PHYTOCHEMICAL INVESTIGATION AND ANTI–BACTERIAL ACTIVITY STUDIES OF THE LEAF OF *LUFFA AEGYPTIACA* MILL (CUCURBITACEAE)

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ABSTRACT

In this study the leaf *Luff aegyptiaca* was investigated for phytochemical and antibacterial properties. The phytochemical screening was quantified using standard procedure while the extract was tested against four bacterial (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*) for their antibacteria property using agar diffusion method. The preliminary phytochemical screening showed positive test for tannins, flavonoids, saponins and glycosides. The result of the antibacterial activity of the leaf extract showed a high activity against bacteria with a zone of inhibition of 21 mm; 19 mm and 13 mm for *S. aureus, B. cereus* and *P. aeruginosa* respectively, while *E. coli* was not affected by the extract. This study showed scientific basis for the use of this plant as a traditional medicine and can therefore be used to source for new antibacterial substances for the treatment of various enteric infections.

Keywords: Agar-well diffusion method, Antibacterial, Cucurbitaceae, *Luff aegyptiaca*, Phytochemical
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INTRODUCTION

Traditional medicine includes all kinds of folk medicine unconventional medicine and indeed any kind therapeutic method that had been handed down by the tradition of community or ethnic group. Almost 80% of people in developing countries depend on traditional medicine for primary healthcare, most of which are derived from the plants [1]. Ethnobotany, the interaction between plants and people involves traditional use of medicinal plants by indigenous communities and management of plant diversity by the aboriginals [2]. Medicinal plants have also been found useful in the treatment of infections caused by fungi, bacteria, viruses, parasitic and certain clinical conditions occurring naturally or resulting from exposure to environmental contaminants [3]. Different plants have been used as a source of inspiration in the development of novel drugs [4]. Plant derived medicine are widely used because they are relatively safer than the synthetic alternative, they are readily available, cheaper and work effectively with little side effect [5]. As a result of the use of medicinal plants in treatments, some of them been found to have triterpenoids which comprise of a large group of devise C-30 natural metabolites having relatively complex cyclic structures, usually tetra or pentacyclic, although acyclic or monocyclic skeletons have also been found [6]. Most of these plants contain alcohols, aldehydes carboxylic acids or esters which are regarded as important class of compounds in phytochemistry [7].

Luffa aegyptiaca [Mill] is a member of the Cucurbitaceae family native of Asia and widely cultivated in several tropical and subtropical regions worldwide for its economical, medicinal and nutritional uses [8]. It is commonly named sponge gourd, vegetable sponge, bath sponge, dish cloth gourd and loofa [9, 10]. Is a tropical running vine with rounded leaves and yellow flowers, which thrives commonly with twinning tendrils. Luffa produces berry like fruit whose colour at tender stage is green and yellow at maturity. The fruit are smooth and cylindrical in shape with white flesh. One mature Luffa sponge will produce at least 30 seeds. *L. aegyptiaca* has alternate and palmate leaves comprising petiole. The leaf is 13cm and 30cm in length and width respectively and has an acute-end lobe. In Nigeria, *L. aegyptiaca* plant grows in the wild and on abandoned building, structure and fenced walls in town and villages [11, 12].

Plants like Luffa and its extracts have proved to be safe because they contain several bioactive chemicals (phytochemicals) responsible for several activities such as antimicrobial, anti-inflammatory, antifungal, antioxidant, anti-cancer and antiviral [13]. Among the potential medicinal plant is *L. aegyptiaca* which is found to be loaded with several secondary metabolites that are safer, natural and useful in maintaining the health of an organism. It also possesses highly medicinal and nutritional property. The plant has also been reported to perform several functions such as antimicrobial [14, 15].

MATERIALS AND METHODS

Collection, identification and preparation of plant material

The whole plant *Luffa aegyptiaca* was collected wild from Zangon Shanu, Sabon Gari L.G.A Kaduna State, Nigeria, in the month of November 2021. The leaves were packaged into a polythene bag. The plant sample material was taken to department of Biological Science, ABU, Zaria for identification where it was compared with an existing specimen with voucher Number ABU3272 by Mal. Sanusi Namadi. The leaves were air-dried at room temperature for 7 days. It was then pulverized using mortar and pestle into fine powdered.

Extraction of plant material

The dried powdered leaves of the plant were extracted using ethanol as solvent by maceration method for three days. The extract was concentrated in oven to dry the moisture and then stored in the refrigerator before use.

Phytochemical screening

The phytochemical analysis of ethanol extracts of *Luffa aegyptiaca* was carried out according to the established procedures [16, 17]. The phytochemicals tested includes: tannins, saponins, flavonoids and glycosides.

Test organisms

The Clinical isolates of *Staphylococcus aureus, Bacillus subtilis, E. coli* and *Pseudomonas aeruginosa* were obtained from the stock culture of the Medical Microbiology Unit, Department of Microbiology, Ahmadu Bello University Zaria.

Preparation of inoculum

Antibacterial preparation of *Luffa aegyptiaca* ethanol extract was tested against Gram - positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and Gram - negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The Gram – positive and Gram-negative bacteria were precultured in Mueller Hinton Broth (MHB) over night in a rotary shaker at 37^0C cell/ml using 0.5 McFarland Standard [18].

Antibacterial screening

Activities of the plant extracts were tested using agar-well diffusion method as explained by Sidney *et al.* [19]. One ml of fresh bacteria culture was pipetted in a center of sterile petri dish. Upon solidification, three wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculum and the other two served as a control. Then 100 µl of the extract was added to respective wells. The plates were placed in the refrigerator for 30 minutes to let the extract diffusion well into the agar. Then the plates were inoculated at 37^0C for 18 hours bacterial activity was detected by measuring the zone if inhibition (including well diameter) appeared after the incubation period.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was determined according to the macro broth dilution technique as described by Sidney *et al*. [19]. Two drops of standardized suspensions of each of the test isolate organism were inoculated separately into a series of sterile test tubes containing 2 mls of nutrient broth each. Then 3 drops of different concentrations of the extracts were added to the tubes. The cultures were incubated at a temperature optimal for growth of the test organism and a period of time sufficient for growth for at least 10 – 15 generations (24hrs at 37°C). The tubes were inspected visually to determine the growth microorganisms by presence of turbidity and the tubes in which the extract is present in minimum concentration sufficient to inhibit the microbial growth which remains clear was noted as MIC. In experimental term, MIC is the concentration of the extracts present in the last clear tube, which is the tube having the lowest extract's concentration in which growth was not observed [20].

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined using the method described by Rotimi *et al.* [14] by assaying the test tubes content resulting from MIC determination. A loopful of the content of each tube was inoculated at 37ºC for 24h after which the plates were observed for microbial growth. The lowest Concentration of the sub-culture with no growth was considered as the Minimum Bactericidal Concentration (MBC) [21].

RESULTS

Phytochemical screening of *L. aegyptiaca* leaf

The phytochemical analysis of *Luffa aegyptiaca* leaf extract revealed the presence of various bioactive constituents which include: tannins and saponins that is present in high amount, then flavonoid and glycoside as shown in Table 1.

Table 1: Result of phytochemical screening of ethanol leaf extract of *Luffa aegyptiaca*

Phytochemicals	Ethanol extract
Flavonoids	++
Saponins	+++
Tannins	+++
Glycosides	++

Antibacterial activity of ethanol extract of *L. aegyptiaca* leaf

The antibacterial susceptibility of the extract was recorded against the test organisms at a concentration of 200 mg/ml with a zone of inhibition of 21 mm, 19 mm and 13 mm for *S. auerus*, *B. cereus* and *P. aeruginosa*, while *E. coli* was not affected by the extract as shown in Table 2.

Table 2: Antibacterial activity of ethanol extract of *L. aegyptiaca* leaf

Test bacteria	Concentration of extract (200 mg/ml)	Controls	
		Ciprofloxacin (5 ug/ml)	Gentamycin (5 ug/ml)
Staphylococcus aureus	21	38	28
Bacillus cereus	19	23	19
Escherichia coli	-	32	23
P. aeruginosa	13	49	28

Minimal inhibitory concentrations (MIC)

The minimum inhibitory concentration was at 12.5 mg/ml for *S. aureus*, 50 mg/ml for *B. cereus*, 200 mg/ml for *P. aeruginosa* and *E. coli* was not determined as shown in Table 3 below.

Table 3: Result of Minimum Inhibitory Concentration (MIC)

Test Bacteria	Concentration of Extract (mg/ml)				
	200	100	50	25	12.5
Staphylococcus aureus	-	-	-	-	+
Bacillus cereus	-	-	+	++	++
Escherichia coli	ND	ND	ND	ND	ND
P. aeruginosa	+	++	+++	++++	++++

Keys: ND Not Determined, - No Turbidity, + Mind Turbidity, ++ Low turbidity, +++ High turbidity, ++++ Very high turbidity

Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was at 25 mg/ml for *S. aureus*, 100 mg/ml for *B. cereus*, 200 mg/ml for *P. aeruginosa* and *E. coli* was not determined as shown in Table 4 below.

Table 4: Minimum Bactericidal Concentration (MBC)

Test Bacteria	Concentration of Extract (mg/ml)				
	200	100	50	25	12.5
S. aureus	-	-	-	+	+
B. cereus	-	+	+	++	++
E. coli	ND	ND	ND	ND	ND
P. aeruginosa	+	++	+++	++++	++++

Keys: ND Not Determined, - No Turbidity, + Mind Turbidity, ++ Low turbidity, +++ High turbidity, ++++ Very high turbidity

DISCUSSION

The preliminary phytochemical investigations of *Luffa aegyptiaca* revealed the presence of some

phytochemical compounds. These secondary metabolites are most likely linked to antimicrobial activity of the plant material. The phytochemicals analysis indicates the presence of secondary metabolites including tannins, flavonoids, saponins, and cardiac glycosides. *Luffa aegyptiaca* leaves extract contain some natural bioactive compounds that may be of medicinal importance as reported [13]. The importance of alkaloids, saponins and tannins as anti - biotics to treat common pathogenic strains has been reported [22]. It has been reported that phytochemical agents play important role in the treatment of diseases; for instance, cardiac glycosides have found to play significant important role in the treatment of congestive heart failure as well as cardiac arrhythmia. Cardiac glycosides work by inhibiting Na^+/K^+ pump thereby increasing Sodium ions level in the monocytes this in turn rises the level of Ca^{2+}. Increase in Ca^{2+} favors the contraction of the heart muscle by improving cardiac output and also reduces distention of the heart [23].

The antibacterial activity was determined using well diffusion method for the alcohol extract. The ethanol extract exhibited antibacterial activity against the microorganisms tested as indicated by zones of inhibition that ranged from 2.0 to 25.0 mm. From these results, it can be deduced that the ethanol leaf extract exhibited a better antibacterial activity against *E. coli* and *S. aureus*, the zones of inhibition of growth of the microorganisms used in this study are function of relative antibacteria activity of the extracts. It has been reported [24] that saponin and tannin are the major properties in medicinal plants that gives them their antibacteria properties. In this study, the highest susceptibility was recorded at 21 mm (200 mg/ml) for *S. aureus*, 19 mm for *B. cereus*, 13 mm for *P. aeruginosa* and for *E. coli* it was not determined. The antibacterial property could be due to the enhanced effect of the plant constituents based on the increased concentration of the extract, which can be said to contain more phytochemical constituents. The highest activity was demonstrated by the standard antibiotic chloramphenicol (control). This is because the antibiotic is in pure state and has refined processes that have established it as a standard antibiotic [25].

The minimum bactericidal concentrations (MBC) of the plant extract in this study indicate higher concentrations than that of the minimum inhibitory concentration (MIC). This observation is based on the fact that the concentration of the crude extract required to completely eliminate an organism must be higher than the concentration required to inhibit the growth, as reported [25]. The MIC was at 12.5 mg/ml for *S. aureus*, 50 mg/ml for *B. cereus*, 200 mg/ml for *P. aeruginosa* and *E. coli* was not determined. The MBC was at 25 mg/ml for *S. aureus*, 100 mg/ml for *B. cereus*, 200 mg/ml for *P. aeruginosa* and *E. coli* was not determined.

CONCLUSION

The antibacterial study of leaves of *Luffa aegyptiaca* provides promising solution in the ethnomedicine practice of disease control. This can be used to augment the synthetic antibiotic used in the treatment of related ailments as phytochemicals from natural resources are generally consider safer, available and affordable compared to the synthetic drugs in the treatment of infectious disease.

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