91^{b}	254.37±35.52^{a}
α -amylase	682.23±30.86^{c}	407.85±25.54^{b}	205.32±23.47^{a}
α -glucosidase	403.52±20.17^{c}	286.80±18.86^{b}	105.32±13.93^{a}

The different superscript letters in the same column of the different fractions shows the significant difference by Duncan's multiple range test ($p < 0.05$)

Table 3 Quantitative phytocompounds from the ethyl acetate fraction of *Lespedeza cuneate* by UPLC-QTOF-MS/MS analysis

RT (min)	Tentative identification	Formula	*m/z* [M-H]-	Mass Error (ppm)	Response	Fragmentation (*m/z*)	Reference
1.19	Vanillic acid 4-O-b-D-glucoside	C14H18O9	329.0881	1.0	6729	167.0349	(Virgen-Ortíz et al. 2016)
1.36	Glucosyringic acid	C15H20O10	359.0985	0.4	3956	166.0001, 197.0458	(Kaszás et al. 2020)
1.48	Hydroxycinnamic acid O-glucoside	C15H18O8	325.0926	1.2	2046	119.0513, 163.0402	(Virgen-Ortíz et al. 2016)
1.67	Ferulic acid glucoside	C16H20O9	355.1040	1.6	3589	134.0377, 193.0503	(Piraud et al. 2003)
1.95	Luteolin di-C-hexose	C27H30O16	609.1458	-0.5	17518	369.0615, 489.1041	(Zhang et al. 2017)
2.03	Taxifolin O-glucopyranside	C21H22O12	465.1044	1.1	2653	285.0407, 303.498	(Bianco et al. 2001)
2.45	Isolariciresinol 9'-O-beta-D-glucoside	C26H34O11	521.2022	-1.1	2338	329.1047, 344.1263, 359.1496	(Zhou et al. 2016)
2.63	Luteolin C-pentosyl-C-hexoside	C26H28O15	579.1356	0.0	54263	429.0828, 459.0937	(Ruan et al. 2019)
2.67	Unknown	C19H30O8	385.1865	-0.4	209207	-	-
2.88	Apigenin C-pentosyl-C-hexoside	C26H28O14	563.1404	-0.4	16373	353.0666, 473.1090	(Ruan et al. 2019)
2.96	Orientin(Iso)	C21H20O11	447.0932	-0.2	407537	284.0323, 297.0403, 327.0509, 357.0614	(Karar &Kuhnert 2015)
3.14	Apigenin di-C-pentose	C25H26O13	533.1302	0.3	219194	353.0666, 383.0771, 443.0986	(Geng et al. 2016)
3.18	Apigenin C-hexoside-O-pentoside	C26H28O14	563.1407	0.1	41366	293.0454, 413.0880	(Bender et al. 2018)
3.22	Quercetin-O-rhamnose-O-glucoside	C27H30O16	609.1462	0.1	60370	300.0274	(Li et al. 2014)
3.39	vitexin(Iso)	C21H20O10	431.0985	0.4	392599	311.0560, 341.0666	(Karar &Kuhnert 2015)
3.43	Kaempferol-3-glucuronide	C21H20O12	463.0884	0.4	37174	285.0404	(Kaszás et al. 2020)
3.44	Nicotiflorin	C27H30O15	593.1515	0.6	67825	285.0404	(Bianco et al. 2001)
3.52	Quercetin-3-O-β-D-glucopyranoside	C21H20O12	463.0884	0.3	65361	300.0276	(Virgen-Ortíz et al. 2016)
3.62	Apigenin di-C-hexose	C27H30O15	593.1522	1.7	3318	353.0674, 473.1096	(Zhang et al. 2017)
3.74	Apigenin O-hexose	C21H20O10	431.0985	0.5	9142	269.0449	(Piraud et al. 2003)
3.82	Luteolin O-rutinoside	C27H30O15	593.1516	0.7	4457	285.0399	(Bianco et al. 2001)

3.95	Isorhamnetin-3-O-β-rutinoside	C28H32O16	623.1611	-1.1	2156	300.0271, 315.0510	(Zhang et al. 2017)
3.99	Secoisolariciresinol-4-O-β-D-glucopyranoside	C26H36O11	523.2187	0.3	15311	346.1426, 361.1656	(Jeong et al. 2020)
4.92	Cuneataside A	C31H36O15	647.1980	-0.2	3334	145.0298, 163.0403, 501.1612	(Zhou et al. 2016)
5.87	Cuneataside D	C28H34O13	577.1928	0.2	3078	145.0300, 163.0404, 341.1051, 415.1418	(Zhou et al. 2016)
7.11	Unknown	C41H56O7	659.3939	-1.6	1659646	599.3732	-
9.58	Triterpene glycoside	C47H76O17	911.5005	-0.5	559477		-
9.63	triterpene glycoside	C49H80O20	987.5159	-1.2	594216		-

Table 4 Molecular docking analysis of selected phytocompound from Lc-EAF against the anti-diabetic target of α-amylase and α-glucosidase.

Table 5 Changes in the Blood glucose, Body weight and organ weight of the control and LcEAF treated experimental mice.

The different superscript letters in the same column of the different group shows the significant difference by Duncan's multiple range test ($p < 0.05$)

Table 6 Influence of LcEAF in plasma lipid profile of control and experimental mice.

The different superscript letters in the same column of the different group shows the significant difference by Duncan's multiple range test ($p < 0.05$)

Table 7 Influence of LcEAF in plasma ALT, AST, ALP, BUN and Creatinine level in control and experimental mice.

The different superscript letters in the same column of the different group shows the significant difference by Duncan's multiple range test ($p < 0.05$).

Figures

Phytocompound	Docking Score Kcal/ mol	Interactive residues of H-bond	Other Interactive residues
α-Amylase			
Vanillic acid 4-O-b-D-glucoside	-8.04	2 (Gln 63, Asp 300)	His 299, Trp 58, Leu 162, Gly 306, Leu 165, Val 163, Tyr 62, Gly 306, Leu 162.
Glucosyringic acid	-8.33	3 (Arg 389)	Gly 455, Thr 463, Thr 376, Asp 456, Arg 392, Asp 375, Thr 377, TRP 388, Glu 390, Ser 390, Val 458.
trans-o-Coumaric acid 2-glucoside	-9.99	7 (Gly304, Arg 346, Thr 314, Asp 317, Arg 267)	Phe 348, Gly 309, Asp 353, Arg 303, Gln 302, Trp 316, Leu 313, Ile 312, Trp 269, Ala 310, Gly 351.
Ferulic acid glucoside	-9.03	4 (Phe 315, Ala 318, Arg 346)	Asp317, Trp388, Trp 344, Ala 345, Arg 343, Asn 347, Phe 348, Trp 316.
Roseoside	-8.72	1 (Arg 392)	Arg 389, Asp 456, Lys 322, TRP 388, Glu 484, Glu 390, Ala 318, Val 318, Arg 387,Thr 376, Thr 377, Asp375.
Isovitexin	-8.08	2 (Lys 200, Glu 240)	Leu 162, Ala 198, Asp 300, Ile 235, Glu 233, Gly 306, Ala 307, Gly 308, Leu237, Tyr 151, His 201, Leu 165, His 101, Tyr 62, Asp 197, Arg 195.
α-Glucosidase			
Vanillic acid 4-O-b-D-glucoside	-8.51	3 (Ser 241, Lys 156, Arg 315)	Tyr 158, Asp 307, Pro 312, His 280, Leu 246, Tyr 158, Asp 242, Ser 240, Leu 177, Ser 157, Thr 310, Phe 303, Ser 311, Phe 314, Leu 313, Gln 279.
Glucosyringic acid	-8.59	7 (Leu 434, Trp 402, Tyr 407, Asn 401, Lys 400)	Phe 399, Ala438, Asn 398, Thr 358, Lu 439, Ile 437, Glu 435, Val 404, Pro 403, His 444.
trans-o-Coumaric acid 2-glucoside	-9.03	2 (Asp 307)	Tyr 158, Phe 159, Arg 442, His 280, Arg 315, Gln 353, Glu 411, Tyr 316, Asn 415, Phe 178, Val 216, Glu 277, Phe 303, Gln 279.
Ferulic acid glucoside	-9.40	3 (Gln 279, Arg 315, Glu 411)	Phe 178, Tyr 158, Phe 314, Asn 415,Tyr 316, Phe 159, Arg 442, His 112, Asp 215, Tyr 72, Asp 69, Val 216, Glu 277, Phe 303, His 280, Phe 314, Asn 415, Tyr 316.
Roseoside	-9.33	1 (Asp352)	Gln 353, His 112, Phe 178,Val 216, Tyr 158, Arg 442, Glu 411, Gln 182, Phe 159, AsP 69, Tyr 72, Arg 446, His 351, Asp 215, Val 109, Arg 213, Leuy 219, Glu 277, His 280, Gln 279, Phe 303, Asp 307, Thr 306, Arg 315.
Isovitexin	-6.86	3(Glu 435, Asn 401, Ala 438)	Lys 400, Leu 439, Leu 318, Trp 402, His 444, Phe 399, Asn 398. Thr 358, Ile 440, Arg 359, Gly 309, Phe 321, Val 319, Pro 320.

Group	Blood glucose		Body weight			Liver weight (g)	Kidney weight (g)	Relative liver weight (g/100g b.wt)
	Initial (mg/dL)	Final (mg/dL)	Initial (g)	Final (g)	Weight gain(g)			
Control	92.23±5.16^{a}	97.14±4.87^{a}	30.92±1.79^{a}	32.46±1.02^{a}	2.26±0.79^{c}	1.63±0.02^{a}	0.64±0.02^{b}	5.02±0.12^{a}
STZ	295.54±15.79^{c}	403.21±17.34^{d}	32.49±1.12^{b}	29.19±1.87^{c}	-3.12±0.93^{d}	1.22±0.27^{d}	0.57±0.05^{a}	4.17±0.81^{d}
STZ+ LcEAF	261.94±18.83^{b}	130.25±12.11^{c}	32.22±1.32^{b}	34.55±1.32^{b}	2.33±0.74^{a}	1.40±0.04^{c}	0.58±0.03^{a}	4.05±0.18^{c}
STZ+ Metformin	269.04±16.58^{c}	116.97±7.48^{b}	34.17±2.03^{b}	36.54±1.27^{b}	2.47±1.68^{a}	1.55±0.07^{b}	0.56±0.04^{a}	4.24±0.24^{b}
LcEAF Alone	94.56±6.48^{a}	102.48±5.28^{a}	35.80±2.00^{b}	37.25±2.15^{c}	1.75±0.40^{b}	1.61±0.08^{a}	0.65±0.0^{b}	4.86±0.30^{a}

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Total Cholesterol/HDL-C ratio	LDL-C/HDL-C ratio
Control	76.00±3.48^{a}	65.08±4.48^{a}	36.97±3.48^{b}	23.38±3.31^{a}	2.05±0.04^{a}	0.63±0.06^{c}
STZ	129.05±8.65^{d}	116.94±5.42^{d}	20.28±3.9^{d}	55.14±3.10^{d}	6.14±0.43^{d}	2.71±0.03^{a}
STZ+ LcEAF	109.66±7.84^{c}	87.99±5.25^{b}	35.56±2.70^{a}	34.93±6.20^{c}	3.08±0.07^{c}	0.98±0.02^{a}
STZ+ Metformin	92.61±4.83^{b}	80.76±7.23^{c}	30.86±2.67^{c}	27.46±4.24^{b}	3.01±0.02^{a}	0.88±0.03^{b}
LcEAF Alone	72.39±2.16^{a}	63.39±2.84^{a}	34.95±2.20^{a}	24.62±3.10^{a}	2.07±0.01^{b}	0.70±0.04^{c}

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
Control	58.30±4.98^{b}	42.06±3.07^{a}	129.00±10.58^{b}	39.36±2.52^{b}	0.58±0.05^{a}
STZ	134.87±10.84^{d}	121.73±8.02^{d}	227.86±15.69^{e}	110.21±6.41^{e}	2.32±0.32^{c}
STZ+ LcEAF	82.53±5.54^{c}	60.24±4.45^{c}	152.76±2.16^{d}	64.23±3.04^{c}	1.13±0.13^{b}
STZ+ Metformin	62.53±4.54^{b}	54.34±3.65^{b}	124.65±8.83^{c}	77.05±4.76^{d}	1.25±0.21^{b}
LcEAF Alone	51.42±3.30^{a}	43.08±2.09^{a}	119.24±7.51^{a}	33.52±2.81^{a}	0.50±0.05^{a}

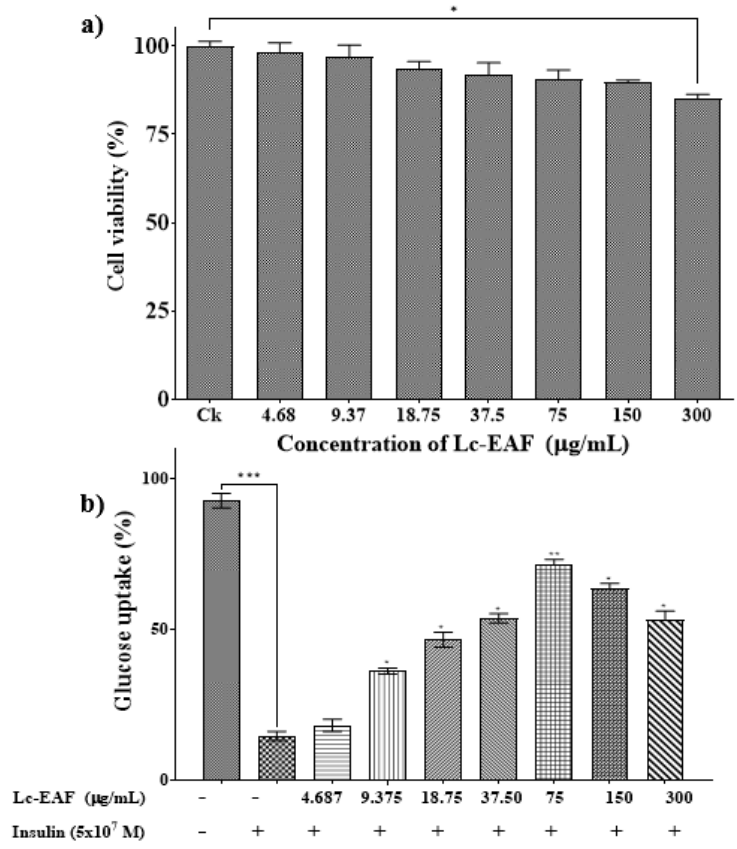

Figure 1

The biocompatibility nature of Lc-EAF was tested against the non-cancerous cell line of NIH3T3 using WST based cytotoxicity assay (a). Lc-EAF stimulate the action of glucose uptake in insulin resistant HepG2 cells (b). The results are presented as the Mean± SEM of three different experiments. *** P< 0.001 **P< 0.01 and *P< 0.05, vs. control.

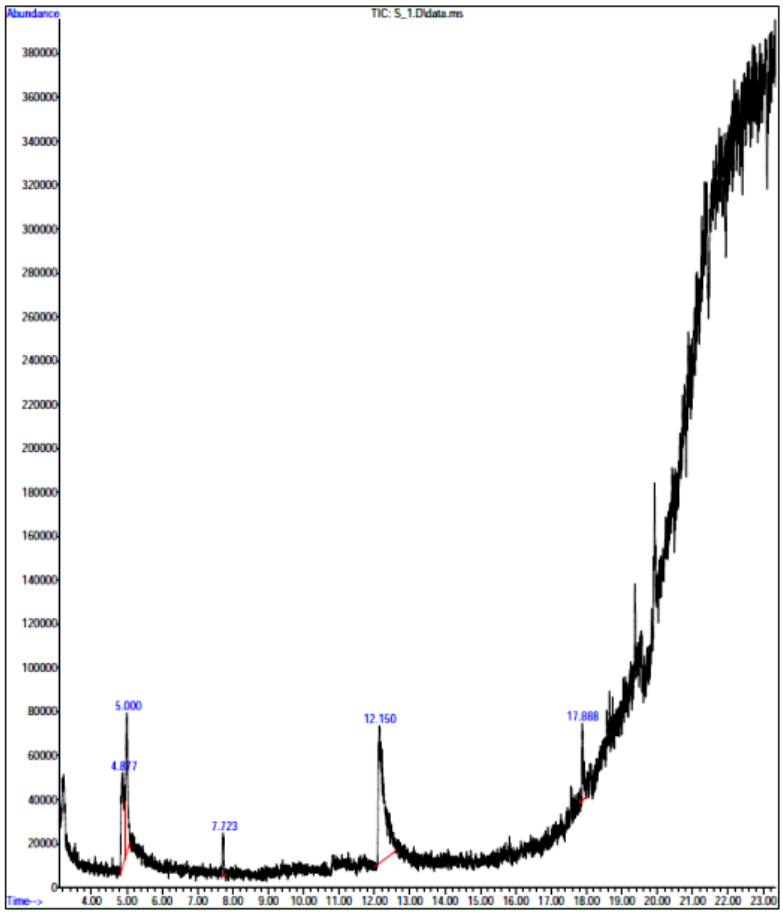

Figure 2

UHPLC-Q-TOF-MS/MS analysis of Lc-EAF.

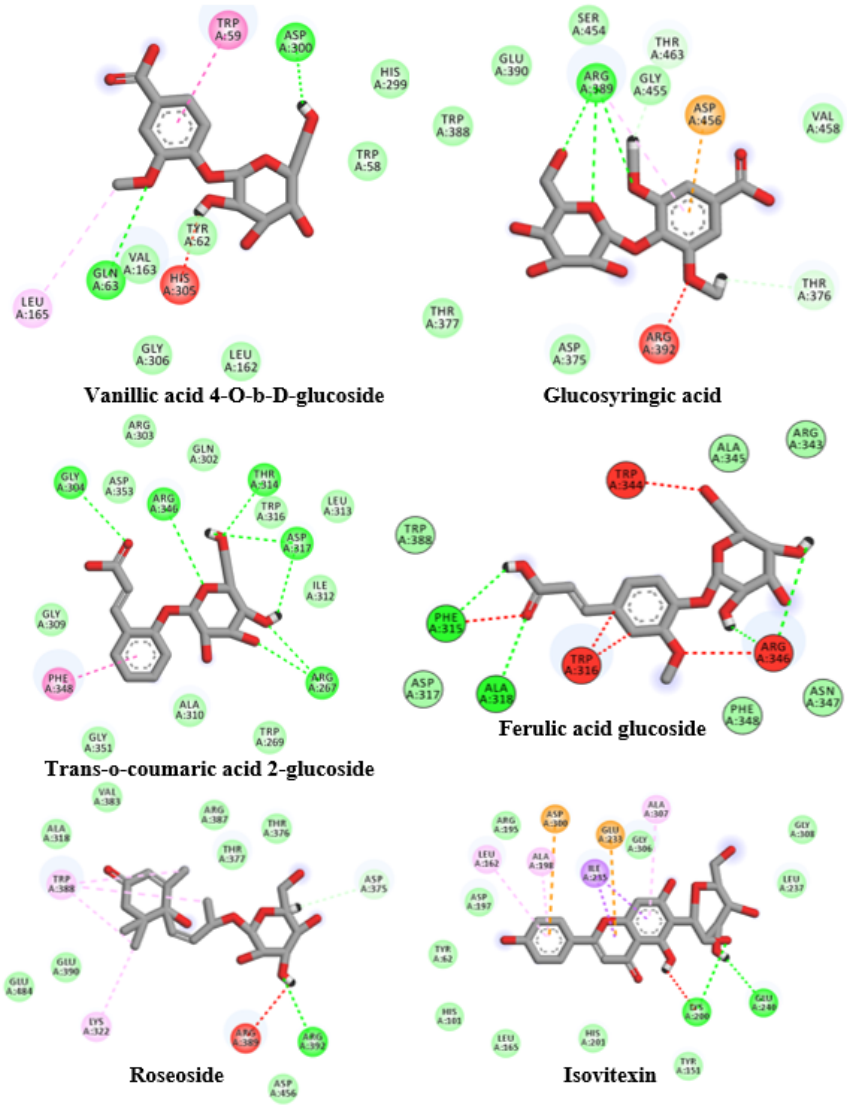

Figure 3

Molecular docking analysis of selected phytocompound from Lc-EAF against the anti-diabetic target of α-amylase.

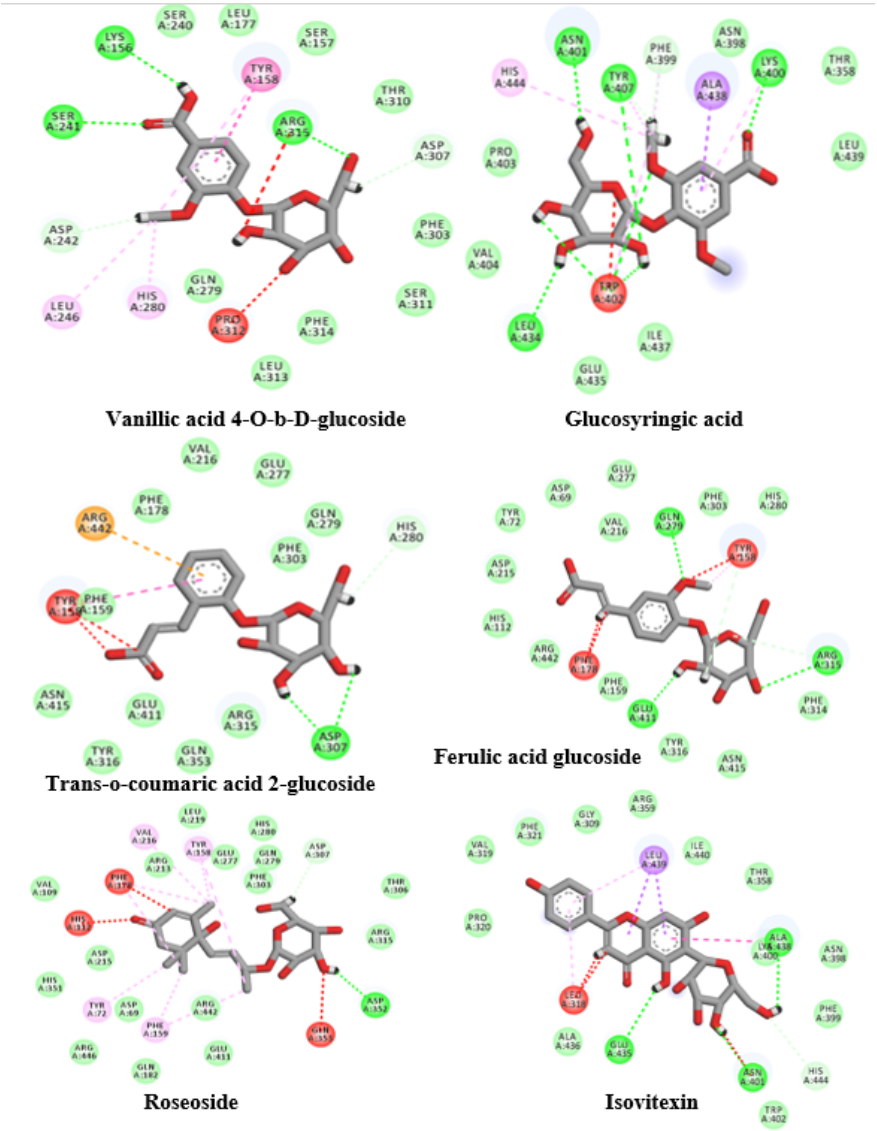

Figure 4

Molecular docking analysis of selected phytocompound from Lc-EAF against the anti-diabetic target of α-glucosidase.

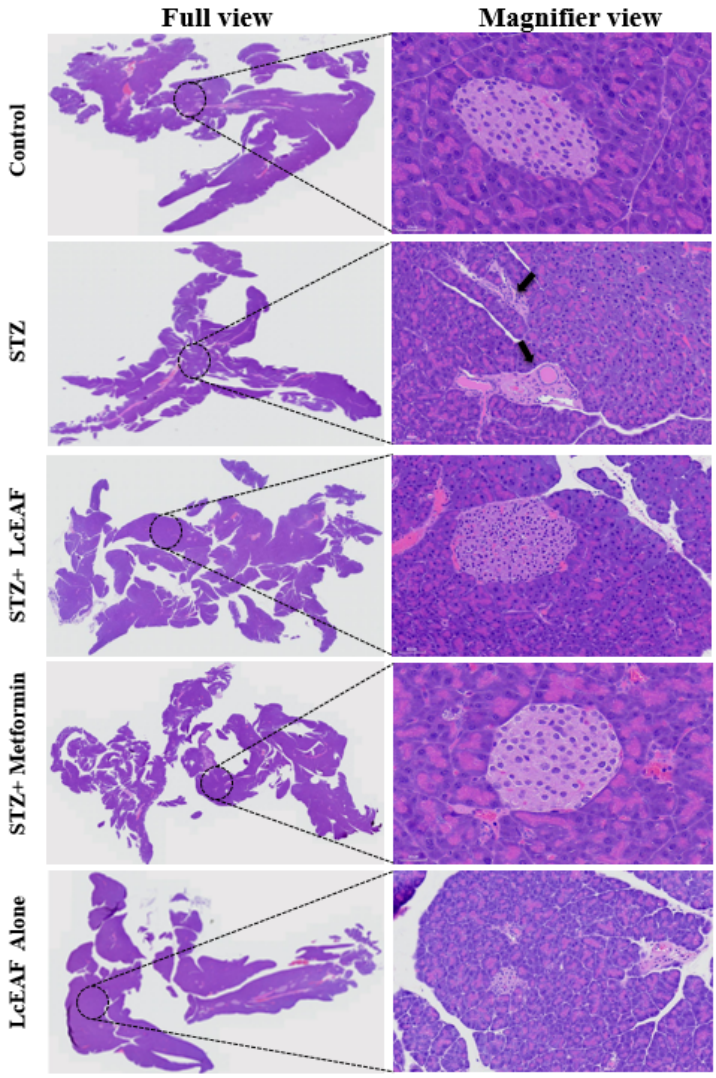

Figure 5

Histopathological changes of the pancrease of STZ and Lc-EAF treated diabetic mice. Hematoxylin and eosin stain (H&E stain). Control and LcEAF alone treated animal's pancreatic cells were appeared as dense staining acini and a light-staining islet of Langerhans. STZ treated animals showing the infiltrated cells, which occurs as the lymphocyte of immune system (Dark blue dots) enter and destroy the beta cells (arrow). STZ with Lc-EAF showed a mild improvement in the pancreatic islets morphology. STZ+ Metformin treated mice showed an improvement in the pancreatic islets morphology and improvement of immune system.

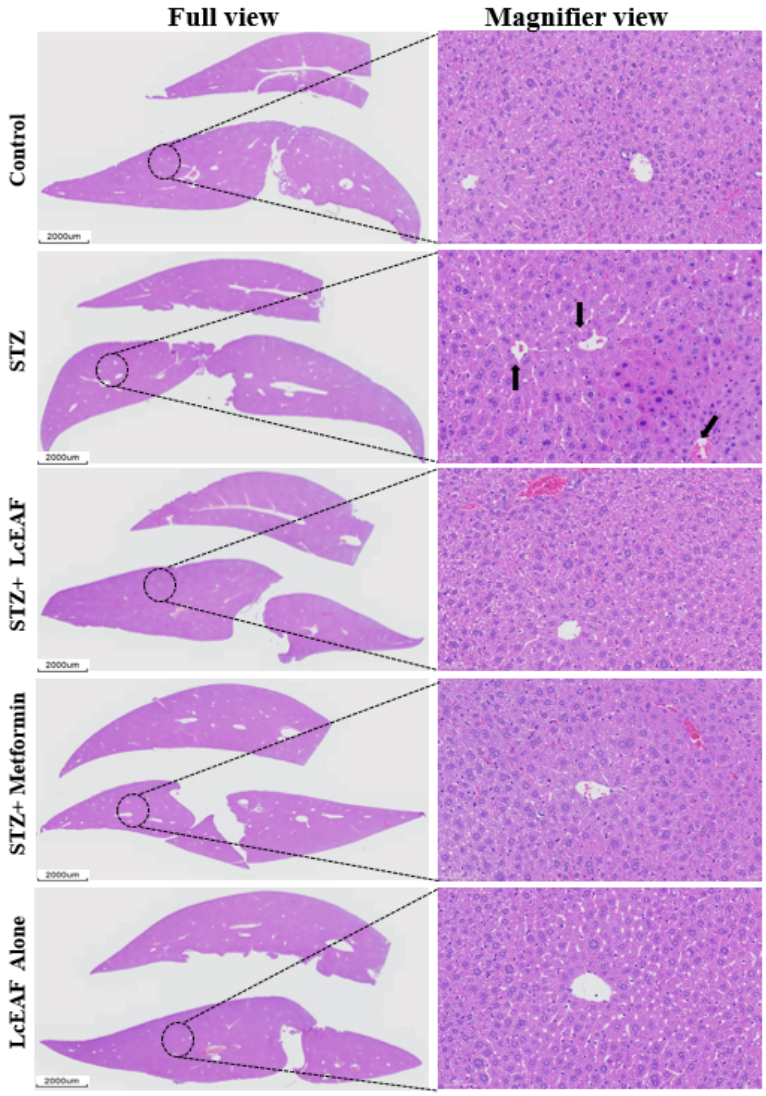

Figure 6

Histopathological changes of the liver section of STZ and Lc-EAF treated diabetic mice (H & E stain). Control and Lc-EAF alone treated animals showing a normal central vein and portal track appearance of liver cells. STZ treated animals showed a fatty change, mild inflammatory infiltrate and mallory bodies due to degeneration of hepatocytes in diabetic rats (marked in arrow). Dilation and congestion of the central veins also appeared. STZ+Metformin treated animals showed a normal central vein and hepatocyte arrangement. STZ+Lc-EAF treated animals showing a mild mononuclear inflammatory.

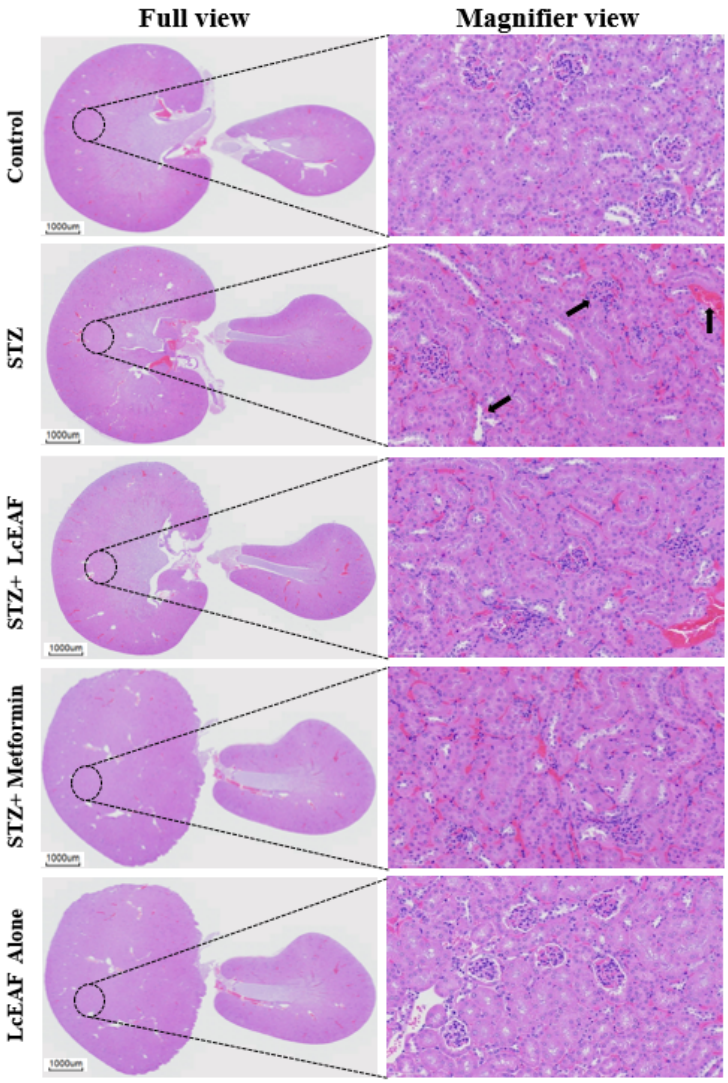

Figure 7

Histopathological changes of the kidney of STZ and Lc-EAF treated diabetic mice. (H&E stain). Control and Lc-EAF alone treated animals showing a normal structure of glomeruli and tubules. STZ treated animals showed lymphocyte infiltration in tubules and fatty infiltration (marked in arrow). STZ+Metformin treated animals showed a glomeruli and renal tubule appears to restored. STZ+Lc-EAF treated animals showed a mild fatty infiltration with mild damage in renal tubules.

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