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COMPARATIVE PHYTOCHEMICAL, PROXIMATE, AND *in vitro* ANTIHYPERGLYCEMIC ANALYSES OF THE ROOT BARK EXTRACTS OF *Nauclea latifolia* Smith AND THE STEM BARK EXTRACTS OF *Azadirachta indica* A. Juss.

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ABSTRACT

Diabetes Mellitus is a systemic metabolic disorder caused by relative or absolute insulin secretion deficiency or resistance, characterized by hyperglycemia. It has affected 5% of the world's population and accounts for about 10 % of total health care expenditure in many countries. The present investigation was carried out to explore the proximate, phytochemical compositions and antihyperglycemic potentials of *Nauclea latifolia* Smith and *Azadirachta indica* A. Juss. The selected plant parts were subjected to solvent extraction using three solvents of varying polarity (hexane, ethyl acetate, and methanol), followed by phytochemical screening. Some of the extracts were analyzed for their antihyperglycemic activity using *in vitro* assays such as α-amylase inhibition assay and glucose uptake by yeast cells. The phytochemical analysis showed the presence of saponins, flavonoids, alkaloids, tannins, steroids, terpenoids, and glycosides in various amounts. The proximate composition revealed the following: moisture content (9.00 %); ash content (5.33 – 8.00 %), crude fiber (23.75 – 33.75 %); oil content (12.50 – 12.86 %) and carbohydrate (6.00 – 31.49 %). The results of the assays suggest that the methanol extract of the stem bark of *Azadirachta indica* A. Juss showed the highest value of inhibition (87.61 %) at 0.1 mg/ml concentration when compared to acarbose (56.0 %) used as the control/standard drug. It is noteworthy that various levels of inhibition are not concentration-dependent. The percent glucose uptake of methanol extract of *Azadirachta indica* A. Juss was recorded highest (49.49 %) at 1.0 mg/ml. This study's finding favors the use of these medicinal plants for the management of diabetes which might be due to the presence of bioactive phytoconstituents inherent in them.

Keywords: *Nauclea latifolia*; *Azadirachta indica*; Phytochemicals; Antihyperglycemic; Root Bark; Stem Bark; Alpha-amylase inhibition; Yeast Cell Glucose Uptake.

INTRODUCTION

Diabetes mellitus is a popular progressive disease and one major killer ailment in recent times. This disease could arise from a deficiency in insulin production by the pancreas, or by the ineffectiveness of the insulin which in turn gives rise to an increased concentration of glucose in the blood which in turn damages the body's vessels and nerves [1]. The steady increase in the proportion of the aged, consumption of caloric rich diet, obesity, and sedentary lifestyle have led to a tremendous increase in the number of diabetics worldwide as there are well over 171 million people or 2.8% of the populace predicted to be suffering from this ailment as of the year 2000, and it is estimated that an increase from 6% in 2005 to 14.2% by the year 2025 [2; 3].

Plants have been used in traditional medicinal practices, and many useful orthodox drugs have been produced. The curative properties of these

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plants have been attributed to be due to some bioactive substances in the plants. Due to the cost, availability and side effects of orthodox drugs, many societies still depend on plants for their healthcare needs [4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 14; 15; 16; 17]. Various species of these medicinal plants have demonstrated anti-hyperglycemic activities, and different parts of these plants individually and in combination with other plant parts have been used in the treatment of diabetes [18; 19; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29; 30; 31; 32; 33; 34; 35; 36; 37; 38; 39; 40; 41; 42; 43; 44; 45; 46; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 57; 58; 59; 60; 61; 62; 63; 64; 65].

For this research, we will be focusing on two plant species; *Nauclea latifolia* Smith and *Azadirachta indica* A. Juss. *Nauclea latifolia* Sm. belongs to the Rubiaceae family, known as *'Ubuluinu, Tafashiya, Egbesi, ''*, or '*'Uvuruilu''* among the Igbos in the Eastern part of Nigeria; the Hausas know it as '*'Tafashiya''*, ''*Marga*'', ''*Tabashiya*'', '*'Tuwonbiri''*. The Yoruba, Ibibios, and the Itsekiris know it as ''*Egbesi*'', '*'Mbong-ibong''*, and '*'Itu''* respectively have been used in the treatment of various ailments by traditional medicine practitioners in different regions of the world [66; 67; 68; 69; 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88].

Many secondary metabolites have been isolated from the plants such as alkaloids, steroids, saponins, tannins, flavonoids, etc. [96; 97; 98]. In addition, many compounds such as β-sitosterol, naucleidinal, angustoline, nauclefoline, naucletine, etc. have also been isolated from various parts of *N. latifolia*. These compounds have shown antimicrobial activities against many human pathogenic organisms, and some folkloric uses of the plant have also been confirmed by the presence of some essential minerals in parts of the plant [89; 90; 91; 92; 93; 94; 95; 96; 97; 98].

Azadirachta indica A. Juss, commonly referred to as neem is a 40-50 ft tree, found in many Asian and African countries. It belongs to the Meliaceae family and is known and called by various names [99]. It has an aromatic garlic-like odor and has been reported to have both insecticidal and gametocidal activities. Various traditional herbal practitioners from various regions of the world use decoctions, extracts, oils, and powder samples of the plant in treating various diseases such as skin disorders, mouth odor, cholera, gastrointestinal disorders, wounds, diarrhea, gingivitis [100; 101; 102; 103; 104; 105; 106].

Triterpenoids, tannins, flavonoids, steroids, saponins, and alkaloids have been detected in the plant and many compounds such as Isomargolonone, n-hexacosanol, flowerone, azadiramide, etc. have been isolated from the plant [107; 108; 109; 110; 111; 112; 113; 114; 115; 116; 117; 118; 119; 120; 121; 122; 123; 124; 125; 126; 127; 128; 129; 130; 131; 132; 133; 134; 135].

Little or no attention has been paid to identifying the antidiabetic potency of either the methanolic stem bark extract of *A. indica* or the ethyl acetate root bark extract of *N. latifolia* which has led to the scarcity of relevant literature on this effect. Hence, the relevance of this study, as it aims to bridge that

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gap and further give room for more research in both areas.

MATERIALS AND METHODS

Both plant samples were collected from the International Center for Ethnomedicinal and Drug Development Nsukka and were identified by Mr. Alfred Ozioko, a senior staff of the herbarium.

The root barks of *N. latifolia* were washed, air-dried, ground into uniform powder, and stored in black polythene bags awaiting analysis. The stem barks of *A. indica* were sliced into very small sizes and sun-dried for 14 days to constant weight. They were ground into a fine powder and stored in air-tight plastic containers awaiting extraction.

Extraction of Phytochemicals

200 g each of both dry powdered samples were soaked in 500 mL of each of the three solvents: methanol, n-hexane, and ethyl acetate. Each of the solutions was shaken and the mixtures were left to stand at room temperature for 48 hours; after which they were filtered using Whatman No.1 filter paper. The filtrates were collected and concentrated by heating on a rotary evaporator and labeled NLRH, NLRE, NLRM, AISH, AISE, and AISM.

Quantitative and qualitative phytochemical analyses were carried out on the powdered samples and extracts respectively using the standard procedures as described in our previous works [136; 137; 138].

Proximate analysis of the powdered samples using the standard method as described by [139]. Due to the aim of trying to close the literature gap associated with this research, and in addition to a high percentage yield, the methanol extract of *A. indica* and the ethyl acetate root bark extract of *N. latifolia* were both selected for carrying out the *in vitro* anti-hyperglycemic potency of these plants.

in vitro Antihyperglycemic Evaluations

Alpha-amylase Inhibitory Assay

The α-amylase inhibitory activities of the ethyl acetate extract of *N. latifolia* and methanolic extract of *A. indica* were evaluated by a previously described method by [140] with some modifications.

About 0.5 mL of both extracts was mixed with 0.5mL of α-amylase solution (0.5 mg/mL) with 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl). The resulting mixture was incubated at room temperature for 10 mins and the reaction was terminated using 1mL of dinitro salicylic acid color reagent. At this time, the test tubes were placed in a water bath (100 °C for 5 min) and cooled until room temperature was attained. The mixture was then diluted with 10 mL of deionized water, and absorbance was determined at 540 nm. 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 calculated using the following equation:

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%inhibition of α−Amylase = $Abs_{control} - Abs_{sample}/Abs_{control} \times 100$ …… equation 1

Where; $Abs_{control}$ corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution and Abs_{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 various concentrations of extracts (1-5 mg /mM) were added to 1mL of glucose solution (5,10 and 25 mM) and further incubated for 10 min at 370 C. reaction was started by adding 100 µl of yeast suspension, vortex and further at 37°C at 60 min, the tubes were centrifuged (2,500×g, 5 min) and glucose was estimated in the supernatant, metformin was taken as the standard anti-diabetic drug used. Absorbance was measured at 540 nm and all experiments were carried out in triplicates. The percentage of increase in glucose uptake by yeast cells was calculated using the following formula:

Activity % = $\underline{Abs_{control} - Abs_{sample} / Abs_{control}}$

Increase in glucose uptake …... equation 2

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 data were subjected to analysis of variance (ANOVA) using statistical package for social science (SPSS) version 23.0. Duncan's new multiple range tests (DNMRT) were used to compare the treatment means and all analyses were carried out in triplicate.

RESULTS AND DISCUSSION

Table 1. Proximate Composition of *N. latifolia* Root Bark and *A. indica* Stem Bark

PARAMETERS	*N. latifolia*	*A. indica*
Moisture Content (%)	9.0000±1.00000	9.0000±1.00000
Ash content (%)	5.3333± 0.57735	8.0000± 0.0000
Crude Fiber (%)	23.7500± 1.25000	33.7500± 1.25000
Oil Content (%)	12.8633± 0.17502	12.5000± 0.50000
Carbohydrates (%)	6.0000± 0.00000	31.4933± 0.07506

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Table 2: Qualitative Phytochemical of *N. latifolia* Root Bark and *A. indica* Stem Bark Extracts.

Phytochemicals	NLRH	NLRE	NLRM	AISH	AISE	AISM
Saponins	+	++	+++	+	++	+++
Flavonoids	+++	+++	+++	++	+++	_
Alkaloids	_	+	+++	_	_	+++
Tannin	+	+	_	++	++	++
Steroids	_	_	+++	_	_	_
Terpenoids	+	++	+++	+	_	+
Glycosides	+	+++	+++	+	++	+++

Key

+++ = Present in high concentration.

++ = Present in moderate concentration.

+ = Slightly or sparingly present.

- = Absent.

NLRH: n-hexane root bark extract of *N. latifolia.*

NLRE: Ethyl acetate root bark extract of *N. latifolia.*

NLRM: Methanol root bark extract of *N. latifolia.*

AISH: n-hexane Stem bark extract of *A. indica.*

AISE: Ethyl acetate Stem bark extract of *A. indica.*

AISM: Methanol Stem bark extract of *A. indica.*

Table 3. Quantitative Phytochemical Composition of *N. latifolia* Root Bark and *A. indica* Stem Bark

Parameter	*N. latifolia*	*A. indica*
Alkaloids (%)	4.3333 ± 0.57735	4.0000± 0.00000
Flavonoids (%)	4.6667 ± 1.52753	3.0000± 0.00000
Phenols (mg/g)	3.4800± 0.03000	0.0090 ± 0.00173
Saponins (%)	7.7000± 0.39686	5.2500 ± 0.25000
Tannins (mg/100g)	5.5333± 0.35119	0.3933 ± 0.01528

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Table 4: α-amylase Inhibitory Activities of *N. latifolia* Root Bark Ethyl Acetate Extract and *A. indica* Stem Bark Methanol Extract.

Concentration of samples (mg/mL)	NLRE	AISM
0.1	50.2033±0.35218	87.6100 ±0.00000
0.2	37.4133± 0.35796	82.0000 ± 0.00000
0.5	44.5833± 0.19630	68.5367 ± 0.46479
1.0	35.7267± 0.18475	60.9400 ± 0.04000
Standard Drug (Control)	56.0000± 0.00000	56.0000 ± 0.00000

Code:

NLRE- Ethyl acetate root bark extract of *N. latifolia.*

AISM- Methanol Stem bark extract of *A. indica*

Table 5: Glucose Uptake in Yeast Cells Treated with *N. latifolia* Root Bark Ethyl Acetate Extract and *A. indica* Stem Bark Methanol Extract.

Concentration of samples (mg/mL)	NLRE	AISM
0.1	6.4267±0.02309	30.3300 ± 0.00000
0.2	7.0000± 0.00000	37.4000 ± 0.00000
0.5	10.6000± 0.51962	43.0000 ± 0.00000
1.0	16.3000± 0.0000	49.4900 ± 0.42579
Standard Drug (Control)	68.4400± 0.00000	68.4400 ± 0.02309

Code:

NLRE- Ethyl acetate root bark extract of *N. latifolia.*

AISM- Methanol Stem bark extract of *A. indica*

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From the results of qualitative phytochemical screening (**Table 2**), it can be deduced that in *A. indica* extracts, saponins, tannins, and glycosides were all present in all three extraction solvents. Flavonoids and steroids, however, were absent in the methanol extract; alkaloids, steroids, and terpenoids were not found in the ethyl acetate extract, same applies to alkaloids and steroids which were also not detected in the n-hexane extract. *N. latifolia* root bark qualitative phytochemical screening on the other hand gave the presence of saponins, flavonoids, terpenoids, and glycosides in all the extracts. Steroids were the only absent compounds in the ethyl acetate extract of *N. latifolia* root bark. Alkaloids and steroids were absent in the n-hexane extract, while tannins could not be detected in the methanol extract. It was also observed that the polarity of the solvents used for extraction might have played a role in the abundance of the metabolites found. The ascending order of abundance (from non-polar to polar solvents) seen in saponins, alkaloids, and glycosides all attest to the solubility of these metabolites increasing with polarity [141; 142]. The presence of alkaloids in the extracts attests to the antimalaria activities of both plants [143; 144; 145; 146; 147; 148]. Tannins were present in all extracts of *A. indica* but were not detected in the methanol extract of *N. latifolia*. This somewhat goes against the report by [149] that the solubility of phenolic compounds is a function of an increase in solvent polarity. Tannins sacs are known to exhibit antiviral, antibacterial, and anti-tumor activities. It was also reported that certain tannins can inhibit HIV replication selectively and are also used as a diuretic. Plant tannins are also the source of commercial tannic acids and tanning agents [150]. Steroids were absent in all extracts of *A. indica*, as well as the n-hexane and ethyl acetate extract of *N. latifolia*; but was highly abundant in the methanol extract of *N. latifolia*. This goes against the hydrophobic nature of steroids as pointed out by [151; 152]. The presence of cardiac glycosides in all extracts of both plant samples gives credence to their antifungal, antibacterial, analgesic, anti-inflammation, antihypertensive, anticancer, muscle relaxation activities, diuretics, and emetics potency, as well as other ethnomedicinal applications of the plant parts [153; 154].

Quantitative phytochemical assay (**Table 3**) of both plant samples gave the content of saponins as the highest concentrations with values of 5.2500± 0.25000 for *A. indica* and 7.7000± 0.39686 for *N. latifolia*. The high content of saponins in plants has been opined by authors and researchers to be responsible for the antidiabetic potency of plants [141; 155; 156; 157]. Saponins from plants have long been employed for their detergent properties. Saponin is used in the manufacturing of shampoos, insecticides, and various drug preparation and in synthesizing steroid hormones. It is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in

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hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss, *etc*. This compound has also been reported to have anti-hyper cholesterol, anti-inflammatory, and cardiac depressant properties, and appears to kill or inhibit cancer cells without killing the normal cells in the process. Saponins have anti-carcinogens properties, immune-modulatory activity, and cholesterol-lowering activity. It is also been reported to have antifungal properties. Some saponins and glycosides are cardiotonic while others are contraceptives and precursors for other sex hormones [158]. *N. latifolia* root bark extracts also recorded higher values in other assayed phytochemicals when compared to *A. indica* stem bark extracts evident in Table 3.

The proximate composition (**Table 1**) of the root bark of *N. latifolia* gave credence to the high nutrient content of the plant which is evident in its carbohydrate (30.88%) and crude fiber (23.75%) contents, which stands to be compared to the most readily consumed seed plants in Nigeria. Proteins, which are considered essential for growth, cell development, repairing worn-out tissues, and increased milk production in animals came in at a percentage of 12.86%. Food/plant proteins are required for the formation of vital hormones, digestive enzymes, DNA and RNA manufacture, building important brain cells, etc. [159]. Although, its protein content is low when compared to other shrubs like *G. latifolium* (27.2%) [160]. The percentage ash content (5.33%) came in as the least abundant. The ash content of the root bark gives information on the number of mineral contents preserved in that plant part. The high carbohydrate content of the root bark means it can serve as a good source of energy together with fats/oil and protein when consumed. The high fiber content of *N. latifolia* root bark gives credence to its role in cleansing digestive tracts, inhibiting the absorption of excess cholesterol, and guarding against metabolic conditions such as diabetes mellitus and hypercholesterolemia [161]. In addition, fiber aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a build-up and lead to several diseases [162]. Adequate intake of dietary fiber can lower the serum cholesterol level, and the risk of coronary heart disease, hypertension, constipation, diabetes, colon, and breast cancer. On the other hand, proximate analysis results from *A. indica* showed crude fiber and carbohydrate came in at the highest concentrations of 33.75% and 32.49% respectively. This high content of fiber has been opined to be associated with a decreased risk of diabetes [163]. Fiber also adds bulk to the diet and prevents the intake of excess starchy food [164], and may guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus [161]. The results also showed oil content of 12.5% of *A. indica* bark, moisture and ash content of 9% and 8% while protein content is the least at 5.25%.

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Alpha-amylase Inhibitory Activities of Methanol Stem bark extract of A. indica and Ethyl acetate Root bark extract of N. latifolia.

Alpha-amylase is a type of intestinal enzyme which play important role in carbohydrate digestion and glucose absorption. Suppression of the activity of digestive enzyme such as α-amylase, would delay the digestion of starch and oligosaccharide, which in turn decreases the absorption of glucose and consequently reduce blood glucose.

Methanol stem bark extract of *Azadirachta indica* was tested for its potency against hyperglycemia by assaying its α-amylase inhibitory potency. Obtained results exhibited positive inhibitory effects; and at a concentration of 0.1 mg/mL, the extract showed its highest value of inhibition (87.61%) when compared to Acarbose (56.0%), used as the standard/control drug. It is also noteworthy to add that the various levels of inhibition are not concentration-dependent as can be seen from the results (**Table 4**). The root bark ethyl acetate extract of *Nauclea latifolia* showed a high level of inhibition (50.20%) at 0.1 mg/mL concentration when compared with the standard drug (Acarbose) used in the treatment of diabetes evident also in **Table 4.** Hence, at an extract concentration of 0.1 mg/mL, ethyl acetate root bark of *N. latifolia* is considered most potent to inhibit alpha-amylase activities *in vitro* when compared to Acarbose (standard drug/control). It can also be deduced from the results that the various levels of inhibition are not concentration-dependent. This technique is one of the anti-diabetic therapeutic approaches to reducing the postprandial glucose level in the blood by inhibiting the activity of the **α**-amylase enzyme and it can be used as a strategy in the management of blood glucose. Regulation of glucose levels in the blood of diabetic patients can prevent numerous complications associated with the disease. The maintenance of plasma glucose concentration for a longer time under variation in the dietary condition is one of the most important and closely regulated processes observed in the mammalian species, especially in type II diabetes characterized by the deficiency of insulin causing increased levels in blood glucose levels and it depends on the uptake of glucose by the cells [165].

Glucose uptake in yeast cells treated with methanol stem bark extract of Azadirachta indica and ethyl acetate root bark extract of Nauclea latifolia

The rate of glucose transport across the cell membrane in the yeast cells system is presented in **Table 5**. After treatment of yeast cells with the methanol extract of *A. indica* stem bark, dose-dependent glucose uptake was observed. The percentage of glucose uptake by the plant sample was recorded highest (49.49%) at a concentration of 1.0 mg/mL. Hence, the percentage of glucose uptake by yeast cells increases with concentrations of *A. indica* stem bark methanol extract. Similar dose-dependent glucose uptake by yeast cells was also observed when ethyl acetate root bark extract of *N. latifolia* was used in treating the yeast cells. The plant sample exhibited the highest glucose uptake (16.30%) at a 1.0mg/mL concentration. The present data suggest that the methanol stem bark extract of *A. indica* and ethyl acetate root bark extract of *N.*

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latifolia are capable of enhancing glucose uptake effectively, which in turn suggests that they are capable of enhancing effective utilization, thereby controlling blood glucose levels as also suggested by other reports. These results further attest to previous works also carried out on the potency of other parts of *N. latifolia* using solvents like ethanol, water, chloroform, and butanol to ascertain the plant's *in vivo* hypoglycemic potency [166; 167; 168]. This further attests to the claims of their use as an antidiabetic drug in traditional medicine [169; 170; 171; 172; 173]. Comparatively, in the present study; the increased ability of the sample to absorb glucose may also be attributed to the dietary fiber (insoluble and soluble fibers) present in the samples [174].

CONCLUSION

This study has tried to bring to light the nutritional, chemical, and medicinal activities of *Nauclea latifolia* and *Azadirachta indica* with their respective analyzed plant parts.

Nutritionally, both plants are abundant in proteins, oils, fibers, and carbohydrates. They also contain phytochemicals such as saponins, alkaloids, tannins, phenols, glycosides, and steroids. All this gives credence to their various dietetic, ethnomedicinal, and pharmacological activities, especially as antidiabetics. The study also shows that methanol is the best solvent for extraction; in line with previous works of literature.

It can be deduced from this work that the methanol extracts of *A. indica* stem bark and ethyl acetate extracts of *N. latifolia* root bark had antidiabetic activities. The observed hypoglycemic activity could be associated with the phytochemicals and phytonutrients and other bioactive compounds present in the analyzed plant parts.

There is also room for conservation efforts to ensure that their continued use is sustainable. There is equally a need to study the toxicological effect of both plants with prolonged usage.

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