International Journal of Applied Chemical and Biological Sciences (2022), 3(2), 50-57
ISSN 2582-788X

RESEARCH PAPER

Article Identifier: https://identifier.visnav.in/1.0001/ijacbs-22c-23009/

Comparative study of in vitro hypoglycemic activity of saponin and flavonoid extracts of *Luffa cylindrica* L.

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Received on: 23 March 2022 *Published on: 13 April 2022*

ABSTRACT

Diabetes mellitus is a long-lasting metabolic disorder in which the use of carbohydrate is impaired and is caused by loss of insulin or insulin resistance. The aim of this study was to determine hypoglycemic activity of crude saponin and flavonoid extracts of *Luffa cylindrica* L. leaf on alpha-glucosidase enzyme inhibition. Phytochemical analysis of aqueous leaf extract, extraction of crude flavonoid and saponin extracts of *L. cylindrica* were carried out using standard procedures. Hypoglycemic activity of crude saponin and flavonoid extracts were determined by in vitro alpha-glucosidase enzyme inhibition. The qualitative phytochemical composition showed the presence of alkaloids, flavonoids, phenols, tannins, saponins and terpenoids while the quantitative phytochemical compositions indicated that flavonoids had the highest concentration of 437.18±0.01mg/100g and terpenoids had the least concentration of 72.88±0.33mg/100g. There was a high significant ($p<0.05$) inhibitory activity of the extracts on alpha-glucosidase enzyme with increase in concentration. Saponins had higher percentage inhibition of 53.32±0.15 % (IC_{50} 49.12±0.35 µg/mL) while flavonoids had (significant) lower inhibition of 44.13±0.30 % (IC_{50} 52.76±0.34 µg/mL) at 200 µg/ml. Metformin percentage inhibition (84.82±0.15 % with IC_{50} 35.57±0.24 µg/mL) was significantly higher than the two extracts at 200µg/ml. The results from this study suggest that saponins and flavonoids extracted from ethanol leaf extract of *Luffa cylindrica* had some hypoglycemic activity which supports its traditional usage in the management of diabetes and its complications.

Keywords: *Luffa cylindrica*, metformin, hypoglycemic, alpha-glucosidase

1. INTRODUCTION

Diabetes is a metabolic disease affecting carbohydrate, protein, and fat metabolism. It results in hyperglycemia as a result of insulin deficiency, insulin action, or both [1]. As one of the most serious endocrine syndromes, diabetes mellitus is considered to be one of the most dangerous. It's a metabolic disorder that's becoming more common around the world. Glucose homeostasis is regulated by insulin, which plays a key role in this process. Insulin deficiency affects the metabolism of carbohydrates, fats, and proteins. The medical community is still struggling to find a way to manage diabetes without causing side effects. Hyperglycemia, glycosuria, hyperlipidemia, a negative nitrogen balance,

and sometimes ketonemia are the symptoms of this condition. When beta-pancreatic cells fail to secrete insulin, it results in type I diabetes [2]. However, type 2 diabetes is characterized by a gradual increase in insulin resistance during the early stages [3].

A lot of plants have been used for a long time as traditional medicine. Though there may not be enough scientific evidence to support their efficiency, some do appear to be effective [4]. Everything was based on experience because there wasn't enough information at the time about the causes of the ailments or which plants could be used as a remedy. It was not long before the discovery of the reasons behind using specific medicinal plants for the treatment of certain diseases. This led to a gradual shift away from the quack framework and towards explanatory facts [5].

Luffa cylindrica (sponge gourd) is one of the medicinal plants used in Shiroro, Niger State, to manage and treat various types of nutrients [3]. It's also known as sponge luffa, climbing okra, and Chinese okra, among others. Full-grown fruit is fibrous and inedible and is used to make scrubbing bath sponges instead of its younger fruit [6]. *Luffa cylindrica*, a member of the Cucurbitaceae family, is a popular vegetable around the world. Its fruit extract has been found to be effective as an excellent hypoglycemic agent [7].

The alpha-glucosidase enzyme is very important in carbohydrate metabolism. It catalyzes disaccharides (byproducts of polysaccharides degradation by alpha-amylase) tomonosaccharides. This enzyme is inhibited by saccharides, which function ashardies in the small intestine. A decrease in glucose absorption is postulated if the alpha-glucosidase activity is inhibited. As a result, the reduction of postprandial blood glucose levels may be enhanced. Hence, reducing post prandial glucose levels in the blood by inhibiting the alpha-glycosidase enzyme can be a primary technique for managing blood glucose levels in the body [8]. This study will emphasize comparative studies on in vitro hypoglycemic activities of saponin and flavonoid extracts of *L. cylindrica*.

2. METHOD AND MATERIALS

2.1. Collection and identification of plant material

Fresh leaves of *Luffa cylindrica* were collected from Dnaiko - Chikwainya village, Shiroro Local Government Area of Niger State in the month of May 2021. The leaves of *L. cylindrica* were identified at the Department of Medicinal Plant Research and Traditional Medicine (MPR & TM), Herbarium, and Ethnobotany Unit at National Institute for Pharmaceutical Research and Development (NIPRD) Idu - Abuja and was given the voucher NIPRD/H/7236. The leaves were washed, air-dried for 6 days, and powdered with pestle and mortar into homogeneous powder. The powdery sample of the plant was stored in a well-labeled and clean container until needed for analysis.

2.2. Preparation of Aqueous Extract

About 20 g of *L. cylindrica* leaf powder sample was added to 200 mL of distilled water at 25 °C and plugged with aluminum foil paper. Cheese cloth was used to filter the mixture and then through the filter paper after 24 hrs. The filtrate was then concentrated in a water bath at 35°C (Gallenkamp Precision Water Bath 186) [9].

2.3. Preliminary phytochemical screening of the aqueous leaf extract of L. cylindrica

Qualitative phytochemical analysis of aqueous extracts of *L. cylindrica* was conducted on the extract using the conventional procedure to determine the contents [10-12].

2.3.1. Quantitative phytochemical screening of aqueous L. cylindrica leaf extract

i. Determination of total flavonoids

Total flavonoid was determined using the aluminum chloride colorimetric technique [13]. Quercetin was used

to generate the calibration curve. 0.5 mL of diluted sample was pipetted to the test tube, which contained 1.5 mL of methanol. This was followed by 2.5 mL of distilled water, which was followed by 0.1 mL of 10 % AlCl3 solution and 0.1 mL of sodium acetate (NaCH3COO). Their reaction combination was measured at 415 nm (VIS–SPECTROPHOTOMETER 721) at room temperature after 30 minutes of incubation. Distilled water was substituted for the 10 % AlCl3 in the blank.

ii. Determination of total alkaloids

The sample of 0.5 g was dissolved in 96 % ethanol and 20 % H_2SO_4 (1:1) [14]. It was then added to 5 mL of 60 % tetraoxosulfate (VI) and left to stand for 5 minutes before being filtered out again. Followed by 0.5 % formaldehyde was added to 5 mL of water, which was left to stand for 3 hours. The reading was taken at a wavelength of 565 nm wavelength, which corresponds to the absorbance. Using vincristine's extinction coefficient (E296, Ethanol ETOH =15136M1cm^1) as a reference alkaloid.

iii. Determination of saponins [15]

The 0.5 g sample was boiled in 1N HCl for 4 hours to extract saponins. Filtered after cooling, 50 mL of petroleum ether was added to the filtrate and evaporated until it was dry. The residue was then treated with 5 mL of acetone ethanol. Three distinct test tubes were filled with 0.4 mL of each sample. It was followed by 2 mL of conc. H2SO4 before adding 6 mL of ferrous sulfate reagent. After 10 minutes of mixing, the absorbance at 490 nm was measured. To create the calibration curve, standard saponin was used as a standard.

iv. Determination of tannins [16]

About 0.02 g of sample was immersed in a water bath at 77-80 °C with 20 mL of 50 % methanol for 1 hour. Shaking it vigorously ensured a homogeneous mixing. A double-layered Whatman No.41 filter paper was used to filter the extract into a 100 mL volumetric flask, to which 20 mL of water, 2.5 mL Folin-Denis reagent, and 10 mL of Na_2CO_3 were added, and the flask was thoroughly shaken. After 20 minutes, the mixture developed a bluish-green color. After color development, the absorbance of the tannic acid standard solutions and samples were measured on a UV-spectrophotometer model 721 at 760 nm.

v. Quantitative test for terpenoids

About 100 mg of dried plant extract (wi) was soaked in 9 mL of ethanol for 24 hours [17-18]. After filtering, the mixture was extracted with 10 mL of petroleum ether. Glass vials were filled with ether extract and allowed to dry completely (wf). When the ether was evaporated, the yield (%) of total terpenoids was quantified by the equation 1

$$wi\text{-}wf / wi * 100 \quad \text{...eq. 1}$$

vi. Determination of total phenols

About 2 g sample was defatted with 100 mL of diethyl ether using soxhlet apparatus over a period of 2 hours [19]. Oil ether was used to extract the phenolic component from the fat-free sample, which was heated for 15 minutes with 50 mL of the solvent. 5 µL was taken from the extract and 10 mL of distilled water was added to the flask. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were also added. In order to develop the color, 30 minutes of reaction time was given to each sample. A 505 nm wavelength was used to measure the wavelength of this reaction mixture. Calibration curves were created using tannic acid.

2.4. Extraction Crude Saponins

About 40 gms of powdered sample was heated for 4 hours at 55 °C with 200 mL 40 % ethanol and then filtered. The residue was re-extracted with 400 mL of 40 % ethanol and filtered as well. The resultant extract was

concentrated in a water bath until the volume was reduced to 40 mL. Then it was combined with 20 mL n-hexane in a separating funnel. The mixture was violently shaken, and the separating funnel was placed on a stand until the aqueous and n-hexane layers developed. The aqueous portion was collected whereas the n-hexane portion was thrown discarded. To the aqueous layer, was added 60 mL of n-butanol, which was mixed vigorously. As a result, all of the n-butanol was extracted four times and then treated with 10ml of 5 % NaCl solution. A water bath was used to concentrate and dry the n-butanol (crude saponin) extract [20].

2.5. Extraction of Crude Flavonoids

The extraction of crude flavonoids was done using the combined methods by Dianur *et al*., (2020) and Awraris *et al*., (2016) [21-22]. The extraction started by mixing 40 g of sample with 200 mL of 1 % HCl in 80 % ethanol (1 % HCl to break glycosidic linkage of flavonoids). The mixture was left in cold maceration for 3 days. The mixture was filtered through cheesecloth and then filter paper. It was concentrated at 40 mL. The mixture was mixed with 20 mL n-hexane in a separating funnel (n-hexane to defat the plant material) and then-hexane fraction was discarded. 60 mL of ethyl acetate was added to the ethanol layer and mixed properly. This process was repeated 4 times and all the ethyl acetate layers were collected and concentrated to dryness in a water bath at 35 °C as flavonoid-rich fraction.

2.6. Determination of alpha-glucosidase enzyme inhibition

To determine the inhibitory activity of alpha-glucosidase, 1 mL of a solution of the starch substrate (2 % w/v maltose), 1 mL 0.2 M Tris-Chloride buffer pH 8.0 and various concentrations of metformin, crude saponin, and flavonoid extracts of the *Luffa cylindrica* leaf were pipetted into each test tube and incubated (Gallenkamp Economy Incubator size 1) for 5 minutes at 37 °C. To the blank test tube, distilled water was added instead of plant extract as a control. To begin the reaction, 1 ml of the alpha-glucosidase enzyme (1 U/mL) was added to each test tube, followed by 40 minutes of incubation at 35 °C. 2 mL of 6N HCl was added to stop the process. This was followed by a measurement of the color intensity at 540 nm (UV-VIS Spectrophotometer 752) [23-24].

To Compute 50 % Inhibitory Concentration (IC50) the percentage of inhibitory activity at five different levels of plant extracts [25]. And it was calculated using the equation 2,

$$\%\text{Inhibition} = \frac{(Ac-As)}{Ac} * 100$$

Where,
Ac = the absorbance of the control and
As = the absorbance of the sample

2.7. Statistical Analysis

The statistical data analysis was performed using Analysis Of Variance (ANOVA) at a level of confidence of 5% ($p<0.05$), using the SPSS analytical program. Means ± Standard Errors of percentage (%) inhibition were used to express the findings of the study.

3. RESULTS AND DISCUSSION

3.1. Qualitative Phytochemical Screening

The preliminary phytochemical screening tests for the aqueous extract of *L. cylindrica* Linn leaf revealed the presence of six phytochemicals (Table 1). Phytochemical compounds such as alkaloids, steroids, flavonoids, phenols, tannins, saponins, and terpenes were screened in the extract.

In the human body, these phytochemical constituents interact directly or indirectly with the body's chemistry. Once the active constituents are absorbed into the blood, these constituents derive the required benefits by circulating and influencing the bloodstream [26]. This is related to the work of Omagha *et al*., who reported the

presence of saponin in *M. indica* [27]. Saponins have been reported by Ng *et al*., (1986) to stimulate the

release of insulin and block the formation of glucose in the bloodstream [28]. Vessal *et al*., (2003) reported that flavonoids suppress the glucose level, reduced plasma cholesterol, and triglycerides significantly, and increased their hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets [29].

3.2. Quantitative Phytochemical Constituents

The quantitative phytochemical composition of *L. cylindrica* leaf aqueous extract is presented in Table 2 in mg/100g. Flavonoids (437.18±0.01 mg/100g) concentration was significantly higher than saponins (123.54±0.02 mg/100g). Terpenoids had the least concentration of (72.88±0.33 mg/100g).

The result from the quantitative analysis shows that flavonoids are the most abundant of the phytochemical constituents of *L. cylindrica* leaf. Saponins were also found in a significant ($p<0.05$) amount and the least among the phyto-constituents quantified in the plant leaf were terpenoids. Phyto-constituents of plants are responsible for their therapeutic properties, saponins and flavonoids have been widely reported to have hypoglycemic properties [28-29]. The presence of a high concentration of flavonoids in *L. cylindrica* leaf could be responsible for the significant alpha-glucosidase inhibition, this is in correlation with the report of Sundar and Vijey, (2017) [8].

3.3. Hypoglycemic Effect of Crude Saponin and Flavonoid Extracts on Alpha-Glucosidase Enzyme

The in vitro hypoglycemic activity of crude saponin and

Table 1. Qualitative compositions of *L. cylindrica* leaf

Phytochemicals	Inference
Alkaloids	Present
Steroids	Absent
Flavonoids	Present
Phenols	Present
Tannins	Present
Saponins	Present
Terpenes	Present

Table 2. Quantitative phytochemical compositions of *L. cylindrica* leaf

Phytochemicals	Concentration (mg/100g)
Flavonoids	437.18 ± 0.01^{a}
Alkaloids	253.00 ± 18.00^{b}
Saponins	123.54 ± 0.02^{e}
Tannins	132.05 ± 0.03^{d}
Terpenoids	72.88 ± 0.33^{f}
Phenols	143.35 ± 0.01^{c}

Table 3. Inhibitory effect of crude saponin and flavonoid extracts on alpha-glucosidase enzyme

Concentration (µg/ml)	Metformin	Saponins (%Inhibition)	Flavonoids
40	28.57 ± 0.22^{e}	8.67 ± 0.08^{e}	6.98 ± 0.30^{e}
80	47.02 ± 0.15^{d}	16.62 ± 0.08^{d}	15.45 ± 0.15^{d}
120	61.76 ± 0.15^{c}	33.05 ± 0.30^{c}	29.73±0.34c
160	76.75 ± 0.26^{b}	42.50 ± 0.11^{b}	41.26 ± 0.34^{b}
200	84.82 ± 0.15^{a}	53.32 ± 0.15^{a}	44.13 ± 0.30^{a}
IC_{50}	35.57 ± 0.24^{f}	49.12 ± 0.35^{f}	52.76 ± 0.34^{f}

Each value is a mean ± standard error based on a three-replicate analysis. A significant difference ($p<0.05$) exists between means in the same column with different superscripts

crude flavonoid extracts of *L. cylindrica* leaf are presented below (Table 3) showing the percentage inhibition of 40, 80, 120, 160, 200 µg/mL respectively using metformin as standard. The inhibitory effect was concentration-dependent. The highest percentage inhibition (53.32±0.15 %) was observed in saponins extract at 200 µg/ml with the IC_{50} 49.12±0.35 µg/ml while flavonoids had 44.13±0.30 %, with the IC_{50} 52.76±0.34 µg/ml. Metformin was significantly higher than the two extracts with inhibitory activity of (84.82±0.15 %, IC_{50} 35.57±24 µg/ml).

The digestive enzyme, alpha-glucosidase is a carbohydrate-hydrolase that catalyzes disaccharides to monosaccharides, which leads to postprandial hyperglycemia [8]. From the result of hypoglycemic activity of the extracts obtained, it showed a dose-dependent increase in percentage inhibitory activity against alpha-glucosidase enzyme for all extracts. There was a highly significant ($p<0.05$) inhibitory activity of the extracts on alpha-glucosidase enzyme with an increase in concentrations. Saponins had higher inhibitory activity on alpha-glucosidase enzyme and thus lower IC_{50} when compared to flavonoids at varying concentrations. This may be due to the fact that saponins are reported by Pan *et al*., to have inhibitory activity on alpha-glucosidase enzyme and decrease glucose uptake through the intestinal epithelium [30]. Metformin inhibitory activity on the enzyme tends to be significantly higher than the two extracts. This could be due to the fact that Metformin is linked to improving peripheral sensitivity to insulin, through a stimulated tissue glucose uptake by a transporter link system [31]. This work was related to the work by Sundar and Vijey, (2017) that showed significant inhibitory activity of the enzyme with an increase in the concentration of ethanolic extract [8].

Furthermore, in both alloxan and fructose-induced hyperglycemia, saponins have been found to elicit a decrease in glucose concentration, which could be attributed to the presence of triterpenoid. According to other research, saponins were effective in reducing the effects of experimental hyperglycemia caused by adrenaline, glucose, and alloxan [32]. Saponins from *Entada phaseoloides* (L) were also employed by Tao and colleagues to treat type 2 diabetic rats with hyperglycemia, hyperlipidemia, and insulin resistance [33].

4. CONCLUSION

The crude saponins and flavonoids extracted from the ethanol leaf extract of *L. cylindrica* showed some hypoglycemic activity at the doses used. This justifies its traditional use in the management of diabetes and its complications, and further supports earlier reports on the use of the leaf in hypoglycemic studies.

It is recommended that further studies on the isolation, purification and identification of active compounds, in vivo hypoglycemic activity, and the mechanism of action of this medicinal plant should be carried out.

5. ACKNOWLEDGEMENT

The author would like to sincerely acknowledge his supervisor for immense effort towards making this work a success. The author would also like to appreciate his parents and siblings for their supports.

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

NA

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