The antihyperglycemic, antioxidant and antimicrobial activities of *Ehretia cymosa*


Abstract

*Ehretia cymosa* Thon. (Family Boraginaceae) is a shrub used by inhabitants of several communities in Ghana to treat diabetes mellitus and treatment of diarrhoea in indigenous poultry species including Guinea fowls (*Numida meleagris*). In this study, the antihyperglycaemic, antioxidant and antimicrobial activities of *Ehretia cymosa* was investigated. A 70% ethanolic extract of the whole plant of *Ehretia cymosa* was assessed for its *in vitro* anti-oxidant and antimicrobial activities by antioxidant and agar diffusion assays respectively. Antihyperglycemic activity was investigated in vivo in Sprague Dawley rats while modulatory effects on intestinal absorption of glucose were also determined *ex vivo*. The extract showed a significant dose independent reduction of the fasting blood glucose level (FBGL) while modulatory effects on intestinal absorption of glucose were also determined *ex vivo*. The extract showed strong antioxidant activity and also exhibited inhibitory activity against *P. aeruginosa*, *E. coli*, *B. subtilis* and *S. aureus*. These findings justify the folkloric use of the extract of *E. cymosa* as an antidiabetic medicine and as well as antibacterial agents for susceptible species.

Keywords: Diabetes mellitus, Antihyperglycaemia, Antioxidant, Antimicrobial, *Ehretia cymosa*, Streptozotocin.

Abbreviations: streptozotocin (STZ), ethanolic extract of *Ehretia cymosa* (EEEC), fasting blood glucose level (FBGL), butylated hydroxytoluene (BHT), Gallic acid equivalents (GAE), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid (AA), minimum inhibitory concentration (MIC)

1. Introduction

The surge of non-communicable diseases in Africa cannot be overemphasized. It is expected that within the next two decades, non-communicable diseases together with diabetes mellitus will overtake infectious diseases as the leading cause of death in Africa [1]. Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin (Type 1 or childhood-onset diabetes), or when the body cannot effectively use the insulin it produces (Type 2 or non-insulin-dependent diabetes). This leads to an increased concentration of glucose in the blood (hyperglycaemia) [2]. The presence of diabetes is associated with a high incidence of hypertension, hyperlipidaemia, nephropathy, and retinopathy and these comorbidities account for increased risk for vascular complications [3]. It is estimated that 347 million people globally have diabetes. In Sub-Saharan Africa, about 8% of the population above 25 years has diabetes [4].

The cost of management of diabetes per patient in Ghana increased from $180 – $420 in 2001 to $1276 - $7660 in 2007 while the economic cost of controlling diabetes using orthodox drugs worldwide is estimated to be $232 billion and could rise to $302.5 billion by 2025 [5-9]. This chronic disease takes an ever-increasing proportion of national healthcare budget, as the number of people with the disease increases. Immediate action is needed to stem the tide of diabetes mellitus and introduce cost effective treatment strategies to reverse this trend [10]. Ethnomedical management of chronic diseases has been encouraged by the World Health Organization as an alternative treatment to orthodox medicine [8]. As such, further research into affordable, available and effective antidiabetic agents is widely encouraged. Accumulated evidence indicates that the generation of reactive oxygen species may play a key role in the etiology of diabetic complications [10]. Many biochemical pathways associated with hyperglycemia such as glucose autoxidation, polyol pathway, prostanoid synthesis and protein glycation can increase the production of free radicals which may quench essential endogenous
biochemicals such as nitric oxide. The deleterious effects of free oxygen radicals may be delayed or reversed by antioxidants and this led to the suggestion that antioxidants limit susceptibility of microvascular and macrovascular complications arising from diabetes.

Patients afflicted with diabetes mellitus are also easily susceptible to infections with increased severity and higher risks of complications. In diabetes mellitus, several aspects of cellular immunity are adversely altered including polymorphonuclear leukocyte function, chemotaxis and phagocytosis. Glycaemic control therefore improves immune function. Another alternative to controlling infection in this group of immunocompromised individuals is using antidiabetic agents with antimicrobial properties. This reasoning justifies further research into medicinal plants which have medicinal constituents capable of possessing multipurpose for the treatment of various disease states and their associated comorbid conditions. Ethnobotanical information suggests that about 800 plants may possess antidiabetic potential. This justified the research into the antihyperglycaemic, antioxidative and antimicrobial activities of the ethanolic extract of Ehretia cymosa. Apart from its documented biological activities, there have been anecdotal reports of the use of the leaves of this plant for its antidiabetic activity.

The plant under study, *E. cymosa* is a shrub that is commonly located in the Savanna and secondary jungle of West Africa. This medicinal plant is used in the management of venereal diseases, epilepsy, dry cough, tonsillitis, typhoid, malaria, asthma, wounds and as an aphrodisiac, along with several others conditions. The leaves are used as febrifuge, laxative, pain-killer, and in paralysis, epilepsy, convulsions and spasm and toothache. The sap from the fresh leaves is used as a mild laxative for children. A decoction of the bark is used to regulate menstrual cycle. Leaf and root decoctions are used in the treatment of hyperthermia, stiffness, and dysentery and tetanus. Bark decoctions are topically applied for the prevention against skin diseases. Ghanaians use the leaf poultices by applying them on fractured bones to promote healing. Leafy twigs of *E. cymosa* are combined with other plant parts in the treatment of gastric ulcers. In Ethiopia, stomach complaints are managed by crushing the roots in water and taking this infusion orally. The people of Maasei use the roots in the treatment of brucellosis. Within the Akans and Ewe ethnic groups in Ghana, there have been anecdotal reports of the use of *E. cymosa* in the management of diabetes. Beside its use in treatment of human diseases, a survey conducted by the team in Volta region of Ghana into use of ethnomedicine in treatment of diseases affecting Guinea fowls (*Numida meleagris*) revealed that *E. cymosa* was one of the key plants ranking third according to frequency of citations. Participating farmers indicated the use of infusion from root and leaves of *E. cymosa* in treatment of diarrhea and loss of appetite (unpublished). In the backdrop of reported ethnomedical and ethnoveterinary use of *E. cymosa* this study aimed at studying antihyperglycaemic, antioxidant and antimicrobial activities of *E. cymosa*.

### 2. Materials and methods

#### 2.1. Plant Material

The whole plant material of *E. cymosa* (Fig 1) was collected in August, 2014 from Tsawula in the Volta region of Ghana. Mr. E. A. Blagoghi, a botanist at Centre for Plant Medicine Research (CPMR) assisted in the identification of the plant. Subsequently, the plant was authenticated at the herbarium in Plant Development Department at Mampong (CPMR) and given voucher specimen number of DPHM/001/04/15. The plant material was air dried for two weeks and pulverised to a coarse powder.

#### 2.2 Extraction by cold maceration

The extraction was done in September, 2014. Five liters of 70% ethanol was used to extract 500 g of the coarsely powdered plant material for 3 days with intermittent shaking. The extract was concentrated using a rotary evaporation at 40 °C. The concentrated extract was pooled together and further evaporated under vacuum to obtain a solvent free semi-solid mass with an extract yield of 2.2097% w/w per dried plant material. The extract was stored in a desiccator at room temperature.

#### 2.3 Phytochemical assay

Phytochemical analysis was carried out on the dried 70% ethanol extract of *E. cymosa* according to the method described by.

#### 2.4 Antimicrobial Assays

Stock solutions of the plant extract were prepared at concentrations of 20 mg/mL using 10% Tween 60. The stock solutions were sterile filtered and subsequently diluted to 10, 5 and 1 mg/mL with sterile water. Molten agar was stabilized at 45 °C and seeded with 0.1 mL inocula of a 24 hour nutrient broth cultured of the test organism. It was rolled in the palm to ensure uniform mixing of the agar and the test organism. This was aseptically poured into a Petri dish and allowed to set. A 10 mm cork borer was used to bore four holes in the agar equidistant from each other. The holes were filled with 200 μL of the respective concentration of the test extract. The Petri dishes were pre-incubated for 30 min and incubated at 37 °C for 24 h. Diameters of the clear zones of inhibition were

Plate 1: *E. cymosa* Thon. 1 × Magnification
measured in millimeters. Gentamicin at a concentration of 10 µg/mL was used as the standard drug for the positive control. This experiment was repeated in triplicate for Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli) NCTC1351, Staphylococcus aureus (S. aureus) NCTC6571 and Bacillus subtilis (B. subtilis).

2.5 Antioxidant assays

2.5.1 DPPH Free Radical Scavenging Activity

To evaluate the antioxidant activity of the extract, the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was performed as described by [19], with few modifications. Various concentrations of 5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, and 0.0391 mg/mL of 70% ethanolic extract of E. cymosa (EEEC) and a stock solution of 0.5 mM DPPH in methanol were prepared. A volume of 100 µL of each extract concentration was added to 100 µL of DPPH solution in a 96 well microtiter plate in increasing concentrations of the extract. The plate was shaken to uniformly mix and incubated for 30 min in darkness, at room temperature. The absorbance was read at λ 517 nm using Infinite M200Pro microtiter plate reader (Tecan, Austria). Each sample was done in triplicate. BHT 0.0391, 0.0781, 0.1563, 0.3125, 0.625, 1.25, 2.5, 5.0 mg/mL were used as positive controls and methanol as the blank.

2.5.2 Total Phenol Content

The total phenol content of the EEEC was determined by the Folin-Ciocalteau reagent method with some modifications [20]. A volume of 100 µL of 5% Folin-Ciocalteau reagent was added to 20 µL of various concentration of EEEC (5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, and 0.0391) mg/mL followed by 80 µL of 7.5 % Na2CO3 in a microtiter plate and the resulting solution was mixed. The solutions were incubated at room temperature in the darkness for 1 hour and the absorbance measured at λ 765 nm using the Infinite M200Pro microtiter plate reader. Gallic acid (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 µg/mL) was used to develop the standard calibration curve. The total phenol content was expressed in milligram of gallic acid equivalents (GAE) per gram of the extract.

2.5.3 Determination of reducing power

The reducing power of the EEEC was determined using the reduction of Fe3+ to Fe2+ [21] with Gallic acid, BHT and ascorbic acid. A quantity of 0.5 mL of various concentration of EEEC (5, 2.5, 1.25, 0.625, 0.3125, 0.1563, 0.0781, and 0.0391) mg/mL was added to 0.5 mL of water and mixed with 1.25 mL of sodium phosphate buffer (0.2M, pH=6.6) and 1.25 mL of 1% potassium ferricyanide. After incubation at 50 °C for 20 min, 1.25 mL of 10% trichloroacetic acid was added and samples centrifuged at 3000 g for 10 min. A 100 µL of supernatant was mixed with 100 µL of distilled water and 100 µL of 0.1% FeCl3. The absorbance of the resulting solutions was measured at λ 700 nm. The blank was prepared in a test tube by adding 0.5 mL of distilled water to 1.25 mL of sodium phosphate buffer and 1.25 mL of FeCl3. The reducing power was expressed as mg of gallic acid equivalents (GAE)/g of the extract.

2.6 Experimental animals

Thirty two (32) adult albino Sprague Dawley male rats, weighing within the range of 175-190 g were obtained from the Centre for Plant Medicine Research (CPMR) at Mampong, Ghana and housed in cages containing saw dust as bedding. Animals were housed under standard conditions of room temperature of 27 °C and supplied with standard pellet food (GAFCO, Ghana) and given tap water ad libitum. The animals were allowed three days acclimatization to this environment with a 12 hour light dark cycle. Handlings of the animals in this study were in accordance with the guidelines published by the National Institute of Health for the Care and Use of Laboratory Animals [23]. The research protocol was approved by the College of Health Sciences Ethics Committee, University of Ghana.

2.6.1 Assessment of Antihyperglycaemic effect of E. cymosa

Baseline fasting blood glucose level (FBGL) of the rats were taken a day before experimentation. The glucose levels of the individual animals were found to be within the range of <6 mmol/L. Diabetes was induced in the rats by a single intraperitoneal dose of streptozotocin (70 mg/kg) after an overnight fast. After a rest period of 48 hours, the fasting blood glucose levels of the rats were measured using an Accu-Chek® glucometer (Roche Diagnostics GmbH, Mannheim Germany). Animals with FBGL ≥10 mmol/L were considered as diabetic and included in the study.

To investigate the antihyperglycaemic effect of the plant extract, the rats were randomly divided into six groups of five animals each: Groups A, B, C, D, E and F. Group A was given distilled water and this group was used as the untreated control group. Groups B, C and D were given high dose, mid-dose and low dose respectively of 90, 60 and 30 mg /kg body weight of the plant extract respectively. The fasting blood glucose levels were determined and monitored hourly for six hours to ascertain the antihyperglycaemic activity of the plant extract. Group E and F were given intramuscular (IM) injections of insulin (1 unit/kg) and glibenclamide (5 mg/kg) dose respectively to serve as the positive control groups [21].

2.6.2 Preparation of intestinal sacs to determine glucose absorption modulation

Eighteen (18) adult albino male Sprague Dawley rats (weighing175-195g) housed under similar conditions as in section 2.6 was used in this experiment. The animals were starved for 12 hours but given water ad libitum. Rats were sacrificed by cervical dislocation and the abdomen opened by midline incision. The entire small intestine was immediately removed by cutting across the upper end towards the duodenum and the lower end of the ileum. The mesenteries were stripped manually. The small intestine was then washed out with normal saline (0.9% w/v NaCl). A thread ligature was then tied around one end of the intestine to facilitate subsequent identification.
2.6.3. Glucose absorption by intestinal sacs

Three (3) sets of intestinal sacs containing two sacs each were prepared as described by [24]. Each intestinal sac was loaded with 0.5 mL of glucose (5.6 mmol) and the ends tied. The sacs were then placed in 25 mL Erlenmeyer flasks containing 5 mL of Krebs-Henseleit bicarbonate buffer (KHB). After oxygenation of the flasks with carbogen (95% Oxygen and 5% CO2) for 1 min, the flasks were tightly stoppered and shaken for a minute at room temperature. Sets 1, 2 and 3 were incubated for 1, 2 and 3 hours respectively. At the end of the incubation period the sac contents were sampled and analysed for glucose using a glucometer.

2.6.4. Effects of E. cymosa on the uptake of glucose across the rat intestinal sacs

To define the effects of the plant extract on intestinal glucose absorption, which is a key step in antihyperglycaemic activity, 10 intestinal sacs (obtained as in section 2.5.2) were randomly divided and treated by the following five groupings: (A) control sacs were loaded with 0.5 mL of glucose (5.6 mmol); (B) the sacs were loaded with 0.5 mL of glucose (5.6 mmol) plus extract at 30 mg/kg; (C) the sacs were loaded with 0.5 mL of glucose (5.6 mmol) plus extract at 60mg/kg; (D) the sacs were loaded with 0.5 mL of glucose (5.6 mmol) plus extract at 90 mg/kg; (E) the fifth group were loaded with 0.5 mL of glucose (5.6 mmol) plus the standard drug, Acarbose at 50 mg/kg.

2.7. Data Analysis

The results were analyzed by two-way ANOVA followed by Bonferroni’s multiple comparison test to establish significance (p >0.005) between the treatment groups and control groups. The data provided were presented as means ± S.E.M.

3. Results and discussion

The phytochemical analysis showed the presence of saponins, reducing sugars, tannins, alkaloids, flavonoids, phenols and glycosides in the ethanolic extract of E. cymosa (Table 1) which are most likely the agents responsible for the observed biological activities in this study. The ethnomedical or ethnoveterinary significance of medicinal plants is commonly as a result of the pharmacological actions of the secondary metabolites present in that medicinal plant. The extract exhibited antihyperglycaemic, antioxidant and antimicrobial activities.

The extract exhibited inhibitory activity against P. aeruginosa, E. coli, B. subtilis and S. aureus (Tabel 2). Escherichia coli was the most susceptible with an MIC of 0.0108 µg/ml and Pseudomonas aeruginosa was least susceptible with an MIC of 0.1744 µg/ml. Some E.coli strains are the most common causal agents of diarrhoea in farm animals and humans. This may explain some of the use reports by poultry farmers including Guinea fowl farmers in treatments of diarrhoea and loss of appetite although some types of diarrhoea is caused by viruses that was not covered in the scope of this current study. Potential use of E. cymosa may be an alternative to emerging antibiotic resistance among poultry species. While antimicrobial effects is important in sole treatment of bacterial infections in animals. Antimicrobial properties of antihyperglycaemic agents are desirable since it will prevent the occurrence of infections or treat opportunistic infections that may occur as a result of hyperglycaemia in humans.

Table 1: Results for phytochemical analysis

<table>
<thead>
<tr>
<th>Plant metabolite</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>2. Saponin Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>3. Cyanogenic Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>4. Cardiac Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>5. Tannin test</td>
<td>+</td>
</tr>
<tr>
<td>6. Pseudotannins</td>
<td>+</td>
</tr>
<tr>
<td>7. Triterpenoids</td>
<td>-</td>
</tr>
<tr>
<td>8. Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>9. Phenols</td>
<td>+</td>
</tr>
<tr>
<td>10. Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + (Present), - (Absent)

Table 2: Antimicrobial activity of ethanolic extract of E. cymosa

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Minimum inhibitory concentrations (%µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>EEEC</td>
<td>0.0211</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>3.4583E-6</td>
</tr>
</tbody>
</table>

A dose dependent DPPH radical scavenging effect was observed with increasing drug concentration (Fig 1). The IC50 value of the extract in the DPPH assay, showed the similar antioxidant ability of the test extract to that of BHT but 15 times less than Ascorbic Acid (Fig 1). Polyphenols such as flavonoids are recognized for their antioxidant properties by scavenging for superoxide radicals [23]. Flavonoids in the ethanolic extract may have the ability to ameliorate or reverse pancreatic lesions that results from hyperglycaemia by preventing or reducing ROS generation or mobbing up the generated ROS in circulation.

In the other antioxidant assays, the reducing power of the extract was negligible as compared to the reference drugs used (Fig 2). Hence reducing power of the extract has negligible contribution to the antioxidant capacity. Other phytochemical constituents present in the extract, may largely be responsible for the antioxidant effect observed. The total phenols of the extract increased with increasing concentration of the EEEC (Fig 3).
Fig 1: DPPH free radical scavenging activity of 70% ethanolic extract of *E. cymosa* (EEEC), butylated hydroxyl toluene (BHT) and ascorbic acid (AA) at λ 517 nm. Each point represents mean ± SEM (n=3). The IC50 of the test extract and the reference compounds were 0.489, 0.403 and 0.032 µg/ml for the extract, butylated hydroxyl toluene (BHT) and ascorbic acid (AA) respectively.

Fig 2: Reducing Power Assay of the 70% ethanolic extract of *E. cymosa* in comparison to gallic acid, ascorbic acid and butylated hydroxyl toluene (BHT) at λ 700 nm. These were then expressed as mg of Gallic acid equivalent per g of extract. Each point represents mean ± SEM (n=8). A non-linear regression for each sample was illustrated and the IC50 of the sample and the two references were compared. IC50 values for EEEC, gallic acid, ascorbic acid and BHT were 13.4000, 0.2030, 0.0037 and 0.1895 µg/ml respectively.

Fig 3: Total Phenols Assay of the 70 % ethanolic extract of *E. cymosa* (EEEC) expressed as mg of Gallic acid equivalent per gram of extract. Each bar represents mean ± SEM (n=4).

The results from the *in vivo* antihyperglycaemic animal experiment showed that the different doses of the extract administered (30 mg/kg, 60 mg/kg and 90 mg/kg) gave statistically significant reduction in FBGL of the rats. Among the doses, the 60 mg/kg and 90 mg/kg had the highest reduction in the area under Curve of FBGL (Fig 4B). Reduction in FBGL was more effective than the glibenclamide treated group. The antihyperglycaemic activity of all the doses of the extract was however less significant than that of insulin. The antihyperglycaemic activity of medicinal plants is known to be mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or due to the facilitation of metabolites in insulin dependent processes [26]. Plants that are frequently implicated as having antidiabetic effect most commonly contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids as their phytochemical constituents [26].

The time-dependent glucose absorption recorded in this study was consistent with those reported by [27]. The *ex-vivo* intestinal rat absorption of glucose served as a useful model to investigate the modulatory effects of the 70% ethanolic extraction intestinal glucose absorption. The high glucose absorption recorded at 3 hours (Fig 5) was because the amount of glucose increases at the brush borders. The linearity of absorption seen over the 3 hours period suggests that glucose absorption did not get to the maximum (Fig 6). This would seem to suggest that glucose transporters were not saturated within the period of 3 hours.

The lower glucose absorption by Acarbose-containing intestinal sacs was to be expected since acarbose is known to inhibit the transport of hexoses at the intestinal brush borders [27]. Thus, the concentration-dependent and the significantly lower glucose levels in the EEEC-containing intestinal sacs (Fig 5, 6) suggest that EEEC like Acarbose inhibited glucose absorption in the intestines. The presence of polyphenols such as flavonoids, tannins and pseudotannins may also reduce intestinal glucose absorption to prevent overshooting plasma glucose and insulin levels in patients with prediabetes and diabetes mellitus type 2 [27].

Polyphenols have been found to interact with intestinal transporters and enzymes that control the absorption of postprandial glucose [28]. These phytoconstituents can reach very high concentrations of hundreds of micromoles in the intestines after being consumed and may therefore be enough to inhibit some of these intestinal enzymes and transporters [27]. Additionally, actions of alkaloids in the gastrointestinal tract [18] may reduce the amount of glucose that is absorbed from the intestines. This may have led to the reduction in the fasting blood glucose level in the extract treated groups. The Saponins are glycosides of both triterpenes and steroids are also known to possess antidiabetic properties [18]. Increasingly, phytochemical constituents or secondary metabolites in plants such as flavonoids and polyphenols have been implicated as key modulators of postprandial blood glucose levels [29].
Fig 4: Antihyperglycaemic activity of the ethanolic extract of *E. cymosa* (EEEC) in STZ-induced diabetic rats. A. Time-course of antihyperglycaemic activity of different doses of EEEC, glibenclamide (5 mg/kg) and insulin (1 unit/kg). B. Total blood sugar level following the various treatment regimens. */" (p ≤ 0.05), **/*/" (p ≤ 0.01), ***/*/" (p ≤ 0.001).

Fig. 5: Time-course study of the glucose absorption level of the small intestinal sacs of normoglycaemic Sprague-Dawley Rats over a 3 h period using the 70% ethanolic extract of *E. Cymosa* when compared to the untreated control group. * p < 0.05, ** (p < 0.01) and *** (p < 0.001) for the 2-way ANOVA followed by Bonferroni’s post hoc test. Values are expressed as mean ±SEM, 3 tissues in each group, (n=4).

Fig. 6: Effects of the ethanolic extract of *E. cymosa* on the inhibition of intestinal glucose absorption of the small intestinal sacs of Sprague Dawley rats. * p < 0.05, ** (p<0.01) and *** (p<0.001), when compared to the control group. Graph is a 1-way ANOVA followed by Dunnett’s multiple analysis. Values are expressed as mean ± SEM, 3 tissues in each group, (n=4).

4. Conclusion
The observed inhibition of glucose absorption by the ethanolic extract of *E. cymosa* could be due phytochemicals such as flavonoids, alkaloids, saponins and phenols. This study revealed that the ethanolic extract of *E. cymosa* had antihyperglycaemic activity in type I induced diabetes mellitus over a 6 hour period and this is being reported for the first time. In addition, the extract has significant anti-oxidant and antimicrobial properties. This research provides scientific support for the folkloric use of the plant in the treatment of diabetes in humans and potential use in treatment of susceptible bacterial diseases in farm animals.

5. Disclosure of conflict of interest
There is no known conflict of interest regarding financial interests in relation to the research work described in this submission.

6. Acknowledgement
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7. References