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Antimicrobial and Physicochemical Evaluation of *Luffa acutangular* Leaf Extracts

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Abstract: Leaves of *Luffa acutangular* are consumed in some parts of Nigeria as part of folk medicine for the treatment of diseases and as a vegetable food source. This study was undertaken to evaluate the phytochemical constituents from ethanol extracts, antimicrobial resistance, proximate and mineral analysis of its leaf extracts. The phytochemical investigation revealed the presence of alkaloids, phenols, saponins, tannins, terpenoids, and triterpenoids. The elemental analysis of the dried leaf revealed the presence of Calcium (58.6 mg/g), Copper (0.6 mg/g), Magnesium (12.4 mg/g), Manganese (0.9 mg/g), Zinc (0.6 mg/g), Sodium (14.4 mg/g) and Potassium (143.6 mg/g) respectively. The samples were screened against *Staphylococcus aureus, Staphylococcus pneumonia, Streptococcus pyrogens, Klebsciella pneumonia, Candida albicans and Candida tropicalis* for their anti-microbial activity using Ciprofloxacin, Streptomycin and Fluconazole as control. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values were determined. Proximate analysis revealed moisture content of 10.6%, ash 6.3%, crude protein 2.6%, crude fiber 4.0%, fat 5.1% and carbohydrate content of 71.4%. The study showed that the leaf extract of *L. acutangular* may be used to manage some common diseases caused by the tested organisms. The major antimicrobial activity is tailored to the phyto-constituents. This confirms the folkloric use of the plants in the management of various diseases.

Keywords: *Luffa acutangular,* Phytochemical Constituents, Antimicrobial Activities, AAS, Proximate Analysis

1. Introduction

In the past decade, clinical drug research programs have turned towards plants as a reservoir of untapped therapeutic agents, primarily used in orthodox medical practices by over 75% of global populace [1]. Such plants contain varied secondary metabolites; phyto-compounds including alkaloids, flavonoids, glycosides, phenols, saponins, terpernoids, minerals, etc which exhibit unique activities that are of pharmaceutical interest [2-4]. These metabolites generally do not participate in plant development and growth, rather in plant defensive mechanisms to combat onslaughts of abiotic and biotic stresses [5-6].

The curative interest in these compounds emanates from their unique mechanisms of resistance against a horde of disease causing organisms by circumventing the host-pathogen interaction which would have otherwise led to the propagation of the vector, ultimately producing a diseased state in the host [7]. It can then be inferred that the unique chemical diversity derived from natural products; phyto-compounds, is considered to be superior whereby the active compounds' framework is absent in its synthetic variants, a factor which could be associated with certain side effects via consumption of the latter.

As a food source, consumption of said plants elicits a cascade of immuno-modulatory events which serves as a

prophylactic, thereby authenticating their use in folk medicine [8]. As a raw material in biopharming, nutraceuticals, etc, several studies have been conducted in many parts of the world to validate their use as antimicrobial agents or biological control agents [9-12]. However, some plants belonging to known families with therapeutic potential have either been neglected nor experienced satisfactory attention. Agricultural expansion strategies in developing countries necessitates exploring the full potential of indigenous or wild food plants as an essential part of our diet [13]. Reports indicate that food crops grown in the wild possess an array of minerals, micronutrients and therapeutic properties that are crucial towards combating nutritional deficiencies especially during periods of food scarcity, diseases, etc [14-15]. One of such crops is *Luffa acutangular* (L.) Roxb var. *amara* (sponge guard) from the Cucurbitaceae family which is widely distributed across China, Korea, India, Japan and Central America and grows wildly in the western and north central parts of Nigeria where it is consumed as a vegetable [16]. There is however a limited amount of scientific information on the nutritional, mineral and phytochemical composition of some indigenous crops, like the diversity of *Luffa acutangular* (L.) Roxb var. *amara* (sponge guard) grown in Nigeria. There is therefore a need to evaluate this in order to fill the knowledge gap and raise the awareness towards cultivation and consumption. This study was therefore aimed at identifying the phytochemical, antimicrobial, nutritional and mineral composition of indigenous *Luffa acutangular* (L.) Roxb var. *amara* (sponge guard) obtained in Nigeria.

2. Materials and Methods

Leaves of *Luffa acutangular* were collected in fresh condition at Sheda and Abakiliki regions of Kogi state, Nigeria. The plants were identified, air-dried and kept in airtight containers until required for further laboratory analysis. Crispy plant leaf sample (100g) was placed and soaked with ethanol in a Soxhlet apparatus for 6-8 hours. The crude extracts were later concentrated using rotary evaporator. Phytochemical screening was then performed using standard procedures [17].

2.1. Mineral Analysis

The metal analysis was determined using an Atomic Absorption spectrometer (iCE 3000, Thermo fisher). Five grams of oven dried samples were weighed into a crucible and transferred to a furnace at 600°C and left to ash for 3 hours. The furnace was cooled to about 120°C and then placed in a desiccator for an hour to cool before weighing. This process was repeated until a constant weight was obtained. The ashed samples (0.5g) were weighed and transferred into the digestion tube. Distilled water, concentrated HNO_3 and perchloric acid (5mL of each) were added and the content mixed. The tubes were placed into the digestion block inside a fume cupboard and the temperature was set at 150°C for 90 minutes. The temperature was then adjusted to 230°C and incubated for another 30 minutes to obtain white fumes. The temperature was then reduced to 150°C, followed by the addition of 1 mL of hydrochloric acid to the tubes within a few minutes. Water was added to the tube to make up to the mark, mixed and filtered.

Elemental analysis of the solution obtained was then performed using an atomic absorption spectrophotometer (AAS) at an appropriate wavelength, temperature and lamp-current for the different elements. The following elements were determined, calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), potassium (K), sodium (Na).

2.2. Proximate Analysis

The moisture, protein, fat, ash, crude fibre and carbohydrate content of the dried leaves were determined. For moisture content, 5g of dried leaf samples was weighed and dried in an oven at 105°C to a constant weight. The percentage weight loss was determined. Fat content was determined by extracting 5g of dried leaf sample with hexane or petroleum ether in a Soxhlet apparatus for 8 hours. The ash content was estimated by incinerating 5g of dried sample in a muffle furnace (Carbolite-RHF 1600) at 550°C for 4 hours, and then the percentage ash content was determined. The micro-Kjedahl method was employed for estimation of crude protein. All experiments were done in triplicate and results were expressed as the averages on dry weight basis.

2.3. Preparation of Inoculum

The bacterial isolates were collected from Medical Microbiology Department of Specialist Teaching Hospital, University of Abuja, F. C. T., Nigeria on a slant Nutrient agar. The isolates were restored on Nutrient broth and confirmed using standard biochemical tests according to the Bergey's manual of Bacteriology [18].

2.4. Biological Screening of Extracts

The crude extract was screened for antimicrobial activity using agar well diffusion technique with little modification. Clinical isolates utilised were *Staphylococcus aureus, Staphylococcus pneumonia, Streptococcus pyrogens, Klebsciella pneumonia, Candida albicans* and *Candida tropicalis.* The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extracts and was done by broth dilution method [19]. Mueller Hinton broth was prepared, dispersed into test tubes and the broth was sterilized at 121°C for 15 minutes, the broth was allowed to cool. Normal saline was prepared, 10mls was dispersed into sterile test tube and the test microbes was inoculated and incubated at 39°C for 6hrs. Dilution of the test microbes was done in the normal saline until the turbidity marched that of the McFarland's standard scale by visual comparison at this point, this test microbes has a concentration of about 1.5×10^8 CFU/ml. Standard antibiotics (Ciprofloxacin, Streptomycin and Fluconazole) used in this study to serve as positive control while Dimethyl

sulfide (DMSO) used as negative control. Minimum bactericidal and fungal concentrations (MBC/MFC) were evaluated by plating the bacterial suspensions from individual well at the beginning and at the end of the experiments on Mueller Hinton agar medium for estimation of MBC. The culture from MIC well was taken and streaked on the surface of fresh Mueller Hinton agar in a 90-mm plate with division and incubated at 37°C for 24 hours (bacteria) after which the plates of the medium was observed for colony growth, the MBC was the plates with lowest concentration of the extract without colony growth.

3. Results

Table 1 represents the phytochemical composition of ethanol extracts from the leaves *L. acutangular*. The powdered leaves tested positive for the presence of certain phyto-compounds such as alkaloids, phenolic acid, saponins, tannins and terpenoids (table 1).

The mineral content analysis of the leaves of *Luffa acutangular* revealed that the most predominant micronutrients were potassium (143.6 mg/g) and calcium (58.6 mg/g) whereas the least concentrations of 0.6 (mg/g) each was determined for zinc and copper (Table 2).

Table 1. *Phytochemical Analysis of leaf sample.*

Phyto-Constituents	Ethanol
Alkaloids	+
Balsams	-
Cardenolides	-
Cardiac glycosides	-
Flavonoids	-
Glycosides	-
Phenolic acid	+
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+
Triterpenoids	+

Key: -= Negative/Absent, += Positive/Present

Nutritional analysis revealed that the leaves possessed a high moisture to ash and fat content at 10.6%, 6.3% and 5.2% respectively (Table 3). The carbohydrate content was highest (71.4%).

The highest zone of inhibition recorded for the alcoholic extracts of *Luffa acutangular* leaves was against *Streptococcus pyrogens* (20.0±0.35 mm), followed by 18.0±0.65 (mm) against *Candida albicans* (Table 4).

The lowest combined MIC and MBC values obtained (70 and 80 mg/ml) was against *Streptococcus pneumonia* and *Streptococcus pyrogens* (Table 5).

The lowest combined MIC and MFC values obtained (70 and 70 mg/ml) was against *Candida albicans* (Table 5).

Table 2. *Mineral Analysis.*

Calcium (Ca) (mg/g)	Iron (Fe) (mg/g)	Magnesium (Mg) (mg/g)	Manganese (Mn) (mg/g)	Zinc (Zn) (mg/g)	Copper (Cu) (mg/g)	Sodium (Na) (mg/g)	Potassium (K) (mg/g)
58.6	8.1	12.4	0.9	0.6	0.6	14.4	143.6

Table 3. *Proximate Composition of leaves.*

Ash *(%)*	Fat *(%)*	Fibre *(%)*	Protein *(%)*	Moisture *(%)*	Carbohydrate *(%)*
6.3	5.1	4.0	2.6	10.6	71.4

Table 4. *Antimicrobial activity of Luffa acutangular leaf extracts against test organisms Zone of inhibition (mm).*

Test microorganisms	Leaf Extracts	Control[1]	Control[2]	Control[3]
Staphylococcus aureus	14.0 ± 0.90	28.0 ± 0.35	24.0 ± 0.35	-
Streptococcus pneumoniae	17.0 ± 0.35	24.0 ± 0.35	30.0 ± 0.35	-
Streptococcus pyrogenes	20.0 ± 0.35	25.0 ± 0.35	18.0 ± 0.35	-
Klebsiclla pneumoniae	14.0 ± 0.30	22.0 ± 0.35	22.0 ± 0.35	-
Candida albicans	18.0 ± 0.65	-	-	20.0 ± 0.35
Candida tropicalis	12.0 ± 0.60	-	-	16.0 ± 0.35

Control[1]= Ciproflaxin (100 µg/g), Control[2]= Streptomycin (100 µg/g), Control[3]= Fluconazole (100 µg/g)

Table 5. *Minimum Inhibitory Concentration (mg/ml ± SD) of L. acutangular leaf extracts against test organisms.*

Test microorganisms	MIC/MBC/MFC	Concentration (mg/g)			
		Plant Extract	Control[1]	Control[2]	Control[3]
Staphylococcus aureus	MIC	80	50	50	-
	MBC	90	60	70	-
Streptococcus pneumoniae	MIC	70	50	40	-
	MBC	80	60	50	-
Streptococcus pyrogenes	MIC	70	50	70	-
	MBC	80	60	80	-
Klebsiclla pneumonia	MIC	80	60	60	-
	MBC	90	70	70	-
Candida albicans	MIC	70	-	-	60
	MFC	70	-	-	70
Candida tropicalis	MIC	ND	-	-	70
	MFC	ND	-	-	80

Control[1]= Ciproflaxin (100 µg/g), Control[2]= Streptomycin (100 µg/g), Control[3]= Fluconazole (100 µg/g). Each value represents mean (n = 3). ND= Not Detected

4. Discussion

Whole plants, herbs and vegetables provide established launch-pads through the field of phyto-chemistry and pharmacognosy for the development of new drugs and their intermediates that have adequate therapeutic uses [20-21]. A plethora of essential molecules in plants is responsible for both their nutritional, energy giving and prophylactic effects [21]. The mode of action of different complex plant products bear a resemblance to ligands, hormones, signal transduction molecules, etc thus have beneficial medicinal effects on humans due to their potential target sites similarities. In this study, the presence of alkaloids, phenolic acid, saponins, tannins, terpenoids and triterpenoids (table 1) suggests that the plant possess a unique antimicrobial range as these individual compounds exert action that thwarts the growth or development of disease causing pathogens [23]. Recent studies have shown that alkaloids, obtained from different plant extracts like *Callistemon citrinus* and *Vernonia adoensis* successfully diminished the growth of *Staphylococcus aureus* when compared to β-lactam antibiotics [24]. Bioactive compounds like tannins has also been studied and found to be active against *Staphylococcus aureus* alongside a host of bacterial pathogens linked with a variety of infections [25-26]. The presence of alkaloids suggests that the leaves of *Luffa acutangular* could be used as neuro-stimulants or in new drug therapies [27]. As a result of the saponin content, ingestion of these leaves may aid in lowering cholesterol metabolism thereby boosting liver function in the body [28-29]. Saponins would also deter Na^+ efflux and trigger the Na^+- Ca^{2+} antiporter in cardiac muscle thereby elevating the cytosolic Ca^{2+} via the influx of calcium, thus strengthening the contraction of cardiac muscles towards abating congestive heart failure [30]. The presence of phenols, flavonoids and tannins indicate that these leaves possess antioxidant potentials that may be applied towards end-products that aid the prevention of stroke, cardiovascular diseases, cancer and other neurological disorders [31-33]. Steroids play a vital role in enhancing the well-being of animals and humans as they function in resisting infiltration by many pathogens owing to their antimicrobial properties [34].

From the data obtained (Table 2), the highest mineral content in the leaves of *Luffa acutangular* is potassium (143.6 mg/g) followed by calcium (58.6 mg/g). This affirms its therapeutic role as either a mineral supplement towards bone growth and linked to the presence of sufficient fiber content, aids in the prevention of osteoporosis [35-36]. Furthermore, consumption of this vegetable would be useful towards bone formation, energy, normal functioning of metabolic and metallo-enzymes needed for nervous transmission and muscle contraction [37- 38]. With the presence of other essential elements, micronutrient malnutrition that affects women and children in developing countries can be averted via the consumption of this leaf as a vegetable. Also, consumption of this plant should reduce the risk of stroke, lower blood pressure, and reduction in the formation of kidney stones due to the amount of potassium in it [39]. Calcium aids in the maintenance of bone and dental health, prevention of colon cancer, reduction of obesity and protects cardiac muscles, since *L. autangula* is so rich in calcium it could be an alternative source of calcium in milk for older people and also added to the diet of children as well [38]. Investigation of its nutritional value (table 3) revealed that the study plant contained a higher percentage of ash (6.3%) than fat (5.1%), fibre (4.0%) or protein (2.6%). The cucurbitaceae family are known to possess high moisture content and *L. acutangula* was not exempted as seen in table 3, consuming

such water rich plants keeps the body hydrated and aids in digestion [38]. This study showed that the leaves extract of *L. acutangula* had antimicrobial and anti-fungal activity. The ethanolic extract of *L. acutangula* leaves exhibited a stronger anti-microbial activity on *streptococcus pyrogens* compared to the controls suggesting a new drug with little or no side effect for the treatment of diseases caused by *streptococcus pyrogens*. The effect of the ethanolic leaf extracts on the other microorganisms and fungi compared to the control was less than the control. Therefore *L. acutangula* leaves can be used as a preventive drug to upper respiratory and urinary tract organisms (table 4).

The antimicrobial screen for the studied plant revealed that *Luffa acutangular* leaves confer a higher degree of resistance to *Streptococcus pyrogenes* (20mm), *Candida albicans* (18 mm) followed by *Streptococcus pneumonia* (17mm), thereby hinting at the possibility of new drug candidates from the plant (table 4). The findings from table 4 compliments the results given in tables 1 and 2 whereby other studies have shown that bioactive compounds detected as well as the presence of some minerals triggers resistance against a range of disease causing pathogens linked with respiratory infections [25, 26]. In particular, interference of bacterial enzyme activity is commonly observed in the presence of tannins also detected in this study [40]. Table 5 data shows the MIC, MBC and MFC of the crude leaf extracts of *L. acutangular*. The data confirms that the crude leaf extracts were more active against *Candida albicans and both Streptococcus* species. The general observation from the displayed activity against the selected microorganisms and detected minerals suggests that the studied plant part could be employed towards alleviating infections as a plant for food and medicine.

5. Conclusion

This study explored the potential of an indigenous medicinal plant in the treatment of infections and its nutritional content towards encouraging the cultivation and consumption of the plant as food and to boost commercialization. The study has provided additional scientific knowledge of the ethnomedicinal ability of the plant, thereby supporting the folkloric use of the plant in the management of infections. It was observed that the plant has a high amount of potassium and calcium, showing that the plant could be nutritional supplement in diet. The anti-microbial activity of the plant extract suggested that the plant could be used as a prophylactic for the management of infections caused by the tested micro-organisms. The plant could be a possible drug candidate for the management of infections caused by *Streptococcus pyrogens,* due to its high anti-microbial activity against the organism compared to streptomycin which was used as a control. Since this plant is one of the forgotten indigenous plants that grows mainly in the wild in Nigeria, cultivation and consumption could be encouraged based on the possibilities obtained from this study.

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