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Research Paper

Proximate Analysis of Luffa Cylindrica Seeds and Phytochemical Screening of the Seed Pods Harvested In Ilorin, Kwara State, Nigeria.

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Abstract
Luffa Cylindrica (Sponge gourd) seed sample was investigated for the proximate composition and the phytochemical composition of the seed pods was also screened. The proximate analysis showed that the Crude fibre, Protein, Fat, Moisture, Ash, and digestible carbohydrate contents were: 9.01, 10.50, 31.05, 2.80, 6.00, and 9.45g%. The phytochemical screening of extracts obtained from four different solvents using Soxhlet method showed that the n-Hexane extract had the presence of Carbohydrate, Alkaloid, Flavonoid, Unsaturated steroid/ Triterpenoids; while the dichloromethane (DCM) extract contained Carbohydrate, Alkaloid and Triterpenoids. In the ethylacetate extract of the seed pod contained only carbohydrate. The ethanol extract contained several phytochemicals- Flavonoid, Tannin, Resin, Saponin and Anthraquinone. The seed is a lesser source of protein than fat. The mineral component is as well low as indicated by the ash content. However, the seed pod contained several phytochemicals that may make it medicinally useful.

Keywords: *LuffaCylindrica, Proximate analysis, Phytochemical screening.*

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I. INTRODUCTION

Luffa cylindrical (Linn). has several names which include; sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd running okra, strainer vine, Chinese okra, California okra, and loofah (James, 2012), and was believed to have originated from America (Mazali and Alves, 2005). *Luffa* is the genus name of a group of gourds and is a member of cucurbitaceous family. The number of species in the genus *Luffa* varies from 5 to 7. Only 2 species *L. cylindrica* and ribbed or ridge gourd [*L. acutangula (L.) Roxb*] are domesticated (Bal et al., 2004). *Luffa cylindrica* is a large climbing vine, with a thin but very tough light green, succulent stem, attaining a length of 10-30 feet. *Luffa* is a sub-tropical plant which requires warm summer temperatures and a long frost-free growing season when grown in temperate regions. The leaves are alternate and palmately lobed, of a light green color and almost destitute of taste (Velmurugan et al, 2011). The fruits, which also have a network of fibres surrounding a large number of flat blackish seeds (Indumathy et al., 2011).

Angled *Luffa* is *L. acutangula*, while the smooth-fruited version is *L. cylindrica*, or the same as *L. aegyptica*. Both luffas have value as food items, but are seldom eaten. Most gardeners grow them for their fibrous interior, which is useful as a rough cloth or sponge for cleaning and scouring. The two species are somewhat similar in appearance. Both are vigorous, climbing, annual vines with several lobed cucumber-like leaves. When crushed, the leaves give off a rank odor (James, 2012).

L. cylindrica is a sub-tropical plant, which requires warm summer temperatures and long frost-free growing season when grown in temperate regions. It is an annual climbing which produces fruit containing fibrous vascular system. It is a summer season vegetable. It is difficult to assign with accuracy the indigenous areas of *Luffa* species. They have a long history of cultivation in the tropical countries of Asia and Africa. Indo-Burma is reported to be the center of diversity for sponge gourd. The main commercial production countries are China, Korea, India, Japan and Central America (Bal et al., 2004; Oboh and Aluyor, 2009).

The pharmacological activities of *L. cylindrica* has been reported to include: *Anti-inflammation*- it inhibited carrageenin-induced plantar edema in rats (Kang et al., 1984); *Anti-fungus*- in vitro, luffacylin inhibited Mycosphaerellaarachidicola and Fusariumoxysporum (Parkash et al., 2002); *Analgesia and sedation*-

inhibited acetic acid-induced writhing, raised the pain threshold in hot plate and electric shock tests, reduced spontaneous activities, and synergized the effects of pentobarbital sodium (Kang et al., 1992; Kang et al., 1993); *Anti-myocardial ischemia*- In a pituitrin-induced acute myocardial ischemia mouse model, it lowered T-wave increase in electrocardiogram, inhibited the decrease of heart rate, inhibited the raise in serum lactate dehydrogenase level and myocardial malondialdehyde level, and enhanced the activity of myocardial superoxide dismutase (Guan et al., 2006); *Anti-hypertriglyceride*- in a hypertriglyceridema rat model, decreased serum cholesterol and triglyceride levels, increased high density lipoprotein-cholesterol, and reduced the body weight (Li et al., 2004); *Immunostimulation*- Oral administration of the petroleum ether fraction of the ethanol extracts of fruits, leaves and stems potentiated the cytophagic action and acid phosphatase activity of peritoneal macrophages in mice (Mao et al., 2004). In the enhancement of the production of interleukin-1, interleukin-2 and tumor neurosis factor-α in mouse thymocytes (Li et al., 2001); *Anti-allergy*- inhibited homologous passive cutaneous anaphylaxis in rats, heterologous passive cutaneous anaphylaxis in mice, Arthus reaction in mice, and sheep red blood cell-induced delayed type hypersensitivity in mice (Kou et al., 2001); *Anti-asthma anti-tussive and expectorant effects*- its extract suppressed SO_2- and ammonium aerosol-induced cough in mice, and increased the respiratory tract phenol red excretion in mice. In guinea pigs, it inhibited histamine induced asthma (Pallavi et al., 2011); It had anti-acute hepatic injury, cardiac stimulation etc. (Ng et al., 1992; Zhang et al., 1990; and Khan et al., 1992).

Its medicinal importance shown from its various uses hasbeen very clearly reported by Pallavi et al (2011) stating that the plant is used as a bitter tonic, emetic, diuretic and purgative and useful in asthma, skin diseases and splenic enlargement. It is used internally for rheumatism, backache, internal hemorrhage, chest pains as well as hemorrhoids. Young fruit can be eaten raw like cucumber or cooked like squash, while the young leaves, shoots, flower buds, as well as the flowers can be eaten after being lightly steamed. The seeds can be roasted as a snack, or pressed to produce oil. Externally, it is used for shingles and boils. The dried fruit fibers are used as abrasive sponges in skin care, to remove dead skin and to stimulate the circulation. The fruits are anthelmintic, carminative, laxative, depurative, emollient, expectorant, tonic and galactagogue (i.e promoting or increasing the flow of mother's milk) and are useful in fever, syphilis, tumours, bronchitis,splenopathy and leprosy. The vine is most commonly grown for the fibrous interior of the fruits. Kernel of seed is expectorant, demulcent and used in dysentery. The seed oil is used in leprosy and skin diseases. Fruit is intensely bitter and fibrous. It has purgative property and is used for dropsy, nephritis, chronic bronchitis and lung complaints. It is also applied to the body in putrid fevers and jaundice.

This paper is not only aimed at exploring the plant in support of the well reported facts of its medicinal and physiological status, but also to concentrate on the nutritional status of the seed possibly as a potential source of animal feed. In addition, it is to attempt to investigate in isolation the medicinal value of the seed pods which rather may be discarded as a non-useful part of the plant.

II. MATERIALS AND METHODS

All reagents used were of analytical grade. The plant, *Luffa cylindrica* sample was collected by picking from an uncultivated land in Ogidi- Oloje area of Ilorin-South Local Government Area, Kwara State of Nigeria.

The seeds were separated from the rest of the plant manually. The removal of each seed from its pod by cracking was also done manually. The seed sample and the pod sample were separately dried under shade for about 3 days before pulverizing each in a ceramic mortar and pestle. The powdered samples were employed for the analyses.

Proximate Analysis

Determination of Crude Fibre: 1g of the defatted powdered gourd seeds was weighed and placed in 500ml flat bottom flask.150ml of prepared 0.5 M H_2SO_4 was added to it and the was allowed to boil under reflux for 30mins. After which it was removed from the set-up and allowed to cool for some minutes. The mixture was filtered. The residue obtained was transferred into another flask with 150ml of 0.5 M KOH and was refluxed for another 30mins.The mixture was removed and allowed to cool for some minutes. It was filtered and the residue was allowed to dry in oven and cooled in dessicator. The dried sample was weighed and the crude fibre computed from the weight.

Mathematically,

$$\% \text{ fibre} = \frac{\text{wt of sample} - \text{wt of sample after refluxing}}{\text{Wt of sample}} \times 100$$

Determination of Ash Content: 5g of the seed sample was weighed into a pre-weighed crucible. The crucible and its content were placed in the furnace and the temperature of the furnace was regulated to about 550^0C.The sample was left in the furnace for 3-5hours.It was repeated for 3 times until the sample turns ash. The ash was removed and placed in a dessicator for cooling. The ash was weighed and the mass was compared with the initial mass of the powdered Gourd seeds.

$$\text{Ash\%} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

W_1 = Wt of crucible; W_2 = wt of crucible + sample; W_3 = wt of after ashing

Determination of Protein Content: 1.00g of the seed sample (i.e. *Luffa cylindrica* seed) was weighed into a 500ml Kjeldahl digestion flask. One Kjeldahl tablet was added to the sample and adding 10mls of concentrated sulphuric acid to the sample to wash any traces of the sample down the glass wall of the flask and the mixture was heated on a heating mantle in the fume cupboard. The digestion was continued until clear solution was obtained and the flask was allowed to cool. The digested sample was transferred into a 250ml volumetric flask and was made up to mark with distilled water. The sample solution was transferred into a 500ml distillation flask, made alkaline with Sodium hydroxide (NaOH) and the nitrogen was distilled off as Ammonia (NH_3). The ammonia is trapped in a 25ml of 0.2M boric acid solution.

$$(NH_4)_2SO_{4(s)} + 2NaOH_{(l)} \rightarrow 2NH_{3(q)} + NaSO_{4(s)} + 2H_2O$$

$$NH_{3(l)} + H_3BO_{3(q)} \rightarrow NH_4^+ + H_2BO_{3(l)}$$

(ammonia) (Boric acid) (Ammonium ion) (Borate ion)

The amount of ammonia and nitrogen in this solution was quantified by titration with a standard Hydrochloric acid (HCl) solution. A reagent blank is carried through the analysis and the volume of HCl titrant required for this blank was subtracted from each determination.

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3$$

This analysis determines the total nitrogen and unusuable nitrogen. This is called crude protein analysis.

$$\%N = \frac{1.4007 \times C \times (V - V_b)}{\text{Sample weight (g)}}$$

Where:

C= Concentration of the standard acid solution i.e. Hydrochloric acid 0.1M/L

V= Consumption of the standard acid in ml (sample)

V_b= Consumption of the standard acid in ml (blank sample)

$$\text{\% Crude or raw protein} = \%N \times 6.25$$

Nitrogen to protein conversion factor for cereals, grains or oil seeds is 6.25.

Determination of Moisture Content: 5g of powdered Gourd seeds sample was weighed. It was placed into a clean, dried and pre-weighed crucible. The sample and the crucible were placed in the oven and dried at 100^0C for 3 hours. The sample was allowed to cool inside dessicator. The heating and cooling were repeated three times. The sample was weighed and reweighed after each heating to obtain a constant value. The percentage moisture content was calculated by expressing loss in weight on dryness as fraction of the initial weight of the sample used and multiplied by 100.

$$\text{\% moisture content} = \frac{M_1 - M_2}{M_1 - M_3} \times 100$$

M_1= Mass of crucible; M_2= Mass of crucible + sample; M_3=Mass after heating

Determination of Fat Content: 10g of the sample was weighed and wrapped in a thimble placed in a soxhlet extractor. 30ml of pre-distilled petroleum ether was used to extract the sample for 3hours. After the extraction, the solvent was recollected by distillation. The remained oil was further heated in order to remove any trace of the solven that might remained in the oil. After the evaporation, the oil was weighed and the value was recorded in gram.

$$\%\text{fat} = \frac{\text{wt. of oil}}{\text{Wt of sample}} \times 100$$

Weight of oil = weight of sample after heating – weight of empty beaker

Determination of Carbohydrate: The determination of total carbohydrate by carried out by the Anthrone Method. Amount of carbohydrate present in 100 mg of the sample:

$$= \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Phytochemical Analysis

Extraction of Sample

20.00g of the coarsely grinded seed pod sample of *Luffa cylindrica* were extracted using 400cm^3 each of *n*-Hexane, Dichloromethane, Ethylacetate and Ethanol in a soxhlet extractor by batch extraction. The seed pod sample was extracted for about 2-3hrs with each solvent, after which the extracted sample was distilled to remove the solvent. Each time the sample was dried and before extracted with the next solvent. The crude extract from each solvent was screened for the presence of the following phytochemicals.

Phytochemical Screening

Test for Carbohydrates: Three different tests were carried out for carbohydrates:

(a) **Molisch's test:** 2ml of the extracts were mixed with 0.2ml of alcoholic solution of α-naphthol and 2ml of sulphuric acid in different test tubes respectively.

(b) **Fehling's test:** 3ml of the extracts were treated with 5ml Fehling's solution A and heated respectively.

(c) **Benedict's test:** 1ml of the extracts and 5ml of Benedict's solution were mixed and heated respectively.

Test for Alkaloids: 1ml of HCl was added to 3 drops of the extracts and 2 drops of Wagner's reagent was also added.

Test for Flavonoids: 2ml of the extracts were mixed with 0.5ml of HCl (10%) and Magnesium metal. Also, 3ml of 1% Hydrochloric acid and the extracts were mixed with Sodium Hydroxide.

Test for Saponins: 2ml of the extracts were mixed with 5ml of water, and were shaked vigorously.

Test for Tannis: 50% Ethanol was added to 2ml of the extracts and 2 drops of Ferric Chloride was also added.

Test for Unsaturated Steriods/Triterpenes: 1ml of Acetic Acid Anhydride was added to 3ml of the extracts, then 2ml of Sulphuric acid was added down the wall of the test tubes.

Test for Resins: 5ml of Ethanol was added to 2ml of the extracts and were boiled for 10mins in a beaker filled with water.

Test for Anthraquinones: 2ml of the extracts were adjusted to pH3 with 1% HC, the solution was washed by diethyl ether. The upper layer of the solution was separated from the aqueous one, the ether solution was then extracted with 5% Sodium bicarbonate solution in a separating funnel, then the alkaline aqueous solution was separated, acidified and then shaken with ether in the separating funnel.

Test for Cardenolides: 2ml of Glacial Acetic Acid, a drop of $FeCl_3$ and 1ml of the extracts were added respectively in two test tubes, then 1ml of conc. H_2SO_4 was added to the mixtures.

III. RESULTS

Table 1: Result of Proximate Analysis of *Luffa cylindrica* seed

Proximal Composition	**Content (%)**
Crude fibre	9.01
Ash content	6.00
Protein content	10.50
Moisture content	2.80
Fat content	31.05
Carbohydrate content	9.45

Table 2: Result of Phytochemical Screening of *L.cylindrica* seed pod

Phytochemical	***n*-Hexane**	**DCM**	**Ethylacetate**	**Ethanol**
Carbohydrates	+	+	+	+
Alkaloids	+	+	-	+
Flavonoids	+	-	-	+
Saponins	-	-	-	+
Tannins	-	-	-	+
Unsat. Steroids	+	+	-	-
Resins	-	-	-	+
Anthraquinones	-	-	-	+
Cardinolides	-	-	-	-

IV. DISCUSSION

The result of the proximate analysis indicates the seed is composed of all the major food component. However, it surprisingly is richer in fat than most other food component. It could be deduced that the oil present in the seeds may be richer in saturated fatty-acids than the unsaturated ones. The seed is also made up of a reasonable amount of inorganic component (i.e. the mineral component) as shown by the ash content. The moisture content suggests that the seed may require mild drying process for preservation.

From the phytochemical screening, the ethanolic extract tend to be the richest in phytochemicals. Carbohydrate seemed to be present in all the extracts as well as Alkaloids. Other phytochemicals showed positive test only in one or two of the extracts.Cardenolides were absent in all the extracts.

V. CONCLUSION

This work has been able to reveal the food composition of the seed and some physiologically active compounds present in the seed pods. The presence of the major food components may make the seed a good candidate for feed. After further work must have been carried out on the edibility of the defatted seed cake and seed oil, the seed may also be fit for human consumption. The presence of several phytochemicals in the seed pod explains the medicinal importance and herbal usage of the plant. Furthermore, in place of disposing as waste, the pods may also be a good candidate for drug lead source in the future.

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