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Evaluation of the phytochemical and elemental constituents and the in vitro anti-bacterial activity of aqueous leaf extract of *Luffa cylindrica* (sponge gourd)

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Abstract

Infections and diseases are the most notable health challenges faced by humans and animals; they lead to health impairments, death and economic losses. The leaf extract of *Luffa cylindrica* plant (sponge gourd) have been noted for its medicinal properties, but its phytochemical and elemental constituents and its anti-bacterial activity has not been reported. The present study evaluated the phytochemical and elemental constituents and the in vitro anti-bacterial activity of aqueous leaf extract of *L. cylindrica.* Leaves of *L. cylindrica* used for the study were collected within the University of Maiduguri premises, Jere Local Government of Borno State, Nigeria. Two hundred grammes of the dried *L. cylindrica* leaves were extracted with water, and subjected to standard qualitative phytochemical and quantitative elemental analysis and in vitro anti-bacterial activity evaluation. Phytochemical screening showed the presence of following phytochemicals: alkaloids, flavonoids, terpenoids, glycosides, carbohydrates, saponins and tannins. The elemental analysis of the leaves revealed the following concentrations of elements: Copper – 2.489 ± 0.132 mg/kg, Iron – 0.787 ± 0.076 mg/kg, Chromium – 0.166 ± 0.009 mg/kg, Nickel – 0.042 ± 0.004 mg/kg, Arsenic – 0.009 ± 0.001 mg/kg and Cadmium – 0.005 ± 0.0 mg/kg; all these concentrations fall within the permissible limit as stated by FAO. In vitro evaluation of anti-bacterial activity showed that the extract was effective against the following tested bacteria, with inhibition zone diameters at the highest concentration of 300 mg extract, as follows: *Salmonella* – 14.5 ± 0.5 mm, *Shigella* – 14.5 ± 1.5 mm, *Escherichia coli* – 15.5 ± 0.5 mm, *Bacillus* – 14.5 ± 1.3 mm, *Klebsiella* – 14.5 ± 1.3 mm, *Proteus* – 15.5 ± 0.5 mm, and *Staphylococcus* – 14.5 ± 1.0 mm. It was concluded that aqueous extract of leaves of *L. cylindrica* is a potential source of safe and effective anti-bacterial agent.

Keywords: Phytochemicals; Elemental analysis; Anti-bacterial activity; *Luffa cylindrica*; Aqueous leaf extract.

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Introduction

Medicinal plants are the stronghold of the economy of some nations. About 80 % of the global populace, mainly in developing countries, rely on plants and plant products for their primary health care needs (Sen *et al*., 2009). Natural products from medicinal plants are widely used globally to treat and cure various diseases. The use of medicinal plants is increasing tremendously, because it is believed to be safer than most synthetic drugs and their use is not commonly associated with some of the adverse effects reported with the use of synthetic drugs (Kamboj, 2009; Ramchoun *et al*., 2009).

The medicinal value of most plants have been reported to depend on the secondary metabolites they produce (Edeoga *et al*., 2005). These chemical constituents are referred to as phytochemicals. Phytochemicals are secondary metabolites of plants, which are chemically bioactive; they include glycosides, saponins, flavonoids, alkaloids, tannins, terpenoids, steroids, carbohydrates etc. (Mallikharjuna *et al*., 2007; Tang *et al*., 2010).

Luffa cylindrica, commonly known as sponge gourd, belong to the family *Cucurbitaceae* (Reynoids, 1996; Lis-Balchin and Deans, 1997). The fruits of the plant have a network of fibers which are surrounded with a large number of flat blackish seeds (Stephen, 2003). The plant has vine-like growth habit, with long slender stem that can reach up to 20 metres in length. The leaves of *Luffa cylindrica* are large, lobed and palmate, providing a dense canopy. The plant produces a yellow flower with separate male and female booms, usually appearing in clusters. The fruits of *Luffa cylindrica* are the most widely used part of the plant. When young, the fruit is edible and consumed in various cuisines, particularly in Asian dishes. As the fruits mature, its fibrous interior becomes tough and sponge like, making it ideal for use as natural scrubber or exfoliator in personal care products. In addition to its culinary and cosmetic uses, *Luffa cylindrica* have been reportedly used also for its medicinal properties (Musibau *et al*., 2013).

It has been reported that *L. cylindrica* have been used in the treatment/cure of sinusitis, asthma, and fever. It has also been reported that it has a potential as a therapeutic agent for the treatment of AIDS patients, because it contains luffaculin, which exhibited ribosome-inhibiting properties on the duplication of HIV infected phagocyte and lymphocyte cells (Ng *et al*., 1987). It has also been established that extract from the stem has been used in the treatment of respiratory disorders, and the seed exhibits emetic action (Bailey *et al*., 1989).

Several phytochemicals have been reported to directly be responsible for the medicinal activity of most plants (Kakate, 1997). Therefore, phytochemical screening of medicinal plants may provide new useful information to the scientific community that will often explain the therapeutic efficacy of such plants. To the best of our knowledge, there are no reports in available literature on the phytochemical and elemental constituents of *L. cylindrica* leaves and its anti-bacterial activity. The present study evaluated the phytochemical and elemental constituents and the in vitro anti-bacterial activity of aqueous leaf extract of *L. cylindrica.*

Materials and Methods

Plant Collection and Identification: Fresh leaves of sponge gourd were collected within University of Maiduguri premises, Jere Local government of Borno State, Nigeria. The leaves were identified to be of *Luffa cylindrica* by a taxonomist. The leaves were latter air dried at room temperature, then crushed using a mortar and pestle into fine particles. The sample was then taken to the Chemistry Laboratory for extraction and phytochemical analysis.

Aqueous Extraction by the Reflux procedure: Two hundred grammes of the plant material was extracted with water. The extract was filtered to remove debris and concentrated to dryness at 40 – 50°C in a hot air oven. The extract obtained was weighed and kept in a desiccator until required for use.

Qualitative Phytochemical Screening: The extract was screened qualitatively for phytochemical constituents using standard procedures (Sofowora, 2008; Evans, 2009). For carbohydrates, the following tests were done on the extract: the Molisch's test, Barfoed's test for monossacharides, Fehling's test for free reducing sugars and test for combined reducing sugars (Sofowora, 2008), Seliwanoff's test for ketoses (Evans, 2009) and tests for pentoses and soluble starch (Vishnoi, 1979). Tannins were qualitatively assayed following the ferric chloride and lead acetate tests (Evans, 2009). The Salkowski's test for cardiac glycosides (Silva *et al*., 1998) and Liebermann-Burchard's test for steroids (Sofowora, 2008) were also done. Tests for phlobatannins, free anthraquinones (Borntrager's test) and combined anthraquinones were done by the methods described by Evans (2009), while terpenoids were tested for by the procedure described by Silva *et al*. (1998). Saponins were qualitatively detected using the methods described by Vishnoi (1979) and Sofowora (2008). Flavonoids were detected using the Shinoda method (Markham, 1987) and sodium hydroxide method (Evans, 2009), while alkaloids were tested for by the Dragendroff's test and Meyer's test (Evans, 2009).

Quantification of the Elemental Components of *Luffa cylindrica* leaves*:* Fifty grammes of the leaves were used to determine the concentration of some elements in the leaves. The leaves were placed in a porcelain crucible and then put into muffle furnace. The temperature was raised to about 500^{o}C. The ashed sample was removed and cooled in a dessicator, then stored in a refrigerator at 4^{o}C until required. Half gramme (0.5g) of the ash sample was transferred into 250 ml beaker, 10 ml of 6 molar hydrochloric was added and swirled. 10 ml of distilled water was further added and it was heated on a hot plate to complete dissolution (Radojevic and Bashkin, 1999). After allowing it to cool, it was filtered with filter paper (Whatman No-541) into 100 ml volumetric flask up to the mark. The solution was then transferred into polyethylene bottle for elemental analysis. The levels of the elements, Iron, Chromium, Nickle, Arsenic, Cadmium and Copper were determined by atomic absorption spectrophotometry (Radojevic and Bashkin, 1999).

Determination of inhibitory effect of different concentrations of plant extract on selected bacteria isolates: The inhibitory effect of different concentrations of the *L. cylindrica* extract were tested separately against different bacteria isolates namely: *Salmonella* spp, *Shigella* sp, *Escherichia coli*, *Bacillus* sp, *Klebsiella* sp, *Proteus* sp and *Staphylococcus* sp using the well diffusion method, a method that replaces discs with wells into which the extract to be tested are introduced. A known antibiotic was used as control.

Each bacterium was emulsified in peptone water and turbidity was compared to that of 0.5% McFarland's standard. 0.5 ml of the bacteria suspension was placed on the surface of nutrient agar and a sterile glass rod was used to evenly spread the suspension over the entire surface of the agar plate. Holes (wells) were however first made on the plates by use of cork borer, one hole at the centre and other holes around the circumference of the plate corresponding to the number of different concentrations of plant extract. The holes around the centre were aseptically filled with 1 ml of the dissolved extract at different concentration (50 mg, 100 mg, 150 mg, 200 mg, 250 mg and 300 mg). Ciprofloxacin was placed at the centre as control and the plates were incubated at 35 ± 2°C for 24 hours. The

experiment for each bacteria isolate was replicated three times. The zones of inhibition around the wells containing the different concentrations of the plant extract and that at the centre containing Ciprofloxacin as control were measured in milimetres and analysed after the incubation period according to Egbere *et al*., (2022). The diameters of the zones of inhibition for the three replicates were used to find the mean inhibition zone diameter of the plant extract for each of the test bacteria isolates. The above procedure was carried out for all the other test bacteria isolates. Bacteria isolates with little or no zones of inhibition were considered to be resistant to the plant extract while isolates with clear zones of inhibition were considered susceptible to the effect of the plant extract at the concentrations showing the zone of inhibition.

Data Analysis and Presentation: Data generated from the study were subjected to descriptive statistics and expressed as mean ± standard deviations (SD).

Results

Yield of the extraction process: The aqueous extraction procedure yielded 25 g of a dark brown, sticky semisolid substance. The percentage yield was 12.5% (25g out of the 200 g pulverized dry leaves).

Phytochemical constituents: Phytochemical analysis revealed the presence of the following constituents: Alkaloids, flavonoids, terpenoids, saponins and tannins (Table 1).

Elemental analysis results: Elemental analysis of the leaves revealed the presence of the following elements: Copper – 2.489 ± 0.132 mg/kg, Iron – 0.787 ± 0.076 mg/kg, Chromium – 0.166 ± 0.009 mg/kg, Nickel – 0.042 ± 0.004 mg/kg, Arsenic – 0.009 ± 0.001 mg/kg and Cadmium – 0.005 ± 0.0 mg/kg (Table 2).

Anti-bacterial activity of aqueous leaf extract of *L. cylindrica*: The aqueous extracts of *L. cylindrica* exhibited varying degrees of inhibition activity against the tested bacteria (Table 3); and the results were expressed in terms of the diameter of the growth-inhibition zone (clear zones). There mean diameter of the inhibition zone ± standard deviation at 300 mg concentration of the extract (highest concentration) were: *Salmonella* – 14.5 ± 0.5 mm; *Shigella* – 14.5 ± 1.5 mm; *Eecherichia coli* – 15.5 ± 0.5 mm; *Bacillus* – 14.5 ± 1.3 mm; *Klebsiella* – 14.5 ± 1.3 mm; *Proteus* – 15.5 ± 0.5 mm; and *Staphylococcus* – 14.5 ± 1.0 mm These mean diameter of inhibition zone at the highest concentration of 300 mg extract was higher than what was recorded for other lower concentrations (Table 3).

Discussion

The detection in the aqueous extract of *L. cylindrica* leaves in the present study of phytochemicals such as terpenoids, flavonoids, tannins, cardiac glycosides, saponins, ketones, and the absence of reducing sugars, cardenolides and anthraquinones in the extract agrees with earlier reports by Adeyeni *et al* (2020) on the phytochemical constituents of ethanolic extract of *L. cylindrica* seed extracts, though they reported the presence of anthraquinones. It had earlier been reported that differences in the extracting solvent can account for variations in the phytochemical constituents of extracts (Seddon and Downey, 2008; Harbertson and Downey, 2009). Moreover, it had been reported that the medicinal value of a plant depends on the secondary metabolites they produce (Edeoga *et al*., 2005).

Table 1. Qualitative phytochemical constituents of aqueous extract of ***Luffa cylindrica.***

Tests	Results
Test for alkaloids	
• Dragendorff reagent phytochemical screening	–
• Meyers reagent	+
Test for flavonoids	
• Shinoda's test	+
• Ferric chloride	+
• Lead acetate	–
• Sodium hydroxide	+
Test for terpenoids	+
Test for cardioglycoside	
• Salkowski's test	+
• Lieberman-burcharid	–
Test for cardenolids	
• Killer-killiani	–
Test for carbohydrate	
• Molish's test	+
• Test for monosaccharide	–
• Test for free reducing sugar	–
• Test for combined reducing sugar	–
• Test for ketone	+
Test for saponins	
• Frothing test	+
Test for tannins	
• Ferric chloride	+
• Lead acetate	–
Test for free anthraquinone	–
Test for combined anthraquinone	–

– = Absence of phytochemical constituent; + = Presence of phytochemical constituent

Table 2. Results for elemental analysis of leaves of *Luffa cylindrica (analyses was done in triplicates – 1, 2 and 3).*

Element	1	2	3	Mean ± SD
Cadmium (mg/L)	0.0050	0.0050	0.0050	0.005 ± 0.0
Chromium (mg/kg)	0.1767	0.1551	0.1659	0.166 ± 0.009
Copper (mg/kg)	2.6503	2.3270	2.4887	2.489 ± 0.132
Iron (mg/kg)	0.7093	0.8901	0.7613	0.787 ± 0.076
Arsenic (mg/kg)	0.0081	0.0102	0.0087	0.009 ± 0.001
Nickel (mg/kg)	0.0424	0.0372	0.0469	0.042 ± 0.004

Table 3. Mean inhibition zone diameter (mm) of the extract of *Luffa cylindrica* on some bacterial organisms.

Organisms	Mean inhibition zone diameter (mm), with standard deviation in brackets						
	Control (Cipro 5 ug)	50 mg extract	100 mg extract	150 mg extract	200 mg extract	250 mg extract	300 mg extract
Salmonella	19.5 (0.5)	12.5 (1.5)	12.5 (0.0)	13.0 (2.0)	13.0 (2.0)	13.5 (1.5)	14.5 (0.5)
Shigella	24.5 (0.0)	12.0 (1.0)	12.5 (3.4)	12.5 (1.5)	13.5 (0.3)	14.0 (1.0)	14.5 (1.5)
Escherichia coli	25.5 (1.5)	11.5 (1.5)	13.0 (2.0)	12.5 (0.5)	13.5 (0.7)	14.5 (0.7)	15.5 (0.5)
Bacillus	17.0 (1.5)	12.5 (1.5)	12.0 (2.0)	13.0 (0.1)	12.5 (0.5)	13.5 (0.5)	14.5 (1.3)
Klebsiella	29.0 (3.0)	11.5 (0.3)	12.0 (1.0)	12.5 (0.5)	12.5 (0.5)	13.5 (0.5)	14.5 (1.3)
Proteus	16.0 (1.0)	12.0 (1.0)	12.5 (1.0)	13.0 (0.1)	13.5 (2.0)	14.5 (0.5)	15.5 (0.5)
Staphylococcus	14.5 (0.5)	11.5 (0.3)	12.0 (0.5)	12.5 (0.5%	13.5 (0.7)	13.5 (0.5)	14.5 (1.0)

It was noted that in the phytochemical screening for alkaloids, the extract gave a negative test with Dragendorff reagent screening (amine group test) and a positive result with Meyer's reagent test (hydroxyl group test); this difference in results is thought to be due to the different molecular structures of the test reagents. Meyer's reagent reacts with alkaloids to generate colored complexes or precipitates that are associated with hydroxyl groups (-OH) within their structures. This is due to the fact that Meyer's reagent only reacts with hydroxyl groups, whereas alkaloids containing nitrogen atoms with a single electron (amine groups, -NH2 or -NH-) will react with the Dragendorff reagent to produce a precipitate of bismuth iodide with color. Dragendorff reagent will not react with alkaloids that lack these amine groups (Raal *et al*., 2020). Also, in the phytochemical screening for flavonoids, the lead acetate test gave a negative result for flavonoids while the Shinoda test, ferric chloride test, and sodium hydroxide tests gave a positive result: this difference is believed to be due to the different chemical properties these test targets. Some flavonoids lack the sulfur-containing groups necessary to react with lead acetate. Instead, most flavonoids are identified by their reactions with specific reagents (like ammonia, ferric chloride, and sodium hydroxide) that interact with their phenolic hydroxyl groups or other characteristic structural elements. These tests are well-established and each targets different chemical features of flavonoids, thus collectively providing robust confirmation of their presence in a sample (Godlewska *et al*., 2023). Further, though the lead acetate test may be negative for tannins due to its reliance

on detecting sulfhydryl groups, the positive result with the ferric chloride test for tannins confirms the presence of tannins based on their specific chemical interaction with iron (Abdelfatah *et al*., 2021).

The concentrations of heavy metals (cadmium, chromium, copper, iron, arsenic and nickel) recorded for the leaves of *L. cylindrica* in this study fall within the permissible limit as stated by the World Health Organization (WHO 1996). This implies that the leaves of this plant can safely be used as food vegetable since the concentration of these heavy metals evaluated were within the WHO permissible level (Mensah *et al*., 2009). Madziga *et al*. (2023) reported comparable values in *Moringa oleifera.*

In this study, *a*ll the bacteria tested were sensitive at all the six different concentrations with the highest concentration (300 mg) showing larger zone of inhibition. From these results, it can be deduced that the aqueous leaf extract of *L. cylindrica* exhibited anti-bacterial activity against all the microbes tested. This is comparable to earlier reports of Aladejimokun *et al*. (2014) on the anti-microbial activity of methanolic extract of both the leaves and flower of *L. cylindrica.*

Conclusion: The aqueous leaf extract of *Luffa cylindrica* contains several phytochemicals of medicinal value, and the heavy metal concentration in the leaves of the plant is within the WHO permissible limit. The extract exhibited in vitro anti-bacterial activity against bacterial organisms such as *Salmonella, Shigella, E. coli, Bacillus, Klebsiella, Proteus* and *Staphylococcus*, in a dose dependent manner.

Conflict of interest

The authors declare no conflict of interest.

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