



Antioxidant Activities, Nutritional Composition and Antinutritional Properties of Two Leafy Vegetables (*Cleome rutidosperma* and *Cassia tora*) Consumed in Adamawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author KKS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Author SAP performed the literature search, phytochemical analysis and other laboratory work. Both authors read and approved the final manuscript.

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ABSTRACT

Antioxidants are agents which scavenge free radicals and prevent cellular damage cause by them. They reduce the damage due to free radicals by neutralizing them before they can attack the cells. The aim of this research was to evaluate the antioxidant, nutritional and antinutritional properties of two leafy vegetables, *Cleome rutidosperma* and *Cassia tora* consumed in Adamawa State, Nigeria. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) models were used to determine antioxidant activities of extracts. Nutritional composition and antinutritional properties were determined using standard procedures. There was a significant ($p=0.05$) decrease in the concentration of DPPH radical due to the scavenging activity of ethanol leaf extract of *Cleome rutidosperma* compared to control/standard. In FRAP also, *C. rutidosperma* ethanolic extract exhibited higher ferric reducing power than *C. tora*. Proximate analysis revealed the nutrients for *C. rutidosperma* and *C. tora* as; crude protein ($31.06\pm 0.00\%$ and $26.24\pm 0.00\%$), fat

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(7.13±0.00% and 6.93±0.10%), ash (15.17±0.61% and 11.60±0.00%), crude fiber (11.06±0.01 and 13.19±0.10%), moisture (9.99±0.00% and 9.12±0.10%), NFE (25.60±0.10% and 32.94±0.10%) respectively. Antinutritionals in ethanolic extracts of *C. rutidosperma* and *C. tora* were; oxalates, saponins, tannins, cyanogenic glycosides, phytate and alkaloids. Both vegetables revealed good percentages of proteins which can be used to compliment other sources of protein. All antinutrients of extracts were within tolerable levels.

Keywords: Antioxidants; nutritional; antinutritional; tolerable levels; leafy vegetables.

1. INTRODUCTION

Antioxidants are agents which scavenge free radicals and prevent cellular damage cause by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA [1]. In other words, antioxidants are substances which protect cells from damage caused by unstable molecules known as free radicals. Antioxidants terminate these chain reactions by removing the free radical intermediates and inhibit other oxidation reactions by being oxidized themselves [2].

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life in metabolism. They are species which have high reactivity and damaging activity towards macromolecules. These species are mainly reactive oxygen species (ROS) and reactive nitrogen species (RNS); they have been implicated in the etiology of diseases such as cancers, atherosclerosis, neurodegenerative diseases, infections, chronic inflammatory diseases, diabetes and autoimmune diseases [3].

Antioxidants are usually classified into two major groups, enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include peroxide dismutase catalyse and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins. They are derived from natural plant sources [4]. In their study of various foods, Paur et al. [5] reported that herbal and traditional plant medicines emerged as many highest antioxidant-containing products in their study of various foods. The search for natural antioxidant source from plants, therefore becomes paramount.

Cleome rutidosperma is a weed found in West Africa, tropical America and Southeast Asia. It is an erect annual herb of up to 90cm tall, found

around waste dumps and grassy places. The stem is cylindrical, soft wooded, greenish with fairly coarse hairs. The leaves are compound, trifoliolate with petioles of up to 5 cm long [6]. The plant has various use in traditional medicine, which include the treatment of paralysis, epilepsy, convulsion, spasm, skin disease and pain. *C. rutidosperma* has also been reported to have antimalarial, analgesic, antihelmintic, antibacterial and anti-inflammatory, diuretic, laxative and antidiabetic activities [7,8]. The leaves are eaten as vegetable or added to soup [9]. In North-East Nigeria, the leaves are also used for making soup, especially among the Kanakuru (Dera) people in Adamawa State, Nigeria. The soup is usually prepared without salt, only water; that is why it is called aragwa (*ara* means soup; *gwa* means water).

Cassia tora commonly called Foetid cassia or *Tafasa* in Hausa, is a plant found in most parts of the West African and in the tropical regions of India. It is an under shrub of 1-7 metres high with yellow flowers in parts or singly. In traditional medicine, the leaf is used for the cure of fever, pneumonia, arthritis, ulcers and cough [10]. The plant is also claimed to repel snakes [11]. In addition, *C. tora* has been reported to exhibit many pathological properties such as, antidiabetic, anti-inflammatory, anticancer, anti-aging and in the treatment of asthma and degenerative eye disease [12,13]. The leaves are used for making soup in some parts of Northern Nigeria [14]. This work aims to investigate the antioxidant, nutritional and antinutritional properties of two leafy vegetables consumed in Adamawa State, Nigeria, with a view to encourage or otherwise the mass consumption of the leafy vegetables.

2. MATERIALS AND METHODS

2.1 Sample Collection and Authentication

Leaves of *Cleome rutidosperma* were collected from Karewa GRA, Yola and fresh leaves *Cassia tora* were collected from Nyibango, Yola,

Adamawa State, both in August 2018. The plants were identified and authenticated by Assoc. Prof. D. F. Jatau of Forest Resources Department, Modibbo Adama University of Technology, Yola, Nigeria, where a voucher specimen was kept. Fresh leaves of experimental plants were air dried under shade at room temperature. The dried samples were separately reduced to powder using a laboratory blender. The powdered form of samples was stored in air-tight containers until the time for extraction and phytochemical screening.

2.2 Preparation of Leaf Extracts and Phytochemical Screening

The air dried powdered leaves of *Cleome ruidosperma* and *Cassia tora* were extracted separately by cold maceration method using successive solvents such petroleum ether, chloroform and ethanol in increasing polarity for 48 hours respectively. Phytochemical screening was done using the method described by Harborne [15]. The leaf extracts were screened for phytochemicals such as saponins, tannins, terpenoids, flavonoids, alkaloids, glycosides, steroids and phenols.

2.3 *In vitro* Antioxidant Activity of Extracts

2.3.1 DPPH radical scavenging

The free radical scavenging activity of the extracts was measured *in vitro* by 1, 1-diphenyl -2 - picryl - hydrazyl (DPPH) assay [16]. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 10 ml of this solution was added to 3ml of the fraction dissolved in methanol at different concentrations (20 – 100 mg/ml). The mixture was then shaken and allowed to stand at room temperature for 30min and the absorbance was measured at 517 nM using a spectrophotometer. The percentage scavenging activity at different concentrations was determined or calculated using the formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Absorbance of Control} - \text{Absorbance of Test sample}) / \text{Absorbance of Control}] \times 100}{1}$$

Ascorbic acid was used as reference standard or control.

2.3.2 Ferric Reducing Antioxidant Power (FRAP)

In ferric reducing antioxidant power assay, 1 ml of test sample of ethanolic extract in different

concentrations (20-100 mg/ml) were mixed with 1 ml of 0.2 M sodium sulphate buffer (pH 6.6) and 1ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in temperature controlled water bath at 50°C for 20 minutes followed by addition of 1ml of hydrochloric acid. The mixture were then centrifuged for 10 minutes at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionized water and 200µl of 0.1% Fe³Cl. The blank was prepared in the same manner as the sample except that 1% ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in the A₇₀₀ after blank subtraction [17].

2.4 Proximate Analysis of Experimental Plants

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

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

2.5 Antinutritional Properties of Experimental Plants

Antinutritional properties which include, oxalate, saponins, alkaloids, tannins, cyanogenic glycosides and phytate, were determined using standard methods/procedures. The saponin content of the samples was determined by

double extraction gravimetric method described by Harborne [15], phytate content of the sample was determined according to the method outlined by Lucas and Markaka [18] and tannins content of the sample was determined using methods described by AOAC [19], with slight modification.

The oxalate content of powdered samples was determined by the modified method of [20], alkaloid content of samples was determined using the gravimetric method [15].

2.6 Data Analysis

The data was analyzed using ANOVA and results expressed as mean and standard deviation. Where applicable, P. values less than 0.05 ($p=0.05$) were considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The Preliminary phytochemical screening of ethanolic leaf extract of *C. rutidosperma* showed the presence of saponins, tannins, flavonoids, alkaloids phenols, while that of *C. tora* showed the presence of saponins, tannins, flavonoids, alkaloids, glycosides, steroids and phenols. Most of the phytochemicals were common to both plants, except glycosides and steroids which were not revealed in *C. rutidosperma*, but showed up in *C. tora* (Table 1). Comparatively, *C. tora* extract had more phytochemicals than *C. rutidosperma*. The extracts were further screened for antioxidant activities.

Phytochemicals are the bioactive non-nutrient plant compounds (in fruits, vegetables and grains) and are known to play a very important role in the prevention and treatment of chronic diseases, such as cancer, aging, cardiovascular

diseases, diabetes mellitus, obesity and neurodegenerative diseases, caused by oxidative stress [21,22]. The presence of saponins, tannins, flavonoids alkaloids and phenols as phytochemicals in both experimental plants' extracts (Table 1) validates their use in traditional medical practice in prevention and cure of diseases. Phytochemicals often possess strong antioxidant activity as well as anti-inflammatory activity, which are the basis of their bioactivities and health benefits [23].

3.2 Antioxidant Activities of *C. rutidosperma* and *C. tora* Leaves

The antioxidant activities of *C. rutidosperma* and *C. tora* ethanolic leaf extracts were determined using DPPH method in different concentrations ranging from 20 to 100 mg/ml by *in vitro* model. The changes in the free radical scavenging ability of the ethanolic extracts of the leaves of *C. rutidosperma* and *C. tora* on the basis of percentage inhibition is presented in Fig. 1. The percentage inhibition of DPPH of *C. rutidosperma* extract at 100mg/ml was $69.83 \pm 0.55\%$, whereas that of *C. tora* at 100mg/ml was 58.37 ± 1.45 , compared to control (ascorbic acid) $67.17 \pm 0.40\%$. It is clearly seen from the figure that the ethanolic of *C. rutidosperma* had the highest scavenging property and *C. tora* had the lowest. There was a significant ($p=0.05$) decrease in the concentration of 1, 1-diphenyl -2 - picryl - hydrazyl radical due to the scavenging activity of ethanolic leaf extract of *Cleome rutidosperma* as compared to standard. Both plants extract showed a dose dependent scavenging activity.

DPPH scavenging assay is based on the reduction of stable free radicals in methanol solution in the presence of hydrogen-donating antioxidants due to the formation of non-radical

Table 1. Phytochemical analysis of samples

Phytochemicals	<i>Cleome rutidosperma</i>	<i>Cassia tora</i>
Saponins	+	+
Tannins	+	+
Terpenoids	-	-
Flavonoids	+	+
Alkaloids	+	+
Glycosides	-	+
Steroids	-	+
Phenols	+	+

Where + = present, - = absent

form of standard stable free radical [24,9]. The radical scavenging assay was found to be $69.83 \pm 0.55\%$ for *C. rutidosperma*, compared to control (Ascorbic acid, 67.17 ± 0.40). The ethanolic extract of *C. rutidosperma* in effect possesses a significant antioxidant activity. This finding agrees with Chakraborty et al. [6] and is similar to Ghosh [9] who recorded an inhibition of $71.29 \pm 0.27\%$ for aqueous extract and $74.53 \pm 0.25\%$ ethanolic extract of *C. rutidosperma*. On the other hand, the ethanolic extract of *C. tora* showed a good antioxidant activity compared to control. The DPPH antioxidant activity of *C. tora* agrees with Arya and Yadav [25]; Sirappuselvi and Chitra [10]. In both cases, the antioxidant activities of plants' extracts is attributable to their phytochemical contents, especially their flavonoid and phenolic contents. [10,11,26].

The Ferric reducing antioxidant power (FRAP) is presented as Fig. 2. There was somehow a

linear increase in reducing power over the concentration range 20 – 100 mg/ml. It could also be observed that *C. rutidosperma* ethanolic extract has higher ferric reducing power than *C. tora*. FRAP assay measures the reducing power/ability of antioxidants against oxidative effects of free radicals, especially reactive oxygen species [27]. In this study, ethanol extract of *C. rutidosperma* exhibited a good reducing power of 0.42 (absorbance at 700 nm) at the concentration of 100 mg/ml compared to ascorbic acid (control) having total reducing power of 0.48. (Absorbance at 700 nm). *C. tora* also exhibited a moderate reducing power of 0.36 (absorbance at 700 nm) compared to control. The reducing power may serve an indicator of a compound's potential antioxidant activity [28]. Both ethanolic extracts of *C. rutidosperma* and *C. tora* showed an increasing trend in activity with increase in extract concentration, as also observed by Ahmed et al. [29].

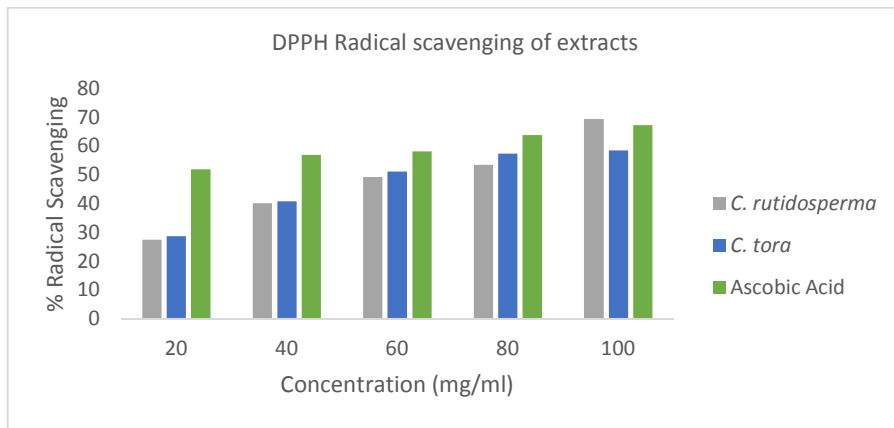


Fig. 1. DPPH radical scavenging activities of plants' extracts

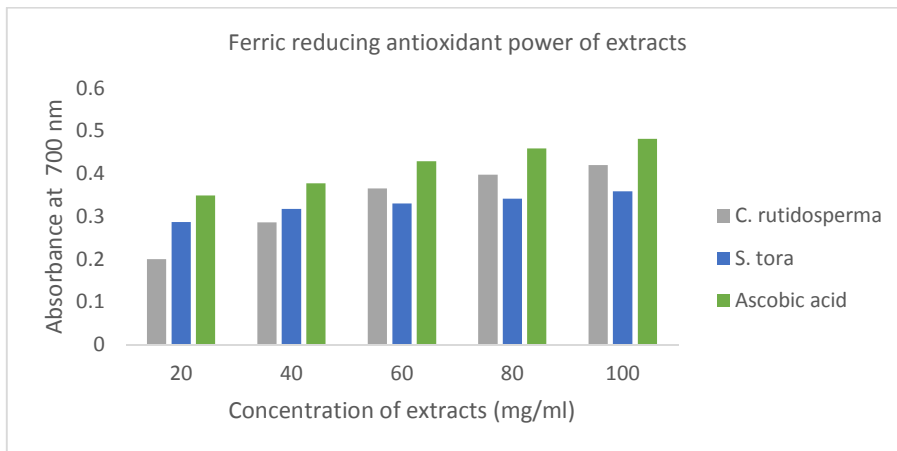


Fig. 2. Ferric reducing Antioxidant Power (FRAP) of plants extracts

Table 2. Nutritional composition of samples

Composition (% Mean \pm SD)		
Nutritionals	<i>Cleome rutidosperma</i>	<i>Cassia tora</i>
Crude protein	31.06 \pm 0.00	26.24 \pm 0.00
Fat	7.13 \pm 0.00	6.93 \pm 0.10
Ash	15.17 \pm 0.61	11.60 \pm 0.00
Crude fiber	11.06 \pm 0.01	13.19 \pm 0.10
Moisture	9.99 \pm 0.00	9.12 \pm 0.10
NFE	25.60 \pm 0.10	32.94 \pm 0.10
NFE=100-(crude protein +fat +Ash +Crude fibre+Moisture)	NFE = Nitrogen free Extractives (carbohydrate)	

3.3 Nutritional Composition of Extracts

The result of composition of plants' extracts is presented in Table 2. The mean protein content of *C. rutidosperma* and *C. tora* recorded are 31.06 \pm 0.00% and 26.24 \pm 0.00% respectively. Both plants seemed to a relatively high protein as compared to other nutrients. Both plants have a relatively low fat contents; *C. rutidosperma* 7.13 \pm 0.00% and *C. tora* 6.93 \pm 0.10%. Both plants' extracts seemed to have moderate amounts of crude fiber, ash and moisture. Leafy vegetables are rich source of carbohydrates, fats and proteins; and they form a major part of human and animal diet in which a cheaper source of energy is gotten [30]. Proximate and nutrient analysis of these vegetables becomes crucial in assessing their nutritional status. In addition, evaluating their nutritional significance can help to understand their worth [31].

The contents of crude protein recorded for *Cleome rutidosperma* and *Cassia tora* showed that the vegetables are good sources of dietary protein and can be used as cheap and abundant source of proteins [32], to alleviate protein deficiency among children, especially in rural communities where animal protein is often lacking. The amount of protein in *C. rutidosperma* tallies with protein in *Parkia biglobosa* -30.06% [33] and in *Ardisia solanacea* -31.25% [34]. Also the amount of protein in *Cassia tora* closely tallies with protein in *Corchorus oliterius* - 27.32 \pm 0.02% [35].

The concentration of fat recorded for *C. rutidosperma* and *C. tora* are 7.13 \pm 0.00% and 6.93 \pm 0.10% respectively. Both vegetables seemed to have moderate amount of fats. Dietary fats yield energy, contain fat-soluble vitamins and essential amino acids, and also increase the palatability of food by absorbing and

retaining flavours [36]. The percentages of ash in *C. rutidosperma* and *C. tora* stand at 15.17 \pm 0.61 and 11.60 \pm 0.00 respectively. *C. rutidosperma* has a significantly higher ash content compared to *C. tora*. Nonetheless, both plant vegetables showed a good ash content. Ash content represents the quantity of mineral elements present in them [30,37]. The percentage of ash in *C. tora* recorded in this study seemed to agree with Kubmarawa et al. [14]. The values of 15.17 \pm 0.61% and 11.60 \pm 0.00% is an indication of how rich the two edible vegetables are in nutritionally important mineral elements. The consumption of these vegetables should be encouraged.

The two experimental plants' leaves showed good percentages of fibre; 11.06 \pm 0.01% and 13.19 \pm 0.10% for *C. rutidosperma* and *C. tora* respectively. The amount of fibre in *C. rutidosperma* differ significantly with Arhoghro et al. [38]. The fibre in *C. rutidosperma* and *C. tora* suggests that both edible vegetables can help in preventing constipation, bowel problems and piles when consumed in large quantities [39]. The two edible vegetables showed almost the same moisture content, 9.99 \pm 0.00% for *C. rutidosperma* and 9.12 \pm 0.10%. This result is in agreement with Rishi et al. [40] who posited that reasonable amount of moisture in most vegetables is 6.0% to 15%. Moisture content is an index of water activity and used as a measure of stability and susceptibility to microbial contamination [41]. This implies that the vegetables can be stored easily without microscopic spoilage. NFE- Nitrogen free extractives (carbohydrates) for *C. rutidosperma* and *C. tora* are 25.60 \pm 0.10 and 32.94 \pm 0.10 respectively. *C. tora* has a significantly higher carbohydrate than *C. rutidosperma*. The result indicates that both plants can a good sources of carbohydrates whose primary function is the production of energy.

Table 3. Antinutritional properties of plant experimental plants

Antinutritional factors	Experimental plants	
	<i>Cleome rutidosperma</i> (Mean±SD)	<i>Cassia tora</i> (Mean±SD)
Oxalate (mg/100g)	220.86±0.00	297.11±0.00
Saponins (%)	1.98±0.10	2.77±0.00
Tannins (Mg/100g)	29.51±0.00	15.31±0.10
Cyanogenic glycosides (mg/100g)	4.29±0.00	6.24±0.00
Phytate (%)	0.77±0.10	0.89±0.28
Alkaloids (%)	0.62±0.45	1.20±0.00

It is worth mentioning here that processing methods such as sun-drying, heating and blanching in some way, affects the nutritional quality and antioxidant properties of leafy vegetables. Shade drying, however results in less loss of nutritional quality as reported by Troare et al. [42].

3.4 Antinutritional Properties of Plants' Extracts

The results of antinutritional properties of *C. rutidosperma* and *C. tora* are presented in Table 3. Extracts of experimental plants showed that each contained various concentrations of antinutritients. Antinutritional compounds otherwise known as antinutritional factors are secondary metabolites which are known to be biologically active and elicit very harmful biological responses [43]. In this research, extracts of experimental plants showed that each contained various antinutritients. The concentration of oxalate (mg/100g) of *C. rutidosperma* and *C. tora* are 220.86±0.00 and 297.11±0.00 respectively. The presence of oxalates in vegetables or any foods above acceptable levels causes irritation and pain in tissues [44]. It can be more painful when the crystals implant themselves in areas where they prevent other material from passing through, such as in digestive tract [45]. The concentrations of oxalate in *C. rutidosperma* and *C. tora* extracts in this work are within acceptable level of < 2-5g/kg body weight [46,47]. To be on a safer side however, moderate consumption of these leafy vegetables is therefore suggested.

The concentration of saponins in experimental plants are; 1.98±0.10% for *C. rutidosperma* and 2.77±0.00% for *C. tora*. Saponins have bitter taste, toxic in high concentrations and may affect nutrient absorption by inhibiting metabolic and digestive enzymes as well as binding with mineral elements such as zinc [48]. The concentrations of saponins in this research is

within acceptable levels of <10% in a diet, which is said to be harmless to the body [49]. Tannins concentrations recorded in this work are, 29.51±0.00mg/100g for *C. rutidosperma* and 15.31±0.10mg/100g for *C. tora*. The concentrations recorded are less than lethal dose of tannins (30 mg/kg) [46]. The consumption of the leafy vegetables under consideration should be encouraged with regard to tannins. Tannins are heat stable and so they interfere with digestion of proteins in man and animals, probably by inhibiting digestive enzymes and increasing fecal nitrogen [50].

Cyanogenic glycosides concentrations in extracts are; 4.29±0.00mg/100g for *C. rutidosperma* and 6.24±0.00mg/100g for *C. tora*. The concentrations are within acceptable levels of 5.3 – 80mg/100g [51] and also much less than the lethal dose of 50-60mg/kg [46]. The percentage of phytate in extracts of experimental plants are; 0.77±0.10% for *C. rutidosperma* and 0.89±0.28% for *C. tora*. The phytate values obtained are below the toxic level of 50-60mg/kg [46,47].

Alkaloids values recorded in this research was 0.62±0.45% for *C. rutidosperma* and 1.20±0.00% for *C. tora*. High level of alkaloids in foods is said to exert toxicity and adverse effects to humans, especially in physiological and neurological activities [52]. The quantities recorded in this research is less than the lethal dose of 20mg/100g [46] and so the consumption of these leafy vegetables in regard to alkaloids should be encouraged.

4. CONCLUSION

The presence of saponins, tannins, flavonoids alkaloids and phenols as phytochemicals in both experimental plants' extracts, validates their use in traditional medical practice in prevention and cure of diseases. The ethanolic extract of *C. rutidosperma* and *C. tora* exhibited a good antioxidant activity compared to control both in

DPPH and FRAP models. In both cases, the antioxidant activities of plants' extracts is attributable to their phytochemical contents, especially their flavonoid and phenolic contents. The leafy vegetables showed varied concentrations of phytonutrients. Both vegetables revealed a good percentage of proteins which can be used to compliment other sources of proteins especially in rural communities where animal proteins are sometimes lacking. All antinutritional contents of vegetables under consideration are within acceptable or tolerable levels.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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