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Pharmacological Activities of Phytochemical and Micro-Element Constituents of Successive Extracts of *Luffa cylindrica* (M.J. Roem) Leaf

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ABSTRACT

Background and Objective: *Luffa cylindrica* is a plant used in the traditional medicine of Nigeria to treat different ailment conditions. Chemical constituents present in plants that are used in traditional medicine were reported to possess pharmacological activities. The present study investigated the pharmacological activities of the phytochemical and microelement constituents of successive extracts of *L. cylindrica* leaf. **Materials and Methods:** Different extracts of dried-powder leaves of *L. cylindrica* were prepared by successive extraction using n-hexane, ethyl acetate, methanol and distilled water. The extracts were screened for the presence of tannins, alkaloids, terpenes, flavonoids, anthraquinones, cardiac glycosides, saponins and phlobatannins using standard methods. Microelements in the dried-powder leaf were determined using atomic absorption spectrophotometer. **Results:** Tannins, alkaloids, terpenes, flavonoids, cardiac glycosides and saponins were present in varying amounts in the different successive extracts. The dried powder leaf contained considerable amount of potassium (9.80±0.97 mg/100 g), sodium (0.32±0.12 mg/100 g), phosphorus (6.17±0.41 mg/100 g), calcium (0.42±0.97 mg/100 g), magnesium (0.12±0.0 mg/100 g), zinc (7.67±0.02 mg/100 g), chromium (0.70±0.01 mg/100 g), vitamins C (6.92±0.03 mg/100 g) and E (0.11±0.01 mg/100 g). **Conclusion:** The selective solubility of phytochemicals in the different solvents is responsible for conferring a wide range of therapeutic and pharmacological activities attributed to *L. cylindrica* thus suggesting the relevance of solvents as a decisive factor for confirming the presence of bioactive principles in plants which could serve as a benchmark in drug discovery. The mineral and vitamin constituents detected in the leaves of *L. cylindrica* suggested that the plant can serve as a source of dietary supplement for boosting the immune system.

KEYWORDS

Luffa cylindrica, leaf, successive extracts, phytochemicals, minerals, pharmacological activities, folk medicine

INTRODUCTION

In the folk medicine practice of 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

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is because medicinal plants contain bioactive chemical constituents that possess therapeutic properties[1,2]. The identification of the bioactive components in medicinal plants plays a strategic role in the phytochemical investigation of plant extracts and respective pharmacological values[3]. Therefore assessment of both organic and inorganic constituents in medicinal plants can provide information on the scope of applications of such plants.

Luffa cylindrica belongs to the family, *Curcubitaceace* and is commonly called sponge gourd. It is a source of food and a known medicinal plant used as a therapeutic in the traditional medicine of Nigeria and some other countries including China and India. Decoctions of the leaves, stems, roots seeds and fruits are used locally to treat fever, malaria, jaundice, tumours, leprosy, wounds, bleeding from bowels or bladders and also to alleviate pain and inflammation[4-6].

Earlier studies had reported the phytoconstituents present in different extracts of *L. cylindrica* leaves using a single solvent extraction method. Oyetayo *et al.*[7] reported the presence of saponins, alkaloids and cardiac glycosides in methanolic and ethanolic extracts of the plant leaf. Sharma *et al.*[8] identified glycosides, terpenoids, steroids, flavonoids and tannins in methanolic extract. Flavonoids, saponins, tannins and cardiac glycoside were identified in the aqueous extract of *L. cylindrica* leaf reported by Mhya and Mankilik[9]. Aboh *et al.*[10] carried out a comprehensive study on hexane, ethyl acetate and methanolic extracts of the leaf and found carbohydrates, sterols, saponins, alkaloids, flavonoids and phenols to be present in the extract of the various solvents while terpenes, tannins, anthraquinones, phlobatanins and resins were reportedly absence in the solvents extract studied. Etim *et al.*[11] identified saponins, phenols, tannins, flavonoids, quinolones, glycosides, terpenoids, steroids and alkaloids in the methanol extract of the plant. A study also carried out by Saliu *et al.*[12] on pharmacological evidence favouring the ethnomedicinal use of *L. cylindrica* leaf in the relief of pain and fever revealed the presence of saponins, tannins, terpenes, phenolics, flavonoids, alkaloids and cardiac glycosides in methanolic extract of the plant leaf.

At the time this study was conducted, there is a dearth of information on the quantification of the bioactive constituents from successive extracts of *L. cylindrica* leaf using solvents of varying polarity in succession. The present study therefore aimed at investigating the phytochemicals in successive leaf extracts of *L. cylindica* obtained in North-West, Nigeria.

MATERIALS AND METHODS

Collection of plant material and authentication: Fresh leaves of *Luffa cylindrica* (Fig. 1) were harvested from Zulle Farms, Suleja, Niger State, Nigeria in July 2015 around 10 a.m. The plant leaf was 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.

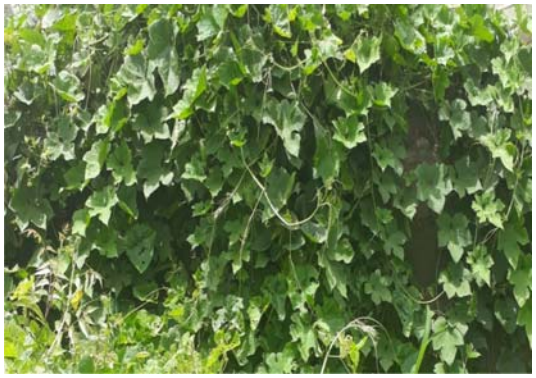

Fig. 1: Leaves of *Luffa cylindrica* harvested from Zulle Farm, Suleja, Niger State, Nigeria

Preparation of successive extracts: The leaves were washed with distilled water to remove dirt and air-dried to constant weight for 21 days. The dried leaves were milled into powder with a mechanical blender (Mazeda Mill, MT 4100, Japan). About 100 g of the dried-powdered leaves was firstly extracted with n-hexane followed by ethyl acetate, methanol and distilled water in succession by the maceration method. Each extract obtained following the successive extraction was filtered with 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 constant using a water bath at 45°C. The percentage yield of each extract was calculated and stored in a refrigerator at 4°C till further use.

Phytochemical screening: Qualitative phytochemical screening viz. saponins, tannins, flavonoids, cardiac glycosides, phenolics, phlobatannins, terpenes, alkaloids, anthraquinones were carried out for each successive extract using standard methods described by Harbone[13], Evans[14] and Sofowora[15]. Quantification was carried out for saponins by the method of Brunner[16], alkaloids by Henry[17], flavonoids, tannins, terpenes, phenolics and phlobatannins by El-Olemy *et al*.[18].

Mineral and vitamins evaluation: Mineral evaluation which includes calcium, potassium, magnesium, sodium, zinc, phosphorus and chromium was carried out on dried-powder leaves of *L. cylindrica* using the multiple-nutrient wet acid digestion method described by AOAC[19]. The minerals were quantified by the 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.

Statistical analysis: Data represent the mean of two replicates±SEM. They were statistically analyzed using SPSS Version 20.

RESULTS

Phytochemical screening of successive extracts: The yield obtained from each successive extract of *Luffa cylindrica* leaf using hexane, ethylacetate, methanol and water was depicted in Table 1. The aqueous extract gave the highest yield followed by methanol, hexane and ethylacetate extracts. The qualitative and quantitative secondary metabolites evaluation of the various successive extracts was presented in Table 2. The phytochemical screening indicated 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 only in the aqueous and methanolic successive extracts, cardiac glycosides in aqueous, methanolic and hexane extracts, flavonoids in methanolic and ethylacetate extracts while tannins only in the methanolic extract. Prominent among these secondary metabolites were terpenes, found to be (44.86±19.4 mg g^{-1}) in hexane, (22.45±0.03 mg g^{-1}) in ethylacetate and (17.45±2.16 mg g^{-1}) in methanolic successive extracts while cardiac glycosides had the lowest concentration (0.05±0.01 mg g^{-1}) among the respective successive extracts. Alkaloids, flavonoids, tannins, phenolics and saponins varied in concentrations among the respective successive extracts.

Microelements composition: The mineral and vitamin composition of *L. cylindrica* leaf was shown in Table 3. The leaf contained potassium (9.80±0.97 mg g^{-1}), phosphorus (6.17±0.41 mg g^{-1}), calcium

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

Organic solvent used	Weight of sample (g)	Extraction time (hrs)	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*

	Composition (mg g^{-1})			
Secondary metabolites	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	-	-	-	-
Cardiac glycosides	0.06±0.01	0.05±0.01	-	0.05±0.02

-: Absent, values are Means±SEM of three replicates

Table 3: Mineral and vitamin constituents in the powdered leaf of *L. cylindrica*

Mineral/vitamin constituents	Composition (mg/100 g)
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

(0.42±0.01 mg g^{-1}), magnesium (0.12±0.01 mg g^{-1}) and sodium (0.32±0.12 mg g^{-1}) as macro elements. Microelements such as zinc (7.62±0.02 mg g^{-1}) and chromium (0.70±0.01 mg g^{-1}) were also present in the leaf of *L. cylindrica*. Vitamin C (6.92±0.03 mg g^{-1}) and vitamin E (0.11±0.01 mg g^{-1}) were present in considerable amounts.

DISCUSSION

The secondary metabolites, mineral and vitamin constituents identified in *L. cylindrica* leaf in this present study could be responsible for the choice of the plant in the folk medicine of some countries like Nigeria as the biological activities exhibited by plants are attributed to phytochemicals. Alkaloids are one of the secondary metabolites in plants majorly used as basic active ingredients in pharmaceutical drugs to kill or relieve pain. Flavonoids are also important bioactive compounds which exhibit a wide range of biological activities among which are antioxidant, anti-inflammatory and antimicrobial activities[20,21]. Flavonoids have been reported to be capable of inhibiting the synthesis of prostaglandin, a metabolite that mediates pain and fever[22]. In addition, flavonoids, saponins and terpenes are also reported to possess analgesic property[23,24] thus, justifying the use of *L. cylindrica* leaves in the treatment of pain and enteric fever in folk medicine. Saliu *et al.*[12] further established the analgesic and antipyretic activities of methanolic extract of *L. cylindrica* leaf which contained alkaloids, flavonoids, saponins and terpenes in animals. Tannins are capable of precipitating proteins and therefore they possess astringent properties, promote wounds healing and inflamed mucous membranes. Plants containing tannins are used for healing wounds, varicose ulcers, haemorrhoids, frostbite and burns[25,26] therefore the use of *L. cylindrica* leaf for the treatment of inflammation and wounds in folk medicine may be attributed to the presence of tannins and flavonoids. In addition, Kanwal *et al.*[27] also established the anti-inflammatory activities of *L. cylindrica* 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 with the ability to inhibit DNA replication in cancerous cells and thus act as anti-tumour and anti-cancer agents. Similarly, flavonoids

have also been reported to be potent against cancer cells[28]. Because of this, several studies establishing the anti-cancer activity of *L. cylindrica* containing saponins and flavonoids were well documented in literature[29-31]. Tannins, saponins, phenolics, alkaloids and flavonoids have been suggested to be involved in anti-bacterial and anti-viral activities.

Bulbul *et al.*[32], Aladejimokun *et al.*[33] reported the anti-microbial activities of *L. cylindrica* leaves against *Staphylococcus species*, *Salmonella typhii*, *Escherichia coli and Aspergillus species*. This supported the traditional use of *L. cylindrica* to treat microbial-related diseases such as leprosy, skin diseases etc., due to the presence of tannins, saponins, phenolics, alkaloids and flavonoids.

The macro minerals content of the powdered leaves of *L. cylindrica* from this study was lower but the micro minerals content was higher than ones reported by Ogunyemi *et al.*[34] in the seed of the plant obtained from the Southwestern region of Nigeria. This suggested that trace minerals may be predominantly present in the leaf than in the seed of the plant. From this study, *L. cylindrica* leaves contain 0.42 mg/100 g of calcium which is lower than the calcium content (2.12 mg/100 g) in the plant seed reported by Ogunyemi *et al.*[34]. The phosphorus content in the powdered leaf was 6.17 mg/100 g as against 30.63 mg/100 g of phosphorus obtained from the seed of *L. cylindrica*[34]. The leaf also contains a smaller amount of magnesium with a value of 0.12 mg/100 g than the seed which contained 28.93 mg/100 g.

Calcium, magnesium and phosphorus are minerals that played a key role in bone mineralization and teeth development. Calcium is important for optimal bone health and assists in the 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 also acts as a laxative against constipation by assisting the movement of stool through the intestine. Phosphorus is involved in energy production, storage and cell signalling via phosphorylation reaction and regulation of normal acid-base balance (homeostasis) by acting as one of the body's most important buffers[35].

The concentration of potassium was 9.80 mg/100 g, which is lower than the 13.86 mg/100 g of potassium concentration recorded in the seed of the plant[34]. Potassium is an important element that acts as a vasodilator, strengthens the elasticity of the blood vessels and thus lowers the risk of developing cardiovascular disease. Sodium whose concentration (0.32 mg/100 g) in the leaf of *L. cylindrica* in this study was lower than the report in the seed (8.18 mg/100 g) was required in the body for regulating blood pressure and blood volume. Sodium also helps in the proper functioning of the muscles and nerves in the body. Although the calcium, magnesium, phosphorus and sodium contents present in the *L. cylindrica* leaf of this study were lower than those reported in the seed but were higher than those reported in another species *L. aegytiaca*[34]. This suggested that the leaf parts of the plants could also be good 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 which are part of reports of our findings also play a significant physiological role in the body. *Luffa cylindrica* dried-powdered leaf contains 7.67 and 0.70 mg/100 g of zinc and chromium respectively which were higher than the concentrations (3.42 and 0.25 mg/100 g for zinc and chromium respectively) accounted for by Ogunyemi *et al.*[34] in the seed. The zinc content (7.67 mg/100 g) in *L. cylindrica* leaf is close to the recommended daily allowance (8 mg/day for women and 11 mg/day for men) implying that the leaf of *L. cylindrica* is a good source of zinc. Zinc stimulates the production of sex hormones and thus promotes 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 factors to activate gene expression[36-38]. Chromium (Cr^{3+}) as an essential trace mineral assists

the body cells in the uptake of glucose by enhancing the body's sensitivity to insulin response. The chromium perhaps acts in conjunction with other phytoconstituents in *L. cylindrica* to promote the anti-hyperglycemic and anti-diabetic activities of the plant[39,40]. The Cr^{3+} also help lower cholesterol levels such as triglyceride, total cholesterol and low-density lipoproteins (bad cholesterol) and increases the level of high-density lipoproteins (good cholesterol) which reduces the risk of developing cardiovascular disease. 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 result in loss of the activity of the enzyme. Vitamins are part of organic molecules required in the body though in small quantities for the proper functioning of the body.

Vitamins such as vitamins C and E can act as antioxidants that help prevent ageing and damage to cells, tissues, proteins and DNA induced by free radicals. Vitamin E is most often referred to as α-tocopherol synergies with Vit. C to acts on free radicals by donating electrons to them and converting them to non-radical molecules. According to our earlier study[41], the presence of vitamin C and E as well as phenolic compounds like flavonoids and saponins contributes to the antimalarial activity demonstrated by *Luffa cylindrica* leaf extracts in rats. Also, previous 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[6,11,12]. In this study, the phytochemical screening of the successive leaf extracts of *L. cylindrica* was obtained using n-hexane, ethyl acetate, methanol and water as solvents for extraction which confirmed the presence of different active ingredients with selective solubility in successive solvents of varying polarities suggest the important role of solvents as a decisive factor[42]. This therefore could provide a guide for the isolation of bioactive ingredients with biological activities in plants for drug development in addressing health challenges.

CONCLUSION

The nature of solubility of the phytochemicals plays a significant role in conferring a wide spectrum of therapeutic and pharmacological activities attributed to plants suggesting the relevance of solvents as a decisive factor for confirming the presence of bioactive principles in plants which could serve as a benchmark in drug discovery. The mineral and vitamin constituents detected in the leaves of *L. cylindrica* suggested that the plant can serve as a source of dietary supplement for boosting the immune system. Also, the study justified the use of the *Luffa cylindrica* in folklore medicine of Nigeria and some other countries in treating different ailments.

SIGNIFICANCE STATEMENT

In this study, the phytochemical investigation of *L. cylindrica* provides insight into the pharmacological activities of the plant. The phytochemicals reported in *L. cylindrica* serve as the basis for the ethnobotanical use of the plant in treating different ailments, especially malaria and could therefore also serve as a promising alternative source for the development of drug(s) to treat different diseases.

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