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Original Article

# *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* antidibetic 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- amylase and α-glucosidase) and lipid (pancreatic lipase) digestive 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 $IC_{50}$ 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.

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## 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.[1] 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.[3-4] 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) Lehm 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.[9-10] 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,

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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 VH04 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 Harbone.[12-13] 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.[14] The reaction mixture consisted of 500 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) in the dark for 72 h. Gallic acid was used as positive control. Absorbance was read at 443 nm using spectrophotometer.

### α-Glucosidase inhibitory activity

α-Glucosidase inhibitory activity of the extracts was investigated following the method described by Rayar and Manivannan.[15] A reaction mixture consisting of 50 µl of phosphate buffer (10 mM, pH 6.8), 10 µl of α-glucosidase (0.5 unit/ml) and 20 µl of the plant extract at various concentrations (0 - 2 mg/ml) were pre-incubated at 37°C for 15 min. Thereafter, 20 µl of 4-nitrophenyl-β-D glucoparanoside (2.5 mM) were added to initiate the reaction. The mixture was then incubated at 37°C for another 20 min. The reaction was terminated by adding 50 µl of sodium carbonate (0.1 M). Absorbance was read at 405 nm using spectrophotometer.

### Pancreatic lipase inhibitory activity

Pancreatic lipase inhibitory activity of the extract was ascertained by the method of Slanc *et al.*[16] The reaction mixture consisting of 125 µl of Tris-HCl buffer (75 mM, pH 8), 75 µl of various concentration of the extract (0 - 5mg/ml) and 50 µl of lipase (sigma) (10 mg/ml) in a beaker (50 ml) was incubated at 37°C for 15 min. Afterwards, 25 µl of 3,3 mM *p*-nitrophenyl palmitate (sigma) was added to initiate the reaction. The reaction mixture was then incubated for 30 min. The blank was made of methanol and water (1:1) whereas or list at served as positive control. Absorbance was read at 405 nm using spectrophotometer.

### Animal experiment

Ethical clearance certificate (UZREC 171110-030 PGD 2014/53) was collected from University of Zululand Ethics Committee for the use of animals for experiment. The procedures for animal experiments were adhered.[17] Sprague–Dawley rats (250 g) were collected from animal house of Department of Biochemistry and Microbiology, University of Zululand. The animals were kept under standard environmental conditions (25°C; 12: 12 lights: dark cycle) with free access to safe drinking water and pellet feeds. The animals were acclimatized for 5 days.

### Intestinal glucose absorption

Intestinal glucose absorption was determined following the method of Said *et al.*[18] Two Sprague-Dawerly rats (250 g) were sacrificed by cervical dislocation and dissected to harvest the intestine. The small intestines were rinsed with distilled water (50 ml) to remove blood stains. Thereafter, the intestines 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 pancreatin (1 %) and 2 ml plant extract (5 mg/ml). Tween 20 served as the negative control. The beakers containing the reaction mixtures 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.

### 1 1, -Diphenyl-2-Picryl Hydrazyl (DPPH) scavenging activity

DPPH scavenging activity of the extract was determined by method of Brad.[19] The DPPH (0.02 mg/ml) was mixed (1:1 v/v) with various concentration 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 517 nm using spectrophotometer. Ascorbic acid and Butylated Hydroxyl anisole (BHA) served as the positive controls.

### ABTS Scavenging activity

Assessment of ABTS scavenging activity of the extract was carried out with the method of Re *et al.*[20] ABTS solution (0.003 g/ml) was mixed in the ratio 1:1 (v/v) with different concentrations of the plant extract. The mixture was left to stand for 14 min at 25°C and the absorbance was read at 734 nm using spectrophotometer. Ascorbic acid and 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 $IC_{50}$ values were calculated using the graph pad prism. The percentage (%) inhibitions of the extract against the measured parameters were calculated using the formula; % Inhibition = $(A_0 - A_1)/A_0) \times 100$. Where, $A_0$ is the absorbance value of the control and $A_1$ is the absorbance of the extracts or essential oils.

## RESULTS

### 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.

**Table 1: Phytochemical constituents of *E. ferox*.**

| Phytochemicals | *Encephalartos ferox* |
|---|---|
| Tannins | + |
| Saponins | - |
| Flavonoids | + |
| Terpenoids | + |
| Alkaloids | + |
| Steroids | - |

Sign notations: + Present, - Absent

**Table 2: The results ($IC_{50}$ values) of *E. ferox* on α-glucosidase and pancreatic lipase inhibition**

| Compound | α- glucosidase (mg/ml) | Pancreatic lipase(mg/ml) |
|---|---|---|
| *E. ferox* | $2.9\times10^{-2}$ , | $179\times10^{-2}$ |
| Orlistat | - | $3.92\times10^{-2}$ |

Data are presented as mean ± SD, n =3.

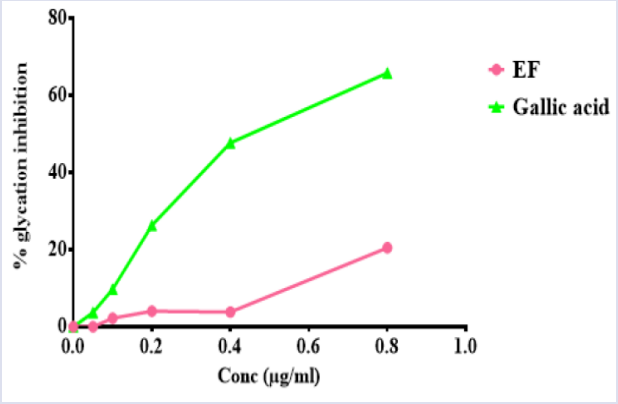


**Figure 1:** The result of the anti- haemoglobin glycation efficacy of *E. ferox*. $IC_{50}$ value of Gallic acid 455 µg/ml. Data expressed as mean ± SD.

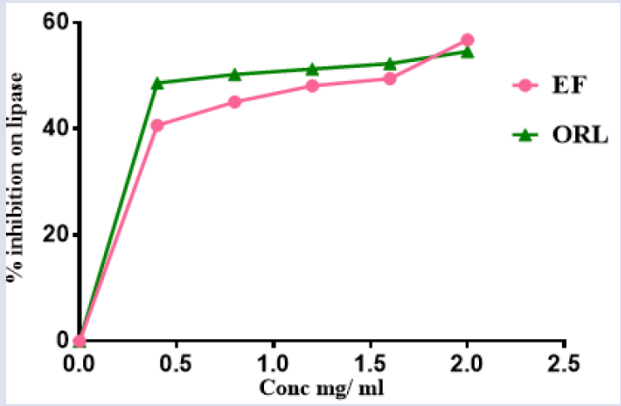


**Figure 3:** Shows the inhibitory activity of *E. ferox* against pancreatic lipase. Data expressed as mean ± SD.

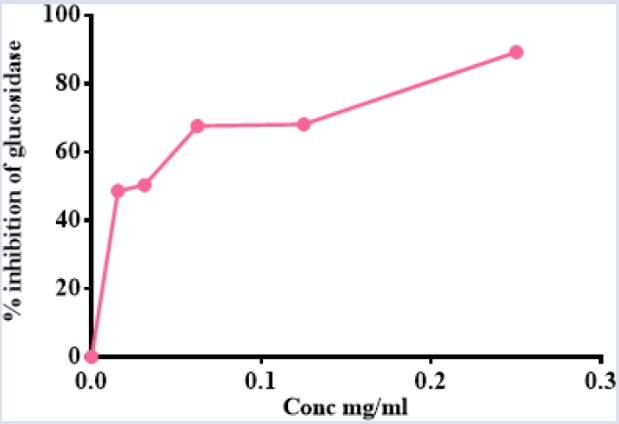


**Figure 2:** The result of the inhibitory effect of *E. ferox* on α-glucosidase. Data expressed as mean ± SD.

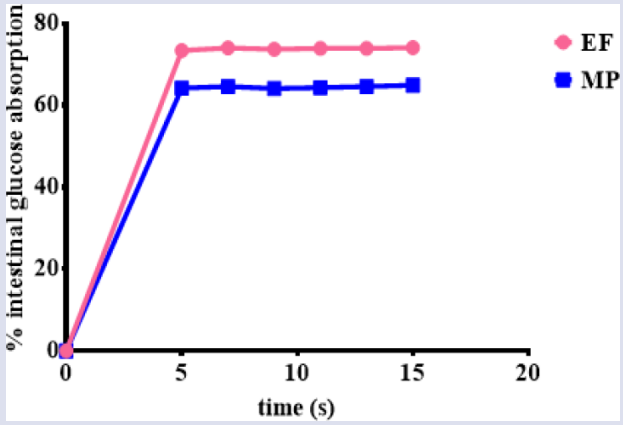


**Figure 4:** Shows the effect of *E. ferox* on intestinal glucose absorption. Data expressed as mean ± SD.

## 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 $IC_{50}$ 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 $IC_{50}$ value of or list at, positive control (1.79 mg/ml) (Table 2).

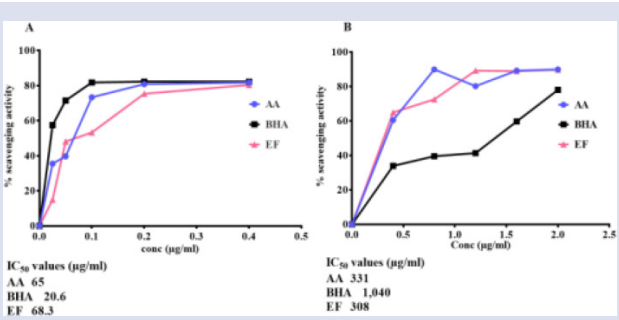


**Figure 5: The results of the scavenging activity of *E. ferox* against ABTS (A) and DPPH (B) radicals with the $IC_{50}$ values. Data expressed as mean ± SD.**

### 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.[21] Modulation of carbohydrate and lipid metabolizing enzymes by dietary supplement are desirable therapeutic approach to abating diabetes and its complications.[3,22] Medicinal plant usage in ameliorating metabolic diseases is gaining more favor in research due to its phytochemical constituents.[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.[24] Likewise, hemoglobin glycation has also been established to be significantly increased in diabetes. Interestingly, the extract displayed its anti-protein glycation potency 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.[25] This finding was in accordance with the report of Hosseini *et al.*[25] in which red clover and alfalfa extracts inhibited hemoglobin glycosylation.

Furthermore, prolonged post-prandial blood glucose spike is associated with diabetes mellitus.[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.[26-27] Likewise, these findings synchronized with the report of Sompong *et al.* and Riyaphan *et al.*[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.[30] Attenuation of pancreatic lipase activity reduced free fatty acids availability for onward absorption into the small intestine, thus reversed hyperlipidemia.[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.[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.[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.[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.[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.[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.[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.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ABBREVIATIONS

**AA:** Ascorbic acid; **ABTS:** 2,2[1]- 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.

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## GRAPHICAL ABSTRACT


## SUMMARY

- The study revealed that *Encephalartos ferox* leaves through inhibition of pancreatic lipase, α-glucosidase, lowering intestinal glucose absorption and mopping of free radicals can prevent diabetes and its complication.

## ABOUT AUTHORS

**Prof. Andy Rowland Opoku1** obtained his PhD in 1977 from the university of Manchester, United Kingdom. He had academic positions in Various universities across Africa. Currently he is working as professor emeritus in the University of Zululand, South Africa with lots of publications.

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