Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Pharmacy

Multidimensional Antidiabetic Potential of *Eleusine indica* Aqueous Extract: Hypoglycemic Action, Antioxidant Power, and Flavonoid Wealth

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DOI: https://doi.org/10.36347/sajp.2025.v14i09.004 | **Received:** 28.09.2025 | **Accepted:** 22.11.2025 | **Published:** 28.11.2025

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Abstract **Original Research Article**

Diabetes is a debilitating disease that is associated with lifelong costs for low-income populations. Glucose intolerance is one of the key developmental stages of type 2 diabetes. Numerous bioactive compounds, such as flavonoids and tannins, have already demonstrated their effectiveness in experimental models of diabetes management. The aim of this study was to evaluate the antidiabetic potential of *E. indica* in relation to its bioactive compound content and its antioxidant activity. The work, therefore, initially involved measuring the total phenolic compounds, flavonoids, and condensed tannins using conventional methods widely described in the published literature. The DPPH test was used for antioxidant analysis, while the glucose tolerance test was used to evaluate the antidiabetic potential of the extract using four groups of six animals each, with glipalamide as a positive control. The results obtained show a total phenolic compound (TPC) content of 121.27 ± 2.61 µg EAG/mg of extract and a total flavonoid (TFT) content of 367.25 ± 5.63 µg EQ/mg of extract. Condensed tannins (CT) indicate a content of 35.33 ± 1.45 µg EC/mg of extract. The antioxidant test using the DPPH method shows an IC50 of 0.7739 ± 0.0033 mg/mL. The tolerance test shows that the 200mg/kg dose exhibits a very significant glucose tolerance compared to the normal control (glucose alone). In fact, blood sugar levels normalized after 60 minutes and stabilized at around a 0% increase, compared to 150 minutes for glucose (glucose alone). Compared to glipalamide, the 200 mg/kg dose of extract normalized blood glucose levels, while glipalamide caused slight hypoglycemia after a much faster normalization. As for the 400 mg/kg dose, it had a similar effect as that of the 200 mg/kg dose, but after 60 minutes it caused a slight increase, indicating a biphasic effect of the extract. This could indicate toxicity at high doses.
Keywords: *Eleusine indica*, glucose tolerance, antidiabetic potential, DPPH, phytochemical determination.

1-INTRODUCTION

Diabetes is a pathological disorder that has become a real public health problem and could become the seventh leading cause of death worldwide in 2030, according to World Health Organization projections. [1]. This pandemic is estimated to affect 536.6 million people worldwide, and is projected to affect 643 million people by in 2030 and 783.2 million worldwide by 2045 [2]. Diabetes is recognized by elevated blood glucose levels even during non-meal times. This persistent elevation of blood sugar levels over time leads to irreversible glycation of hemoglobin. The glycated hemoglobin is a risk indicator for diabetes complications. These complications include, for example, the development of dyslipidemia, which is a real risk factor for cardiovascular disease [3]. It has been documented that patients with glucose intolerance or impaired fasting glucose levels have an increased risk of developing diabetes [4]. In addition to these prediabetic individuals, there is another category of people who are highly susceptible to diabetes but are rarely suspected of having it. In fact, it has been reported that nearly 40% of those who developed type 2 diabetes had normal glucose tolerance in the previous five years. [5]. This shows that diabetes is a very complex and silent disease that requires special attention. Pharmaceutical companies have made considerable efforts to provide modern medicine with oral or injectable antidiabetic drugs to provide relief to patients. However, these long and costly treatments often remain inaccessible to people with modest incomes, particularly in poor countries. Furthermore, prolonged use of these synthetic antidiabetic drugs may present toxicity risks [6], hence the need for researchers to develop alternative, accessible antidiabetic treatments based on plants.

Indeed, plant extract-based medicines would be both effective and less toxic than synthetic molecules due to the complex interactions between the different elements in the extract. [7]. Plants are rich in complex bioactive phytochemical compounds, such as polyphenols and flavonoids, which have many natural bioactive properties and less side effects. [2]. Eleusine indica is a herbaceous plant widely used in traditional African medicine to treat various conditions, including high blood pressure, arthritis, kidney problems, diarrhea, dysentery, epilepsy, etc. Numerous other researchers have demonstrated the wide-ranging properties of different parts of this plant, highlighting its pharmacological potential [8]. This study aims to evaluate the antidiabetic potential of E. indica in relation to its bioactive compound content and its antioxidant activity.

2-MATERIALS AND METHODS:

2.1. Sample collection and plant extraction

Fresh leaves of E. indica were obtained during the rainy season (under the guidance of a botanist) in western Burkina Faso, precisely in Bobo-Dioulasso. The fresh leaves obtained were cleaned and dried at room temperature in the shade. The dried leaves were then ground into a fine powder and macerated in distilled water for 48 hours. The macerate was then filtered and centrifuged, and the supernatant obtained was distributed into Petri dishes and dried in an air oven set at 40°C. The dry extract obtained was stored at -4°C, protected from moisture and light.

2-2-Animals

To conduct this experiment, we needed thirty Wistar rats divided into five groups of six animals each. The different groups of animals were randomized to avoid significant differences in weight between the groups. Each group was placed in an individual cage and fed protein-rich pellets (29%). The Animals had free access to water and food before the beginning of the test. A 12-hour light/dark cycle was imposed.

2-3-Test de tolérance au glucose

To evaluate the effects of EAEi on oral glucose tolerance, we randomized 24 rats into five groups of six rats each. After a 12-hour fast, baseline blood glucose levels were first recorded for all groups of animals. The animals were then immediately pre-treated via gavage as follows: Group 1 received distilled water (10 mL/kg body weight), Group 2 received glibenclamide (10 mg/kg), and Groups 3 and 4 respectively received doses of 200 and 400 mg/kg body weight of the aqueous extract of E. indica. Thirty (30) minutes after this pretreatment, the animals in all groups received a single dose of glucose (4g/kg body weight each). Immediately after glucose administration, blood glucose levels were measured every 30 minutes for two hours using the SD CodeFree glucometer. The variation in blood glucose levels in relation to time was expressed in percentage terms using the following relationship:

Variation in blood glucose levels at time t (%) = $[(G_t - G_0) \times 100]/G_0$

G_0: basal blood glucose level at T0 and Gt blood glucose level at time t. [9]

2-4-Evaluation of antiradical activity

The antiradical activity of the aqueous extract of *E. indica* was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method as described by Karanga et al. in 2024 [10]. Thus,50 µL aliquots of the extract were dissolved in methanol at different concentrations, and then incubated with 200 µL of DPPH● solution (0.1 mM) in the dark for 10 min. The absorbance was measured at 517 nm using a SPECTROTOstar NANO spectrophotometer (BMG LABTECH, Germany). Trolox was used as the reference standard. DPPH●+ methanol was considered the positive control, while methanol alone was considered the blank control. The median inhibitory concentration (IC50), required to scavenge 50% of free radicals, was determined using a linear regression curve.The tests were repeated three times. A lower IC50 value indicates higher antiradical activity

2-5-Phytochemical screening:
total phenolic compound quantification

The total phenolic compounds were measured using the Folin-Ciocalteu reagent method reported by Masfria and Dalimunthe in 2018. [11] In this, 0.1 mL of EAEi concentrated at 200 µg/mL was mixed with 0.1 mL of 50% reagent, then with 2 mL of a 2% sodium carbonate solution. After 30 minutes of incubation in the dark, the absorbance was determined at 750 nm. The phenolic compound content was calculated relative to a gallic acid standard curve and expressed as gallic acid equivalent (µg GAE/mg extract).

Dosage of total flavonoids:

The total flavonoid content was measured using a method based on that reported by Ben-Moussa et al. in 2022 [12]. To this purpose, 250 µL of diluted EAEi was mixed successively with 1 mL of distilled water and 75 µL of 5% sodium nitrite ($NaNO_2$), and after 5 minutes of incubation, this solution was then mixed with 75 µL of 10% aluminum chloride ($AlCl_3$). After 6 minutes, 500 µL of 1N sodium hydroxide (NaOH) and 600 µL of distilled water were added to the mixture. The absorbance of the colored complex formed was measured at 415 nm. The total flavonoid content was determined according to the quercetin calibration curve. The results were expressed in milligrams of quercetin equivalent per gram of dry plant substance (mg EQ/g of extract).

Determination of condensed tannins:

For the titration of condensed tannins, the spectrophotometric method using vanillin reported by Ali-Rachedi (2017) [13] was used. The experiment consisted of mixing 50 µL of EAEi with 1500 µL of 4% vanillin/methanol solution, followed by the addition of 750 µL of concentrated hydrochloric acid. The mixture obtained was incubated for 20 minutes at room temperature. The absorbance was then measured using a spectrophotometer at a wavelength of 550 nm. The tannin content was determined using the equation of a linear calibration curve produced with catechin (0-1000 µg/mL) as the standard. The results were expressed as catechin equivalent per gram of dry extract.

RESULTS

1- Glucose tolerance of control groups

Glibenclamide is an oral antidiabetic drug. In this study, it is considered our positive control. In this confirmatory test, we observed that animals receiving glucose alone (normal control) had hyperglycemia persisting (significantly) longer over time (60, 90, 120, and 180 minutes) compared to those receiving glibenclamide.

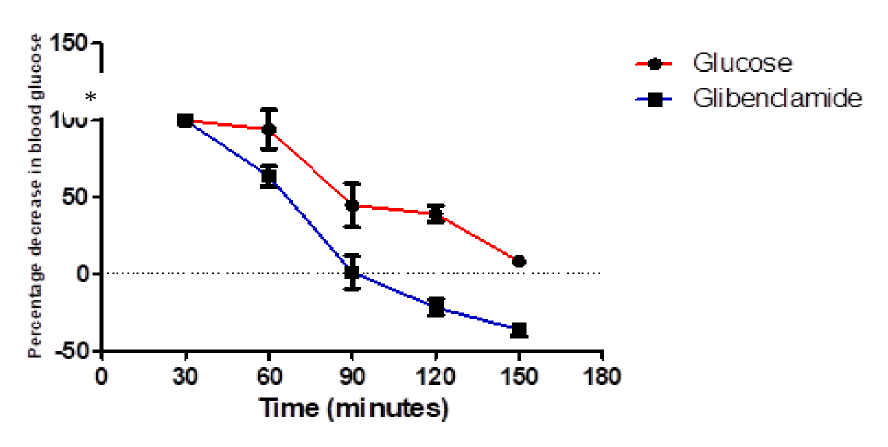

Figure1: Oral glucose tolerance curve for control groups

The results are presented as mean ± standard deviation of the mean (n=6). The significance of the statistical difference was determined by two-way ANOVA analysis followed by Bonferroni post-hoc tests... ***p<0.001 significant decrease compared to glucose

2- Effect of Eleusine indica aqueous extract on glucose tolerance

In Figure 2 below, we can observe that doses of 200 mg/kg and 400 mg/kg of EAEi normalized blood glucose levels more quickly (1 hour after the peak blood glucose level) compared to animals that received glucose alone, which took 150 minutes to recover normal blood glucose levels. However, the 400 mg/kg dose significantly increased blood glucose levels again after 60 minutes.

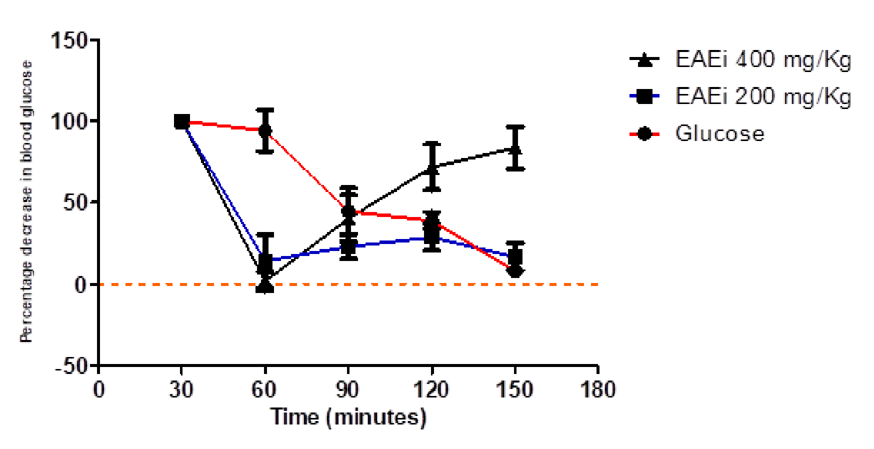

Figure2: Glucose tolerance curve of EAEi 200 and 400 mg/kg body weight doses

The results are presented as mean ± standard deviation of the mean (n=6). The significance of the statistical difference was determined by two-way ANOVA analysis followed by Bonferroni post-hoc tests... ***p<0.001 significant decrease compared to glucose only;. *###p* p<0.001: significant increase compared to glucose.

3- Hypoglycemic effect of Eleusine indica extract compared to glibenclamide

In the figure presented below (Figure 3), EAEi (200 mg/kg) more rapidly reversed hyperglycemia induced by high sugar intake compared to glibenclamide 60 minutes after the peak blood glucose level. This blood glucose level remained more or less stable at around zero percent increase. In contrast, glibenclamide induced a significant decrease below 0% increase after 120 and 150 minutes when the animals were not fed.

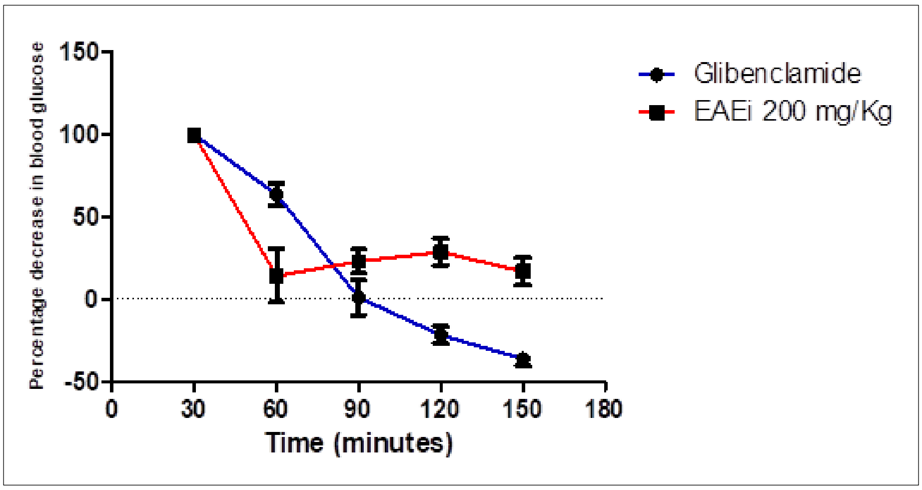

Figure 3: Hypoglycemic effect of EAEi (200 mg/kg) compared to glipalamide

The results are presented as mean ± standard deviation of the mean (n=6). The significance of the statistical difference was determined by two-way ANOVA analysis followed by Bonferroni post-hoc tests... ***p<0.001 significant difference compared to Glibenclamide.

Table1: Quantitative phytochemical profile of AEEi

Phytochemicals	Results	Methods
Total Phenolic Compounds (TCP)	121,27 ± 2,61 µg EAG/mg of extract	Folin-Ciocalteu method. Gallic Acid Equivalent.
Total Flavonoids (TF)	367,25 ± 5,63 µg EQ/mg of extract	Colorimetric method using $AlCl_3$.; Quercetin equivalent
Condensed Tannins (CT)	35,33 ± 1,45 µg EC/mg of extract	Vanillin method.; Catechin Equivalent

Antioxidant activity of AEEi

DPPH test : (IC_{50})	0,7739 ± 0,0033 mg/mL	Inhibitory concentration to sequester 50% of DPPH free radicals.

The table presented above, resulting from quantitative phytochemical analysis, shows a particularly high content of total flavonoids. Antioxidant activity provides an IC_{50} value of 0.7739 mg/mL. The results are presented as mean ± standard deviation (n=3).

DISCUSSION

The present study evaluates the antidiabetic activity of *Eleusine indica* aqueous extract (EAEi) in relation to its phytochemical profile and antioxidant capacity. The glucose tolerance results show that *EAEi* at a dose of 200 mg/kg causes a very significant decrease in blood glucose levels compared to the normal control (glucose alone). In the presence of EAEi (200 mg/kg), blood glucose levels have been normalized after 60 minutes, versus 150 minutes for the normal control. Furthermore, when compared with our positive control (glibenclamide), we observed that the 200 mg/kg dose simply canceled out the hyperglycemia induced in rats, while glibenclamide normalized blood glucose levels 90 minutes after the peak of hyperglycemia and continued to lower them, inducing slight hypoglycemia. This result shows that this dose of EAEi has beneficial effects (for the correction of hyperglycemia) comparable to those of glibenclamide and suggests an insulinotropic mechanism for our extract at this concentration. However, 400 mg/kg showed a biphasic effect: up to 60 minutes, this dose normalized blood glucose levels in a similar way to the 200 mg/kg dose. However, after 60 minutes, it caused a significant increase in blood glucose levels. This decrease in the effectiveness of the 400 mg/kg dose compared to the 200 mg/kg dose, as well as the two-stage response of the 400 mg/kg dose, is often described as a biphasic response, one of the characteristics of complex plant extracts in the analysis of mechanisms of action in pharmacology [14]. These observations could be explained at least in two ways: firstly, the presence in the extracts of compounds with antagonistic effects, of which the most toxic would have an activity threshold, could partly explain these observations [15]. Secondly, saturation of its receptors by an active principle could reduce its expected activity at higher doses or may inhibit these receptors or activate other pathways with opposite effects to those expected. [16]. Thirdly, it has already been reported that certain phytochemical compounds such as tannins (in high concentrations) could reduce the bioavailability of many compounds. Thus, if this interference affects the bioavailability of the active compound(s), this could explain at least part of this result [17]. In review of this result, we can conclude that the management of hyperglycemia with *EAEi* can only be optimized within a specific concentration range. Quantitative phytochemical analysis revealed a total phenolic compound (TPC) content of 121.27 ± 2.61 µg EAG/mg of extract and a total flavonoid (TFT) content of 367.25 ± 5.63 µg EQ/mg of extract. Condensed tannins (CT) yielded a content of 35.33 ± 1.45 µg CT/mg of extract. Furthermore, the DPPH method test of the antioxidant power of EAEi indicated an IC50 of 0.7739 ± 0.0033 mg/mL. The highly remarkable presence of flavonoids could partly explain the anti-hyperglycemic activity of *EAEi*. In fact, phytochemical compounds, particularly polyphenols and especially flavonoids, are frequently mentioned for their antidiabetic properties. This efficacy may depend on several mechanisms, including the inhibition of digestive enzymes such as α-amylase and α-glucosidase, interaction with glucose transporters, and their ability to neutralize free radicals and reduce oxidative stress [2]. In terms of molecular mechanisms, the adenosine monophosphate-activated protein kinase (AMPK) pathway has been reported as one of the main pathways of action for flavonoids. Flavonoids bind to the α1 subunit of AMPK, stimulating a cascade of reactions that improve insulin sensitivity for their receptors (and promote better glucose absorption by cells) and reduce both neoglucogenesis and hepatic glycogenolysis [18]. It is also well documented that the antidiabetic action of flavonoids also involves, among other factors, reduction in glycated hemoglobin levels, activation of glucose transporter (GLUT4) synthesis and translocation, and inhibition of tyrosine kinase activity [1]. The antioxidant activity of *EAEi* shows an IC50 of 0.77 mg/mL, comparable to that of *Mentha spicata* (0.75 mg/mL), a well-known medicinal plant [19]. Diabetes is known to be associated with oxidative stress, which releases free radicals with toxic effects on pancreatic β cells [20]. Monitoring biomarkers of oxidative stress and implementing therapies based on plant-derived antioxidants can improve diabetes management and reduce its complications. Monitoring biomarkers of oxidative stress and implementing therapies based on plant-derived antioxidants can improve diabetes management and minimize complications. [21]. *E. indica* therefore, remains a promising prospect for the integrated management of diabetes due to its remarkable flavonoid content, well-known for its antidiabetic and antiradical properties.

CONCLUSION

This preliminary analysis highlights the potential of E indica for integrated diabetes management through its hypoglycemic activity and antioxidant properties, which are conferred by its high flavonoid content. However, it should be noted that for optimal diabetes management with EAEi, it is important to adopt a strict concentration range to avoid adverse effects from potential overdose, due to the biphasic effects observed at high doses

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