In-vitro Anti-diabetic and Antioxidant Efficacy of Methanolic Extract of Encephalartos ferox leaves

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ABSTRACT

Background: Diabetes mellitus has been identified as one of the global cause of disability and death. Objectives: The study aim to investigate the in-vitro antidiabetic and antioxidant activities of methanolic extract of Encephalartos ferox leaves. Materials and Methods: The plant was screened for its Phytochemical composition. The plant material was extracted with methanol and the methanolic extract was screened (in-vitro) for its antioxidant activity using ABTS and DPPH assays. The potential antidiabetic activity of the plant extract was evaluated against some carbohydrates (α- amylose and α-glucosidase) and lipid (pancreatic lipase) di-gestive enzymes. The inverted intestinal sac model was also used to investigate the effect of the extract on intestinal glucose absorption. The anti-protein glycation activity of the extract was determined using haemoglobin. Results: The phytochemical screening revealed the presence of most of the phytochemicals (Tannins, Flavonoids, Terpenoids, Alkaloids etc) that were screened for. The crude extract exhibited the antidiabetic potential as it significantly (P < 0.05) inhibited α-glucosidase and pancreatic lipase in a dose dependent fashion. The extract also effectively reduced intestinal glucose absorption. The extract further showed antioxidant activity by efficiently scavenging ABTS and DPPH radicals with IC₅₀ values of 68.3 µg/ml and 308 µg/ml, respectively. The extract also inhibited haemoglobin glycation, thus displaying the anti-protein glycation potential. Conclusion: It is apparent that E. ferox extract could serve as scaffold for diabetic therapy. For future study, cytotoxicity profile and in vivo investigation of the antidiabetic activity of the crude extract are essential.

Key words: Diabetic, Hypoglycaemic, Protein- glycation, Flavonoids, Hyperglycaemia, Hyperlipidemia.

INTRODUCTION

Diabetes Mellitus (DM) is a progressive metabolic disorder of carbohydrates and lipids that is characterized by hyperglycemia.¹ DM is known to result from defect in insulin secretion, action or both.² Poor management of diabetes mellitus can degenerate into debilitating conditions including heart attack, stroke, kidney failure, leg amputation, vision loss, nerve damage and erectile dysfunction.³ The inability of the cells to assimilate glucose formed via stepwise catabolism of carbohydrates with α-amylase and α-glucosidase, leads to hyperglycemia. Likewise, hyperactivity of pancreatic lipase results to increase in lipid metabolism which also triggers hyperlipidemia.⁴ Therefore, hyperglycemia and hyperlipidemia continue to be the underlying factors in the on-set of diabetes mellitus and its complications.⁵ In addition, reactive oxygen species also play crucial role in the pathogenesis of diabetes mellitus.⁶ Despite the potency of currently used diabetic drugs such as acarbose, Voglibose, Miglitol, insulin mimetics and secretagogues, they still are associated with adverse side effects.⁷ Therefore, the search for alternative remedy from plant origin has become paramount. Medicinal plants are known to possess less or no aftermath effect, inexpensive and easily available especially to rural dwellers.⁸ Encephalartos ferox (G. Bertol) Lehmann is a small cycad which belongs to the family Zamiaceae and endemic in northern KwaZulu Natal. E. ferox contains pinnately compound leaves that can grow up to two metres in length and a trunk that is a metre long which lies under the surface of the earth. E. ferox grows easily on a well-drained soil, moderate temperatures and abundant of water. E. ferox propagates using cones that are sexually dimorphic. It is commonly called Tongaland broodboom in Afrikaans, Umthobane and Uthobani in Zulu and Chihanga in Tonga.⁹ Ten In the past, E. ferox stems were used as meal while the leaves were used for the treatment of estrogen-dependent tumor.¹¹ Contemporarily, Encephalartos ferox leaves are also used by traditional healers in KwaZulu Natal province of South Africa to manage diabetes and its complications, especially diabetic wounds. However,
the medicinal folklores usage still lacks scientific validation. This is the first report on the in-vitro anti-diabetic and anti-oxidant potentials of methanolic extract of *E. ferox* leaves to the best of our knowledge.

**MATERIALS AND METHODS**

**Chemicals**

All the chemicals and kits used in this study were of analytical grades and were purchased from Sigma Aldrich Co.Ltd (Steinheim, Germany).

**Plant identification**

The leaves of *Encephalartos ferox* were collected in May, 2017 from Mbazwana (27 4937° S, 32 5882° E) KwaZulu-Natal, South Africa. The plant’s sample was taken to Department of Botany, University of Zululand and was authenticated by Dr. N.T Ntuli. The plant’s sample with specimen number V104 has been deposited at the University herbarium.

**Plant extraction**

The leaves of *Encephalartos ferox* were air dried and pulverized into fine powder. Pulverized sample (50 g) was extracted with methanol (1:5 w/v) using mechanical shaker (150 rpm; 25°C) for 72 h. The extract was filtered using Whatman filter paper 1 and concentrated using a Heidolph rotor evaporator (45 rpm, 40°C) to yield 8.4 % of crude extract.

**Phytochemicals screening**

The phytochemicals screening was conducted on the pulverized samples following the method described by Odebiyi and Sofowara and Harborne. The following phytochemicals were screened for; saponins, tannins, flavonoids, alkaloids and terpenoids.

**Haemoglobin glycation**

The haemoglobin glycation inhibitory activity of the extracts was determined using the method of Pal and Dutta. The reaction mixture consisted of 50 μl of various concentrations (0 - 2mg/ml) of the extract, haemoglobin (0.06 %), ciprofloxacin (0.02 %) and fructose (2 %). All the components of the reaction mixture were prepared in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated (37°C for 15 min. Thereafter, the mixtures were inverted and the bottom part was tied up before being filled with the Kerbs-Henselleit buffer. The upper part of the intestine was also tied after it has been filled. The tied intestinal sacs were then placed in a beaker (100 ml) containing 7 ml of starch solution (1 %), 2 ml pancreatic (1 %) and 2 ml plant extract (5 mg/ml). Tween 20 served as the negative control. The beakers containing the reaction mixture were then incubated at 37°C for 2 h. Glucose oxidase assay kit (Sigma) was used to estimate the amount of glucose in the intestinal sac and beaker. The amount of glucose obtained in the beaker represented the amount of starch digested while the glucose inside the sac represented the amount of glucose absorbed by the intestine.

**RESULTS**

**ABTS Scavenging activity**

Assessment of ABTS scavenging activity of the extract was carried out with the method of Re et al. ABTS solution (0.003 g/ml) was mixed in the ratio 1:1 (v/v) with various concentrations of plant extract (0-4 mg/ml). Each mixture was made to stand for 60 min at 25°C and the absorbance was read at 734 nm using spectrophotometer. Ascorbic acid and Butylated Hydroxy anisole (BHA) served as the positive controls.

**Data analysis**

All the data were triplicated and expressed as mean ± standard-deviation. The data was analyzed using one-way Analysis of variance (ANOVA). The *p*-value was calculated using the graph pad prism. The percentage (% inhibition) of the extract against the measured parameters were calculated using the formula: % Inhibition = (A0 – Aj)/A0 x 100. Where, *A*<sub>0</sub> is the absorbance value of the control and *A*<sub>j</sub> is the absorbance of the extracts or essential oils.

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**Phytochemical studies**

The phytochemical constituent of *E. ferox* is depicted in Table 1. The results revealed that the plant consist of tannins, flavonoids, terpenoids, alkaloids, but deficient in saponins and steroids.
Haemoglobin- glycation
The percentage haemoglobin- glycation inhibitory potential of *E. ferox* was represented by Figure 1. The *E. ferox* significantly (*P* < 0.05) inhibited haemoglobin- glycation with the optimal inhibitory activity observed at 0.4 mg/ml. In addition to this, *E. ferox* (0.4 mg/ml) possessed similar anti-haemoglobin glycating activity to gallic acid, the positive control.

α- glucosidase activity
The results revealed that *E. ferox* inhibited α-glucosidase activity in a dose dependent manner as depicted by Figure 2. The optimal inhibitory activity was observed at the highest concentration 0.25 mg/ml, whereas the minimum inhibitory activity was at 0.0312 mg/ml. The IC₅₀ values of *E. ferox* was 0.0293 mg/ml (Table 2).

Pancreatic lipase activity
The inhibitory efficacy of *E. ferox* against pancreatic lipase was presented in Figure 3. *E. ferox* displayed high inhibitory activity that is concentration dependent. Furthermore, it was observed that *E. ferox* showed twice the IC₅₀ value of orlist at, positive control (1.79 mg/ml) (Table 2).
Intestinal glucose absorption

The result revealed that the crude extract of *E. ferox* significantly *(P < 0.05)* reduced glucose absorption in the intestine compared to control as presented in Figure 4. Addition to this, it was also observed that at 5 secs *E. ferox* decreased intestinal glucose absorption however further exposure time seem not to influence glucose absorption.

ABTS and DPPH activity

The results of the ABTS and DPPH scavenging activity of the crude extract of the plant are given in Figure 5. The extract showed antioxidant activity by efficiently scavenging ABTS and DPPH radicals in concentration dependent manner. The IC_{50} values of the extract were 68.3 µg/ml and 308 µg/ml, against ABTS and DPPH, respectively.

DISCUSSION

Diabetes mellitus connotes starvation of cells amidst abundance of plasma glucose.\textsuperscript{23} Modulation of carbohydrate and lipid metabolizing enzymes by dietary supplement are desirable therapeutic approach to abating diabetes and its complications.\textsuperscript{3,22} Medicinal plant usage in ameliorating metabolic diseases is gaining more favor in research due to its phytochemical constituents.\textsuperscript{23} In this present study, *E. ferox* is known to possess tannins, flavonoids, terpenoids, alkaloids.

During diabetes condition, Advanced Glycated End-products (AGEs) become more pronounced. AGEs stimulate the production of pro-inflammatory cytokines which further aggravate diabetic complications.\textsuperscript{24} Likewise, hemoglobin glycation has also been established to be significantly increased in diabetes. Interestingly, the extract displayed its anti-protein glycation activity by preventing hemoglobin glycation (Figure 1). The extract's anti-haemoglobin glycation activity could be linked to its antioxidant potential, since the glycosylation of proteins are oxidation process.\textsuperscript{25} This finding was in accordance with the report of Hosseini et al.\textsuperscript{26} in which red clover and alfalfa extracts inhibited hemoglobin glycosylation.

Furthermore, prolonged post-prandial blood glucose spike is associated with diabetes mellitus.\textsuperscript{4} Alpha-glucosidase is pivotal in increasing plasma glucose after the consumption of carbohydrate rich meal therefore, inhibiting the action of α-glucosidase is therapeutic in managing diabetes complications. In this study, *E. ferox* attenuated α-glucosidase activity (Figure 2). This activity could be linked to its alkaloids and terpenoids components (Table 1). In previous studies, alkaloids and terpenoids were reported to inhibit α-glucosidase activity and α-amylase.\textsuperscript{26-27} Likewise, these findings synchronized with the report of Sompong et al. and Riyaphan et al.\textsuperscript{28-29} in which some plants extract inhibited carbohydrate and lipid metabolism enzymes activities.

High level of triglycerides and cholesterol have been implicated in the onset of type 2 diabetes.\textsuperscript{30} Attenuation of pancreatic lipase activity reduced free fatty acids availability for onward absorption into the small intestine, thus reversed hyperlipidaemia.\textsuperscript{31} The study revealed that *E. ferox* inhibited pancreatic lipase activity (Figure 3). This implies that *E. ferox* could effectively reduce post-prandial free fatty acid and subsequently ameliorate diabetes symptoms. The anti-hyperlipidemic activity of the extract, could be attributed to its phenolic constituents. Phenol compounds have been demonstrated to inhibit pancreatic lipase by competitively binding to the enzyme active site.\textsuperscript{21} In addition, *E. ferox* effectively reduced intestinal glucose absorption into the blood (Figure 4). This indicates that *E. ferox* disrupts glucose absorption mechanism in the small intestine. Some plants extracts have also been reported to attenuate intestinal glucose absorption into the blood.\textsuperscript{32-34}

Oxidative stress caused by the imbalance between free radicals and cellular oxidants scavengers in favour of free radicals has been implicated in the etiology of insulin resistance and diabetic complications.\textsuperscript{35} ABTS and DPPH assay were widely used to monitor the antioxidant potential of the extract. These methods accommodated for Single Electron Transfer (SET) and Hydrogen Atom Transfer (HAT) activities.\textsuperscript{36-37} In this study, the extract displayed good antioxidant activities by scavenging free radicals (Figure 5). These findings were in accordance to previous studies in which medicinal plants were regarded as effective antioxidant agents.\textsuperscript{36} The better antioxidant activity of the extract than AA and BHA in DPPH assay further affirms its potency (Figure 5). The extract antioxidant activity could be attributed to its phenolic constituent. Phenolic compounds have been established to confer on medicinal plants their antioxidant potential, based on the hydroxyl groups in their chemical structure.\textsuperscript{23}  

CONCLUSION

The antidiabetic efficacy of *E. ferox* can be attributed to the scavenging of antioxidants, attenuation of carbohydrate and lipid metabolizing enzymes, inhibition of glucose absorption and prevention of protein glycation. All these activities were based on the extract’s phytochemical constituents. Therefore, the extract could be a promising therapeutic in management of diabetic complications. Addition to this, folklore usage of this plant as diabetes remedy was justified. For further study, cytotoxicity profile, *in vivo* antidiabetic activity and isolation of bioactive compounds are essential.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AA: Ascorbic acid; ABTS: 2,2′- Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); BHA: Butylated hydroxy anisole; DM: Diabetes mellitus; DPPH: 2,2-diphenyl-1-picrylhydrazyl; *E. ferox*: Encephalartos ferox; HAT: Hydrogen electron transfer; IC_{50}: Inhibition concentration; SET: Single electron transfer.

REFERENCES


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