Proximate and mineral analysis and antinutrient and antimicrobial properties of *Talinum triangulare* (*Talinaceae*) and *Celosia argentea* (*Amaranthaceae*)

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Abstract. *Talinum triangulare* and *Celosia argentea* are two common underutilized leafy vegetables in Nigeria. This work was carried out to determine the mineral, proximate, and antinutritional contents of the plants, which were estimated by standard methods. Antibacterial activity of the leaf aqueous and ethanolic extracts was also evaluated against some bacteria. Results obtained from the study have shown that the two plants had constituents that show them as a good source of nutritional and medicinal benefits.

Key words: *Celosia argentea*, food, nutrients, *Talinum triangulare*, vegetables, Water Leaf

Introduction

In Africa, there has been a switch to consumption of local, abundantly available, but neglected and underutilized foods, particularly vegetables (Kimbonguila & al. 2010; Ukpong & Idiong 2013). Vegetables have biologically active compounds, which make them a potential source of therapeutic agents (Oyewole & Kalejaiye 2012). Interest has been evinced in the use of natural products, particularly in the developing countries, because several common chronic diseases have evidenced that most of these conditions are related to diet and lifestyle (Borokini & al. 2017). Plumed or silver, Cockscomb (*Celosia argentea* L.) and other related forms is a leafy vegetable widely cultivated in tropical Africa (Hayakawa & al. 1998; Denton 2004). Water Leaf (*Talinum triangulare* L.) is consumed as a leafy vegetable in vegetable soups in Nigeria. It is highly nutritious and contains calcium, magnesium, potassium, and vitamins (Oguntona 1998). While commonly used leafy vegetables have been studied for their nutrients, less popular ones have not (Kola 2004). Underutilization of popular available vegetables might be due to lack of information on mineral, phytochemical and proximate contents. Three types of *Celosia argentea* are cultivated in Nigeria. Among these, only the green variety is popular and widely consumed. The red variety, popularly known as *soko pupa*, is less cultivated and consumed, partly due to the anthocyanin pigmentation of the leaf blades and part of the stem which colours the soups in red. The nutritional values of *C. argentea* have been widely studied by different researchers. The work of Adegbaju & al. (2019) has reported maturity impact on the nutritional composi-

tion of *C. argentea* at three different stages of growth and development. Ayodele & Olajide (2011) have found adequate amino acid contents of the plants, as compared to the WHO recommended values. However, none of these works have reported the nutritional value of the *soko pupa* red variety, which is usually regarded as weed and, therefore, is less consumed. Ogbonnaya & Chinedum (2013) have found *Talinum triangulare* to contain considerable amounts of vitamins and carotenoids, while Adeniyi (1996) has found different concentrations of minerals in the plants that were collected from dumpsites and control sites. However, information is lacking on the antinutrient and antibacterial activity of this vegetable.

This study was, therefore, undertaken to determine the proximate, mineral, antinutritional, and antimicrobial properties of *C. argentea* (Red variety) and *T. triangulare,* with a view of understanding their medicinal and nutritional values.

Material and methods

Fresh leaves of *T. triangulare* and *C. argentea* were collected from a vegetable farm in Osogbo, Osun State, Southwest Nigeria. Leaf samples were thoroughly washed, dried to a constant weight at 55 °C in a convection oven (model OV-160, Gallenkamp BS, England) and separately ground into fine powder prior to analyses.

Proximate contents analysis

Moisture content

Moisture content was determined by the AOA C method (2006). Two grams of the sample were dried in a pre-weighed crucible at 105 °C in a convection oven (Gallenkamp) for 24 h. The percentage of moisture content was calculated as follows:

$$\% \text{ moisture} = W_1 - \frac{W_2 \times 100}{W_1},$$

where W_1 = initial weight of sample; W_2=weight of the dried sample.

Crude protein content

Crude protein was determined from the total organic nitrogen by the macro-Kjeldhal method (Koyuncu & al. 2014).

Crude fiber content

Crude fiber was analyzed by the AOAC method (2006). One gram of the extracted sample was added to 3 mL of H_2SO_4, followed by 17 mL of hot 1.25 % H_2SO_4. The solution was gravity filtered through filter paper for 30 min and the insoluble residue washed with hot water to drain the acid. Three mL of 0.313 M NaOH was added to the residue, followed by of 17.0 mL of 0.313 M hot NaOH. The mix was shaken for 30 min and gravity filtered through filter paper. The residue was washed with 1 % HCl, followed by boiling water. The residue was washed with ethanol and ether before drying in a convection oven (Gallenkamp) at 100 °C. Fiber content was calculated by dividing sample loss in weight by weight of the sample.

Fat content

Fat content was determined by the AOAC method (2006). Two grams of the sample were extracted with petroleum ether for 12 h. The extract was reduced to half with a rotary evaporator and dried at 105 °C in a convection oven (Gallenkamp) until a constant weight was obtained. The percentage of fat content was calculated by dividing the weight after drying by the weight of the sample (Kumari & al. 2017).

Total lipid

Total lipid was extracted according to AOAC (2006) from the sample with petroleum ether (60–80 °C) in a Soxhlet apparatus for about 6–8 h. The residual solvent in a pre-weighed beaker was evaporated by keeping the beaker in a warm water bath (50 °C). Increase in weight of the beaker provided the total lipid (Kumari & al. 2017).

Ash content

For ash content, samples were heated in a muffle furnace at 550 °C until becoming white, or grayish-white and ash was obtained and weighed (AOAC 2006).

Energy content

Energy content was estimated by the Atwater general factors system. This method involves multiplying the percentage of carbohydrate content by 4 %, of the protein content by 4 % and of the lipid content by 9 %. Energy is then calculated as follows:

Energy = (%CP × 4) +) + (%CHO × 4) + (%CLP × 9),

where %CP = percentage of crude protein;

%CLP=percentage of crude lipid; %CHO=percentage of carbohydrate (Hassan & al. 2011).

Carbohydrate content

Carbohydrate content was calculated from differences in the sum of protein, fat, moisture, and ash content from 100 (Igbabul & al. 2014)

Mineral content analysis

An atomic absorption spectrophotometer was used for estimation of leaf mineral contents (AOAC 2005). Half a gram of crushed composite sample was digested in concentrated HNO_3 and $HClO_4$ at 115 °C for about 1 h to generate the digest solution. An aliquot of the digest solution was used to estimate the contents of calcium, magnesium, sodium, copper, zinc, iron, and phosphorus. The titration method (Day & Underwood 1986) was applied to estimate the antinutrient content. One gram of sample was weighed into a 100 mL conical flask. Seventy-five mL of 3M H_2SO_4 were added and the mix was continuously agitated for 1 h with a magnetic stirrer. The mix was gravity filtered through Whatman No 1 filter paper. Twenty-five milliliters of the filtrate were titrated while hot against 0.05M $KMnO_4$ solution, until a faint pink color persisted for at least 30 sec. The oxalate content was calculated according to Ihekoronye & Ngoddy (1985); Chinma & Igyor (2007). Phytate was determined according to Wheeler & Ferrel (1971). One hundred milliliters of sample were extracted with 3 % trichloroacetic acid. The extract was treated with a $FeCl_3$ solution and the iron content of the precipitate determined by an atomic absorption spectrophotometer (model 2900, Cye Unicam). A 4:6 Fe/P atomic ratio was used to estimate the phytic acid content. Cardiac glycoside content was evaluated using Buljet's reagent (El-Olemy & al. 1994). One gram of the powdered sample was soaked in 10.0 mL of 70 % alcohol for 2 h and filtered. The extract was treated with lead acetate and Na_2HPO_4 solution before adding freshly prepared Buljet's reagent (95.0 mL aqueous picric acid and 5.0 mL 10 % aqueous NaOH). The difference between intensity of colors of experimental and blank (distilled water and Buljet's reagent) samples provided absorbance and was proportional to concentration of glycosides. Saponin content was estimated by a modified method of Fenwick and Oakenfull (1981). Saponin was extracted for 2 h in a reflux condenser containing pure acetone. Exhaustive re-extraction over a heating mantle with methanol in the Soxhlet apparatus took place for 2 h. The extract was weighed after allowing the methanol to evaporate. The saponin content was calculated. Tannin content was estimated by the Folin-Denis Colorimetry method (Harborne 1998). A weighed sample (5.0 g) was mixed with distilled water in the ratio of 1:10 (w/v). The mix was shaken for 30 min at room temperature and gravity filtered through filter paper to obtain the extract. A standard tannin acid solution was prepared. Two mL of the standard solution and an equal volume of distilled water were dispensed into separate 50.0 mL volumetric flasks. Then 2.0 mL of each sample extract was put in respective flasks and labeled. The content of each flask was mixed with 35.0 mL distilled water and 1.0 mL of the Folin-Denis reagent. This was followed by addition of 2.5 mL of saturated Na_2CO_3 solution. The contents of each flask were diluted to the 50.0 mL mark with distilled water and incubated for 90 min at room temperature. Absorbance was measured at 760 nm in a colorimeter with the reagent blank at zero. The tannin content was calculated following Ojinnaka & Agubolum (2013).

Antibacterial activity

Bacterial inocula were prepared from 24 h old cultures of isolates of the bacteria *Proteus mirabilis ATCC7002, Bacillus subtilis NCTC8263, Klebsiella pneumonia ATCC 43816, Corynebacteria spp ATCC 13812, Pseudomonas aeruginosa ATCC 10145,* and *Escherichia coli ATCC 35150.* All isolates were from medical samples which have been emulsified in sterile normal saline and adjusted to 0.5 McFarland standard (by comparing the desired inoculum with a 0.5 McFarland standard).

Extraction procedures for antibacterial activity

Preparation of aqueous extract

In accordance with the method of Olanrewaju & al. (2019) with some modifications, fresh aerial leaves of *T. triangulare* and *Celosia argentea* were air-dried at 27 °C for seven days and milled into powder in a mechanical grinder. The powdered material (40 g) was extracted by maceration with 400 ml of distilled water at room temperature. After filtration and evaporation of the solvent under reduced pressure, the resulting residue was re-extracted thrice with distilled water. The filtrate was pooled together, filtered with Whatman number 1 filter paper. The filtrate was then con-

centrated at 50 °C using a Speed Vac (Model 7811001; Labconco, USA) and stored until further use.

Preparation of ethanolic extract

Fresh aerial leaves of *T. triangulare* and *Celosia argentea* were air-dried at 27 °C for seven days and milled into powder in a mechanical grinder. The powdered material (40 g) was extracted by the addition of 400 ml of methanol and allowed to stand for 48 hrs. The residue was then transferred into a Soxhlet apparatus with ethanol for 48 hrs and evaporated to dryness in a water bath. The extract was diluted with 10 % dimethyl sulfoxide (DMSO) and sterilized via filtration with Whatman number 1 filter paper. The filtrate was then concentrated at 50 °C using a Speed Vac (Model 7811001; Labconco, USA) and stored until use.

Antibacterial activity was determined by the agar well diffusion method. Each aqueous or ethanol extract of *T. triangulare* and *C. argentea* was assigned a solidified Mueller-Hinton agar plate. Three plates were prepared for each bacterium isolate and solutions of each bacterium isolate were inoculated onto each plate. Holes were bored on each of the agar plates and filled with both aqueous and ethanol extracts from the plants. An amount of 0.25mg/l of the extracts was utilized for antibacterial activity in agar well. Amoxicillin was chosen as positive control. Plates were incubated at 37 °C for 24 h. Zone of inhibition was measured and determined by a standardized chart.

Statistical analysis

Data obtained from the study were processed with Microsoft Excel and conveyed as mean ±standard error (SE), with three replicates.

Results and discussion

Proximate contents of the vegetables varied (Table 1). Protein content of *C. argentea* and *T. triangulare* were within the range reported in other leafy vegetables (Uusiku & al. 2010). Plants with more than 12 % of total calorie value from protein were considered good sources of protein (Pearson 1976). Ash content of *T. triangulare* was similar to that reported in *Hibiscus esculentus* (8.00 %) (Akindahunsi & Salawu 2005). This indicates that similarly to *H. esculentus* the plant was rich in organic matter, which is convertible to oxides and water at heating (Akinwumi & Omotayo 2016). This was also an indication that the plant contained a high amounts of essential and valuable minerals necessary for normal body development (Habtamu & al. 2016). Crude fiber content of *T. triangulare* was comparable to that reported by Akubugwo & al. (2007) in *Amaranthus hybridus*. The tested plants may not be beneficial for people with cholesterol-related problems, as their crude fiber contents were low (Kadiri & Fasidi 1990). High level of fiber is known to be an antitumorigenic and hypocholestrolaemic agent (Okoro & Achuba 2012). Fat content of *C. argentea* and *T. triangulare* was comparable to that reported in *Ocimum bassillium* L., *O. viride* L. and *Piper guineens* (Schumach. & Thonn.) (Udosen 1995). The high carbohydrate content in *C. argentea* and *T. triangulare* indicated that they were good sources of energy and as such could be consumed as staple or supplementary foods. The low lipid contents in both plants agreed with earlier reports (Oulai & al. 2014, Ejoh & al. 1996). The plants may help control obesity.

The mineral content analysis of the vegetables indicated that they contained a considerable amount of minerals required by the human body (Table 2). In both plants, the values of sodium, calcium and potassium were high and the levels of magnesium were below the recommended daily intake (NAFDAC 2010). Copper, zinc and phosphorous were present in low amounts in both plants.

Levels of antinutrients in these vegetables were below the toxic limits (Birgitta & Gullick 2000) (Table 3). Food value of plants could be limited by the presence of antinutrients, since they prevent absorption of essential minerals. Phytate toxicity was due to a high binding affinity for calcium, iron and zinc, which reduces bioavailability as a result of the formed insoluble precipitates, which are less able to be absorbed (Prom-U-Thai & al. 2006).

Table 1. Proximate content of *Celosia argentea* and *Talinum triangulare*.

Parameter (g/100g DW)a	*C. argentea*	*T. triangulare*
Dry matter	93.41±0.02	93.26±0.01
Carbohydrate	51.95±0.08	53.99±0.05
Crude protein	22.58±0.12	19.97±0.02
Ash	12.85±0.03	8.03±0.05
Moisture concentration	6.59±0.02	6.73±0.02
Crude fiber	4.50±0.01	8.97±0.02
Lipid	1.63±0.19	2.30±0.01
Fat	1.50±0.02	0.34±0.01
Energy value ($g \cdot cal^{-1}$)	1.27±0.05	1.57±0.05

Note: [a] except for energy value. Values are mean±SD of triplicate determinations.

Table 2. Mineral composition of *Celosia aregentea* and *Talinum triangulare*.

Element (mg/100g DW)	*C. argentea*	*T. triangulare*
Na	42.21±0.01	52.34±0.02
Ca	32.10±0.04	60.31±0.02
Mg	23.03±0.03	21.22±0.02
K	58.22±0.02	61.22±0.01
Mn	0.03±0.00	0.01±0.00
Cu	0.04±0.00	0.06±0.00
Fe	0.52±0.01	0.20±0.01
Zn	0.06±0.00	0.09±0.00
P	0.12±0.01	0.37±0.02

Note: Values are mean±SD of triplicate determinations.

Table 3. Antinutrient composition of *Celosia aregentea* and *Talinum triangulare*.

Compound (mg/100g)	*C. argentea*	*T. triangulare*
Tannins	3.43±0.15	2.51±0.01
Cardiac glycoside	32.37±0.29	26.1±0.09
Phytate	2.79±0.03	4.85±0.61
Oxalate	1.79±0.02	3.23±0.03
Saponin	15.01±0.12	17.3±0.07

Note: Values are mean±SD of triplicate determinations.

Oxalate combines with the divalent metallic cations Ca^{2+} and Fe^{2+} to form oxalate crystals, which are excreted in urine. These crystals can lead to kidney disease as a result of obstruction of kidney tubules (Coe 2005). Tannins bind and precipitate proteins and reduce their availability. Permissible levels of this antinutrient in food depend on the level of protein in food. Presence of saponins in plants reduces nutrient absorption, utilization and conversion efficiency (Sen & al. 1998). They inhibit the digestive enzymes trypsin and chymotrypsin, which ultimately affects protein digestibility (Shimoyamada & al. 1998). This is an indication that, though present in these vegetables, the antinutrients will not interfere with absorption of essential minerals, when consumed.

Extracts from *T. triangulare* were used against some bacterial isolates such as *Proteus mirabilis ATCC7002, Bacillus subtilis NCTC 8263, Klebsiella pneumonia ATCC 43816, Corynbacterium spp ATCC 13812, Pseudomonas aeruginosa ATCC 10145* and *Escherichia coli ATCC 35150*, as presented in Table 4. The results have shown that the extracts do not have an inhibitory effect against the tested bacterial isolates.

Also, the extracts from *C. argentea* were used against the same bacterial isolates listed for *T. triangulare* above and the results are presented in Table 5. The results obtained have shown that the aqueous extract had inhibitory effects only against *E. coli,* while the ethanol extract did not have any inhibitory effect against any of the bacterial isolates. The aqueous extracts of *C. argentea* showed antibacterial activity only against *E. coli,* while the ethanol extract did not. The antimicrobial activity of *C. argentea* aqueous extract can be attributed to the presence of phytochemical components, which include saponins, tannins, phenols, and cardiac glycosides that have all been implicated in antimicrobial ectivity. Fiori & al. (2013) reported that tannins produce antimicrobial activity. The results of this study have indicated that the aqueous leaf extract of *C. argentea* has potential for treatment of various infections caused by *E. coli.*

Finally, the two vegetables contain enough carbohydrates, caloric value, lipids, proteins, and minerals so that they could be good sources of nutrients in human diets. The antinutrient contents were negligible. If consumed in sufficient amounts, the plants might contribute to nutritional requirements for good health in humans.

Table 4. Antibacterial activities of *T. triangulare* extracts against some bacterial isolates.

Bacterial isolate	Diameter of zone of inhibition(mm/25µl)		
	Aqueous extract	Ethanol extract	Control (Amoxicilllin)
Proteus mirabilis	no inhibition	no inhibition	no inhibition
Bacillus subtilis	no inhibition	no inhibition	15mm
Klebsiella pneumoniae	no inhibition	no inhibition	no inhibition
Corynbacterium	no inhibition	no inhibition	no inhibition
	–	no inhibition	4mm
Pseudomonas aeruginosa	no inhibition	no inhibition	9mm
Escherichia coli	no inhibition	no inhibition	no inhibition

Table 5. Antibacterial activities of *C. argentea* extracts against some bacterial isolates.

Bacterial isolate	Diameter of zone of inhibition(mm/25µl)		
	Aqueous extract	Ethanol extract	Control (Amoxicillin)
Proteus mirabilis	no inhibition	no inhibition	no inhibition
Bacillus subtilis	no inhibition	no inhibition	9mm
Klebsiella pneumoniae	no inhibition	no inhibition	no inhibition
Corynbacterium	no inhibition	no inhibition	no inhibition
Pseudomonas aeruginosa	no inhibition	no inhibition	4mm
Escherichia coli	15mm	no inhibition	no inhibition

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