PHYTOCHEMICAL SCREENING AND ANTIDIABETIC ACTIVITY OF METHANOLIC LEAF EXTRACT FROM *Kigelia africana* (Lam.) Benth.

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ABSTRACT

Diabetes mellitus represents a complex array of disorders that disrupt the metabolism of carbohydrates, fats, and proteins, posing significant health challenges. In the pursuit of alternative treatments, plant-based medicaments have gained prominence for addressing various human ailments. This study centred on *Kigelia africana*, a plant from the Bignoniaceae family and collected from the Calicut University Botanical Garden with official approval. The main objective of the present investigation included uncover the phytochemical composition of *Kigelia africana* leaves and evaluate their potential antidiabetic properties. Utilizing methanol as the primary solvent for cold extraction, the preliminary analysis revealed the presence of essential metabolites such as carbohydrates, proteins, and amino acids, with the absence of sterols. Alkaloids, flavonoids, steroids, terpenoids, saponins, phenols, cardiac glycosides, and tannins were shown to be secondary metabolites. An alpha-amylase inhibition experiment was used to assess the anti-diabetic qualities at three different concentrations. The most striking result was a 23.3% inhibition at 200 ml. This result point to a potential function for *Kigelia africana* in the treatment of diabetes.

Keywords- *Kigelia Africana,* Phytochemical analysis, Methanol, Cold extraction process, Anti-diabetic properties

Introduction:

Diabetic mellitus is a complex and a diverse group of disorders that disturbs the metabolism of carbohydrates, fat and protein.[1] The use of herbal medicines for the treatment of diabetes mellitus has gained importance throughout the world and there is an increased demand to use natural products with anti-diabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents.[2]

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Kigelia is a genus of flowering plants in the family Bignoniaceae. The genus comprises only one species, *Kigelia africana*, which occurs throughout tropical Africa from Eritrea and Chad south to northern South Africa, and west to Senegal and Namibia. The genus name comes from the Mozambican Bantu name, kigeli-keia, while the common name Sausage Tree refers to the long, sausage-like fruit. Its name in Afrikaans Worsboom also means Sausage Tree, and its Arabic name means "the father of kit bags".[3] The *K. africana* plant has many medicinal properties due to the presence of numerous secondary metabolites. So, the current investigation has been focused on preliminary analysis and anti-diabetic property from methanolic leaf extract of *Kigelia africana.*

Material and Methods: Plant collection:

The leaves were collected from the Calicut university botanical garden with special permission. The authenticity of the plant was carried out by Dr. Asma V.M, Associate Professor, Research & P G Department of Botany, MES Asmabi College, P. Vemballur, Kodungallur, Thrissur (Plate 1).

Plate 1: Morphology of the Plant- *Kigelia Africana*

Extraction procedure: The leaves were shade dried and grinded in homogenizer in to coarse powder. The samples were extracted using the solvent methanol. For the preparation of extract the 20 g of plant powder were dissolved in 200 ml of methanolic solvent. Then it was kept in shaker for 48 hrs to shake well for uniform mixing. The content of flask were filtered through four layers of muslin cloth. The extract obtained was stored under refrigerator and used for further analysis.

Preliminary phytochemical analysis: The leaf extract of the plant Kigelia Africana underwent analysis to detect a range of components, including flavonoids, steroids, cardiac glycosides, alkaloids, phenols, proteins, carbohydrates, amino acids, saponins, sterols, and tannins. This assessment was conducted using established and standardized procedures to identify and quantify the diverse constituents present in the plant.[4]

Carbohydrates: About 0.5 ml of the filtrate was taken to which 0.5 ml of Benedict's reagent is added. This mixture was heated for about 2 minutes in a boiling water bath. The appearance of red precipitate indicates the presence of sugars.

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Proteins: Add 4% NaOH and few drops of 1% CuSO4 solution to 3 ml of the extract. Formation of violet or pink colour indicates the presence of proteins.

Aminoacids: To the extract add 0.25% Ninhydrin reagent and boil for a few minutes. Formation of blue colour indicates the presence of aminoacid.

Alkaloids: Methanolic extract was warmed with 2% H2SO4 for 2 minuts . It is filtered and a few drops of dragendroff's reagent were added and the red precipitate indicates the presence of alkaloids.

Flavonoids: A portion of crude powder was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. The presence of flavonoid is indicated by a yellow colouration.

Steroids: 0.5 g of crude powder was dissolved in 5 ml of methanol. 1 ml of the extract was treated with 0.5 ml of acetic acid anhydride and cooled in ice . This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulphuric acid was then added carefully by means of a pipette. At the separate level of the two liquids, a reddish- brown ring was formed as an indication of the presence of steroids.

Sterols: The extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) is carefully added to form a layer.Shake well and allow to stand for some times . Red colour appears in lower layer as the indication of the presence of sterol but if it not there indicated absence of sterol.

Terpenoids: The extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) is carefully added to form a layer. A reddish-brown coloration of the interface is formed indicate the presence of terpenoids.

Saponins: 0.5 mg of extract was vigorously shaken with few ml of distilled water. The formation of frothing is positive for saponins.

Phenols: Add 2 ml of test solution in alcohol is added with 1 drop of neutral ferric chloride (5%) solution . Formation of an intense blue color indicates the presence of phenols.

Cardiac glycosides: 5 ml of each methanolic extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution (FeCl3) followed by the addition of 1 ml concentrated sulfuric acid.Brown ring was formed at the interface which indicate the presence of deoxysugar of cardenolides. A violet ring may appear beneath the brown ring . While in the acetic acid layer, a greenish ring may also form just gradually through out the layer, indicate the presence of cardiac glycosides.

Tannins: About 0.5g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black colouration indicates positive test for tannins.

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Enzyme inhibitory effects of *Kigelia africana* extract.

Alpha-amylase inhibition assay: The investigation into α-amylase inhibitors in *Kigelia africana* adhered to the specified research protocols.5 1000µL of starch solution was mixed with 1000 µL of α-amylase enzyme (purchased from Hi Media) in 5 test tubes. 10, 20, 50 and 100µL of extract were added to 4 test tubes (Test samples) and one without extract was kept as control. All the test tubes were incubated for 3 minutes. After incubation 500µL of 96mM DNS reagent (0.438g in 20mL distilled water) was added to all the test tubes and kept in incubation for 15minutes. Then solutions in each test tube were made up to 6ml with distilled water.Optical density of these samples was measured at 540nm. Then a set of another 4 test tubes were prepared with 4 different concentrations of the sample (10, 20, 50 and 100µL). All test tubes were made up to 8mL with distilled water and labeled as extract control. Blank was prepared with 1000µL starch and 500µL DNS reagent. These samples are also made up to 6ml with distilled water. Optical density of the samples was measured at 540nm.

Inhibition of enzyme activity was calculated as;

Inhibition of enzyme activity(%) = AC 540 – AT 54 x 100

where , AC= Absorbance of control

AT= Final absorbance of Test sample

AT= Absorbance of test sample – Absorbance of extract control

Results: Preliminary phytochemical screening: The analysis of methanol leaf extract from *Kigelia africana* unveiled the presence of a variety of significant phytochemicals, including alkaloids, tannins, glycosides, phenolic compounds, and others. The detailed findings are presented in Table 1.

Inhibitory activities of *Kigelia africana* leaf extract on α-amylase: The results of the alpha-amylase inhibition assay for methanolic extracts of *Kigelia africana* leaves are presented in Table No.2 & Fig.1. At a concentration of 50 ml, the alpha-amylase inhibition percentage is recorded at 6.66%, which increases to 13.33% at 100 ml, and significantly rises to 23.33% at 200 ml of the sample. This indicates a notable increase in alpha-amylase inhibition with higher concentrations of the solution. Additionally, the optical density of the sample solution shows a decreasing trend from 0.028 at a low concentration (50 ml) to 0.023 at a high concentration (200 ml). The data suggests a correlation between concentration and both alpha-amylase inhibition percentage and optical density, emphasizing the potential effectiveness of *Kigelia africana* leaf extracts in inhibiting alpha-amylase activity.

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Table: 1. Preliminary phytochemical analysis from methanolic leaf extract of *Kigelia africana*

SL.NO.	TEST	RESULT
1	Carbohydrates	+
2	Proteins	+
3	Amino acids	+
4	Alkaloids	+
5	Flavonoids	+
6	Sterols	-
7	Steroids	+
8	Terpenoids	+
9	Saponin	+
10	Phenol	+
11	Cardiac glycosides	+
12	Tannins	+

+ indicates the presence of the compound, -indicates the absence of the compound

Table: 2. Inhibitory effect of α- amylase by *Kigelia africana* leaf extract at various concentrations

Concentration(µl)	OD of Test	OD of control	% of Inhibition
50	0.028	0.03	6.66
100	0.026	0.03	13.33
200	0.023	0.03	23.33

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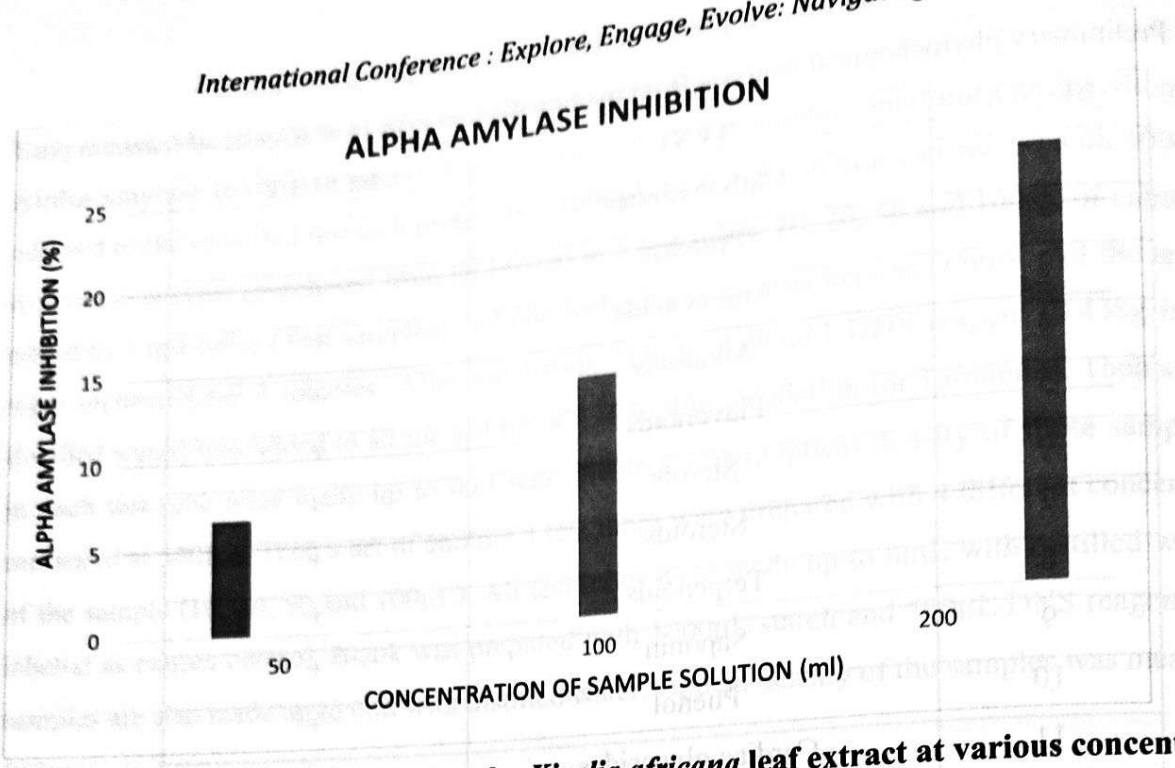

Fig: 1 Inhibitory effect of α- amylase by *Kigelia africana* leaf extract at various concentrations

Discussion:

The results obtained in this study suggest that the leaf extract of *Kigelia africana* harbor a diverse array of phytochemicals, playing a role, either directly or indirectly, in influencing the biological activity of the extracts. Preliminary phytochemical analysis involved the application of various tests to examine the composition of the leaf extract of *Kigelia africana*. The screening revealed the presence of both primary and secondary metabolites. Primary metabolites, essential for cellular growth and function maintenance, encompass carbohydrates, proteins, and amino acids. These metabolites typically carry out vital physiological functions in organisms. On the other hand, secondary metabolites, which act as defence chemicals in plants, do not adversely impact plant health when absent. The study identified carbohydrate, protein, and amino acids as the primary metabolites. Interestingly, sterol was the sole secondary metabolite absent in the *Kigelia africana* leaf extract. Conversely, alkaloids, flavonoids, steroids, terpenoids, saponins, phenols, cardiac glycosides, and tannins were among the secondary metabolites identified in the extract.

The methanolic extract of *Kigelia africana* leaves showed a trend in alpha-amylase inhibition percentages across concentrations (6.66% at 50 ml, 13.33% at 100 ml, and 23.33% at 200 ml) that indicates a positive correlation between sample solution concentration and alpha-amylase inhibitory activity. The inhibitory action on alpha-amylase increases with concentration, suggesting a possible dose-dependent connection. Furthermore, the measurements of optical density offer significant understanding of the behaviour of the sample solutions. The optical density is 0.028 at a low concentration (50 ml) and drops to 0.023 at a greater concentration (200 ml). The inverse relationship

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between concentration and optical density suggests that the optical density falls with increasing sample solution concentration.

Despite the availability of anti-diabetic medications on the market, medicinal herbs are often effective in the treatment of diabetes.[7] Herbal treatments and plant components with low toxicity and no side effects are notable treatment options for diabetes around the world. Nowadays, medicinal plants are used to treat diseases such as diabetes because they include phytoconstituents such as flavonoids, terpenoids, saponins, carotenoids, alkaloids, and glycosides, which may have anti-diabetic properties. Furthermore, the combined action of biologically active compounds (e.g., polyphenols, carotenoids, lignans, coumarins, glucosinolates, etc.) leads to the potential beneficial properties of each plant matrix, which can serve as a starting point for understanding their biological actions and beneficial activities.[6] pancreatic α-amylases hydrolyze the internal 1,4-glycosidic bond of starch to produce maltose and glucose.[8] [&9].The World Health Organization (WHO) has substantiated the utilization of herbal remedies for the management of diabetes.[10]

The comprehensive analysis of *Kigelia africana* reveals the presence of all metabolites, excluding sterol. This observation suggests substantial anti-diabetic potential in the plant, indicating promising prospects for future diabetes treatments. Furthermore, the observed escalation in the plant's anti-diabetic efficacy with higher sample concentrations, as evidenced by the alpha-amylase assay, emphasizes the substantial promise of *Kigelia africana* in the domain of anti-diabetic therapies.

Conclusions:

The findings derived from the present study reveal a promising correlation between the methanolic leaf extract of *Kigelia africana* and its potential anti-diabetic properties. This suggests that the leaf extract from the *Kigelia africana* plant may serve as an effective treatment for certain manifestations of diabetic mellitus. The study's results emphasize the significant therapeutic potential embedded in the natural components of this plant, highlighting its role as a valuable resource in the development of alternative approaches for managing diabetes. Further research and exploration into the specific mechanisms underlying these anti-diabetic effects could provide deeper insights and contribute to the advancement of novel therapeutic interventions for diabetes.

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References

[1] Poojari, S., Porika, R., & Mamidala, E. (2014). Phytochemical analysis and in vitro antidiabetic activities of *Physalis angulata* fruit extracts. National Journal of Integrative Research in Medicine, 5, 34-38.

[2] Akhtar, M., Rashid, M., Wahed, M. I., Robiul Islam., Shaheen, S., Ariful Islam., Amran., & Ahmed, M. (2007)."Comparison of long-term antihyperglycemic and hypolipidemic effects between *Coccinia cordifolia* (Linn.) and *Catharanthus roseus* (Linn.) in alloxan-induced diabetic rats," Research Journal of Medical Sciences, vol. 2, pp. 29–34.

[3] Roodt, V. (1992). *Kigelia Africana* in The Shell Field Guide to the Common Trees of the Okavango Delta and Moremi Game Reserve. Gaborone, Botswana: Shell Oil Botswana

[4] Harborne, J. B. (1984). Methods of plant analysis. In Phytochemical methods: a guide to modern techniques of plant analysis (pp. 1-36). Dordrecht: Springer Netherlands.

[5] Xiao, Z., Storms, R., & Tsang, A. (2006). A quantitative starch? Iodine method for measuring alpha-amylase and glucoamylase activities. Analytical biochemistry, 351(1), 146-148.

[6] Salehi, B., Ata, A., V. Anil Kumar, N., Sharopov, F., Ramírez-Alarcón, K., Ruiz-Ortega, A., & Sharifi-Rad, J. (2019). Antidiabetic potential of medicinal plants and their active components. Biomolecules, 9(10), 551.

[7] Colagiuri., R. (2010). Diabetes: a pandemic, a development issue or both?. Expert review of cardiovascular therapy, 8(3), 305-309.

[8] Mwakalukwa, R., Amen, Y., Nagata, M., & Shimizu, K. (2020). Postprandial hyperglycemia lowering effect of the isolated compounds from olive mill wastes–An inhibitory activity and kinetics studies on α-glucosidase and α-amylase enzymes. ACS omega, 5(32), 20070-20079.

[9] Zanzabil, K. Z., Hossain, M. S., & Hasan, M. K. (2023). Diabetes Mellitus Management: An Extensive Review of 37 Medicinal Plants. Diabetology, 4(2), 186-234.

[10] Bailey, C. J., & Day, C. (1989). Traditional plant medicines as treatments for diabetes. Diabetes care, 12(8), 553-564.