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Phytochemical analysis, proximate composition and antinutritional factors of *Corchorus oliterius* plant

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ABSTRACT

Leafy vegetables play a major in meeting the dietary requirement of an average Nigerian. The knowledge of nutritional and antinutritional properties of these local plant resources therefore becomes necessary. This study was to conduct a phytochemical analysis, determination of proximate composition and antinutritional factors of *Corchorus oliterius* using standard procedures. The qualitative phytochemical analysis revealed the presence of saponins, terpenoids, flavonoids, alkaloids and phenols. Proximate analysis, which partitioned the nutrients into six components revealed the nutrients, thus, moisture (8.84±0.00%), ash (11.18±0.00%), crude protein (27.32±0.02%), crude fat (5.64±0.01%), and crude fibre (5.84±0.02, and nitrogen free extractives (NFE) (41.16±0.00%). Antintritional factors were recorded, thus; oxalate (241.96±0.0 2mg/100g), saponins (0.68±0.00%), tannins (18.16±0.00 mg/100g), cyanogenic glycosides (2.78±0.01 mg/100g), and phytate (0.80±0.00%). The presence of phytochemicals such as terpenoids, flavonoids alkaloids and phenols validates the use of *C. olitrius* in traditional and alternative medicines since phytochemicals found in fruits and vegetables are generally known for being responsible for protective health benefits in man and animals. This result showed that the vegetable was a promising source of protein in human diet if well processed. All the concentrations of antinutrients were found to be within acceptable levels for human and animal consumption. However, the levels of antinutrients can be reduced by traditional processing techniques such as boiling, steaming, cooking, to make it safer for human consumption.

Keywords: Phytochemicals, proximate analysis, antinutrients, acceptable levels.

INTRODUCTION

Phytochemicals are secondary metabolites found in most plants, medicinal and nutritional alike. They are naturally occurring compounds thought to be largely responsible for protective health benefits in plant based foods and beverages beyond those conferred by their vitamins and minerals contents (Webb, 2013). These phytochemicals are said to be responsible for the color, flavor, and odor of plant foods, such as blue berries' dark hue, broccoli's bitter taste, and garlic's pungent odor. Research seemed to strongly suggest that consuming foods rich in phytochemicals provides health benefits, but there are gaps of information to make specific recommendations for phytochemical intake

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especially in Yola, North-east Nigeria, where vegetables are consumed in large quantities.

Phytochemicals are known to function as immunomodulators and may exhibit antioxidant, anti-inflammatory, anticancer, antimalarial and antimicrobial properties (Sadat et al., 2017). Phytochemicals are also referred to as phytonutrients and are classified according to their chemical structures and functional properties. The phytochemical analysis of the plants is very important so that the phytonutrients would be known so as to give credence or validate the consumption of the plant.

Every human being requires a good nutrition as a basic need for proper development and wellbeing. In developing countries where resources are sometimes scarce, one of the ways of achieving good nutrition is through the exploitation of available local resources, as observed by Atasie et al. (2009). Knowledge of the proximate composition of these local plant resources is therefore very necessary in order to encourage increase cultivation and consumption of these plant sources.

Antinutritional factors are chemical compounds synthesized in natural foods by the normal metabolism of species and by different mechanisms, for example, the inactivation of some nutrient, and diminution of the digestive process or metabolic utilization of food which exerts effects contrary to optimum nutrition (Soetan, 2008). One major factor limiting the wider food utilization of many tropical plants is the universal occurrence in them of a diverse range of natural compounds capable of precipitating/eliciting harmful effects in man and animals which act to reduce nutrient utilization or food intake, often referred to as antinutritional factors (Gemede and Ratta, 2014). Antinutrients found in foods have been described as having both adverse and beneficial effects in humans. Their concentration-dependent effects may be manipulated in such a way that advantage is taken from their health-related benefits (Zang et al., 2015). Nonetheless, there is a fear that high intake of foods that are rich in antinutrients may expose the body to these potentially harmful compounds.

C. oliterius is an erect herbaceous plant and grows about 1.5 meters high, and can attain up to 4 meters height. The plant has a hairless green stem with a faint red-brownish hue (Fondio and Grubben, 2011). It has serrate acute leaves, 6 to 10 cm long and 2 to 4 cm wide. The stem can be branched or unbranched. It is an edible vegetable known to be found widely in Africa and Asia. It is a shrub of the family Malvaceae. Its vernacular names are Jew's mallow, jute mallow, krinkrin, tossa jute, bush okra, West African sorrel (En) and a host of others (Fondio and Grubben, 2011). The vegetable is said to have originated from either Asia or Africa. Irrespective of its origin, the plant has been under cultivation for a long period of time in the two continents and grows wild or as a crop in every country in tropical Africa (Wikipedia Encyclopedia, 2019). However, the presence in Africa of more wild *Corchorus* species and the larger genetic diversity within *C. olitorius* point to Africa as the first center of origin of the genus, with a secondary center of diversity in the Indo-Burmese region. At present *C. olitorius* is widely spread all over the tropics, and it probably occurs in all countries of tropical Africa (Fondio and Grubben, 2011).

The traditional medicinal use of the plant in Egypt and India dates back to some 2500 years, where it was used to treat fever, diarrhea and vomiting (Islam, 2012). The antioxidant and analgesic activities of the plant leaves have been reported by Sadat, et al., (2017) and Ipav et al. (2018). In Nigeria, bush okra *C. oliterius* is consumed by almost every ethnic group, especially in South-West, North Central and Northeastern Nigeria. Its nutritious young leaves are cooked into paste and eaten with starchy staples. Young leaves can also be cooked with okra and used as sticky sauce for easy consumption. Bush okra has abundant levels of β-carotene, iron, calcium, vitamin C, and has been listed as one of the seven highly valued indeginous leafy vegetables in Nigeria (Adebooye et al., 2003; Akaneme et al., 2014).

The aim of this work was to analyze the phytochemical content, determined the proximate composition and the antinutritional factors and the properties of the plant leaves. This study was also to scientifically establish or understand the nutritional content for the purpose of encouraging cultivation and mass consumption of the vegetable in the North-east Nigeria.

MATERIALS AND METHODS

Sample collection

Leaves of *C. oliterius* was collected from Nyibango area, Yola, Adamawa State, in August 2018. The plant was identified and authenticated by Dr. D. F. Jatau of Forest Resources Department, Modibbo Adama University of Technology, Yola, Nigeria, where a voucher specimen was kept.

Fresh leaves of experimental plant was air dried under shade at room temperature ±25 ^{0}C. The dried sample was reduced to powder using a laboratory blender. The powdered form of sample was stored in air-tight container until the time for extraction and phytochemical screening.

Preparation of leaf extract

The air dried powdered leaves C. *oliterius* was extracted by cold maceration method using successive solvents such petroleum ether, chloroform and ethanol in increasing polarity for 48 hours respectively.

Qualitative phytochemical analysis of extract

The qualitative phytochemical screening of the sample was carried out using the method described by Harborne (1984). The leaves of *C. oliterius* was screened for saponins, tannins, terpenoids, flavonoids, alkaloids, glycosides steroids and phenols.

Test for Tannins

To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish color indicates the presence of tannins.

Test for Saponins

To 1ml of plant extract, 5-10 ml of distilled water was added and shaken in a graduated cylinder for 15 min. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Terpenoids

Five (5) ml of aqueous extract of the plant sample was mixed with 2 ml of $CHCl_3$ in a test tube and then 3 ml of concentrated H_2SO_4 was carefully added to the mixture to for a layer. Formation of redish brown coloration indicates that a terpenoid constituent was present.

Test for Flavonoids

i. To 1 ml of plant extract, 1ml of aqueous NaOH solution was added and observed for the formation of yellow-orange coloration.
ii. Two (2) ml of plant extract was treated with 4 drops of concentrated H_2SO_4 and observed for the formation of orange color.

Test for Alkaloids

To 2 ml of plant extract 2 ml of conc. Hydrochloric acid was added. The 3 drops of Mayer's reagent was added. The presence of green color or white precipitate indicates the presence of alkaloids.

Test for Glycosides

To 2 ml of plant extract, 1 ml of glacial acetic acid and 5% ferric chloride were added. To these, 3 drops of conc. H_2SO_4 was added. The presence of greenish blue color indicated the presence of glycosides.

Test for Steroids

To 1 ml of plant extract, equal volume of chloroform and 3 drops of conc. H_2SO_4 was added. Formation of brown ring indicated the presence of steroids.

Test for Phenols.

To 1 ml of extract, 2 ml of distilled water followed by 5 drops of 10% ferric chloride was added. The formation of blue or green color indicated the presence of phenols.

Proximate analysis and anti-nutritional properties

Proximate analysis of experimental plant

Proximate analysis refers to the determination of the major constituents of leave extract. The analysis partitions nutrients into six components: moisture, ash, crude protein, ether (crude fat), crude fiber and Nitrogen free extractives (NFE). The moisture content was determined by as loss in weight that resulted from drying a known weight of sample to constant weight at 100 ^oC. The ash content was determined by ignition of a known weight of the food sample at 550 ^oC until all carbon was removed. The residue was ash and is taken to represent the inorganic contents of the food sample.

The protein content was calculated from the nitrogen content of the food sample, determined by a modification of technique originally devised by Kjeldahl over 100 years ago. The crude fat was determined by subjecting the food sample to a continuous extraction with petroleum ether for a defined period. The residue after evaporation of the solvent was the crude fat.

When the sum of the amounts of moisture, ash, crude protein, crude fat and crude fiber expressed in percentages was subtracted from 100, the difference was designated as the nitrogen-free extractives (NFE).

Antinutritional factors of experimental plant

Antinutritional factors are compounds which act to reduce the nutrient utilization/bioavailability or food intake. In effect, they play a great role in limiting the wider use of many plants. They include, oxalate, saponins, alkaloids, tannins, cyanogenic glycosides and phytate.

The saponin content of the sample was determined by double extraction gravimetric method described by Harborne (1984). Phytate content of the sample was determined according to the method outlined by Lucas and Markaka (1975). Tannin content of the sample was determine using methods described by OAOC (2005). The method was however, slightly modified.

The oxalate content of powdered sample was determined by the modified method of Abeza et al. (1968). Alkaloid content of samples was determined using the gravimetric method of Harborne (1984). Cyanogenic glycosides were determined using the method described by AOAC (2005).

Data analysis

The data was analyzed by ANOVA and results expressed as means and standard deviation.

RESULTS

Phytochemical analysis

Qualitative phytochemical analysis of *C. oliterius* leaves revealed the presence of saponins, terpenoids, flavonoids, alkaloids and phenols (Table 1). Glycosides and steroids were found to be absent in the plant sample.

Proximate composition of *C. oliterius* leaves

The result of proximate composition of *C. oliterius* is presented in Table 2, with Crude protein 27.32±0.02%, Fat 5.64±0.13%, Ash 11.18±0.00%, Crude fibre 5.84±0.02% and Moisture 8.84±0.00%. Crude protein had the highest percentage whereas fat had the least percentage in the plant (Table 2).

Antinutritional factors of *C. oliterius*

The result for the antinutritional content of the vegetable is presented in Table 3. The oxalate content recorded was 241.96±0.2 mg/100g and showed the highest concentration compared to tannins and cyanogenic glycosides all recorded in mg/100g of sample. On the other hand, alkaloids had the least percentage concentration compared to saponins and phytate. The concentrations of all the antinutrients in the plant happened to be within acceptable levels.

Table 1: Phytochemicals in *C. oliterius.*

Phytochemicals	C. oliterius
Saponins	+
Terpenoids	+
Flavonoids	+
Alkaloids	+
Glycosides	-
Steroids	-
Phenols	+

NFE – Nitrogen Free Extractives, values are expressed as mean±standard deviation (SD), n=2.

Table 2: Proximate composition of *C. oliterius* leaves.

Parameters	Percentage (%) composition
Crude protein	27.32±0.02
Fat	5.65±0.01
Ash	11.18±0.00
Crude fibre	5.85±0.02
Moisture	8.85±0.00
NFE	41.16±0.00

NFE – Nitrogen Free Extractives, Values are expressed as mean±standard deviation (SD), n=2.

Table 3: Antinutritional content of *C. oliterius.*

Antinutrients	Composition
Oxalate (mg/100g)	241.96±0.02
Saponins (%)	0.68±0.00
Alkaloids (%)	0.53±0.00
Tannins (mg/100g)	18.16±0.00
Cyanogenic glycosides (mg/100g)	2.78±0.01
Phytate (%)	0.80±0.00

Values are expressed as mean±standard deviation (SD), n=2.

DISCUSSION

The result of qualitative phytochemical screening which revealed the saponins, terpenoids, flavonoids, alkaloids and phenols, somehow tallies with Sadat et al. (2017), where they reported also the presence of cardiac glycosides in the work. Phytochemicals found in fruits and vegetables are generally known for being responsible for protective health benefits in man and animals (Webb, 2013). The phytochemical content found in this analysis justifies the use of the plant in traditional medical practice for cure or prevention of diseases such as in wound healing, and antidiabetic activity, lowering of cholesterol level (Shrmila et al., 2007).

Saponins, from recent evidence seem to possess hypocholesterolemic, immunostimulatory and anticarcinogenic properties. In addition, they reduce the risk of heart diseases in humans (Gemede and Ratta, 2014). Terpenoids are said to help in preventing metabolic disorders, fight cancer and exert antiaging benefits. As phytochemicals, terpenoids are responsible for a wide variety of flavors and aromas, and have been found to possess analgesic, anti-imflammatory, anti-fungal, anti-microbial, anti-viral and anti-parasitic properties (Mercola, 2017).

Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, vasodilatory, antiarrhythmic, analgesic antibacterial and antihyperglycemic activities. Many have found use in traditional or modern medicine, or as starting points for drug discovery (Wikipedia Free encyclopedia, 2019). Flavonoids are a large family of polyphenolic plant compounds and have a wide spread occurrence in the plant kingdom. They are also known to exhibit various pharmacological activities; antimalarial, anti-inflammatory, antioxidant (Liu, 2013) and a host of others. The occurrence of these phytochemicals in *C. oliterius* gives the plant a nutritional advantage.

The main functions of proteins in the human body are growth and the replacement of damaged tissues. With 27.32±0.02% crude protein in *C. oliterius*, the vegetable can be considered as a high protein vegetable, especially when compared to *Amarantus spinosus* (4.6%), *Vernonia amigdalina* (5.80%), *Teiferia occidentalis* (5.2%), *Talinum triangulare* (2.6%), Okra pods (23..4g/100g) and *Moringa oleifera* (15.04±0.18%) (Agbaire, 2011; Ogungbenle and Omosola, 2015; Soetan and Aiyelaagbe, 2016). The crude protein content of this work slightly differs with Daniel (2014) who had 24.14±0.21% crude protein. On the other hand, this result is similar to the protein content in *Crassocephalum crepdioides* (27.13±0.01%) reported by Adjatin et al. (2013). The result, in any case, shows that *C. oliterius* can be a good source of protein to meet up with the low quality protein affecting human and animal population in the developing countries (Soetan and Aiyelaagbe, 2016). In addition, it could play a significant in the provision of cheap and affordable protein for rural populations (Ndlovu and Afolayan, 2008).

Fat had the value of 5.64±0.01%. Fats are secondary plant products that yield more energy per gram than carbohydrates (Ilodibia, et al., 2014). They are important not only because of their energy value, but the fat-soluble vitamins and essential fatty acids contained in the fat of natural foods. Dietary fats also increase the palatability of food by absorbing and retaining flavours (Antia et al., 2006; Ilodibia et al., 2014). The relatively low fat in *C. oliterius* shows that the leaves can be recommended as weight reducing diet and can therefore be consumed in large quantities with safety without risk of cardiovascular disease, obesity and other related diseases (Okon et al, 2017; Loucou et al., 2018). The ash content of a plant part is a reflection of its mineral elements. The value of 11.18% in *C. oliterius* leaves shows how rich the vegetable is in nutritionally important minerals, such as potassium, calcium, sodium, magnesium, phosphorus, zink, copper, iron and selenium. Minerals are utilized by the body in many ways and they play vital roles in the nutritional development of humans and animals (Rahman et al., 2014). The result of this work differed from Onwordi et al. (2006) who had 21.20±0.80% as ash content. Reason for difference could due to processing or soil and climatic conditions.

Crude fibre in *C. oliterius* leaves had the value of 5.84±0.02%. This to some extent agrees with Daniel (2014) who had 6.64±0.01% and Onwordi et al. (2006) who reported 6.70±1.40% as crude fibre content. Dietary fibre promotes growth and protects beneficial intestinal flora. Furthermore, high intake of fiber is said to promote digestion and reduce the risk of colon cancer (Dawczynski et al., 2007; Gemede et al., 2015). Plants with high fibre content are used for the treatment of obesity, diabetes, cancer, and gastrointestinal disorders (Ibironke, 2013). Though the percentage of fibre is moderate (5.84%), it is nonetheless a diatary advantage to its consumers knowing that it assist digestion and limits cholesterol absorbtion as observed by Ngaha et al. (2016).

Moisture content is an integral part of proximate composition analysis of food (Gemede et al., 2015). It is an index of its water activity and is used as a measure of stability and susceptibility to microbial contamination (Uyoh et al., 2013). This study recorded moisture content as 8.84±0.00%. This value is consistent with Rishi et al. (2012) who reported that reasonable amount of moisture in most vegetable is 6% to 15%. This implies that *C. oliterius* leaves can be stored easily and the moisture content can contribute in slowing down growth and development of microorganisms and in hindering hydrolysis of components present in plant material (Ngaha et al., 2016).

The presence of oxalate in foods or vegetables above acceptable levels causes irritation in the mouth and the lining of the gut (Gemede and Ratta, 2014) and also hinders the absorption of divalent minerals, particularly calcium (Ola and Oboh, 2000). This in effect makes calcium inaccessible by the body, especially for maintenance of strong bones, teeth, co-factor in enzymic reactions, nerve impulse transmission and blood clothing (Unuofin et al., 2017). However, the concentration of oxalate in this work (241.96±0.02 mg/100g) is below acceptable level of 250 mg/100g fresh sample (Oguchi et al., 1996). The consumption of *C. oliterius* leaves in effect may not be harmful or toxic, especially that the vegetable goes through heat processing before use.

The concentration of saponins recorded here stood at 0.68±0.00%. Saponins in high concentrations exhibit bitter taste and astringency in dietary plants (Gemede and Ratta, 2014). The bitter taste is considered a major factor that limits the use of saponins. In addition, saponins have been found to reduce the bioavailability of nutrients and decrease enzyme activity (Liener, 2003). The concentration of saponins recorded in this work (0.68±0.00%) is within acceptable levels. At levels < 10% in a diet is said to be harmless to the body (Igile et al., 2013); and so the consumption of the vegetable with regard to saponins should be encouranged, especially for its health benefits.

The amount of alkaloids recorded here stands at 0.53±0.00%. Alkaloids are phytochemicals and sometimes are considered to be antinutrients because of their action on the nervous system, where they disrupt electrochemical transmission when they are consummed in large quantities (Gemede and Ratta, 2014). Inuwa et al. (2011) reported that consumption of large concentration of alkaloids could be toxic especially when it exceeds the lethal dose of 20 mg/100g. Fortunately, the quantity recorded here is 0.53±0.00% which is much less than the lethal dose of 20 mg/100g. Therefore, the consumption of *C. oliterius* leaves should be encouraged in regard to the concentration of alkaloids. The quantity of alkaloids recorded in this work seemed to differ from Efemeje et al. (2014) who reported the amount of alkaloids in *C. oliterius* as 7.21±0.04%. Differences could be due to environmental and processing factors.

The concentration of tannins recorded here is 18.16 mg/100g. This is much less than lethal dose of tannins (30 mg/kg) reported by Inuwa et al. (2011), and is higher than the concentration of tannins in *C. oliterius* reported by Ifemeje et al. (2014) in which they recorded the concentration of tannins as 1.45±0.03%. The dietary use of the vegetable should therefore be encouraged as it would not cause any harm, going by the lethal dose of tannins. Tannins are known to be heat stable and they interfere with the digestion of protein in humans and animals, probably by making protein partially unavailable or by inhibiting digestive enzymes and increasing fecal nitrogen. Tannins present in food products have been found to inhibit the activities of trypsin, chemotrypsin, amylase and lipase. They also reduce protein quality of foods (Felix and Mello, 2000).

The leaves of *C. oliterius* showed the level of cyanogenic glycosides to be 2.78±0.01 mg/100g. This level is within the permissible level of 5.3 to 80 mg/100g (Wobeto et al., 2006), and it closely agrees with Musa and Ogbadoyi (2014). This suggests that the leaves of the vegetable are safe for consumption with regard to cyanogenic glycosides. High level of cyanogenic glycosides, however, has been implicated for cerebral damage and lethargy humans and animals (Agbaire, 2012).

The phytate level/value recorded in this work is 0.80±0.00%. The value is slightly higher the phytate in *Garcinia kola* (0.634%) reported by Dike and Nnamdi (2012). All the same, the value obtained is below toxic levels and so does not pose any danger to consumers, with respect to *C. oliterius* leaves. Phytic acids are found in abundance in fiber-rich foods and are recommended because they protect human from cardiac vascular diseases and some form of cancer (Norhaizan and Nor-Faizadatul-A, 2009; Akaneme et al., 2014). With this advantage, yet phytic acid reduce the bioavailability of minerals because of its strong binding affinity to them. They chelate metal ions such as calcium, copper, iron, zinc, magnesium and molybdenum forming insoluble complexes that are poorly absorbed from gastrointestinal tract (Bello et al. 2008; Adebiyi et al., 2015).

Conclusion

The phytochemicals recorded in this work gave the vegetable a nutritional advantage and also validated the use of the plant leaves in traditional and alternative medicines. The large concentration of crude protein in *C. oliterius* indicated that the plant leaves can be a good source of protein in human and animal foods. The concentrations of antinutrients were found to be within acceptable levels for human and animal consumption. However, traditional processing techniques should be used to reduce the levels of antinutrients for safe consumption. Also, a moderate consumption of the vegetable is recommended as they are vital in improving health conditions in humans.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

KKS conceived the idea of the research, collected sample for laboratory work and contributed in the writing of the manuscript. GPC and SPA contributed in the laboratory and data analysis. All the authors participated in the review of the manuscript.

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