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Comparative Assessment of Enzyme inhibiting Properties of Selected Ethnomedicinal Cereals (Panicum sumatrense and Eleusine coracana) used in The Management of Diabetes Mellitus

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ABSTRACT

This study comparatively evaluated the inhibitory effects of two ethnomedicinal cereals, Panicum sumatrense and Eleusine coracana, on carbohydrate-hydrolyzing enzymes associated with postprandial hyperglycemia. Aqueous extracts of the cereals were prepared and tested for their inhibitory activities against α-amylase and α-glucosidase enzymes at concentrations ranging from 1–5 mg/ml. Acarbose served as the standard reference drug. The results showed that both extracts exhibited inhibitory activities against α-amylase and α-glucosidase enzymes in a concentration-dependent manner. For α-amylase inhibition, Panicum sumatrense and Eleusine coracana showed IC_{50} values of 3.25 mg/ml and 5.00 mg/ml respectively, while acarbose exhibited a lower IC_{50} of 1.28 mg/ml. In the α-glucosidase assay, Eleusine coracana demonstrated stronger inhibitory activity (IC_{50} = 3.87 mg/ml) compared to Panicum sumatrense (IC_{50} = 4.30 mg/ml), although both were less potent than acarbose (IC_{50} = 0.30 mg/ml). The findings suggest that these ethnomedicinal cereals possess significant enzyme inhibitory activities which may contribute to their traditional use in the management of diabetes mellitus.

Keywords: Ethnomedicinal cereals, Aqueous extracts, Enzyme inhibitory, Diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM), also known simply as diabetes is a complex metabolic disorder characterized by hyperglycemia, a physiologically abnormal condition represented by continued elevated blood glucose levels. Hyperglycemia results from anomalies in either insulin secretion or insulin action or both and manifests in a chronic and heterogeneous manner as carbohydrate, fat, and protein metabolic dysfunctions. Diabetes follows a progressive pattern with complex pathogenesis and varied presentation (American Diabetes Association, 2014 & 2018).

Hyperglycemia and its associated carbohydrate, fat, and protein metabolic dysfunctions affect multiple organs of the body and disrupt their normal functioning. These disruptions progress gradually and arise mostly due to the adverse effects of hyperglycemia and its associated metabolic anomalies on the normal structure and functioning of micro- and macrovasculature, which lie at the core of organ structure, and function throughout the body. The structural and functional disruptions in organ system vasculature led to micro- and macrovascular complications. Organ damage, dysfunction, and, ultimately, organ failure characterizes these complications and affect body organs, which include, in particular, eyes, kidneys, heart, and nerves. Eye-related complications result in retinopathy with progression to blindness. Kidney-associated complications lead to nephropathy and potential renal failure. Heart-related complications include hypertension and coronary heart disease. Nerve-associated complications lead to neuropathy, which can be autonomic and/or peripheral. Cardiovascular, gastrointestinal, and genitourinary (including sexual) dysfunctions are characteristic manifestations of autonomic neuropathy, whereas foot infections including ulcers requiring amputations and Charcot joint (osteoarthropathy) are often associated with long-term peripheral neuropathy. (American Diabetes Association, 2018; Rawshani et al., 2017). The cerebrovascular disease, peripheral arterial disease, and coronary heart disease, together termed as atherosclerotic cardiovascular disease, are of common occurrence in diabetes and constitute one of the major causes of diabetes-associated morbidity and mortality. (American Diabetes Association, 2014

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& 2018; Rawshani et al., 2017). Several comprehensive reviews have been made on the nutritional, bioactive, industrial and climate resilient potentials of the lesser grains like small millet and finger millet , their rich nutritional profiles and their potentials to act as nutraceutical and functional foods that may be used to address malnutrition and food insecurity have been highlighted. Most of these wellness advantages are due to the dietary fibres, polyphenols especially phenolic acids and tannins (Simardeep Kaur et al., 2024) 81% of polyphenols in finger millet for instance is made up of benzois derivatives, flavonoids, cinnamic acids, phytates, phenols and tannins, ferulic acid (p-coumaric acids, p-hydroxybenzoic acid, proanthicyanines, Gallic acid, vanillic acid), quercetin, and ferulic rich arabinoxylan (Abioye et Al., 2022). The gluten free nature of these millet varieties place them on the recommended dietary list for people with inflammatory bowel disease (Simardeep Kaur et al., 2024). The essential amino acid profile of these lesser grains place them at the top of the list when compared with other grains. The protein content of finger millet for instance falls between 4.88to 15.58% and essential amino acid make up 44.7% of its amino acid profile (Abioye et Al., 2022). Despite the aboundant information on the nutritional and phytochemical components of these millets, their is sparsity of information as to how these bioctive compound rich foods can be harnessed for the prevention, management or treatment of type II Diabetes . The root cause of Diabetes is inflammation which is often precipitated or aggravated by the perpetually raised blood sugar levels found in type II Diabetes patients. The constant blood sugar spikes experienced in this condition is also a factor that worsens both insulin resistance and the inflammatory response. Developing diabetic diets which will not only eliminate blood sugar spikes but also provide the additional benefits attending to the root cause of the disease, which is chronic inflammation could provide brake through answers to the question of development of natural approach to the prevention and treatment of type II Diabetes through reversal of insulin resistance and the inflammatory markers.Panicum sumatrense, small millet helps to keep blood sugar levels stable for a long time in diabetic patients. It is also helpful for diabetes patients because it has a comparatively small glycemic index that helps steadily digest and contain glucose at a slower pace than other foods (Saini et al., 2021). This will help healthy blood sugar levels for long stretches.

Finger millet-based food formulations and preparations show lower glycemic index and induce lower glycemic response (Shobana et al., 2007; Shukla and Srivastava, 2014). Intake of dietary calcium and magnesium was suggested to reduce the type-2 diabetes risk in two independent studies (Pittas et al., 2006; van Dam et al., 2006). This study therefore aims at evaluating the inhibitory capacity of extracts of Panicum sumatense and Eleusine coracana on two digestive enzymes implicated in postprandial hypoglycaemia.

MATERIAL AND METHOD

Plant materials

The fresh plant of Panicum sumatrense and Eleusine coracana, was collected from local markets in Anambra State. The taxonomic identity of the plant was determined by a qualified plant taxonomist at the laboratory of Applied Biochemistry, Nnamdi Azikiwe University, Awka. The plant was washed 2 to 3 times with running tap water and oven dried at le temperature (45°c) before use.

Plant extracts preparation

Cold methanol extracts of ground plant materials were prepared as follows: 200 grams of the dried grain powder of plant materials were soaked separately in methanol for 10 days, and then filtered. The extracts were concentrated in a vacuum rotary evaporator under reduced pressure at a temperature of 46°C to give a residue (extract), which was further suspended in water to get aqueous solutions of the extracts.

All the extracts were concentrated again using rotary flash evaporator. After complete solvent evaporation, each of these extracts was weighed, labelled and stored in airtight bottles for further use.

Alpha amylase inhibition assay

This was carried out using the modified method described and published by **(**Miller, 1959; Worthington, 1993 and Kazeem et al.,2013**).** The assay determines the ability of a test compound (e.g. plant extract, synthetic drug) to inhibit the enzymatic breakdown of starch into reducing sugars. Alpha amylase catalyzes the hydrolysis

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of α 1,4-glycosidic bonds in polysaccharides (starch, glycogen), producing maltose and glucose. The released reducing sugars react with 3,5-Dinitrosalicylic acid (DNS) under alkaline conditions forming a red orange colour complex measurable at 540nm. A reduction in color intensity indicates enzyme inhibition.

Starch + Alpha amylase = Maltose + Glucose

Three set of tubes were labeled control, extract and standard. To the extract tubes, 100µl of 0.005% alpha amylase, 25µl of extract (1-5mg/ml concentrations) and 100µl of 1% starch solutions were mixed. In the control tubes, 100µl of 0.005% alpha amylase, 25µl of phosphate buffer and 100µl of 1% starch solutions were mixed.

The standard tubes contained 100µl of 0.005% alpha amylase, 25µl of acarbose (1-5mg/ml) and 100µl of 1% starch solution. These were incubated at 37°c for 10 minutes and the reaction stopped by addition of 250µl of DNS. The tubes were then heated for 5-10 minutes at 100°c for colour development and then diluted by addition of 2.5ml of phosphate buffer (or distilled water). The absorbance was then read at 540nm and percentage inhibition calculated thus:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Alpha glucosidase inhibition assay

The method described by Kim et al., 2004 was adopted with slight modifications. This assay is based on measuring the ability of a test compound or extract to inhibit the enzyme alpha glucosidase, which catalyzes the hydrolysis of carbohydrates to glucose by cleaving the alpha 1,4-glycosidic linkages.

In the assay, the enzyme acts on a synthetic substrate usually p-nitrophenylphosphate-α-D-glucopyranoside (pNPG), instead of a natural carbohydrate. The intensity of the yellow color measured at 405nm is proportional to enzyme activity. Inhibitors reduce the formation of p-nitrophenol, hence lower absorbance.

p-nitrophenylphosphate-α-D-glucopyranoside (pNPG) → Alpha glucosidase → p-niNitrophenol + Glucose

Three set of tubes were labeled control, extract and standard. To the extract tubes, 500µl of 0.1U/ml α-glucosidase in 0.1M phosphate buffer (pH 6.8), 100µl of extract (1-5mg/ml) and 200µl of 0.1M phosphate buffer (pH 6.8). In the control tubes (Blank), 500µl of 0.1U/ml α-glucosidase in 0.1M phosphate buffer (pH 6.8), 100µl of distilled water and 200µl of 0.1M phosphate buffer (pH 6.8).

The standard tubes contained, 500µl of 0.1U/ml α-glucosidase in 0.1M phosphate buffer (pH 6.8), 100µl of acarbose (1-5mg/ml) and 200µl of 0.1M phosphate buffer (pH 6.8). These were incubated at 37ºC for 10 minutes and then 400µl of 5mM pNPG in 0.1M phosphate buffer pH 6.8 was added.

The tubes were then incubated for 30 minutes at 37ºC and the reaction stopped with 800µl of 0.2M Na_2CO_3. The absorbance was then read at 405nm and percentage inhibition calculated thus:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences among the extracts and the standard drug at different concentrations. The IC_{50} values were estimated from concentration–response curves generated by nonlinear regression analysis.

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RESULTS

Alpha amylase inhibition

Panicum sumatrense extract exhibited moderate α-amylase inhibition across the concentrations tested in Table 1. The inhibition ranged from 39.47% to 60.18%. The highest inhibition occurred at 2 mg/ml (60.18%), while the lowest occurred at 3 mg/ml (39.47%). The IC_{50} value obtained was 3.25 mg/ml, indicating a moderate inhibitory effect.

In Table 1, Eleusine coracana extract demonstrated comparatively higher inhibitory activity with inhibition values ranging from 54.91% to 71.23%. The highest inhibition occurred at 5 mg/ml (71.23%), suggesting stronger enzyme inhibition at higher concentrations. However, the IC_{50} value was 5.00 mg/ml, indicating a weaker potency compared to Panicum sumatrense.

The standard drug acarbose showed the highest inhibition across all concentrations (72.69% – 79.47%). Its IC_{50} value was 1.28 mg/ml, indicating stronger inhibitory potency compared to the plant extracts.

Inhibition of alpha glucosidase assay

Panicum sumatrens: The percentage inhibition ranged from 21.09% at 1 mg/ml to 48.48% at 4 mg/ml in Table 2, after which it slightly decreased to 35.89% at 5 mg/ml. The gradual increase in inhibition from 1 mg/ml to 4 mg/ml indicates that the extract becomes more effective at higher concentrations, suggesting a concentration-dependent inhibitory effect. The IC_{50} value of 4.30 mg/ml indicates the concentration required to inhibit 50% of the enzyme activity, reflecting moderate inhibitory potency.

In Table 2, Eleusine coracana, the inhibitory activity ranged from 21.51% at 1 mg/ml to 61.02% at 5 mg/ml. The inhibition increased steadily with increasing concentration, especially between 3 mg/ml and 5 mg/ml, where the activity rose sharply.

This indicates a stronger enzyme inhibition at higher concentrations compared to Panicum sumatrense. The IC_{50} value of 3.87 mg/ml suggests that Eleusine coracana has slightly higher inhibitory potency against α-glucosidase than Panicum sumatrense.

The standard drug acarbose exhibited the highest inhibition among all tested samples, with values ranging from 71.35% at 1 mg/ml to 85.73% at 5 mg/ml. Its IC_{50} value of 0.30 mg/ml indicates very strong inhibitory activity, which is expected because acarbose is a purified pharmaceutical inhibitor specifically designed to block carbohydrate-digesting enzymes.

Overall, the results show that both cereal extracts possess moderate α-glucosidase inhibitory activity, with E. coracana demonstrating slightly stronger inhibition than Panicum sumatrense. Although their activity is lower than that of acarbose, the findings suggest that these ethnomedicinal cereals may contribute to the dietary management of diabetes by slowing carbohydrate digestion and reducing glucose release into the bloodstream.

Table 1: Average percentage inhibition of alpha amylase of the various extracts at varying concentrations with their IC_{50}.

Labels/concentration (mg/ml)	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	IC_{50} (mg/ml)
Panicum sumantrese	59.47±6.84	60.18±3.92	39.47±5.09	44.85±2.43	50.85±4.91	3.25
Eleusine coracana	59.47±0.18	54.91±3.51	69.91±1.49	61.93±7.19	71.23±0.35	5.00
Acarbose	72.69±4.77	79.47±2.28	76.96±4.64	75.50±2.63	74.74±5.96	1.28

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Figure 1: Scatter plot showing average percentage inhibition of alpha amylase of the various extracts at varying concentrations.

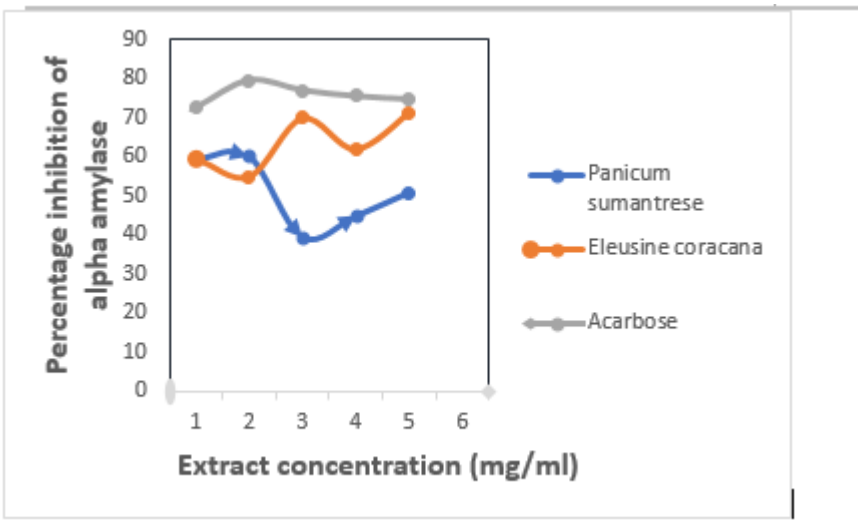
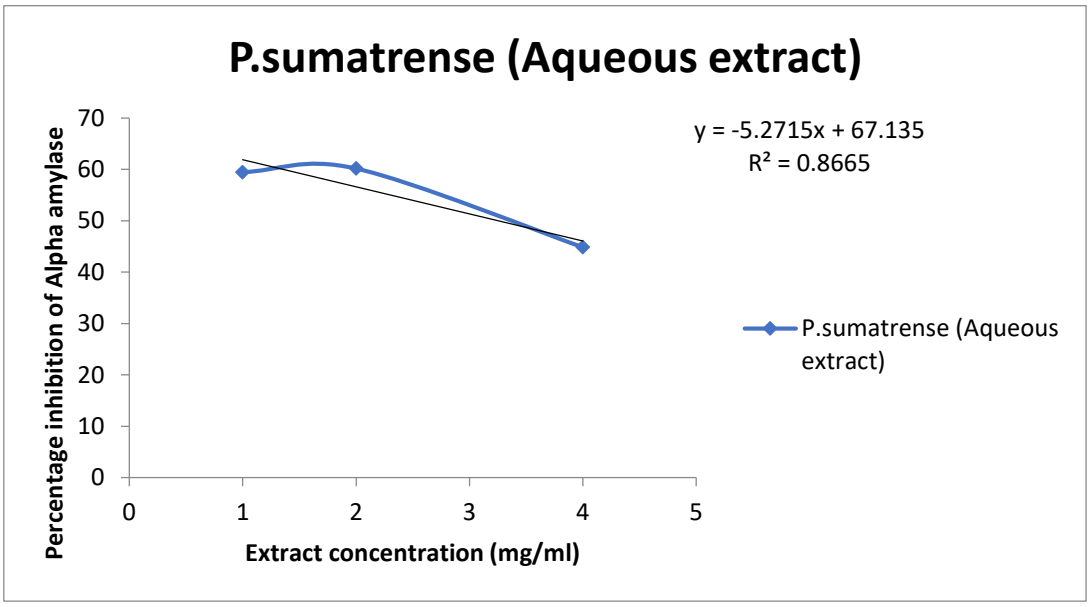
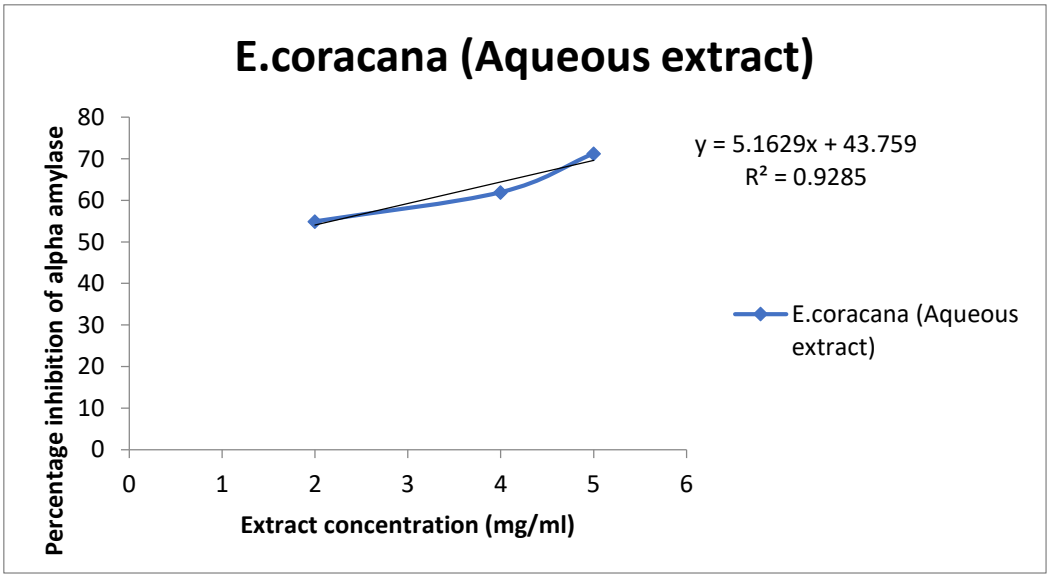


Figure 1.1 Individual scatter plots showing equation of the curve and thus used to estimate IC_{50}





Inhibition of alpha glucosidase assay

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Table 2: Table showing percentage inhibition of alpha glucosidase by the extracts

Labels/concentration (mg/ml)	1mg/ml	2mg/ml	3mg/ml	4mg/ml	5mg/ml	IC_{50} (mg/ml)
Panicum sumantrese	21.09±0.73	22.15±0.37	38.02±1.44	48.48±7.27	35.89±0.51	4.30
Eleusine coracana	21.51±1.35	19.91±1.15	37.27±0.45	60.44±3.20	61.02±4.67	3.87
Acarbose	71.35±0.32	82.11±0.28	82.11±1.04	83.23±0.56	85.73±0.14	0.30

Figure 2: Scatter plot showing percentage inhibition of alpha glucosidase by the extracts

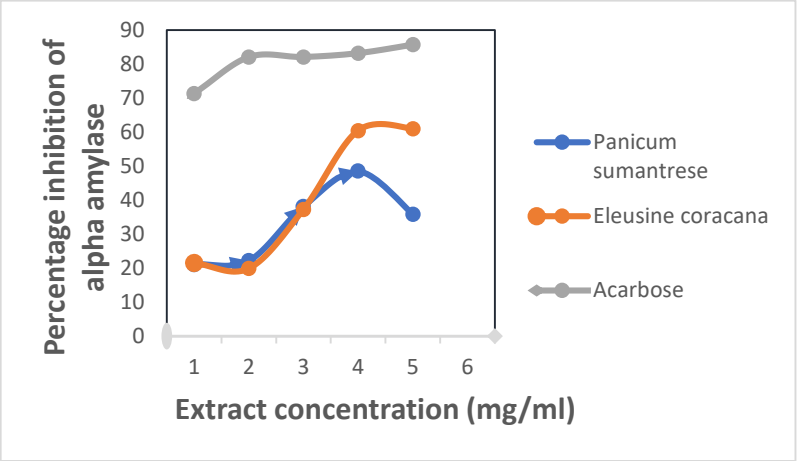
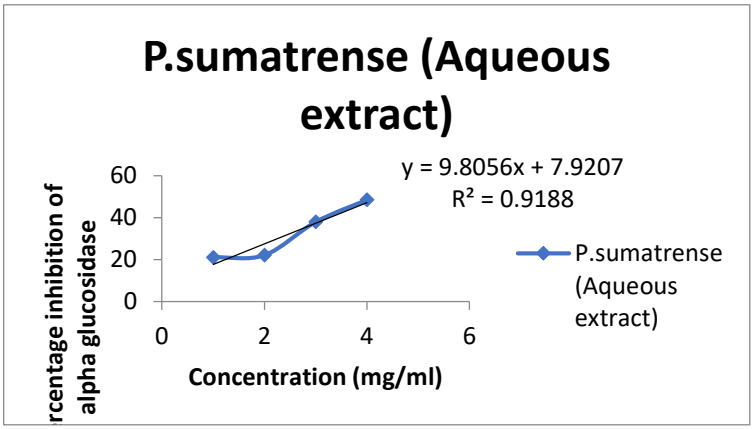
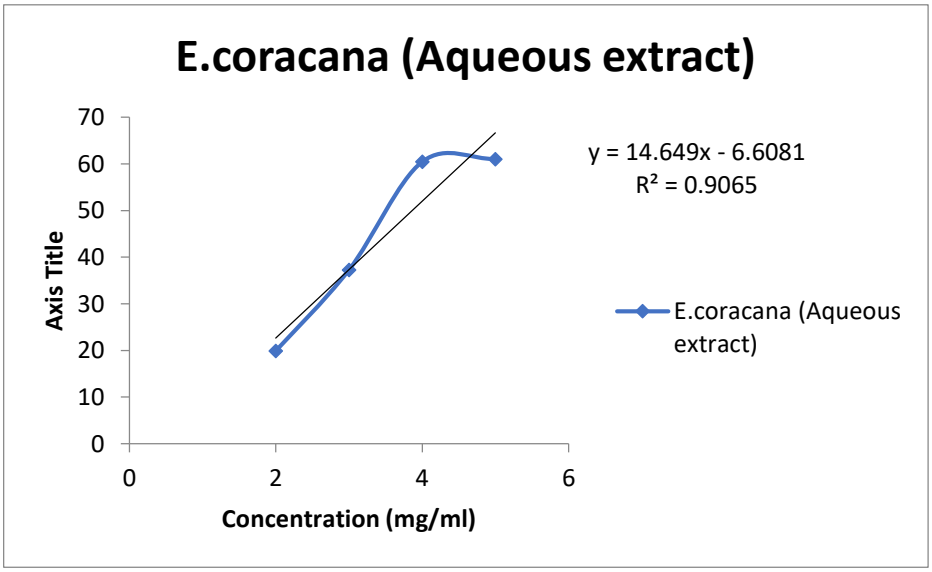


Figure 2.1 Individual scatter plots





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DISCUSSION

The inhibitory potential of Panicum sumatrense and Eleusine coracana extracts against two carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, which are involved in the digestion of dietary carbohydrates. Inhibition of these enzymes is an effective therapeutic approach for controlling postprandial hyperglycemia in diabetic patients.

The results obtained in the α-amylase assay revealed that both plant extracts exhibited significant enzyme inhibitory activity. Eleusine coracana showed higher percentage inhibition at higher concentrations compared to Panicum sumatrense. However, the IC_{50} value of Panicum sumatrense (3.25 mg/ml) was lower than that of Eleusine coracana (5.00 mg/ml), suggesting that Panicum sumatrense possesses relatively stronger inhibitory potency against α-amylase. This is reflected in the higher inhibition activity (60.18%) that P. sumatranse showed at 1.00mg/ml concentration compared with Eleusine coracana. It is quite possible that the percentage inhibitory activity at 5.00mg/ml (50%) observed for P. sumatranse may be actually higher than the value observed in this result, and that P. sumatranse may be actually more effective at inhibiting amylase at 5.00mg/ml concentration compared with Eleusine coracana. The lower inhibition values observed for P.sumatanse at 3mg/ml and 4mg/ml concentration may be due to pipetting errors. In the α-glucosidase inhibition assay, Eleusine coracana exhibited stronger inhibitory activity with an IC_{50} value of 3.87 mg/ml compared to Panicum sumatrense (4.30 mg/ml). This indicates that Eleusine coracana may be more effective in delaying glucose absorption in the small intestine.The fluctuations in the glucosidase inhibition levels observed at 5.00mg/ml concentration for P. Sumatranse and at 2mg/ml concentration for E. Coracana may be attributed to technical errors in handling. Phenolic compounds of finger millet have been recognized as effective inhibitors of α-amylase and α-glucosidase enzymes. In addition, by inhibiting the protein glycation process, the key molecular basis of diabetic complications, phenolic compounds help in the prevention of diabetic complications (Mathanghi and Sudha, 2012, Kumar et al., 2016, Singh et al., 2016).

The observed enzyme inhibitory activities may be attributed to the presence of phytochemicals such as polyphenols, flavonoids, and other bioactive compounds commonly found in these cereals. These compounds are known to interact with carbohydrate-digesting enzymes, thereby reducing glucose release and absorption. This may explain the reason why incidence of diabetes is rare among the populations that consumes small millet as staple diet. The millet protein characterization showed that its protein concentrate is a potential functional food ingredient and the essential amino acid pattern suggests possible use as a supplementary protein source to most cereals because it is rich in lysine (Ravindran, 1992).

Although the plant extracts demonstrated significant inhibitory effects, their activities were expectedly lower than that of acarbose, the standard antidiabetic drug. This suggest that intake of the cereals will not precipitate sudden decline in blood glucose levels and that they may be freely included as part of the diabetics menu without the fear of precipitation of hypoglycaemia. This difference is expected since acarbose is a purified pharmaceutical inhibitor, while the plant extracts contain complex mixtures of compounds.

Nevertheless, the results support the traditional use of these cereals in the management of diabetes mellitus and suggest that they may serve as potential sources of natural antidiabetic agents.

CONCLUSION

This study demonstrated that the extracts of Panicum sumatrense and Eleusine coracana possess inhibitory effects against α-amylase and α-glucosidase enzymes. Both extracts exhibited moderate enzyme inhibitory activities, although their potency was lower than the standard drug acarbose. Among the two cereals studied, Panicum sumatrense showed stronger α-amylase inhibition, while Eleusine coracana exhibited better α-glucosidase inhibitory activity. These findings indicate that the studied ethnomedicinal cereals may contribute to the management of diabetes mellitus by reducing carbohydrate digestion and glucose absorption. Further studies are recommended to isolate and characterize the specific bioactive compounds responsible for these effects and to evaluate their therapeutic potential in vivo.

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Recommendation

Cereal formulations containing both grains may be formulated and marketed as a diet that will help maintain controled blood sugar levels in diabetics. This formulation may synergistically achieve a controled alpha amylase and alpha glucosidase inhibition which may help eliminate the very much undesirable blood glucose spikes experienced by diabetic patients. In vivo anti-diabetic, analgesic and anti-inflammatory studies should be carried out on these extracts to concretize these findings.

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