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Phytochemical, Proximate and Metal Content of *Ricinus communis* (Castor Plant), *Caladium bicolor* (Elephant Ear) and *Gloriosa superba* (Climbing Lily) in Umudike, Abia State, Nigeria

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ABSTRACT

The world health organisation (WHO) reported that three quarters of the world population depend on herbal medicine for the treatment of different ailment and diseases because it is affordable and contain active ingredients. Notwithstanding this, the quality of the plants in relation to mineral and metal composition is very important since it is used for human health care. This study, therefore, assessed the phytochemical, proximate, and metal content of Ricinus communis, Caladium bicolor and Gloriosa superba sampled from Umudike in Ikwuano Local Government area of Abia State. A factorial experiment in randomised complete block design (RCBD) was used to collect leaf samples from R. communis, C. bicolor and G. superba. The samples were put in envelopes, labelled appropriately, placed in well cleaned, large ASEPA cellophane bag and taken to the laboratory for pretreatment and analysis. The result indicate that C. bicolor had the highest significant ($p<0.05$) content of ash (9.87±0.01%), fibre (6.96±0.01%), lipid (5.47±0.01%), alkaloid (4.44±0.01%), flavonoid (6.5±0.01%), phenol (25.19±0.01mg/100g), and Zn (10.70±0.01mg/kg while protein (28.49±0.01%), calorific value (366.42±0,01kcal), saponin (4.25±0.01%), tannin (84.18±0.01mg/100g) and copper (19.10±0.01mg/kg) were significantly ($P<0.05$) highest in R. communis. Consequently, C. bicolor and R. communis are promising plant species for herbal treatment and are recommended for manufacturing of drugs by pharmaceutical industries in Nigeria since the two plant species had higher content of phytochemical and proximate composition, while their metal content is below the permissible limit by FAO/WHO.

1. INTRODUCTION

According to World Health Organisation, about three quarters of the world population depends on traditional remedies (mainly herbs) for their health care (WHO, 1996). Herbal plants have not only provided food and

shelter but have also helped humanity to cure different dysfunctions. Indeed, a wide array of diseases including life threatening ones such as cancer, diabetes, hypertension etc. are being treated successfully with medicinal plants (Ahmed *et al*., 2017). It has been used extensively in the developing countries as a more available and affordable alternative to pharmaceutical drugs. The use of plant species, plants extracts or plant-derived pure chemicals to treat disease in Nigeria has become a therapeutic modality that has stood the test of time.

Minerals are essentially required for tissue functioning (Zafar *et al*., 2014), osmotic adjustment and to activate enzymes (Aslam *et al*., 2005). Man do not synthesize minerals rather, we obtain minerals from our diet (Anjorin *et al*., 2010) and major minerals such as Ca, Mg, Cl, Na, S, K, and P serve as structural components of tissues, take part in cell metabolism and acid-base balance (Smith, 1998). Most trace elements aid in prevention and treatment of diseases (Prasad, 1993).

Protein in plants are very essential since proteins form the basic building blocks for cells and tissue repairs in the body and its content influences the nutritional quality of plants. Ash content plays an important role to reflect the mineral elements of a food sample (Bhat and Sridhar, 2008; Mbatchou and Dawda, 2013). It also gives an idea to determine the levels of essential minerals present in the food (Edeogu *et al*., 2007). Fibres have the potential to decrease the blood cholesterol and sugar levels after meals in diabetics (Yeager, 1998), reduce the risk of bowel disorders and fights against constipation (FAO/WHO, 1998; Ahmed *et al*., 2012). The high incidence of a wide range of diseases in man may have a relationship with the absence or low fibre in diet (Eastword, 1974). The presence of fibre in diet increases the bulk of faeces, which has a laxative effect in the gut (Mbatchou and Dawda, 2013).

Ricinus communis belongs to the spurge family Euphorbiaceae, *Caladium bicolor* belongs to the family *Araceace* while *Gloriosa superba* Linn belongs to the family *Colchicaceae*. These three (3) plant species (*R. communis*, *C. bicolor* and *G. superba*) are used for medicinal purposes at Umudike in Abia State, Nigeria. The composition of these plants is not known to the local or rural dwellers that use the plants for therapeutic purposes. The awareness on nutritive value and health benefits of these plant species is of vital importance in order to increase the consumption of these medicinal plants by the rural people. Presently, there is paucity of literature on the phytochemical, proximate and metal composition of these three plants (*R. communisa, C. bicolor* and *G. superba*) in South east Nigeria. This study, therefore, was carried out to provide information on the phytochemical, proximate and metal composition of these plants in South east, Nigeria.

2. MATERIALS AND METHODS

2.1. Study Area

The study on phytochemical, proximate and metal content in *R. communis, C. bicolor* and *G. superba* was carried out in Umudike in Ikwuano Local Government Area of Abia State, Nigeria. Umudike is located 9 km South-east of Umuahia, the Capital of Abia State. It is situated on latitude 5°23' to 5° 23' N and longitude 7° 9' to 7°9'30 E with an altitude of 122 m above sea level. The climate of Umudike is made up of wet and dry seasons. The wet season starts from March and ends in November while the dry season starts by December and end in February. The wet season is characterized by clouds driven by light winds, relatively constant temperatures, frequent rains and high humidity while the dry season is notably dry with little or no rainfall, hotter days, cooler nights, and lower humidity (Chukwu and Ajuamiwe, 2013). The weather clears rapidly as the northeast trade wind shifts to become the dusty 'Harmatan' bringing in the drier air from the Sahara desert (Chukwu and Ajuamiwe, 2013).

2.2. Sample Collection

A reconnaissance survey was carried out with the assistance of a Taxonomist to identify three (3) medicinal plant species (*R. communis, C. bicolor and G. superba)* that are common in the study area but are underutilised by the locals due to lack of information on the nutritive and health benefits of these plant species. Fresh mature leaves were collected separately in the month of June 2017 from five plant species each of *R. communis, C. bicolor, and G. superba* in the study area. The leaves were sampled from different parts of each plant species with well clean secateurs and each sample were stored separately in envelopes, labelled well and taken to the laboratory for pre-treatment and analysis of phytochemical, proximate and metal content.

2.3. Phytochemical Screening

Standard methods for phytochemical screening (phenols, alkaloids, flavonoids, saponins, tannins, and phytates) was employed.

2.3.1. Determination of total phenol by spectrophotometric method

The sample (10 g) was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. The extract (5 ml) was pipetted into a 50 ml flask, then 10 ml of distilled water was added. Thereafter, 2 ml of 0.1M ammonium hydroxide solution and 5 ml of 20% concentrated alcohol (ethanol) was also added. The samples were left to react for 30 min for colour development. The absorbance was measured with Spectrophotometer at 505 nm (Adewole *et al*., 2015).

2.3.2. Determination of alkaloid

The standard methods of analysis of the Analytical Methods of Committee of Royal Society of Chemistry (2002) was adopted to determine alkaloid as modified by Momoh *et al*. (2012). Exactly 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and then allowed to stand for 4 hrs. This was filtered and the extract was concentrated in a water bath to the quarter of the original volume. Ammonium hydride (0.5M) was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid that was dried and weighed.

2.3.3. Determination of tannin

The procedure of Van Burden and Robinson (1981) was adopted. The sample (500 mg) was weighed into a 50 ml plastic bottle then 50 ml of distilled water was added and shaken for 1 hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask. Thereafter, 5 ml of the filtered solution was pipetted into a test tube and mixed with 2 ml of 0.13M $FeCl_3$, 0.11M HCl and 0.008M potassium ferocyanide. The absorbance was measured at 120 nm within 10 min.

2.3.4. Flavonoid determination

The standard methods of analysis of analytical methods of Committee of Royal Society of Chemistry (2002) was adopted to determine flavonoid as modified by Momoh *et al*. (2012). The sample (10 g) was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed.

2.3.5. Saponin determination

The procedure of Obadoni and Ochuko (2001) was adopted to determine the saponin content. The sample (20 g) was measured into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hrs with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts was reduced to 40 ml over water bath at 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. n-butanol (60 ml) was added and this was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. The saponin content was calculated and expressed as percentage.

2.3.6. Determination of percentage phytate

Following the procedure of Rahila *et al.* (1994)**,** a known weight of the grounded sample was soaked in 100 ml of 2% HCl for 5 hrs and filtered. The filtrate (25 cm^3) was poured into a conical flask and 5 cm^3 of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of 0.008M $FeCl_3$ until a brownish yellow colour persisted for 5 min. The concentration of the $FeCl_3$ was 1.04% w/v. Calculation mole ratio of Fe to phytate = 1:1.

$$\text{Concentration of phytate phosphorous} = \frac{titre\ value \times 0.064}{weight\ of\ sample} \quad (1)$$

2.4. Determination of the Proximate Composition

The proximate analysis (moisture content, crude fat, crude protein, total ash and carbohydrate, nitrogen) was determined using the standard procedure of AOAC (1990).

2.5. Determination of Metal Content in Plant

The plants were rinsed in distilled water, separated into leaves, stems and roots and oven-dried at 80°C for 48 hrs. The dried samples were milled to fine powder (<1 mm) in an agate mortar with pestle. Thereafter, the grinded powder was taken to laboratory for analysis of heavy metal content in the plants. Metal content was determined by Atomic Absorption Spectrophotometer (AAS) as described by James (1995). Following the ashing of samples, the resulting ash was dissolved in 10 ml of hydrochloric acid, HCl, filtered with Whatman 42 filter paper and extract was used for the analysis using atomic absorption spectrophotometer (AAS).

2.6. Experimental Design and Statistical Analysis

A simple factorial experiment in randomised complete block design was used to conduct the study on phytochemical, proximate and metal content in *R. communis*, *G. superba* and *C. bicolor*. Data obtained from this study was subjected to appropriate statistical analysis using the Statistical Package for Social Science (SPSS) version 16. Means were separated with Duncan multiple range test (DMRT) at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Proximate Composition of Leaves of *Ricinius communis*, *Gloriosa superba* and *Caladium bicolor*

The results of the proximate composition of leaves of *R. communis*, *G. superba* and *C. bicolor* is presented in Table 1. The highest values of ash (9.87 ±0.001%), fibre (6.96 ±0.01%) and lipid (5.47± 0.01%) were obtained in *C. bicolor* and these values are significantly ($p<0.05$) higher than values obtained in *R. communis* (7.10 ± 0.01, 5.12 ± 0.01 and 3.06 ± 0.01%) and *G. superba* (8.46 ±0.01, 6.02 ± 0.01 and 4.47 ± 0.0%). The highest content of ash, fibre and lipid in *C. bicolor* is attributed to its inherent ability. Inherent ability is the natural quality or characteristics of plant species that enabled the plant (*C. bicolor*) to generate more ash, fibre and lipid than *R. communis and G. superba*. The value of ash content in the leaves of *C. bicolor* (9.80%) was relatively higher than 9.56% in bitter leaf but lower than 13.01% in scent leaf reported by Asaolu *et al.* (2012). This inferred that the leaves of *C. bicolor* has better content of ash than the leaves of *R. communis* and *G. superba* since high ash content is an indication of the level of inorganic minerals and organic matter present in the leaves. The highest crude fibre content of 6.96±0.01 obtained in *C. bicolor* was well below the ranged (8.50-20.90%) for some Nigerian vegetables (Isong and Idiong, 1997). Dietary fibre helps to prevent bowel problem, piles and constipation (Adewole *et al*., 2015). The highest lipid value of 5.47±0.01 in leaves of *C. bicolor* shows that the plant contains more fat than the leaves of *R. communis* and *G superba*. This value is higher than the value (1.65 ± 0.62%) obtained in *Solanium* species (egg plants) by Odetola *et al.* (2004).

The highest value of protein (28.49±0.01%) and calorific values (366.42+0.01 kcal) were recorded in *R. communis* which are significantly ($p<0.05$) higher than values obtained in *C. bicolor* (19.25±0.01 and 360.03±0.01%) and *G. superba* (17.70 ± 0.01 and 364.43 ± 0.01%) while the highest content of carbohydrate, CHO was obtained in *G. superba* (63.34 ± 0.01 %). The crude protein content of 28.49±0.01% in *R. communis* was higher than 11.29% reported for *Momordica balsamina* (Ali, 2009). Since these plants are edible, their protein contents could be used to supplement the proteins from staple food. This result show that *R. communis* is a good source of protein more than *C. bicolor* and *G. superba*. The carbohydrate content (63.34 ± 0.01 %) obtained in *G. superba* was higher than 8.65% reported by Asaolu *et al.* (2012) in bitter leaf.

Table 1: Proximate contents of the leaves of *Ricinius communis*, *Gloriosa superba* and *Caladium bicolor*

Sample	Ash (%)	Fibre (%)	Protein (%)	Lipid (%)	CHO (%)	Calorific value (kcal)
Ricinius communis	7.10^{c}±0.01	5.12^{c}±0.01	28.49^{a}±0.01	3.06^{c}±0.01	56.23^{c}±0.01	366.42^{a}±0.01
Caladium bicolor	9.87^{a}±0.01	6.96^{a}±0.01	19.25^{b}±0.01	5.47^{a}±0.01	58.45^{b}±0.01	360.03^{c}±0.01
Gloriosa superba	8.46^{b}±0.01	6.02^{b}±0.01	17.70^{c}±0.01	4.47^{b}±0.01	63.34^{a}±0.01	364.43^{b}±0.01

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p<0.05$. Mean separation was done using the Duncan multiple range test (DMRT)

3.2. Phytochemical Content of *Ricinius communis, Gloriosa superba* and *Caladium bicolor*

The results of the phytochemical content in leaves of *R. communis*, *C. bicolor*, and *G. superba* are presented in Table 2. The highest values of alkaloid (4.44±0.01%), flavonoid (6.52±0.01%) and phenol (25.19±0.01%) were obtained in *C. bicolor and* these values are significantly higher than ($p<0.05$) values obtained in *R. communis* (2.91±0.01%, 3.70±0.01% and 16.41±0.01%) and *G. superba* (3.12±0.01%, 5.42±0.01% and 16.03±0.01%). The high content of alkaloid, flavonoid and phenol in *C. bicolor* may be attributed to the inherent ability of the plant (Ogbonna *et al*., 2018) to produce these phytochemicals more than *R. communis* and *G. superba*. The highest value 6.52±0.01% of flavonoid recorded in this study (*Caladium bicolor*) is relatively lower than the values (7.20±0.87%) reported by Ekanem *et al.* (2013) in the leaves of *C. bicolor.*

Similarly, the value of flavonoid obtained in *G. superba* (5.42±0.01%) is well below the value (55.92±4.21%) reported by Gopi (2016) for *G. superba* in India. The higher value of *G. superba* in India may be attributed to locational and/or environmental difference between India and Nigeria. Active substances contained in the same plant species may be different in types, contents, and proportions of the constituents because of the environmental differences in growing locations (Liu *et al*., 2016). Active substances are the result of the interaction between plants and the environment in the long evolution process, and its production and changes have a strong correlation and association with the environment (Peñuelas and Llusià, 1997). The highest value for saponin (4.25±0.01%), tannin (84.18±0.01 mg/100g) and phytate (43.32±0.01 %) was recorded in *R. communis* and the values were significantly($p<0.05$) higher than the values obtained in *C. bicolor* (3.40±0.01%, 42.94±0.01 mg/100g and 42.94±0.01 %) and *G. superba* (1.44±0.01, 36.31±0.01 mg/100g and 36.31±0.01 %). The highest content of saponin, tannin and phytate in *R. communis* may be attributed to its inherent ability to produce more of these phytochemical (saponins, tannin and phytate) than *C. bicolor* and *G. superba.* However, Ekanem *et al.* (2013) reported 6.25±0.90% for saponin in *C. bicolor* which is relatively higher than 3.40±0.01 obtained in this study. However, the value of tannin (84.18±0.01%) in this study is well above 3.33±0.01 mg/100g reported for tannin in *Vernonia amygdalina* by Adewole *et al.* (2015). Similarly, the content of phytate in this study (43.32±0.01 %) is well above 19.73±0.04 % obtained in *Vernonia amygdalina* (Adewole *et al*., 2015). Saponins and tannins exhibit cytotoxic effects and growth inhibition, thus, making them suitable as tumour inhibiting agents (Akindahunsi and Salawu, 2005; Asl and Hossein, 2008). Saponin and flavonoids are very useful in formulation of drugs in food, drinks and beverage industries as foaming agents (Fenwick *et al.*, 1983), as antioxidants, preservatives and flavouring agents (You *et al*., 1993; Fenwick *et al*., 1983).

Table 2: Phytochemical composition of *Ricinius communis, Gloriosa superba* and *Caladium bicolor*

Sample	Alkaloid (%)	Flavanoid (%)	Saponin (%)	Phenol (mg/100g)	Tannin (mg/100g)	Phytate (%)
Ricinius communis	2.91^{c}±0.01	3.70^{c}±0.01	4.25^{a}± 0.01	16.41^{b}±0.01	84.18^{a}±0.01	43.32^{a}±0.01
Caladium bicolor	4.44^{a}±0.01	6.52^{a}±0.01	3.40^{b}± 0.01	25.19^{a}±0.01	42.94^{b}±0.01	42.94^{b}±0.01
Gloriosa superba	3.12^{b}±0.01	5.42^{b}±0.01	1.44^{c}± 0.01	16.03^{c}±0.01	36.31^{c}±0.01	36.31^{c}±0.01

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p<0.05$. Mean separation was done using the Duncan multiple range test (DMRT)

3.3. Metal Concentration in *Ricinius communis, Gloriosa superba* and *Caladium bicolor*

The results on the concentration of metals in medicinal plants is summarised in Table 3. From the results, the highest value of copper (19.10±0.01 mg/kg) was obtained in *R. communis* which is significantly ($p<0.05$) higher than values obtained for *C. bicolor* (18.80±0.01 mg/kg) and *G. superba* (14.20±0.01 mg/kg). The highest value of Cu in *R. communis* is attributed to the inherent ability of *R. communis* to take up Cu from the soil more than *C. bicolor* and *G. superba.* The highest value of Zn (10.70±0.01 mg/kg) and Mn (41.20±0.01 mg/kg) in *C. bicolor* may be attributed to the inherent ability of the plant (*C. bicolor*) to absorb and translocate these metals (Zn and Mn) to the leaves more than *R. communis* and *G. superba.* The values are significantly ($P>0.05$) higher than values obtained in *R. communis* (0.05±0.01 mg/kg and 23.40±0.01 mg/kg) and *G. superba* (10.40±0.01 mg/kg and 30.60±0.01 mg/kg). Similarly, the highest value of iron Fe (55.60±0.01 mg/kg) was obtained in *G. superba* and it is not significantly ($P>0.05$) higher than the value of Fe in *C. bicolor* (55.30±0.01 mg/kg) but it is significantly ($P<0.05$) higher than the value obtained in *R. communis* (46.20±0.01 mg/kg). The range of Fe in this study is 46.20±0.01–55.6±0.01 mg/kg which is lower than 81.25–1101.22 mg/kg reported in mint, parsley, chamomile, basil sage, oregano and thyme by Martin and Griswold (2009). The World Health Organisation (WHO) permissible limit for Fe in medicinal plant is yet to be established (Dghaim *et al*., 2015). Iron (Fe) plays an important role in the body through oxygen supply, energy production, enhancing body immunity (Martin and Griswold, 2009), bone development and haemoglobin production (Nelson and Cox, 2005; Helena, 2008).

The permissible limit set by FAO/WHO (1984) for Cu in edible plants is 2 mg/kg. The acceptable limit for human consumption of Cu is 10 mg/kg (Nair *et al*., 1997). When Cu exceeds its safe level concentration, it causes hypertension, sporadic fever, coma (Nair *et al*., 1997). However, the WHO limits for medicinal plants have not yet been established for manganese (Jabeen *et al*., 2010).

The concentration of Zn in this study range from 0.05±0.00 – 10.70±0.01 mg/kg and this is lower than 12.65-146 mg/kg reported in mint, parsley, chamomile, basil, sage, oregano and thyme by Martin and Grisword (2009). Similarly, the value of Zn in this study is well below the permissible limit of 50 mg/kg set by FAO/WHO (1984). Zn is an essential trace element for DNA synthesis, blood clotting, proper growth, thyroid function and protein synthesis (Fosmire, 1990). Zinc intake beyond the permissible limit will affect negatively blood lipoprotein, immune system and copper level (Martin and Grisword, 2009).

Table 3: Metal composition of *Ricinius communis, Gloriosa superba* and *Caladium bicolor*

Sample	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
Ricinius communis	19.10^{a}±0.01	0.05^{c}±0.00	23.40^{c}± 0.01	46.20^{b}±0.01
Caladium bicolor	18.80^{b}±0.01	10.70^{a}±0.01	41.20^{a}±0.01	55.30^{a}±0.01
Gloriosa superba	14.20^{c}±0.01	10.40^{b}±0.01	30.60^{b}±0.01	55.60^{a}±0.01

Values are mean± standard deviation of three determinations. Mean values within the same column with different superscripts are significantly different at $p < 0.05$. Mean separation was done using the Duncan multiple range test (DMRT)

4. CONCLUSION

This study showed that that *C. bicolor* had the highest content of ash, fibre, lipid, alkaloid, flavonoid, phenol, Zn and Mn which is attributed to the inherent ability of the plant to generate these phytochemicals and proximate more than *R. communis* and *G. superba.* The highest content of protein, calorific value, saponin, tannin, phytate and Cu were observed in *R. communis*. The high composition of proximate and phytochemical contents in *C. bicolor* and *R. communis* indicate that the two plant species are very promising for the manufacturing of drugs. It is therefore recommended that people should use *C. bicolor* and *R. communis* for medicinal purposes.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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