RESEARCH ARTICLE

Diabetes Management

Assessment of phytochemical, antibacterial and antidiabetic properties of extract of *Luffa cylindrica*

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ABSTRACT

Traditional medicine also known as indigenous or folk medicine comprises of knowledge systems that developed over generations within various societies before the era of modern medicine. Plants are generally used for treatment of bacterial infections though they are not clinically regulated due to lack of awareness and enough data to support the reported therapeutic claims. The aim of this research was to investigate chemical composition, antidiabetic and antibacterial activities of *Luffa cylindrica*. The proximate composition of Luffa cylindica leaf and stem were carried out. There was a relatively high carbohydrate content but *Luffa cylindrica* stem had the highest percentage of carbohydrate content. The leaf had a low fat content but the stem had the lowest percentage. The luffa leaf had more protein content than the stem and also the crude fiber content of luffa stem was higher than the leaf. The ash content of luffa leaf is relatively higher than the stem. The quantitative analysis of *Luffa cylindrica* indicated that alkaloids were highly present, flavonoids, tannins, antioxidant ORAC, terpenoids, saponins, steroids, oxylates, phytates and there was a very low presence of cardiac glucosides and phenols. The metal composition of luffa leaf and stem was carried out and the result indicated the presence of Ca^{+}, Fe^{++}, Mg^{+},Zn^{+}, Mn^{+}, K^{+}, Po^{+} but Ca^{+} had the highest presence and Zn^{+} had the lowest presence in the metal composition of Luffa leaf and stem. The alpha glucosidase and amylase *in-vitro* anti-diabetic test was carried out. This was done to check for antidiabetic constituents of *Luffa cylindrica* leaf and stem of which *Luffa cylindrica* stem shows more antidiabetic contents than the leaf. Antibacterial effect of methanol extract of *Luffa cylindrica* leaf and stem was carried out on some bacterial isolates (*Salmonella typhi* ATCC 13311, *Shigella dysentariae* ATCC 49556, *E. coli* ATCC 35928, *Streptococcus pneumoniae* ATCC 49619, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Based on the results obtained in this research study, it can be said that the plant has a good antidiabetic efficacy. However, there is need for good hygiene practice during preparation.

Introduction

Luffa cylindrica is derived from the cucumber and marrow family and originates from America. *Luffa cylindrica* is commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, is a member of cucurbitaceous family. Luffa sponge is a lignocellulosic material composed mainly of cellulose, hemicelluloses and lignin. The fibers are composed of 60% cellulose, 30% hemicellulose and 10% lignin. The fruits of *L. cylindrica* are smooth and cylindrical shaped. In oriental medicine, *L. cylindrica* has effect on the treatment of fever, enteritis and swell etc. The extracts from vines alive are used as an ingredient in cosmetics and medicine. They are used for bathing, removing toxins and regenerating the skin. They help varicose veins and cellulite by stimulating circulation. Immature fruit is used as vegetables, which is good for diabetes [1].

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KEYWORDS

- *luffa cylindrica*
- proximate analysis
- leaf
- stem
- constituents

Methodology

Collection and extraction of samples

Luffa cylindrica leaves and stem used for this research were obtained from Ibuji forest along Akure road, Ondo state. The extraction of *Luffa cylindrica* leaf and stem were carried out separately using methanol as solvent. They were washed and air dried for 30 days. After which the washed *Luffa cylindrica* leaf and stem were ground to fine powder separately, with the use of an electric blender [2].

Proximate analysis: Crude fat, Carbonhydrate, protein, Moisture content, Crude Fibre and Ash.

***Phytochemical analysis*:** Phytochemical analysis of the extract was carried out quantitatively and qualitatively. Test on the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, Carotenoids, steroids, saponins, phytates were conducted accordingly [3].

Determination of anti-oxidant capacity: Antioxidant capacity of free and bound phenolic extracts was measured by using DPPH scavenging as previously described.

Determination of antinutrients: This was carried out using the method.

Determination of *in-vitro* antidiabetic assay: Alpha α-amylase inhibitory assay and Alpha-glucosidase inhibitory assay using the method.

Determination of minerals and vitamins: Ascorbic acid, Thaimin, Ribloflavin was determined.

Structural elucidation of the plant extracts: Structural elucidation of the crude extract was determined using GC-MS adopting the method.

Screening of test organisms

Bacterial isolates obtained were collected from preserved nutrient agar slant in microbiology laboratory of Multisystem hospital of Afe babalola University Ado-Ekiti, Ekiti state, Nigeria. The isolates include *Escherichia coli* ATCC 35928, *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, S. dysentariae* ATCC 49556, *S. typhi* ATCC 13311, *S. pneumoniae* ATCC 49619 and *Klebsiella*. The stock cultures were inoculated into freshly prepared nutrient agar to test for their viability. They were stored at controlled temperature for subsequent antibacterial testing. Further sub-culturing was carried out until pure cultures of the isolates were obtained [3,4].

Antibacterial susceptibility testing: The test of Antibacterial susceptibility test was performed by agar-well diffusion method using Mueller-hinton agar.

Antibiotics susceptibility testing: This test was carried out in order to determine whether or not an etiological agent is sufficiently sensitive to a particular antimicrobial agent to permit its use for treatment. The test was carried out with disc containing known concentrations of the antibiotics to be used for both gram positive and negative bacteria. The test was carried out by making an even spread of the pure islotes on prepared Mueller-hinton agar using sterile swab sticks and aseptic placement of the particular disc meant for gram positive and negative bacteria. The plates containing the disc were then incubated at 37°C for 24 hours. The plates are evaluated based on the sizes of the zones of inhibition of each of the antimicrobial agents [5,6].

Statistical analysis

All experiments were carried out in triplicates, the data are mean ± SD error and the mean values were analyzed.

Results

Proximate composition of *Luffa cylindrica* leaf and stem

The proximate composition of Luffa cylindica leaf and stem were carried out (TABLE 1). There was a relatively high carbohydrate content of both samples, but *Luffa cylindrica* stem had the highest percentage of carbohydrate content. Both samples had low fat content but the stem had the lowest percentage. The luffa leaf had more protein content than the stem and also the crude fiber content of luffa stem was higher than the leaf. The ash content of luffa leaf is relatively higher than the stem [7].

TABLE 1: Proximate composition of *luffa cylindrica* leaf and stem.

Parameters	Leaf	Stem
Moisture content	8.7 ± 0.1	8.17 ± 0.15
Protein	24.47 ± 0.15	11.87 ± 0.15
Ether extract (fat)	0.73 ± 0.15	0.3 ± 0.1
Ash	9.17 ± 0.21	6.77 ± 0.15
Crude fiber	10.57 ± 0.15	18.47 ± 0.15
Carbohydrate	46.37 ± 0.40	54.43 ± 0.42

■ Phytochemical composition of *Luffa cylindrica* leaf and stem

Phytochemical composition was carried out on both *Luffa cylindrica* leaf and stem (TABLE 2) to check for the presence of phytochemical constituents which included alkaloids, phenols, saponins, tannins, cardiac glucosides, cyano glycosides, terpenoids, phytates, protease inhibitors, oxalates, and steroids. The result of luffa stem and leaf showed that alkaloids were highly present; flavonoids were present, tannins, antioxidant ORAC, terpenoids, saponins, steroids, oxalates, phytates and a very low presence of cardiac glucosides. Cyano glycoside and protease inhibitors were not detected in the phytochemical composition for *Luffa cylindrica* leaf and stem [7].

TABLE 2: Phytochemical composition of *luffa cylindrica* leaf and stem.

Parameters	Leaf	Stem
Alkaloids	1463.33 ± 20.82	761.67 ± 12.58
Flavonoids	388.38 ± 7.64	35.00 ± 5.00
Tannins	566.67 ± 7.64	536.67 ± 10.40
Phenol	63.43 ± 0.20	43.73 ± 0.20
Antioxidant ORAC	53.17 ± 0.21	45.17 ± 0.15
Terpenoids	126.67 ± 7.64	65.00 ± 5.00
Saponins	168.33 ± 7.64	65.00 ± 5.00
Cardiac glycosides	1.17 ± 0.29	2.17 ± 0.29
Cyano glucoside	ND	ND
Phytates	70.00 ± 5.00	30.00 ± 5.00
Protease inhibitors	ND	ND
Oxylates	46.67 ± 7.64	23.33 ± 2.89
steriods	90.00 ± 5.00	45.00 ± 5.00

■ Metal composition of *Luffa cylindrica* leaf and stem

The metal composition of luffa leaf and stem were carried out (TABLE 3) and the result indicated Ca^{+}, Fe^{++}, Mg^{+},Zn^{+}, Mn^{+}, K^{+}, Po^{+} but Ca^{+} had the highest presence and Zn^{+} had the lowest presence in the metal composition of Luffa leaf and stem.

TABLE 3: Metal composition of *luffa cylindrica* leaf and stem.

Parameters	Leaf	Stem
Ca^{+}	180.00 ± 5.00	136.67 ± 7.64
Fe^{++}	10.83 ± 0.21	3.70 ± 0.1
Zn^{+}	0.4 ± 0.1	0.13 ± 0.06
Mg^{+}	73.33 ± 2.89	43.33 ± 2.89
Mn^{+}	0.03 ± 0.01	0.02 ± 0.01
K^{+}	43.33 ± 2.89	40.00 ± 5.00
Po^{+}	14.33 ± 2.89	85.00 ± 5.00

■ Mineral and vitamin composition of *Luffa cylindrica* leaf and stem

The mineral and vitamin composition of Luffa leaf and stem were carried out (TABLE 4) and the results indicated the presence of ascorbic acid, B-carotine, thiamin, riboflavin, niacin but B-carotine had the highest presence and riboflavin had the lowest in the mineral and vitamin composition of Luffa leaf and stem [8].

TABLE 4: Mineral and vitamin composition of *luffa cylindrica* leaf and stem.

Parameters	Leaf	Stem
Ascorbic Acid	27.57 ± 0.21	12.57 ± 0.15
β-carotene	1856.67 ± 15.28	185.00 ± 5.00
Thiamin	0.23 ± 0.15	0.05 ± 0.01
Riboflavin	0.17 ± 0.02	0.04 ± 0.01
Niacin	1.55 ± 0.02	0.23 ± 0.02

■ Alpha glucosidase and amylase *in-vitro* antidiabetic assay of *Luffa cylindrica* leaf and stem

The alpha glucosidase and amylase *in-vitro* antidiabetic test were carried out. This was done to determine the antidiabetic activities of *Luffa cylindrica* leaf and stem; *Luffa cylindrica* stem showed more antidiabetic activities than the leaf. *Luffa cylindrica* stem at 30 and 50 mg/ml was having higher antidiabetic activity than the control [9].

■ The structural elucidation of the plant's extracts

Erythritol had the highest percentage in *Luffa cylindrica* leaf followed by Guanosine with 14.26 and 12.63% respectively (TABLE 5). *Luffa cylindrica* stem had more chemical compounds

than the leaf whereby 1,11-Bicyclopropyl-2-octanoic acid 2 hex, 2R, 3S-1-1,3,4-Trihydroxy-2-butoxymethyl and Erythritol had the highest amount compared to other chemical compounds with 32.30, 17.15 and 10.42% respectively (TABLE 6).

TABLE 5: Alpha amylase*in-vitro* antidiabetic assay of *Luffa cylindrica* and leaf and stem.

Mg/ml	*Luffa cylindrica* leaf	*Luffa cylindrica* stem	Control (Acarbose)
10	4.15	5.89	5.11
20	12.46	15.02	13.74
30	16.93	16.29	18.21
40	16.61	21.08	22.04
50	19.8	30.99	31.63

TABLE 6: Alpha glucosidase antidiabetic inhibitory assay of *Luffa cylindrica* leaf extract.

Mg/ml	*Luffa cylindrica* leaf	*Luffa cylindrica* stem	Control (Acarbose)
10	10.36	25.29	16.01
20	19.27	26.99	23.89
30	23.39	32.92	33.06
40	23.73	32.65	37.09
50	26.85	43.6	40.32

Betulin, Lanosterol and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-had the highest percentage of about 11.25, 7.58 and 5.45% respectively (TABLE 7).

TABLE 7: Structural Elucidation of *Luffa cylindrica* leaf extract.

Peak	R. Time	Area%	Name of chemical compounds
1	6.325	2.13	7-methylenebicyclo[3,2,0] hept-3-en-2-one
2	7.812	7.81	1-[2-butyl]oxy-1-methyl-1-silicyclopentane
3	8.43	14.26	Erythritol
4	9.328	3.71	2-methoxy-4-vinylphenol
5	11.846	12.63	Guanosine
6	13.316	13.54	[1,1-Bicyclopropyl]-2-octanoic acid, 2-hex
7	14.029	18.01	[1,1-Bicyclopropyl]-2-octanoic acid, 2-hex
8	16.022	4.29	9,12,15-octadecatrionic acid, 2-[trimethy]

■ Antibacterial susceptibility test for methanol extract of *Luffa cylindrica*

Antibacterial effect of methanol extract of *Luffa cylindrica* leaf and stem was carried out on the bacterial isolates (TABLE 8). The results indicated that there was no zone of inhibition whereby showed resistance on all the different organisms [10].

TABLE 8: Structural Elucidation of *Luffa cylindrica* Stem extract.

Peak	R. Time	Area%	Name of chemical compounds
1	4.752	0.75	5-Hexen-2-ol, 5-methyl-
2	5.002	0.36	4,4-Ethylenedioxy-pentanenitrile
3	5.338	0.43	2-Nonenoic acid
4	6.968	5.35	Isorbide dinitrate
5	7.795	9.79	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-
6	8.675	10.42	Erythritol
7	9.331	2.24	2-methoxy-4-vinylphenol
8	10.648	17.15	2R,3S-1-[1,3,4-Trihydroxy-2-butoxymethyl
9	11.837	7	Guanosine
10	13.326	32.3	[1,11-Bicyclopropyl]-2-octanoic acid,21-hex

■ Antibiotic susceptibility test for the test organisms

The antibiotics susceptibility test was carried out on nine bacterial isolates and it was discovered that *Salmonella typhi* ATCC 13311, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella* were susceptible to Cefotaxime, Ciprofloxacin, Cefuroxime, Tetracycline, Streptomycin, Azithromycin, Amoxicillin, Ceftazidime and Nalidizic acid (excluding *Shigella dysenteriae* ATCC 49556, *Escherichia coli* ATCC 35928, *Streptococcus pneumoniae* which was resistant [11,12] (TABLES 9 and 10).

TABLE 9: Antibacterial susceptibility test for methanol Extract of *Luffa cylindrica* leaf and stem.

S/N	Organism	Stem	Leaf
1	*Salmonella typhi* ATCC 13311	R	R
2	*Shigella dysentariae* ATCC 49556	R	R
3	*Escherichia coli* ATCC 35928	R	R
4	*S. Pneumoniae* ATCC 49619	R	R
5	*B. subtilis*	R	R
6	*P.aeruginosa*	R	R
7	*S. aureus*	R	R

TABLE 10: Antibiotic Susceptibility Test for the Test organisms.

Organisms	CTX	CIP	CXM	TE	S	AZM	AMC	CAZ	OX	NA
S. typhi ATCC 13311	R	34	R	12	18	27	9	R	R	16
S. dysenteriae ATCC 49556	R	R	R	R	R	R	R	R	R	R
E.coli ATCC 35928	R	R	R	R	R	R	R	R	R	R
S. pneumoniae ATCC 49619	R	R	R	R	R	R	R	R	R	R
B .subtilis	14	32	12	21	27	29	16	13	R	20
P. aeruginosa	R	19	R	12.0	24	25	R	R	R	11
S. aureus	13	25	R	20	13	26	22	21	R	12
Bacillus	R	R	R	R	R	R	R	R	R	R
Klebsiella	R	R	R	R	R	R	R	12	R	R

Note: R=resistant

Discussion and Conclusion

In this study, results showed that *Luffa cylindrica* contain low amount of moisture which was about 8.7%, too much of moisture in any sample has been proved to cause caking and determine the storage and viability of the growth of microorganisms. This finding is in support with who described the low moisture content of the leaf and stem extracts indicates its low perishability.

The crude fibre content especially for the stem may also implies that they can serve as a source of dietary fibre and can also be used in the treatment of diabetes, obesity and gastrointestinal tract diseases. Ash contents give an idea about the inorganic content and they are also expected to facilitate the metabolic processes, growth and development. Phytochemicals including alkaloids, flavonoids, phenols and tannins, they have been reported by several authors to possess antimicrobial activities. That the extracts from plants containing chemicals with antibacterial properties had been useful in the treatment of bacterial infections.

Saponins, alkaloids and cardiac glycosides were present in the extracts while anthraquinones, tannins were not detected in any of the extracts. The presence of secondary metabolites have been proven to be medicinal in nature as they have various protective and therapeutic effects essential to prevent diseases and in the maintenance of a state of wellbeing. Alkaloids are

useful in novelty medicare, and have been found to inhibit microbial growth by interfering with cell division.

The extracts were found to have antidiabetic activity, whereby *Luffa cylindrica* stem have a highest activity compared to leaf and control (Acarbose). This is an indication that the extracts have the potentials and ability to treat diabetes. It has been advocated that the use of medicinal plant, extracts or natural products, either alone, comibined or together with antibiotics can increase their efficacy. This suggests that the extract of *Luffa cylindrica* can be used in combination with other natural product or antibiotics for more effectiveness.

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