# PHYTOCHEMICAL AND MINERAL CONSTITUENTS OF SUCCESSIVE EXTRACTS OF *LUFFA CYLINDRICA* (M.J ROEM) LEAVES

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## Abstract

**Objective:** The present study aimed to assess the phytochemical constituents present in successive leaf extracts of *Luffa cylindrica* and the mineral and vitamin constituents of the dried powdered leaves.

**Methods:** The extracts of the powdered leaves of *L. cylindrica* were obtained using n-hexane, ethylacetate, methanol and distilled water in succession by maceration method and were tested for the presence of tannins, alkaloids, terpenes, flavonoids, anthraquinones, cardiac glycosides, saponins and phlobatannins using standard methods for phytochemical screening. The micro, macro minerals and vitamins content in the powdered leaves were also determined.

**Result:** The results showed the presence of tannins, alkaloids, terpenes, flavonoids, cardiac glycosides and saponins in the extracts of the selected solvents (from non-polar to polar) used in succession. The dried powdered leaves of *L. cylindrica* contained potassium ($9.80 \pm 0.97$mg/100g), sodium ($0.32 \pm 0.12$mg/100g), phosphorus ($6.17 \pm 0.41$mg/100g), calcium ($0.42 \pm 0.97$mg/100g), magnesium ($0.12 \pm 0.01$mg/100g), zinc ($7.67 \pm 0.02$mg/100g), chromium ($0.70 \pm 0.01$mg/100g), vitamins C ($6.92 \pm 0.03$mg/100g) and E ($0.11 \pm 0.01$mg/100g).

**Conclusion:** The selective solubility of the phytochemicals is probably responsible for conferring a wide range of therapeutic and pharmacological activities attributed to the leaves of *L. cylindrica* suggesting the relevance of the solvent as a decisive factor for confirming the presence of a phytoconstituent which could serve as a benchmark for drug development from bioactive principle(s) of plant origin with biological activities. The data on the mineral and vitamin constituents detected in the leaves of the plant suggest that the plant could serve as a source of dietary supplement for boosting the immune system. The data also justify the medicinal uses of the *Luffa cylindrica* in folklore medicine of Nigeria and some other countries for the treatment of different ailments.

## Introduction

In folk medicine practice in Nigeria and other African countries, medicinal plants are used locally to treat a myriad of ailments such as jaundice, diabetes, gastrointestinal disorders, hepatitis and malaria. This is because medicinal plants contain bioactive chemical constituents possessing therapeutic properties (Ray *et al*., 2004; Negi *et al*., 2011). The identification of the bioactive principles in medicinal plants play a strategic role in the phytochemical investigation of plant extracts and is so essential with regards to their potential pharmacological effects (Pascual *et al*., 2002). Therefor assessment of both organic and inorganic constituents in medicinal plants can be relevant in the efficacy of the medicinal plants in treating various diseases as well as provide an in-depth understanding of their pharmacological actions. *Luffa cylindrica,* (Family *Curcubitaceace*) sponge gourd as it is commonly called is a source of food and medicinal plant used as a therapeutic in traditional medicine in Nigeria and some other countries like China and Indian. Decoctions of the leaves, stems, roots seeds and fruits are used locally to treat fever, malaria, jaundice, tumors, leprosy, wounds, bleeding from bowels or bladders and also to alleviate pain and inflammation (Pal and Manoj, 2011; Azeez *et al*., 2013; Khan *et al*., 2013a). Earlier studies had reported the phytoconstituents present in the extracts of *L. cylindrica* leaves using a single solvent (Salman *et al*., 2013**,** Sharma *et al*., 2014**,** Etim *et al*., 2018; Saliu *et al*., 2019) but information on the screening of bioactive principles in successive extracts of the *L. cylindrica* leaves using solvents of varying polarities in succession is yet to be reported. The present study therefore aimed at investigating the phytochemicals in successive leaf extracts of *L. cylindica* using n-hexane, ethylacetate, methanol and water solvents in succession.

**Figure 1:** Leaves of *Luffa cylindrica*
**Source:** Zulle Farm, Suleja, Niger State

## Materials and Methods

### Collection of plant material and authentication

Leaves of *Luffa cylindrica* (Fig. 1) were collected from Zulle Farms, Suleja, Niger State, Nigeria in the month of July, 2015 around10 a.m and authenticated at the National Institute for Pharmaceutical Research and Development, (NIPRD), Abuja, where a voucher specimen (NIPRD/H/6650) was deposited at the herbarium of the institute.

### Preparation of successive extracts

The leaves were washed with water to remove dirt and air-dried to a constant weight for three weeks. Leaves were milled into powder with mechanical blender (Mazeda Mill, MT 4100, Japan). 100 g of the dried leaf powder was extracted firstly using n-hexane followed by ethylacetate, methanol and distilled water in succession by maceration method. Each extract obtained following successive extraction was filtered using Whatman No 1 filter paper, concentrated in a rotary evaporator (RE-300B model, product of Henan Touch Science, China) except the aqueous extract and subsequently dried to a semisolid mass using water bath at 45°C. The yield of each extract thus obtained was recorded and stored in a refrigerator at $4^0$C till further use.

**Phytochemical analysis**

Qualitative phytochemical screening viz. saponins, tannins, flavonoids, cardaic glycosides, phenolics, phlobatannins, terpenes, alkaloids, anthraquinones was carried out for each successive extract using standard methods described by Harbone (1973); Trease and Evans (1989) and Sofowora (1993). The quantification was carried out for saponins (Brunner, 1984), alkaloids (Henry, 1973), flavonoids, tannins, terpenes, phenolics and phlobatannins (El-Olemy *et al*., 1984).

**Mineral analysis**

Mineral evaluation such as calcium, potassium, magnesium, sodium and phosphorus were carried out on the powdered leaves of *Luffa cylindrica* using the multiple-nutrient wet acid digestion method described by AOAC (1980). The minerals were quantified by Flame photometric method on Jenway Digital Flame Photometer (PFP7 Model) using filters corresponding to each mineral element. Determination of trace elements Cd, Cr, and Zn was carried out with Atomic Absorption Spectrophotometer (AAS) Buck 211 Model.

**Results**

**Table 1** depicts the yield obtained from each successive extracts of the leaves of *Luffa cylindrica* using hexane, ethylacetate, methanol and water. The aqueous extract gave the highest yield followed by methanol, hexane and ethylacetate. The qualitative and quantitative secondary metabolites evaluations of the various successive extracts are presented in **Table 2**. The phytochemical tests employed detected the presence of alkaloids in all the successive (aqueous, methanolic, ethylacetate and hexane) extracts, terpenes in methanolic, ethylacetate and hexane extracts alone, saponins and phenolics were present only in the aqueous and methanolic successive extracts, cardaic glycosides in aqueous, methanolic and hexane extracts, flavonoids were present in methanolic and ethylacetate extracts while tannins was present only in the methanolic extract. Prominent among these secondary metabolites is terpenes, found to be (44.86 ±19.4 mg/g) in hexane, (22.45 ± 0.03 mg/g) in ethylacetate and (17.45 ± 2.16 mg/g) in methanolic successive extracts while cardaic glycosides had the lowest concentration (0.05 ± 0.01 mg/g) among the respective successive extracts. Alkaloids, flavonoids, tannins, phenolics, saponins varied in concentrations among the respective successive extracts.

The mineral and vitamin analyses of *L. cylindrica* powdered leaves showed elements like K (9.80 ± 0.97 mg/g), P (6.17 ± 0.41 mg/g), Ca (0.42 ± 0.01 mg/g) Na (0.32 ± 0.12 mg/g), Zn (7.62 ± 0.02 mg/g) and Mg (0.12 ± 0.01 mg/g) (**Table 3**). The leaves also contain minute amounts of Cr (0.70 ± 0.01 mg/g). Vitamin C (6.92 ± 0.03 mg/g) and Vitamin E (0.11 ± 0.01 mg/g) were also found to be present in the powdered leaves in considerable amount

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**Table 1:** Yield and colour of successive extract of *Luffa cylindrica* leaves

| Organic solvent used | weight of sample (g) | Extraction time (hours) | Yield (g) | colour of sample |
|---|---|---|---|---|
| n-hexane | 100 | 14 | 5.2 | Dark brown |
| Ethylacetate | 95 | 12 | 3.8 | Dark brown |
| Methanol | 91 | 10 | 5.6 | Black |
| Aqueous | 86 | 10 | 9.2 | Dark green |

**Table 2:** Secondary metabolites of successive leaves extracts of *Luffa cylindrica*

| Secondary Metabolites | Composition (mg/g) | | | |
|---|---|---|---|---|
| | **Aqueous Extract** | **Methanolic Extract** | **Ethylacetate Extract** | **Hexane Extract** |
| Saponins | 1.32 ± 0.18 | 0.90 ± 0.03 | - | - |
| Tannins | - | 3.58 ± 0.90 | - | - |
| Anthraquinones | - | - | - | - |
| Terpenes | - | 17.45 ± 0.18 | 22.45 ± 0.03 | 44.86 ± 19.4 |
| Phenolics | 7.79 ± 0.22 | 9.16 ± 0.12 | - | - |
| Flavonoids | - | 7.45 ± 0.49 | 26.07 ± 0.18 | - |
| Alkaloids | 0.10 ± 0.01 | 11.35 ± 0.04 | 18.24 ± 0.02 | 13.28 ± 0.01 |
| Phlobatannins | - | - | - | - |
| Cardaic Glycosides | 0.06 ± 0.01 | 0.05 ± 0.01 | - | 0.05 ± 0.02 |

- **absent**

Values are means ± SEM of three replicates

**Table 3:** Mineral/vitamin constituents present in powdered leaf of *L. cylindrica*

| Mineral/Vitamin Constituents | Composition (mg/100g) |
|---|---|
| Calcium | 0.42 ± 0.01 |
| Potassium | 9.80 ± 0.97 |
| Phosphorus | 6.17 ± 0.41 |
| Magnesium | 0.12 ± 0.01 |
| Sodium | 0.32 ± 0.12 |
| Zinc | 7.67 ± 0.02 |
| Chromium | 0.70 ± 0.01 |
| Cadmium | - |
| Vitamin C | 6.92 ± 0.03 |
| Vitamin E | 0.11 ± 0.01 |

- **absent**

Values are means ± SEM of three replicates

**Discussion**

The secondary metabolites, mineral and vitamin constituents identified in *L. cylindrica* leaves in this present study could be responsible for the medicinal use of the plant in the folk medicine of some countries like Nigeria as well as the biological activities scientifically attributed to the plant. Alkaloids are one of the secondary metabolites in plants majorly used as basic active ingredients in pharmaceutical drugs to kill or relief pains. Flavonoids are also important bioactive compounds which exhibit a wide range of biological activities among which are antioxidant, anti-inflammatory and antimicrobial activities (Ayoola *et al*., 2008; Panche *et al*., 2016).

Flavonoids have been reported to be capable of inhibiting the synthesis of prostaglandin, a metabolite that mediates pain and fever (Manthey, 2000). In addition, flavonoids, saponins and triterpenes are reported to also possess analgesic property (Borgi *et al*., 2008; Biswas *et al*., 2009) thus, justifying the use of *L. cylindrica* leaves in the treatment of pain and enteric fever in folk medicine. Saliu *et al*. (2019) further established the analgesic and antipyretic activities of *L. cylindrica* leaves in animals. Tannins are capable of precipitating proteins and therefore they possess astringent properties, promote wounds healing and inflamed mucous membrane. Plants containing tannins are used for healing of wounds, varicose ulcers, hemorrhoids, frost- bite and burns (Ibrahim and Fagbohun, 2012; Kigigha *et al*., 2015) therefore the use of *L. cylindrica* leaves for the treatment of inflammation and wounds in folk medicine maybe attributed to presence of tannins and flavonoids. In addition, Khah *et al*. (2013b) have also established the anti-inflammatory activities of *L. cylindrica* leaves in experimental animals. Saponins constitute a group of triterpenes which are bioactive compounds generally known to be produced by plants for defense against pathogens and herbivorous animals. Apart from their role in plant defense, saponins are of pharmacological importance having the ability to inhibit DNA replication in cancerous cell thus act as anti-tumor and anti-cancer agents. Similarly, flavonoids are also reported to be potent against cancer cells (Ravishankar *et al*., 2013). In view of this, several studies establishing the anti-cancer activity of *L. cylindrica* containing saponins and flavonoids are well documented in literature (Abdel-Salam *et al.,* 2018; Garai *et al*., 2018; Abdel-Salam *et al*., 2019).

Tannins, saponins, phenolics, alkaloids and flavonoids have been suggested to be involved in anti bacterial and anti-viral activities. Bulbul *et al*. (2011); Mankilik and Mikailu (2014) reported the anti-microbial activities of *L. cylindrica* leaves against *Staphylococcus species*, *Salmonella typhii*, *Escherichia coli,* and *Aspergillus species*. This is evident for the traditional use of the plant to treat microbial related diseases such as leprosy, skin diseases etc. due to the presence of tannins, saponins, phenolics, alkaloids and flavonoids in the plant. The macro minerals content of the powdered leaves of *L. cylindrica* from this study is lower but the micro minerals content is higher than ones reported by Ogunyemi *et al*. (2020) in the seed of the plant obtained from South-Western region of Nigeria suggesting that the trace minerals maybe predominantly present in the leaves than the seed. From this study, the plant leaves contained 0.42 mg/100g of calcium which is lower than calcium content (2.12 mg/100g) in the plant seed reported by Ogunyemi *et al.* (2020). The phosphorus content of the powdered leaves was 6.17 mg/100g as against 30.63 mg/100g of phosphorus obtained from the seed of *L. cylindrica*. The leaves also contained a smaller amount of magnesium with a value of 0.12 mg/100g than the seed which contained 28.93 mg/100g. Calcium, magnesium and phosphorus are minerals that plays key role in bone mineralization and teeth development. Calcium is important for optimal bone health, assists in transmission of nerve impulses, necessary for muscle contraction and blood clotting. Magnesium like calcium assists in the proper function of nerves, muscles and many other parts of the body. Magnesium also acts as an antacid for heartburn by neutralizing stomach acids and is also helpful as a laxative for constipation as it moves stool through the intestine. Phosphorus is involved in energy production, storage and cell signaling via phosphorylation reactions, and regulation of normal acid-base balance (homeostasis) by acting as one of the body's most important buffers (Knochel, 2006).

The concentration of potassium was 9.80 mg/100g, which is lower to 13.86 mg/100g of potassium concentration recorded in the seed of the plant. Potassium is an important element that acts as a vasodilator, strengthening the elasticity of the blood vessels and thus lowers the risk of developing cardiovascular disease. Sodium whose concentration (0.32mg/100g) in the leaf of *L. cylindrica* in this study is smaller to the value reported in the seed (8.18 mg/100g) of same plant is required in the body for regulating blood pressure and blood volume. It also helps in the proper functioning of the muscles and nerves in the body. Though the calcium, magnesium, phosphorus and sodium contents present in *L. cylindrica* leaves were lower to the recommended daily allowance (Ca-800 to 1200mg, K- 1600mg, Mg- 400mg, Na-2000mg, P-700mg/day), it still suggests that the plant may be good as a therapy for mineral-deficiency related diseases like osteoporosis, clogged arteries and hereditary heart disease. These macro minerals (calcium, potassium, magnesium, phosphorus and sodium) are not only the minerals that promote the proper function of the body, trace minerals like zinc, iron and chromium also plays significant physiological role in the body. *L. cylindrica* powdered leaves contained 7.67 mg/100g and 0.70 mg/100g of zinc and chromium respectively which were higher than the concentrations (3.42 mg/100g and 0.25

mg/100g for zinc and chromium respectively) accounted for by Ogunyemi *et al*. (2020) in the seed of *L. cylindrica.* The content of zinc (7.67 mg/100g) in *L. cylindrica* leaves is close to the recommended daily allowance (8mg/day for women and 11mg/day for men) implying that the leaf of *L. cylindrica* is a good source of zinc. Zinc stimulates the production of sex hormones thus promote fertility. It also promotes wound healing inferring the use of *L. cylindrica* as a choice in folk medicine for the treatment of wounds. Zinc also plays a major role as a regulator of gene expression by binding to transcription factor to activate gene expression (Cousins, 1994; Dalton *et al*., 1997; Cousins *et al*., 2003). Chromium ($Cr^{3+}$) as an essential trace mineral assists the body cells in the uptake of glucose by enhancing their sensitivity to insulin response. The chromium perhaps act in conjunction with other phytoconstituents to promote the anti-hyperglycemic effect exhibited by *L. cylindrica* as the anti-diabetic activity of the plant have been established (Balakrishnan and Sharma, 2013; Akther *et al*., 2014). $Cr^{3+}$ also help lower cholesterol levels such as triglyceride, total cholesterol, low density lipoproteins (bad cholesterol) and increase the level of high density lipoproteins (good cholesterol) which reduces the risk of developing cardiovascular diseases. Calcium, magnesium, and zinc generally are components of some antioxidant enzymes acting as co-factor in enhancing their activities against free radicals that causes damage to biological membranes by oxidative stress. Many other enzymes also depend on some of these minerals for their catalytic action as their removal could results in loss of the enzymes activity. Vitamins are part of organic molecules required in the body though in small quantity for proper functioning of the body. Vitamins like Vitamin C and Vitamin E can act as antioxidants that helps prevent aging, damage to cells, tissues, proteins and DNA induced by free radicals. Vitamin E most often referred as α-tocopherol act on free radicals by donating electrons to them to form a radical tocopherol which can spontaneously be converted back to non-radical tocopherol using other non-enzymatic antioxidants like vitamin C. The presence of vitamin C and E and other phenolic compounds like flavonoids and saponins further supports the earlier report of Saliu *et al.* (2020) on *L. cylindrica* as having free radical and reactive oxygen species scavenging property. Earlier studies on preliminary screening of secondary metabolites have documented the presence of these secondary metabolites identified in the successive leaf extracts of *L. cylindrica* employing a single solvent (Khan *et al*., 2013; Etim *et al*., 2018; Saliu *et al*., 2019). However, in this study, the phytochemical test of successive leaf extracts of *L. cylindrica* obtained by using n-hexane, ethylacetate, methanol and water as the solvents for extraction infers the presence of different active ingredients having selective solubility in successive solvents of varying polarities used in succession thus suggesting the important role of the solvent as a decisive factor (Koruthu *et al*., 2011). This could be guide for isolating bioactive ingredients of plant origin with biological activities for the purpose of drug development.

## Conclusion

The nature of solubility of the phytochemicals is probably responsible in conferring a wide spectrum of therapeutic and pharmacological activities attributed to the leaves of *L. cylindrica* suggesting the relevance of the solvent as a decisive factor for confirming the presence of a phytoconstituent which could serve as a benchmark for drug development from bioactive principle(s) of plant origin with biological activities. The data on the mineral and vitamin constituents detected in the leaves of the plant suggest that the plant could serve as a source of dietary supplement for boosting the immune system. The data also justify the medicinal uses of the *Luffa cylindrica* in folklore medicine of Nigeria and some other countries in treating different ailments.

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