

COMPARATIVE PHYTOCHEMICAL, PROXIMATE, AND *in vitro* ANTIHYPERGLYCEMIC ANALYSES OF THE STEM BARK EXTRACTS OF *Kigelia africana* (Lam.) Benth. AND *Annona senegalensis* Pers.

Abstract

The present research aimed to investigate the phytochemical bioactive constituents and proximate composition of the methanol extract of *Kigelia africana* and *Annona senegalensis* stem bark, as well as its *in vitro* antidiabetic potential. The results suggest that the presence of bioactive compounds may be responsible for the numerous medicinal capacities of these plants, including their antidiabetic abilities. The methanol stem bark extracts of *K. africana* and *A. senegalensis* demonstrated strong α -amylase and yeast cell glucose absorption inhibition, respectively. The stem bark methanol extract of *A. senegalensis* exhibited the highest level of inhibition (87.61%) at 0.1 mg/ml concentration when compared to conventional acarbose medication (56.0%) on the α -amylase enzyme. The percentage of glucose uptake by both methanol stem bark extract of *Kigelia africana* and *Annona senegalensis* was recorded maximum (31.40%) at a concentration of 0.5 mg/ml. The present research establishes the antidiabetic effect of methanol stem bark extract of *Kigelia africana* and *Annona senegalensis* by *in vitro* assays.

Keywords: *Kigelia africana*; *Annona senegalensis*; Phytochemicals; Antihyperglycemic; Stem Bark; Alpha-amylase inhibition; Yeast Cell Glucose Uptake.

Introduction

Diabetes mellitus is a complex chronic metabolic disorder that affects carbohydrate, fat, and protein metabolism. It is a non-infectious illness that affects 5% of the worldwide population [1-4] and accounts for nearly 10% of total healthcare costs in several countries [5]. It is caused by the pancreas producing insufficient or inadequate insulin, resulting in a rise in the concentrations of glucose. Many biological systems, including blood vessels and nerves, have been shown to be negatively affected by it [6]. One of the treatment techniques is to minimize postprandial hyperglycemia, by retarding the absorption of glucose by inhibiting carbohydrate-hydrolyzing enzymes, such as α -amylase and α -glucosidase [7, 8]. Despite being the backbone of diabetes treatment and successful in treating hyperglycemia, oral hypoglycemic medications and insulin have noticeable side effects and do nothing to change the course of diabetic complications [10];

therefore, there is a need to create strong, safe, and cost-effective drugs for diabetes management. These effective, safe, and inexpensive drugs might be developed by using medicinal plants that have been used by humans to treat or cure disorders such as diabetes [9, 10, 11]. There is available literature that shows more than 400 plant species with hypoglycemic activity [12, 13] however, only a few of these medicinal plants have received scientific scrutiny, even though the World Health Organization has recommended that medical and scientific examinations of such plants should be conducted [14]. This study focuses on two plant species; *Kigelia africana* (Lam.) Benth and *Annona senegalensis* Pers.

Kigelia africana (Lam.) Benth (also known as the sausage tree) is one of the tree species that has been extensively used for its medicinal properties [15]. It is the only species in the genus *Kigelia* and belongs to the family Bignoniaceae. It is also known to have several local names such as Rawuya (Hausa), Pandoro (Yoruba), Uturubein (Igbo), Mranaa (Swahili), Worsboom (Afrikaans) etc. This plant has been used to treat a variety of ailments, including skin diseases like eczema, fungal infections, psoriasis, and boils, as well as more serious diseases like leprosy, impetigo, syphilis, skin cancer, elephantiasis, dysentery, swellings, constipation, piles, malaria, diabetes, pneumonia, worm infestations, venereal diseases, rheumatism, psoriasis [16, 17, 18, 19, 20, 21, 22]. Several chemical constituents have been isolated from various parts of *Kigelia africana* by several researchers. Secondary metabolites such as alkaloids, steroids, saponins, tannins, flavonoids, etc. [23] have been identified in *K.africana*. Also, phenylpropanoid, stigmasterol, β -sitosterol, ferulic acid, lapachol, iridoids, kigelinole, kigelin, pinnatal, isopinnatal, verminoside, and other chemical compounds have been found in *K. africana* [24, 25, 26, 27].

Annona senegalensis Pers. commonly known as wild custard apple, is a member of the Annonaceae family and is widely distributed throughout Nigeria and is known locally as Gwándàndààj (Hausa), Dukuu-hi (Fulani), Uburuocha (Igbo), Arere or Abo (Yoruba) [28]. Numerous ethnomedicinal applications have been linked to various parts of *A. senegalensis*, as well as its usage as food and food additives. All parts of the plant have been found useful for traditional medicine applications in the treatment of yellow fever, tuberculosis, and smallpox [29]; snakebite and hernia [30]; difficulty in swallowing, gastritis, male sexual impotence, erectile dysfunction, tuberculosis [31,32,33]; chicken pox, kwashiorkor, and marasmus [34]; antidotes for venomous bites and diabetes [35]; malaria and open sores [36], etc. According to some research, the primary bioactive constituents of the plant include tannins, flavonoids, saponins, alkaloids, glycosides, steroids, volatile acids, and anthocyanin [37, 38, 39, 40, 41]. It was also found to contain ascorbic acid and amino acids, as well as minerals including Ca, K, Mg, Zn, Fe, Cu, Mn, Pb, and Cr, making it a significant source of nourishment. [42].

The objective of the current work is to examine the *in vitro* anti-diabetic activity of methanol extracts of *A. senegalensis* and *K. africana* stem bark and to comprehend the impact of the plant extracts on yeast cell glucose absorption and α -amylase inhibitory activities.

MATERIALS AND METHODS

Both plant samples were collected from the International Center for Ethnomedicinal and Drug Development in Nsukka and were identified by Mr. Alfred Ozioko, a taxonomist and senior staff member of the herbarium. The stem barks of *A. senegalensis* and *K. africana* were washed, sliced into very small sizes, sun-dried, ground into a fine powder, and stored in air-tight containers for further extraction.

Extraction of Phytochemicals

Each of the powdered samples of *K. africana* and *A. senegalensis* were partitioned into three 500g portions. These portions were then soaked separately in 500ml of methanol, n-hexane, and ethyl acetate. Each of the solutions was agitated vigorously for 48 hours at room temperature. The extracts were filtered through Whatman No. 1 filter paper. The filtered extracts were concentrated using a rotary evaporator, under decreased pressure at about 55°C, and kept in sealed containers at -20°C.

The extracts were subjected to quantitative and qualitative phytochemical analysis to determine the presence or absence of flavonoids, alkaloids, terpenoids, saponins, carbohydrates, resins, tannins, reducing sugars, glycosides, and proteins using standard phytochemical procedures described by Anarado *et al.*, [43-45].

in vitro Antihyperglycemic Evaluations

Alpha-amylase Inhibitory Assay

The alpha-amylase inhibitory test of methanol extracts of *A. senegalensis* and *K. africana* was performed using a protocol previously reported by Ranila *et al.* [46], with minor modifications. In summary, 0.5ml of extracts were mixed with 0.5ml of α -amylase solution (0.5 mg/ml) in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl). After 10 minutes at room temperature, 0.5ml of starch solution (1%) was added. The resultant mixture was incubated at room temperature for 10 minutes before being stopped with 1 ml of dinitro salicylic acid color reagent. The test tubes were then immersed in a water bath (100°C for 5 minutes) and cooled until room temperature was reached. The mixture was then diluted with 10ml of deionized water, and absorbance was determined at 540nm. The absorbance of blank (buffer instead of extract and amylase solution) and control (buffer instead of extract) samples were also determined. Acarbose was used as a standard drug. The inhibition of α -amylase was determined using the following equation:

$$\% \text{inhibition of } \alpha\text{-Amylase} = \frac{\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{Sample})}}{\text{Abs}_{(\text{control})}} \times 100.$$

Where;

$\text{Abs}_{\text{control}}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution, and

$\text{Abs}_{\text{sample}}$ corresponds to the solution with extract and α -amylase solution.

in vitro Evaluation of Yeast Cell Uptake of Glucose

Commercial baker's yeast was washed by repeated centrifugation (3,000 ×g, 5 min) in distilled water until the supernatant fluid was clear and a 10% (v/v) suspension was prepared in distilled water. Different concentrations of extracts (1-5 mg/ml) were added to 1 ml of glucose solution and further incubated for 10 min at 37⁰ C. The reaction was started by adding 100µl of yeast suspension, vortexing it, and incubating it at 37°C for 60 minutes. The tubes were centrifuged (2,500 ×g, 5 min) and glucose was estimated in the supernatant. Metformin is taken as a standard antidiabetic drug. All tests were carried out in triplicates, and absorbance was measured at 540 nm. The formula below was used to determine the percentage increase in glucose uptake by yeast cells.

$$\% \text{ increase in glucose uptake} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100.$$

Where,

Abs_{control} is the absorbance of the control reaction (containing all reagents except the test sample), and

Abs_{sample} is the absorbance of the test sample.

STATISTICAL ANALYSIS

All experimental data were subjected to analysis of variance (ANOVA) using Statistical package for social science (SPSS) software (version 23.0). Duncan's new multiple range tests (DNMRT) were used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $p < 0.05$. All analyses were carried out in triplicates.

Results and Discussion

Table 1: Proximate Composition of *A. senegalensis* and *K. africana* Stem Bark Extracts

PARAMETERS	<i>Annona senegalensis</i>	<i>Kigelia africana</i>
Moisture Content (%)	10.0000 ^d ± 0.00000	10.6667 ^{c a} ± 1.15470
Ash Content (%)	6.0000 ^e ± 0.00000	6.0000 ^f ± 0.00000
Crude Fiber (%)	35.0000 ^b ± 0.0000	26.2500 ^b ± 1.25000
Protein (%)	6.8667 ^e ± 0.04041	9.7533 ^{cd} ± 0.04509
Oil Content (%)	8.5000 ^c ± 0.50000	8.0000 ^e ± 0.0000

Carbohydrate (%)	33.9300 ^a ± 0.74940	38.9933 ^a ± 1.29501
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Table 2: Qualitative Phytochemical of *K. africana* and *A. senegalensis* Stem Bark Extracts

Phytochemicals	KaSH	KaSE	KaSM	AsSH	AsSE	AsSM
Saponins	-	+	+	+	-	++
Flavonoids	+	++	-	-	-	-
Alkaloids	-	-	+	+	+	+
Tannin	-	-	+++	-	++	+++
Steroids	++	+++	-	-	-	-
Terpenoids	+++	+++	-	+	++	++
Glycosides	++	++	-	++	++	+++

Key

+++ = Present in high concentration.

++ = Present in moderate concentration.

+ = Slightly or sparingly present.

-

=

Absent.

KaSH = n-hexane stem bark extract of *K. africana*

KaSE = Ethyl acetate stem bark extract of *K. africana*

KaSM = Methanol stem bark extract of *K. africana*

AsSH = n-hexane Stem bark extract of *A. senegalensis*

AsSE = Ethyl acetate Stem bark extract of *A. senegalensis*

AsSM = Methanol Stem bark extract of *A. senegalensis*

Table 3: Quantitative Phytochemical Composition of *K. africana* and *A. senegalensis* Stem Bark

Parameter	<i>Kigelia africana</i>	<i>Annona senegalensis</i>
Alkaloids (%)	12.0000 ^b ± 0.00000	2.5667 ^c ± 0.51316
Flavonoids (%)	21.1667 ^a ± 0.28868	13.0000 ^a ± 0.00000
Phenols (mg/g)	2.1867 ^e ± 0.02309	2.5567 ^c ± 0.01155
Saponins (%)	4.0000 ^d ± 0.00000	3.5833 ^b ± 0.14434
Tannins (mg/100g)	6.6367 ^c ± 0.51481	0.7967 ^d ± 0.06429

The proximate composition of *K. africana* and *A. senegalensis* samples presented above in **Table 1** showed that carbohydrates (38.99%) were higher in concentration in *K. africana* extract than in *A. senegalensis* extract with a concentration of 33.93% and crude fiber (35.00%) was seen to

have a higher concentration in *A. senegalensis* extract than in *K. africana* with a concentration of 26.25%, while a concentration of 6.00%, ash content was the least abundant in both plant samples.

From the result of the qualitative phytochemical analysis (**Table 2**), it can be deduced that the methanol extract of *K. africana* stem bark extract showed the presence of secondary metabolites such as saponin, alkaloid, and tannins, which is in agreement with the phytochemical screening reported on the same plant by [47], while n-hexane and ethyl acetate extracts showed the presence of glycoside, terpenoid, steroids, and flavonoids, respectively. In n-hexane and ethyl acetate extracts, alkaloids and tannins were not detectable. Likewise, flavonoids, steroids, terpenoids, and glycosides were not detected in the methanol extract. The lack of steroids in the methanol extract is not unexpected, given they are lipophilic compounds [43]. On the other hand, *A. senegalensis* stem bark qualitative phytochemical screening confirmed the presence of alkaloids, terpenoids, and glycosides in all three extraction solvents. The methanol extract of the plant showed the presence of saponins, alkaloids, tannins, terpenoids, and glycosides, which is in agreement with the phytochemical screening described by [48]. Flavonoids and steroids were absent in all the extraction solvents. The absence of tannins in n-hexane extract contradicts the finding [40]. The higher amount of tannins in the methanol extract of both the plant samples is in agreement with our previous report, as tannins, being a polar compound, should be more in methanol. The presence of cardiac glycosides in all extracts of both plant samples except in the methanol extract of *K. africana* stem bark offers credibility to their antifungal, antibacterial, analgesic, anti-inflammation, antihypertensive, anticancer, diuretic, and emetic potency [49, 50]. The result of the quantitative phytochemical analysis of the methanol stem bark extract of *K. Africana* and *A. senegalensis* (**Table 3**) indicated that flavonoids were more abundant in both plants.

Table 4: α -amylase Inhibitory Activities of Methanol Extract of *K. africana* and *A. senegalensis* Stem Bark.

Concentration of samples (mg/mL)	<i>K. africana</i>	<i>A. senegalensis</i>
0.1	30.0000 ^d ± 0.00000	87.6100 ± 0.00000
0.2	23.7667 ^e ± 0.05774	82.0000 ± 0.00000
0.5	38.4333 ^c ± 0.30551	68.5367 ± 0.46479
1.0	39.9067 ^b ± 0.00577	60.9400 ± 0.04000
Standard Drug (Acarbose; 2.5mg/ml)	56.0000 ^a ± 0.00000	56.0000 ± 0.00000

α -amylase is a type of intestinal enzyme that plays a vital function in carbohydrate digestion and glucose absorption. Suppression of the activity of digestive enzymes such as α -amylase, would delay the digestion of starch and oligosaccharides, which in turn inhibits the absorption of glucose and consequently reduces blood glucose. This study looked at the *in vitro* α -amylase inhibitory activity of methanol extracts of *K. africana* and *A. senegalensis* stem bark. Acarbose is a standard drug for α -amylase inhibitors. Acarbose at a concentration of 2.5mg/ml, showed α -amylase inhibitory activity of 56.0%. It is also important to note that the various levels of inhibition are not concentration-dependent, as indicated in **Table 4**. The stem bark extract of *A. senegalensis* demonstrated the highest level of inhibition (87.61%) at a 0.1 mg/ml concentration when compared to the standard drug (Acarbose). The methanol stem bark extract of *A. senegalensis* was found to be the most effective at inhibiting α -amylase enzymes *in vitro*. The plant extract might be utilized as a starch blocker as it limits or delays the absorption of starch into the body, mainly by preventing the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltotriose, and other simple sugars. This inhibitory action could be credited to the presence of flavonoids and alkaloids, which play a vital role as antidiabetic agents [51]. The data revealed that the *A. senegalensis* extract is capable of inhibiting glucose absorption efficiently, which is similar to the result obtained by Beverly and Eschwège [52]. Hence, validating the claim of its usage as an antidiabetic drug in traditional medicine.

Table 5: Glucose Uptake in Yeast Cells Treated with Methanol Extract of *K. africana* and *A. senegalensis* Stem Bark.

Concentration of samples (mg/mL)	<i>K. africana</i>	<i>A. senegalensis</i>
0.1	11.3667 ^c ± 0.35119	0.4133 ^b ± 0.34641
0.2	12.0000 ^d ± 0.00000	2.0000 ^b ± 1.15470
0.5	17.6333 ^c ± 0.30551	31.4000 ^{ab} ± 0.47258
1.0	23.3333 ^b ± 0.35119	13.3333 ^b ± 0.17321
Standard Drug (Acarbose; 2.5mg/ml)	68.4400 ^a ± 0.00000	68.4400 ^a ± 0.023090

The results of the present study showed that the methanol stem bark extract of *K. africana* and *A. senegalensis* increased glucose uptake in yeast cells at varying glucose concentrations. The result of glucose transport across the cell membrane in the yeast cell system is shown in **Table 5** after the treatment of yeast cells with methanol stem bark of *K. africana* and *A. senegalensis*. No dose-dependent glucose uptake was observed in *A. senegalensis*, whereas dose-dependent glucose

uptake was seen in *K. africana* which indicates an increase in concentration would enhance the capacity of yeast cells to absorb more glucose. The methanol stem bark extract of *A. senegalensis* showed the maximum glucose uptake (31.40%) at a 0.5 mg/ml concentration, while *K. africana* recorded 23.33 % of glucose uptake at a 1.0 mg/ml concentration. According to previous research [53, 54], the methanol stem bark extracts of *K. africana* and *A. senegalensis* are capable of improving glucose uptake and thus controlling blood glucose levels.

Conclusion

The results of this research suggest that *K. africana* and *A. senegalensis* stem bark have antihyperglycemic potential and might be used as an alternative medicine to treat diabetic patients by significantly decreasing the dose of conventional drugs. The plant extracts under study were shown to exhibit inhibitory activity against the α -amylase enzyme and glucose uptake in yeast cells; as a result, their therapeutic potential can be employed in the management and treatment of diabetes mellitus as well as a dietary health supplement. The bioactive compounds in the stem bark of *K. africana* and *A. senegalensis* that are responsible for the inhibitory activity must be isolated, purified, and characterized. Further research is needed to determine if the plant has antidiabetic potential *in vivo* as well as a comprehensive pharmacological study to evaluate the likely toxicological implications of these antidiabetic plants.

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