COMPARATIVE PHYTOCHEMICAL, *in-vitro* ANTI-HYPERGLYCEMIC ACTIVITIES OF *Napoleona vogelii Hook & Planch* STEM BARK AND *Cassia occidentalis* L. ROOT EXTRACTS

Abstract

This work investigated the comparative phytochemical, proximate and anti-hyperglycemic activities of stem bark of *Napoleonaea vogelii* and root of *Cassia occidentals* extracts. Stem bark and *N. vogelii* root of *C. occidentalis* were cold extracted with methanol, ethyl acetate, and n-hexane. The extracts were subjected to proximate, phytochemical analyses and anti-hyperglycemic assays. The result of proximate analysis revealed that stem bark of *N. vogelii* was made up of 40.28 ± 0.49 % crude fibre, 37.05 ± 0.05 % carbohydrates, 7.45 ± 0.45 % protein and more than 6.00 % oil content. whereas 22.33±0.08 % crude fibre, 44.70± 0.93 % carbohydrates, 10.72± 0.05 % protein and 11.00± 0.00 % oil contents in roots of *C. occidentalis*. Alkaloids and terpenoids were present in all the extracts of *N. vogelii* and *C. occidentalis*. Flavonoids were also found in all extracts of *N. vogelii*, whereas tannins and glycosides were also found in all extracts of *C. occidentalis*. *In vitro* antidiabetic screening of the methanol extracts indicated that both plants showed a good concentration-dependent percentage glucose uptake. In all concentrations, extracts of *C. occidentalis* exhibited better glucose uptake than *N. vogelii* extracts. comparable to the activity of the standard drug- metronidazole (68.44±0.00 %), From 0.10 – 0.50 mg/mL, the methanol extracts of both plants also showed a dose-dependent α-amylase inhibitory effect. But the inhibition decreased as the concentration was increased to 1.00 mg/mL. At 0.50 mg/mL, *C. occidentalis* and *N. vogelii* showed percentage inhibitory effect of 50.02±0.035 and35.61±0.01. Both plants' extracts showed good anti-hyperglycemic activity, which may be due to the presence of alkaloids, terpenoids, saponins and tannins in the extracts, validating the folkloric use of both plants in the treatment of diabetes, and thus are good anti-diabetic agents.

Keywords: Phytochemical, proximate, anti-hyperglycemic, alkaloids, terpenoids, flavonoids.

1. Introduction

“More than half a billion people are living with diabetes worldwide, affecting men, women, and children of all ages in every country, and that number is projected to increase to more than 600 million in 2030, and 700 million in 2045” [1,2,3]. “According to the ninth edition 2019 of the International Diabetes Federation (IDF) Diabetes Atlas released by the IDF, as of 2019, the total adult population in the age group of 20–79 years stands at 463 million who live with diabetes, which is set to increase to 578 million by 2030. There is one patient who dies of diabetes mellitus every 6 s, this rate is higher than death rates from human immunodeficiency virus (HIV) (1.5 million), tuberculosis (1.5 million) and malaria (0.6 million), combined” [4].

Several Plants have been used in the treatment of diabetes and several antidiabetic pharmaceuticals commonly used today are structurally derived from natural compounds that are found in these plants [5]. *Napoleonavogelii* and *Cassia occidentalis*are some of the plants used in the treatment of diabetes in Nigeria. [6, 7, 8]

There is an urgent need for effective interventions using medicinal plants in the management of this prevalence, and thus this research which is aimed at studying the phytochemical, proximate and anti-hyperglycemic activities of methanol, ethyl acetate and n-hexane extracts of *Napoleona vogelii* stem bark and *Cassia occidentalis* root extracts.

Fig: 1 *Napoleona vogelii*

Fig: 2 *Cassia occidentalis*

2. Methods

The stem bark of *Napoleona vogelii* and root of *Cassia occidentalis* were collected from Awka, Anambra State, Nigeria and were identified by taxonomists in the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria. The herbarium specimens were deposited in the Department's herbarium for future references. Both were air-dried
for 2 weeks and pulverized to powder. The powdered samples were stored to be used for analysis.

Extraction of the Metabolites

Exactly 10 g each of the powdered *N. vogelii* and root of *C.occidentalis* were soaked separately in 100 ml of each of the three solvents: methanol, ethyl acetate and n-hexane. Each of the three solutions was shaken and the mixtures were left to stand at room temperature for 48 hours after which they were filtered with Whatmann No. 1 filter paper. The filtrates were collected and concentrated by heating on a rotary evaporator. The concentrated extracts were then used for the analysis.

Proximate Analysis

The proximate screening was conducted on the powdered samples using standard procedures;

Moisture Content Determination

"Moisture content was determined according to the standard method of Association of official analytical chemists" [9]. "A petri dish was washed and dried in the oven. Exactly 2g of the sample was weighed into petri dish. The weight of the petri dish and sample is noted before drying. The petri-dish containing the sample was put in the oven and heated at 100 ^{0}Cfor 1 hour, the result was noted and then heated another 1 hour until a steady result was obtained and the weight noted". [9]

The drying procedure was continued until a constant weight was obtained.

% moisture content = $\frac{(W_1 - W_2)}{W1} \times 100$ **Equation 1**

Where:

W_1 = weight of petri dish and sample after drying

W_2 = weight of petri dish

Wt = weight of sample

Ash Content Determination

"Ash content was determined according to the standard method of Empty platinum crucible was washed, dried and the weight noted, exactly 2 g of wet sample was weighed into the platinum crucible and placed in a muffle furnace at 500 ^{O}C for 3 hours. The sample was cooled in desiccators after burning and weighed". [10] % Ash content = $W_3 - W_1$ x 100: **Equation 2**
$W_2 - W_1$
Where,
W_3 = weight of crucible and ash
W_2= weight of crucible and sample
W_1 = weight of crucible

Fiber Content Determination

Fiber content was determined according to the standard method of AOAC 2010 [9]. About 2 g of the sample was defatted with petroleum. The sample was boiled under reflux for 30 minutes with 100ml of a solution containing 1.25% of H_2SO4 per100 mL of solution. The solution was filtered through several layers of cheese cloth on a fluted funnel, washed with boiling water until the washings were no longer acid.

The residue was transferred into a beaker and boiled for 30minutes with 100 mL of a solution containing 1.25NaOH per 100 mL. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, dried in an electric oven and weighed, incinerated, cooled and weighed. The loss in weight after incineration x 100 is thepercentage of crude fiber
% crude fiber = weight of fiber x 100 **Equation 3**
Weight of sample

Protein Content Determination

"Protein content was determined according to the standard method of Anarado*et al.*, 2021b" [11]. "Exactly 0.5 g of sample was weighed into a 30 mL kjehdal flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were stoppered and shaken). Then 0.5 g of the kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire

until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling, about 100 mL of distilled water was added to avoid caking, 50 mL was transferred to the kjedahl distillation apparatus. A 100 mL receiver flask containing 5mL of 2 % boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1drop of methylene blue was placed under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution. The 5mL of 40 % sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops got intothe receiver flask, after which it was titrated to pink colour using 0.01 N Hydrochloric acid". [11]

Calculations:

$$\% \text{ Nitrogen} = \frac{\text{Titre value} \times 0.01 \times 14 \times 100}{\text{Weight of sample}} \quad \textbf{Equation 4}$$

% Protein = % Nitrogen x 6.25

Fat Content Determination

"Fat content determination was determined according to the standard method of Anarado*et al.*, 2021a" [10]. "Exactly 250 mL clean flask was dried in oven at105 to 110 ^{0}C for about 30 minutes. It was transferred into a desiccator, allowed to cool andthe weight noted. The flask was filled with about300 mL of petroleum ether (boiling point 60 ^{0}C), the extraction thimble was plugged lightly with cotton wool and soxhlet apparatus assembled. The set-up was allowed to reflux for about 6hours. The thimble was removed with care and petroleum ether collected in the top container of the set-up, drained into a container for re-use. When flask was almost free of petroleum ether, it was removed and dried at 105 to 110 ^{0}C for 1hour, transferred from the oven into a desiccators and allowed to cool". [10] The % of the fat was calculated as:

$$\% \text{ fat} = \frac{\text{weight of fat} \times 100}{\text{Weight of sample}} \quad \textbf{Equation 5}$$

Carbohydrate Content Determination

Carbohydrate content was determined according to the standard method of AOAC (2010) [9]. % Carbohydrate = 100 – (% Protein + %Moisture + % Ash + % Fat + % Fibre): **Equation 6**

Qualitative and Quantitative Analyses

Qualitative and Quantitative tests were carried out on the samples to determine the presence of the phytochemicals in the powdered samples.

- Alkaloids- Mayer's Test
- Flavonoids- Ammonium Test
- Terpenoids-Salkowski test
- Steroids-Liebermann-Burchard's test
- Glycosides-Keller Killiani test
- Saponins-Frothing Test
- Tannins- Ferric chloride Test [12,13].

The quantitative phytochemical screening was done using standard methods as described by [12].

Anti-Hyperglycemic Assay

The alpha-amylase inhibitory test of stem bark of *Napoleona vogelii*and root of *Cassia occidentalis* was performed using a protocol previously reported by [14], with minor modifications. In summary, 0.5 mL 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: % inhibition of α−Amylase = Abs (control) – Abs (Sample) × 100 / Abs (control) Where; Abscontrol corresponds to the absorbance of the solution without extract (buffer instead of extract) and with α -amylase solution, and Abssample corresponds to the solution with extract and α -amylase solution.

In vitro evaluation of yeast cell uptake of glucose The yeast cell uptake of glucose by the methanol extracts of both plants was done using a procedure previously reported by Anarado *et al*., 2022b [15]. 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 37 ^{o} 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.

% increase in glucose uptake = Abscontrol – Abssample × 100 / Abscontrol

Where, Abscontrol is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbanceof the test sample [15].

3. Results and Discussion

Table 1: Qualitative phytochemical composition of different extracts of *N.vogelii*and of *C. occidentalis*.

Phytochemicals	NvSM	NvSE	NvSH	CoRM	CoRE	CoRH
Saponins	+	-	-	+	-	-
Flavonoids	+	+	+	-	+	+
Alkaloids	+	++	++	+++	++	+
Tannins	++	+	-	+++	++	+
Steroids	-	+	+	-	-	-
Terpenoids	++	++	+	+++	+	++

Glycosides	+	-	-	++	+	+

Table 2: Quantitative phytochemical composition of *N. vogelii* stem bark and *C. Occidentalis* Root

Parameter	***N. vogelii* stem bark**	***C. Occidentalis*Root**
Alkaloids(%)	14.07±0.12	2.50±0.50
Flavonoids(%)	3.00±0.00	1.75±0.25
Phenolics (mg/g)	7.19±0.85	2.48±0.40
Saponins (%)	8.00±0.00	4.50±0.25
Tannins (mg/100g)	4.13± 0.50	5.56± 0.06

Table 3: Proximate composition of *N. vogelii* stem bark and *C. Occidentalis* Root

Parameter	***N. vogelii* stem bark**	***C. Occidentalis*Root**
Moisture content	4.00±0.0000	5.40±0.60
Ash content	5.00±0.00	6.00±0.00
Crude fiber	40.2833±0.49	22.33±0.08
Protein	7.4500±0.45	10.72±0.05
Carbohydrates	37.0500±0.05	44.70±0.93
Fats/oil content	6.50±0.50	11.00±0.00

Table 4: α-amylase inhibitory effect of *N. vogelii* stem bark and *C. occidentalis*root extracts

Concentration of sample (mg/mL)	**NvSM (%)**	**NvSE(%)**	**NvSH(%)**	**CoRM(%)**	**CoRE(%)**	**CoRH(%)**

0.10	23.00±0.00	10.74±0.77	15.05±1.02	38.27±0.46	21.40±0.50	15.14±0.02
0.20	31.40±0.00	13.24±2.15	21.42±0.58	49.15± 0.22	38.68±1.30	16.25±0.15
0.50	36.04± 0.03	22.58±1.66	21.90±0.00	50.02± 0.03	45.00±0.00	20.08±1.25
1.00	35.61± 0.01	28.09±0.16	3318±1.20	47.35± 0.24	46.80±0.20	31.11±2.03
Metronidazol	56.00±0.00	56.00±0.00	56.00±0.00	56.00± 0.00	56.00±0.00	56.00±0.00

Table 5: Glucose uptake in yeast cells treated with *N. vogelii* stem bark and *C. occidentalis* Root extracts

Concentration of extract (mg/mL)	**NvSM (%)**	**NvSE(%)**	**NvSH(%)**	**CoRM (%)**	**CoRE(%)**	**CoRH(%)**
0.10	22.33±0.16	17.44±0.46	05.37±1.21	37.60±0.35	22.56±0.70	30.90±2.00
0.20	27.00 ± 0.00	19.50±0.55	06.88±0.43	41.33±1.15	27.58±1.40	36.08±1.10
0.50	27.70 ± 0.35	32.04±1.35	08.57±2.12	47.53±0.47	49.98±0.34	36.66±0.00
1.00	30.33± 0.35	46.00±0.00	11.80±0.60	53.20±0.17	50.99±2.22	44.17±0.40
Metronidazol	68.44±0.00	68.44±0.00	68.44±0.00	68.44±0.00	68.44±0.00	68.44±0.00

Note: NvSM, NvSE, NvSH- methanol, ethyl acetate and n-hexane stem bark extracts of *Napoleonavogelii*respectively **.**

CoRM, CoRE, CoRH- methanol, ethyl acetate and n-hexane root extracts of*Cassia occidentalis.*

Alkaloids and terpenoids were present in all the extracts of both plants. Flavonoids were present in all extracts of *N. vogelii,* whereas tannins were present in all extracts of *C. occidentalis*.

Ploarity of the solvents played a vital role in the constituents present in each extract in both plants. Steroids which are known to be hydrophobic were found in only non-polar n-hexane extract of *N. vogelii*, but were found absent in methanol and ethyl acetate extracts. This report supported our prvious findings [11] . The

presence of saponins in the methanolic extracts of both plants gave credence to the reports of Widyawati *et al.*, 2014 and Ghasemzadeh *et al.*, 2011 who revealed that saponins increased with increase in the polarity of the solvent [16,17] .

N. vogelii stem bark contained crude fibre most, whereas *C. occidentalis* root contained carbohydrates most.

All the extracts from both plants showed α-amylase inhibitory activity in a concentration-dependent manner, except the methanol extract of *C. occidentalis.*

All the extracts from both plants also exhibitedglucose uptake activity in a concentration-dependent manner.

Cassia occidentalis root extracts showed more anti-diabetic activity than the stem bark extracts of *N. vogelii* in all concentrations. This result is in line with the work of Verma *et al.,* (2010) [18] who analysed the "Petroleum ether, chloroform and aqueous extract of whole plant of *Cassia occidentalis* at the concentration of 200 mg/kg for hypoglycemic effect in normal and alloxan-induced diabetic rats. They reported that Aqueous extract of *C. occidentalis* produced a significant reduction in fasting blood glucose levels in the normal and alloxan-induced diabetic rats. Apart from aqueous extract, petroleum ether extract showed activity from day 14 and chloroform extract showed activity from 7 days". "Alloxan-induced diabetic mice orally administered with methanolic extracts of *C. occidentalis* leaves at the dose of 300 mg/kg produced significant fasting blood glucose lowering activity in 6 and

12 hour samples compared with the control. An increase in the dose of *C. occidentalis* to 450 mg/kg showed more observable hypoglycemic activity in the diabetic mice" [19] . Our result is also in line with the work of Arya *et al.*, 2013 [20] , they reported that "aqueous extract of *Cassia occidentalis* exhibited significant anti-hyperglycemic/antidiabetic activity in normal and alloxan-induced rats. In normal rats, administration of aqueous extract showed 6.50%, 10.29%, and 7.21% decline in the blood glucose levels on 1, 2 and 3 h, respectively. Alloxan-induced diabetic rats administered with aqueous extract showed 4.15%, 6.52%, and 8.56% decline in the blood glucose level on 1, 2, and 3 h, respectively, whereas they showed 12.63%, 22.38%, 30.41%, and 38.19% decline in the blood glucose level on 4, 5, 6, and 7th day, respectively".

Tannins, alkaloids and flavonoids found in the extracts may be responsible for their antidiabetic activities and folkloric use of the plants in the treatment of diabetes[21,22,23] . Alkaloids have also been reported to exhibit antihyperglycemic effect by lowering of blood glucose level due to similarity in structure and action with b3-AR agonists and improving the action of insulin by triggering AMPK; reducing insulin *in vivo* [24].

The presence of terpenoids could also be responsible for the antidiabetic activities of the extracts [25] .

4. Conclusion

The methanol, ethyl acetate and n-hexane extracts of *N. vogelii* and *C. occidentalis* contained some important phytochemicals such as tannins, alkaloids and flavonoids which have been reported to have antidiabetic properties. The extracts also showed anti-hyperglycemic activities *in vitro* by inhibiting the α-amylase as well as increased the glucose uptake, this justified the ethnomedicinal use of the plants in treating diabetes and thus the plants extracts could be used as antidiabetic agents.

5. References

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