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Preliminary Anti-Diabetic and Antioxidant Evaluation of an Herbal Formulation of Stinging Nettle, *Laportea aestuans* (L.) Chew (Urticaceae)

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Abstract

Metabolic disorders associated with non-communicable diseases are among the highest causes of death and disability worldwide. They increase the risk of diseases such as Diabetes mellitus (DM). The cost of currently available medications for DM has raised a need for more affordable and available alternatives to manage this ailment. This study investigated the antioxidant and anti-diabetic activity of a Stinging Nettle, *Laportea aestuans* (L.) Chew [Urticaceae] dried root formulation. Qualitative and quantitative screening of phytochemicals were done using standard tests and ultraviolet (UV) spectrophotometry. Antioxidant evaluation was done using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) inhibition, total antioxidant capacity (TAC) and ferric reducing antioxidant capacity (FRAP) assays while alpha-amylase and alpha-glucosidase inhibitory activity was carried out to estimate anti-diabetic potential. Phytochemicals present in the crude extract of LAF included reducing sugars, cardiac glycosides, steroids, tannins, phenols and flavonoids. The extract of LAF had TP of 42.82 ± 0.19 mg/g gallic acid equivalent (mg/g GAE) and TF of 61.67 ± 0.33 mg/g rutin equivalent (mg/g RE). The crude extract of LAF had DPPH inhibition, TAC and FRAP of 91.31 ± 0.20 %, 167.42 ± 6.48 mg/g ascorbic acid equivalent and 22.15 ± 0.25 µM Fe^{2+}/ml of extract respectively

Keywords: *Laportea aestuans,* alpha-amylase, alpha-glucosidase, phytochemicals, spectrophotometry.

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Introduction

Metabolic syndrome is defined as a collection of pathological conditions such as increased waist circumference, high blood pressure, elevated triglycerides, low high density lipoprotein - cholesterol (HDL), elevated total cholesterol and elevated blood glucose- that predispose to a person being affected by cardiovascular disease, stroke and type 2 diabetes (Dobrowolski *et al*., 2022).

Diabetes mellitus is a metabolic disorder characterised by persistently elevated blood glucose (chronic hyperglycaemia). It is caused by many factors such as insufficient insulin secretion or impaired glucose utilisation by cells consequent upon impaired action of insulin (Dobrowolski *et al*., 2022). This causes a myriad of effects on adipose tissues, skeletal muscles and vital organs such as the liver (Dobrowolski *et al*., 2022). Symptoms characteristic of this malady includes polydipsia (increased thirst), polyuria (increased urinary frequency), polyphagia (increased appetite and food intake), excessive weight loss and problems with sight. A lot of patients in early stage of disease may be asymptomatic (Dobrowolski *et al*., 2022).

Diabetes mellitus is broadly divided into two primary categories: Type 1 and Type 2. Type 1 diabetes (T1D) can be identified before insulin secretion becomes critically impaired, and it is characterized by a reduced responsiveness of β-cells to glucose (Antar *et al*., 2023). Following diagnosis, insulin levels continue to drop progressively. T1D is further divided into two subtypes: idiopathic and fulminant. The idiopathic subtype is uncommon, has no autoimmune origin, and tends to be less severe. Those with this form may develop ketoacidosis alongside insufficient insulin production, and it occurs more frequently in individuals of Asian or African descent (Antar *et al*., 2023). Fulminant T1D, first described in 2000, is also non-immune-mediated. In this subtype, ketoacidosis develops following the onset of hyperglycemia, and β-cell destruction is so rapid and complete that virtually no insulin remains. It is primarily attributed to genetic factors (Antar *et al*., 2023).

The hallmark of Type 2 diabetes is impaired insulin secretion. Insulin output varies considerably in response to changes in insulin sensitivity as the body attempts to sustain normal blood glucose levels (Galicia-Garcia *et al*., 2020). A progressive loss of β-cell function is also a defining characteristic of T2D advancement (Wysham and Shubrook, 2020). Diabetes arising during pregnancy raises the risk of illness and death for both the mother and the child (Guertsen *et al*., 2019). This risk exists regardless of whether the hyperglycemia stems from pre-existing Type 2 diabetes. Children born to mothers with gestational diabetes face a greater likelihood of developing diabetes later in life (Hollander *et al*., 2007).

Antioxidants are present in many natural sources in form of phytochemicals such as phenolics, flavonoids, carotenoids, proanthocyanidins, terpenoids and many others (Gulcin, 2025). They react with free radicals and reactive oxygen species to quench and reduce oxidative stress (Gulcin, 2025). These antioxidants work by free radical Scavenging: by donating electrons to free radicals to make them stable thereby reducing cellular harm, act as enzymes to catalyse reactions that neutralise free radicals and inactivate free radicals to inactive molecules such as oxygen and water, help regenerate other antioxidants and reduce the production of pro-inflammatory cytokines, truncating the inflammatory process that can lead to oxidative damage (Gulcin, 2025).

Laportea aestuans (L.) Chew (Urticaceae) also known as West Indian wood nettle is a perennial plant commonly found in temperate regions (Burkill. 1985). It is known for its sharp, stinging hairs that can cause a painful, burning sensation upon contact with skin. These hairs contain chemicals such as histamine, formic acid, and acetylcholine, which can irritate the skin (Burkill, 1985). Despite its painful sting, West Indian wood nettle has many benefits and uses, both in traditional medicine and modern applications. The plant gets its name from the stinging sensation it causes when touched due to tiny, hair-like structures on the leaves and stems. These structures contain a mixture of chemicals, including histamine, which irritate the skin and cause a burning, itching sensation (Burkill, 1985). They are dioecious, meaning they have separate male and female plants. The plant also produces small, greenish flowers that grow in clusters. Despite its painful sting, nettle has a long history of being used in herbal medicine, textiles, and food. It is rich in nutrients, including vitamins A, C, and K, iron, calcium, and magnesium (Burkill. 1985). Nettle has long been used in folk medicine to treat conditions like arthritis and joint pain due to its anti-inflammatory properties. It has been used as a diuretic to manage urinary tract issues. Nettles are used in soups, teas, pesto, and even as a spinach substitute in dishes. Stinging nettle has a long history of use in traditional medicine. It is known for its anti-diuretic and detoxifying properties. It has been used to treat conditions kidney stones, and urinary tract issues. Nettle has been used for its ability to reduce inflammation, making it useful in treating rheumatoid arthritis. Today, nettle is commonly found in herbal supplements, often marketed for its benefits in treating hay fever, blood sugar, improving joint health, and supporting prostate health (Burkill, 1985). Some of its key pharmacological effects include anti-inflammatory, antioxidant, analgesic, diuretic, antihistamine and antimicrobial activities (Musa *et al*., 2026). Stinging nettle contains a variety of bioactive chemical constituents, which contribute to its medicinal and nutritional properties. Some of the key constituents include flavonoids such as quercetin, kaempferol, and isorhamnetin, which have antioxidant and anti-inflammatory effects (Burkill, 1985), phenolic acids such as chlorogenic acid, caffeic acid and ferulic acid, known for their antioxidant properties (Burkill, 1985), tannins that have astringent properties (Burkill, 1985), terpenoids such as essential oils like Eucalyptol, which possess antimicrobial and anti-inflammatory effects (Burkill, 1985), saponins,

minerals, vitamins, amino acids and carbohydrates such as hemicellulose (Burkill, 1985).

Materials and Methods

Plant collection, identification and extraction

The whole plant was purchased from Mushin market, Lagos, Nigeria. The plants were identified and authenticated at the Lagos University Herbarium (LUH) domiciled in the Department of Botany, Faculty of Life sciences, University of Lagos. A voucher specimen with assigned voucher number (LUH 100526) was deposited at the herbarium. The whole plant was garbled, washed and dried in ambient temperature. The roots were cut from the whole plant and placed into a laboratory oven at 40 °C until dried (Oiseoghaede *et al*., 2024, Oiseoghaede *et al*., 2025). The dried roots were ground with a mechanical grinder and stored in plastic containers until used. The dried plant material (10 g) was macerated with 500 mL of ethanol (95 % v/v) for 72 h and concentrated *in vacuo* to obtain the crude extract.

Qualitative phytochemical screening

The crude extract was tested for the presence of phytochemicals using standard assay methods as described by Ayoola *et al*., 2008.

Quantitative analysis

Estimation of phenolic compounds

The crude extract was quantified for total phenolic content using Folin-Ciocalteu's reagent (FCR) method. The standard (Gallic acid) at varying incremental concentrations was prepared to construct a calibration plot with the experiment based on a protocol used by Oiseoghaede *et al* (2018). Total phenolic content of the extract was extrapolated from the calibration plot and expressed in terms of milligrams of gallic acid equivalent per gram of dry weight of extract after reading out absorbance at 765 nm.

Total flavonoid determination

The crude extract was quantified for total flavonoid content. Varying incremental concentrations of the standard (Rutin) was prepared to construct a calibration plot. The experiment was done using a protocol established by Oiseoghaede *et al* (2018). Total flavonoid content of the extract was extrapolated from the calibration plot and expressed in terms of milligrams of rutin equivalent per gram of dry weight of extract after reading out absorbance at 510 nm.

Antioxidant assays

Determination of Antioxidant Activity Using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

The ability of the extract to reduce the absorbance of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical expressed as a violet colour in comparison with Ascorbic acid standard was the basis of this experiment (Oiseoghaede and Odukoya, 2015). The antioxidant activity was expressed as the equation:

% Inhibition = [(Abs control - Abs sample)/Abs control] × 100 % Abs = Absorbance

Determination of Total antioxidant capacity (Phosphomolydate determination of total antioxidant capacity)

The total antioxidant capacity of the crude extract was evaluated by the Phosphomolybdenum method as described by Prieto *et al* (2008) and modified by Oiseoghaede *et al* (2026). The antioxidant activity was expressed in number of equivalents of ascorbic acid after absorbance was read out at 695 nm.

Determination of Antioxidant Activity Using the Ferric Reducing/Antioxidant Power (FRAP) Method

The assay was conducted following the method described by Benzie and Strain (2006) with minor modifications (Oiseoghaede *et al* 2026).

Anti-diabetic assays

Inhibition of alpha-amylase enzyme

A protocol established by Al-Baidhani *et al* (2020) was used for this experiment. The same procedure was carried out for standard, Acarbose and values compared with that of the extract. Percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{(OD value of control - OD value of samples)}}{\text{(OD value of control of control)}}$$

OD = Optical density

Inhibition of alpha-glucosidase enzyme

A method of Al-Baidhani *et al* (2020) was used for this experiment employing Acarbose as the standard. Percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{(OD value of control - OD value of samples)}}{\text{(OD value of control of control)}}$$

OD = Optical density

Statistical analysis

Analysis of data was done using Microsoft Excel and Analysis of variance (ANOVA) with multiple comparisons at 95%

significance level in conjunction with Dunnett posttest analysis using GraphPad Prism 9 (San Diego, CA)

Results

Percentage yield

The percentage yield after extraction with 95 % ethanol was 0.22 g (2.20%).

Phytochemical screening

The crude extract was shown to contain saponins, reducing sugars, cardiac glycosides, steroids, tannins, phenolics and flavonoids (Table 1).

Table 1: Phytochemicals present in herbal drug samples

Phytochemical	Test	Presence/Absence
Alkaloids	Mayer's Test	-
	Dragendorff's Test	-
	Wagner's Test	-
Saponins	Frothing Test	+
Reducing Sugar	Fehling's Test	++
Cardiac glycosides	Killer Killani's Test	+
Triterpenoids	Liebermaan – Burchard	-
Steroids	Salkowki Test	++
Tannins	Ferric Chloride Test	+++
Phenolic Compounds	Lead acetate Test	++
Flavonoids	Shinoda's Test	+++
Anthraquinone	Borntragers Test	-

Note: Heavily detected: + + +; Moderately detected: + +; Slightly detected: +' Not detected:

Total phenolic and total flavonoid content

The crude extract had TP of 42.82 ± 0.19 mg/g GAE and TF of 61.67 ± 0.33 mg/g RE. The total phenolic content was determined using the calibration plot (Figure 1), y = 0.0093x - 0.0063, R^2 = 0.9994 while total flavonoid content was determined using the calibration plot (Figure 2), y = 0.0041x + 0.0494, R^2 = 0.9999.

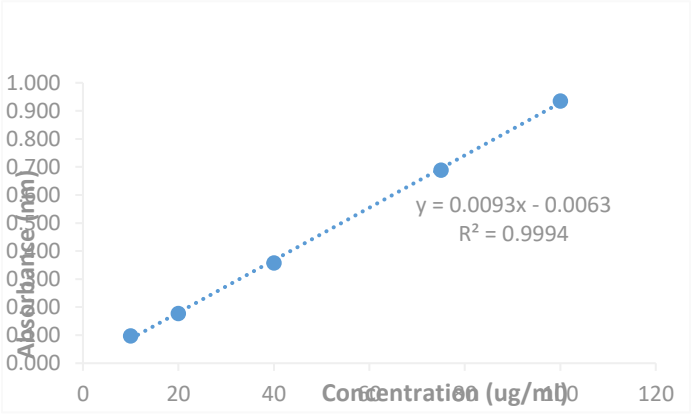

Figure 1: Calibration plot of Total phenolics (mg/g Gallic acid equivalent)

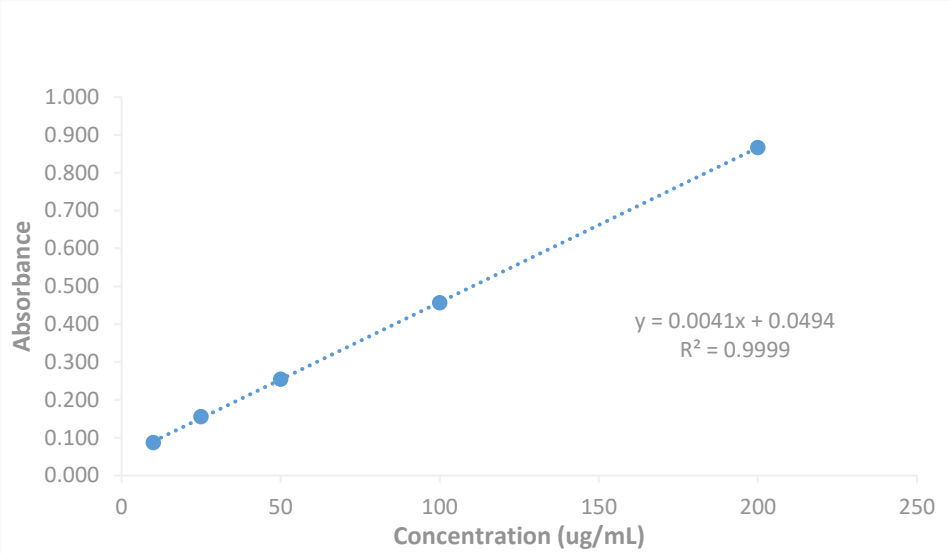

Figure 2: Calibration plot of Total flavonoids (mg/g Rutin equivalent)

Antioxidant assays

The crude extract had peak DPPH inhibition of 91.31 ± 0.20 %, TAC of 37.56 ± 0.22 mg/g ascorbic acid equivalent and FRAP of 22.15 ± 0.25 µM Fe^{2+}/ml of extract. The FRAP was estimated using the calibration curve, y = 0.0073x + 0.1512, R^2 = 0.9812 (Figure 3).

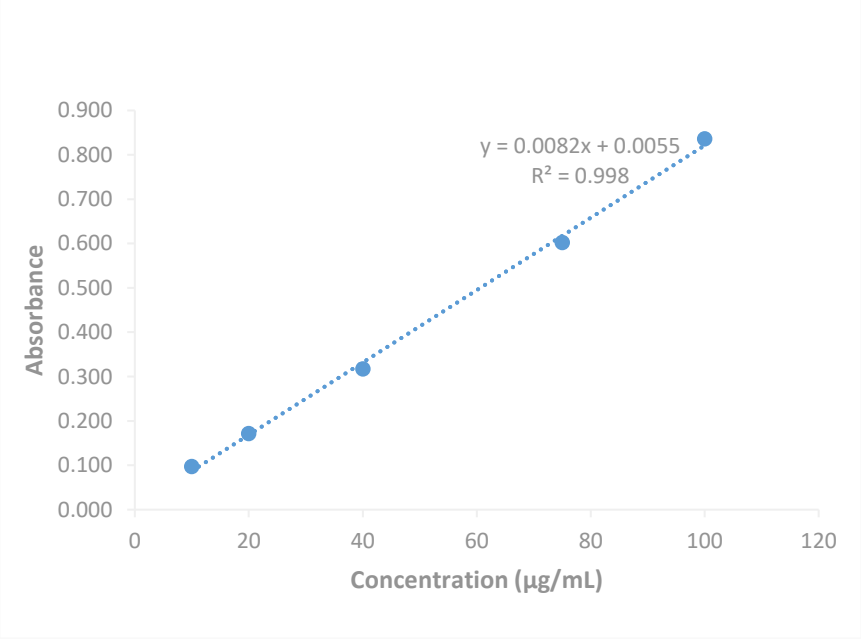

Figure 3: Calibration plot of Total antioxidant capacity (mg/g ascorbic acid Equivalent)

Antioxidant assays

The crude extract had appreciable antioxidant activities (Table 2). However, the standard in all 3 antioxidant models were significantly better than the crude extract.

Anti-diabetic assays

The extract had good alpha-amylase and alpha-glucosidase inhibition as seen (Table 3). Peak alpha-amylase and alpha-glucosidase activities were 85.35 ± 0.28 % and 85.90 ± 0.27 % which were adequate but significantly lower than standard, Acarbose

Table 2: Antioxidant activity

Concentration (µg/ml)	10	25	50	100	200
% DPPH inhibition					
Extract	31.95 ± 0.33****	51.72 ± 0.13****	77.83 ± 0.16****	90.41 ± 0.23****	91.31 ± 0.20****
Ascorbic acid	66.55 ± 0.04	89.21 ± 0.06	91.84 ± 0.07	93.00 ± 0.04	93.18 ± 0.07
Total antioxidant capacity (mg/g Ascorbic acid equivalent)					
Extract	37.56 ± 0.22****				
Quercetin	167.42 ± 6.48				
Ferric reducing aantioxidant power (µM Fe^{2+}/ml of extract)					
Extract	22.15 ± 0.25****				
Ascorbic acid	174.60 ± 1.33				

**** P < 0.0001 compared to Ascorbic acid, Quercetin

Table 3: Percentage alpha-aamylase and alpha-glucosidase inhibition

Concentration (µg/mL)	100	200	500	750	1000
Alpha-amylase inhibition					
Extract	27.61 ± 0.34****	48.70 ± 3.50****	54.50 ± 0.21****	73.14 ± 0.17****	85.35 ± 0.28****
Acarbose	74.68 ± 0.17	88.31 ± 0.04	91.39 ± 0.08	91.94 ± 0.00	92.06 ± 0.12
Alpha-glucosidase inhibition					
Extract	30.36 ± 0.33****	50.65 ± 3.37****	56.23 ± 0.20****	74.16 ± 0.17****	85.90 ± 0.27***
Acarbose	77.93 ± 0.08	85.83 ± 0.14	89.40 ± 0.11	90.88 ± 0.07	91.41 ± 0.10

*** P < 0.001, **** P < 0.0001 compared to Acarbose

Discussion

Compared to a previous study that extracted aerial parts of the plant with 80 % methanol, the yield in our study was higher (Faloye *et al*., 2024). The lower yield from the extraction indicates that 95 % ethanol is a poor choice of solvent for extracting the plant material.
The phytoconstituents of the root extract were similar to those of a previous study (Adetunji *et al*., 2021). This is in agreement with evidence in literature (Adetunji *et al*., 2021) which also shows a good DPPH antioxidant inhibitory activity attributable to the presence of flavonoids and phenolics (Adetunji *et al*., 2021) present in the extract. However, the crude extract lacked alkaloids which were present in another study (Omolola *et al*., 2018) which may be due to difference in area of collection. This suggests that the extract could be useful in treatment of ailments resulting from oxidative stress such as Diabetes mellitus (Momo *et al*., 2007). Extracts from related species (*Laportea ovalifolia*) have also been shown to have good antioxidant activity (Momo *et al*., 2007).

In the evaluation of the anti-diabetic potential of the extract, though Acarbose gave a significant activity in both models the extract had an appreciable alpha-amylase and alpha-glucosidase activity with over 80 % inhibition. This indicates that the extract has potential as an anti-diabetic agent, which agrees with the findings from previous study (Adetunji *et al*., 2021).

Conclusion

The root of L*aportea aestuans* exhibited appreciable anti-diabetic activity as well as antioxidant activity which could be explored in the management of oxidative stress and accompanied disorders such as diabetes. This study validates the ethnobotanical use of these L*aportea aestuans* in managing diabetes.

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