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# Investigations of Phytochemical and Nutritional Composition of *Ruspolia Hypocrateriformis* Leaf

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## ABSTRACT

*Phytochemical and nutritional compositions of Ruspolia hypocrateriformis leaf were investigated in this study. The phytochemical, proximate and selected minerals were analyzed using standard methods whereas the concentrations of some selected vitamins were evaluated using HPLC method. The preliminary qualitative phytochemical screening revealed the presence of phenols, flavonoids, terpenoids, phlobatanins and saponins and absence of alkaloids, tannins, steroids, anthraquinones and cardiac glycosides. The quantitative values (in mg/100g) were found to be 41.42±0.20; 3.11±0.11 and 2.18±0.18 for phenols, saponins, and flavonoids, respectively. Proximate analysis showed carbohydrate, proteins, crude fat, moisture, ash and crude fibre values to be 54.18±0.23, 7.40±0.08, 0.85±0.02, 13.17±0.06, 4.51±0.06, and 19.90±0.24 respectively. The results of vitamin analysis (in mg/100g) revealed that the values of vitamin A, vitamin $B_2$, vitamin $B_1$, vitamin $B_9$, vitamin C, vitamin D and vitamin E corresponds to 125.02±6.76, 32.26±0.49, 0.42, 20.63, 1220.74±486.86, 62.17±26.52 and 28.47±11.82, respectively. The concentrations of the minerals in (mg/100g) of phosphorus, copper, zinc, Iron, calcium, potassium and sodium were 26.19, 6.20, 15.07, 14.62, 34.75, 29.52, and 38.29 respectively. This study shows that Ruspolia hypocrateriformis leaf phytochemicals, minerals and minerals of considerable medicinal and nutritional benefits; suggesting that the plant could be useful in management/treatment of diseases and food supplements.*

**Keywords**: *Ruspolia hypocrateriformis* leaf, phytochemical, proximate, vitamin, mineral, HPLC.

## INTRODUCTION

Generally, the overall benefits of plants to man cannot be overemphasized. They include amongst others, ornamental, medicinal, as well as nutritional. Man hence depends majorly on plants and their materials for survival [1]. The main research interest in plants most times has been to unveil the presence of some active components in them. Several researches have shown that these active components have inherent medicinal potentials for the treatment of various infections and diseases [2]. Identification and evaluation of these active components, also known as phytochemical of rarely used plants could help to generate a data base that would be useful in the development of new drugs [3].

Apart from medicinal relevance, nutritional potentials in most plants have been useful in supplying most basic nutritional needs of humans and other animals. The nutritional importance stems basically from their rich contents of essential amino acids, carbohydrate, lipids, vitamins and minerals [4].

The use of HPLC technique for vitamin separation still remains the most acceptable technique due to its high degree of selectivity [5]. Furthermore, solid-phase extraction is usually carried out before HPLC to exclude possible interfering compounds [6].

*Ruspolia hypocrateriformis,* popularly known as red rose belongs to the family of Acanthaceae. It is locally called Cameroun tea or blood tonic plant. It is a straggling shrub of over one metres high found mostly at the savanna and deciduous forest areas [7]. In the Southeastern Nigeria, it is normally seen growing around homes and gardens. The flowers are borne in showy terminal inflorescences with coral-red tubular flowers 3.75 cm long. Plants belonging to acanthaceae have been reported to have antifungal, cytotoxic, anti-inflammatory, anti-pyretic, antioxidant, insecticidal, hepatoprotective, immunodialatory, anti-platelet aggregation and antiviral activities [8]. In Nigeria, infusion of this plant is traditionally taken as blood tonic. However at present, there is paucity of literature on this plant and to the best of our knowledge, there is no published literature on chemical constituents of this plant. There is the need to understand its aptness for food, medicine or fodder. A good knowledge of its proximate, phytochemical and nutrient constituents will reduce the over dependence of many communities and industries on few known plants. This present study therefore was designed to evaluate the phytochemical, proximate and nutritional composition of *Ruspolia hypocrateriformis* with the aim of exposing and exploring its potential benefits.

## MATERIALS AND METHODS

**Sample Collection and preparation:** *Ruspolia hypocrateriformis* leaves were collected from a location in Abakaliki, South-East Nigeria and *were* identified by Mr. Ozioko of the International Bioresources and Research Centre, Nsukka, Nigeria. The leaves were removed, cleaned and air dried at room temperature of ($25^{0}$C-$28^{o}$C) for about 30 days before they were ground to powder using a blending machine and used in the analysis.

**Figure 1:** *Ruspolia hypocrateriformis* (Red Rose) plant.

**Chemicals and Reagents**: HPLC-grade solvents were used for analysis. Analytical reagent-grade acetonitrile and methanol were obtained from Lab-Scan (Tedia Company, USA). The water used for HPLC and sampling was prepared with a Millipore Simplicity instrument (Millipore, Molsheim, France). All vitamin standards were of chromatography grade and were purchased from Sigma Chemical Co. (Poole, Dorset). All other chemicals and reagents were of standard analytical grades.

**Phytochemical Screening:** The ground leaves of *Ruspolia hypocrateriformis* were analyzed for the presence of alkaloids, saponins, anthraquinone, steroids, tannins, flavonoids, phlobatanin, terpenoids, phenol and cardiac glycosides according to standard methods [9],[10],[11].

**Quantification of Phytochemical Composition:** Quantitative phytochemical analysis to determine the level of saponins, phlobatanin, phenol and flavonoids using standard

methods as described by Trease and Evans (1985), Ball (2006), Van-Burden, and Robinson, (1981) and Sofowora (2008)[12],[13],[14],[15] were carried out.

**Proximate Analysis:** The determination of carbohydrate content, moisture content, ash content, crude fiber, proteins and crude fat was done using Official Method of Analysis of the Association of official Analytical Chemists[16].

**Determination of Vitamins**: High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model-SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data were recorded using LC-solutions software.

## MINERAL ANALYSIS

Mineral contents of *Ruspolia hypocrateriformis* leaves were determined using UNICAM solar 969 Atomic Absorption Spectrophotometer for elements such as P, Fe, K, Na, Zn, Ca and Cu as described by [17].

## RESULTS

## PHYTOCHEMICALS

The results of the qualitative and quantitative analysis of ***Ruspolia hypocrateriformis***is are represented in the Tables 1 and 2 below. Proximate, vitamins and mineral compositions are presented in the Tables 3, 4 and 5 respectively.

## PHYTOCHEMICALS

**Table 1: Table showing the qualitative composition of phytochemicals in *Ruspolia hypocrateriformis* leaf**

| Phytochemicals | Profile |
|---|---|
| **Phenol** | + |
| **Flavonoids** | + |
| **Phlobatanin** | + |
| **Tannins** | - |
| **Alkaloids** | - |
| **Terpenoids** | + |
| **Steroids** | - |
| **Saponins** | + |
| **Anthraquinone** | - |
| **Cardiac glycoside** | - |

KEY: Presence (+), Absence (-).

**Table 2: Quantitative Compositions of Phytochemicals Present in *Ruspolia hypocrateriformis* leaf**

| Phytochemicals | Quantity (mg/100g) |
|---|---|
| Phenol | **41.42 ±0.20** |
| Flavonoid | **2.18 ±0.18** |
| Saponin | **3.11 ± 0.11** |
| Alkaloid | **Non-detectable** |

**Table 3: Proximate Content of *Ruspolia hypocrateriformis* leaf**

| Proximate | Concentration (100%) |
|---|---|
| **Carbohydrate** | **54.18±0.23** |
| **Protein** | **7.40±0.08** |
| **Crude fat** | **0.85±0.02** |
| **Moisture** | **13.17±0.06** |
| **Ash** | **4.51±0.06** |
| **Crude fibre** | **19.90±0.24** |

Data are presented as the mean ± standard deviation ($n$ = 3).

**Table 4: Vitamins Composition of *Ruspolia hypocrateriformis* Leaf**

| Vitamins | Composition (mg/100g) |
|---|---|
| Vitamin C | 1220.74±49.86 |
| Vitamin $B_2$ | 32.26±0.49 |
| Vitamin $B_1$ | 0.42±0.08 |
| Vitamin A | 125.03±6.76 |
| Vitamin D | 62.17±26.52 |
| Vitamin E | 28.47±11.82 |
| Vitamin B9 | 20.63±4.12 |
| | |

Data are presented as the mean ± standard deviation ($n$ = 3).

**Table 5: Minerals Composition of *Ruspolia hypocrateriformis* Leaf**

| Minerals | Composition (mg/100g) |
|---|---|
| Phosphorus | 26.19±7.30 |
| Copper | 6.20±2.11 |
| Zinc | 15.07±0.95 |
| Iron | 14.62±1.97 |
| Calcium | 34.75±5.87 |
| Potassium | 29.52±1.26 |
| Sodium | 38.29±3.12 |

Data are presented as the mean ± standard deviation ($n$ = 3).

## DISCUSSION AND CONCLUSION

Phytoconstituents are responsible for the diverse identified activities of plant species, hence, the assessment of chemical constituents of plants offers germane information that can be exploited for discovery of new drugs [18]. The preliminary phytochemical analysis of the leaf of *Ruspolia hypocrateriformis* showed the presence of phenols, flavonoids, terpenoids, phlobatanin and saponins.

From the quantitative results, *Ruspolia hypocrateriformis* leaves have high content of phenols (41.415±0.195mg/100g). This is quite significant in comparison to many popular plant species known for high phenolic content. The flavonoids content was found to be 2.18±0.180mg/100g. The potent antioxidant activity of flavoniods is their ability to scavenge hydroxyl radicals superoxide anions and lipids peroxy radicals and may be the most important function of flavoniods [19]. These bioactive compounds (flavonoid, alkaloids, phenolics and saponnins) are known to exhibit medicinal activity as well as physiological activity (Sofomora, 1993). Flavonoids have been shown to have anti-mutagenic, antibacterial, anti-inflammatory, antiallergic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity [20].

The quantity of saponins was found to be 3.105±0.105mg/100mg. Saponins have been reported to possess anti-hyperglycaemia, anti-hypercholesterolaemia and anti-hypertension activities (Trease and Evans, 1985)[12]. They possess antibiotic and anti-inflammatory properties and aids in wound healing (Krishnaiah *et al.*, 2009)[21]. Saponins have natural tendency to ward off microbes, this makes them effective therapy for fungal, bacterial and yeast infections. Thus they could be used as natural antibiotics, which help the body to fight infections and microbial actions. Studies have indicated that saponins though non toxic can generate harmful physiological effects in animals that ingest them, they show cytotoxic effect and growth inhibition against a variety of cells making them have anti-inflammatory and anticancer properties (Igile *et al.*, 2013)[22]. The presence of saponins in *Ruspolia hypocrateriformis* makes it a potential for the production of novel drugs. The presence of terpenoids supports the possible use of *Ruspolia hypocrateriformis* in the treatment of bacterial infections because terpenes are active against bacteria. Similarly, phlobatanins were also detected. Tannin was however absent.

The potential of a particular food or plant is determined primarily by its nutrient composition. The nutritional evaluation of *Ruspolia hypocrateriformis* revealed that the carbohydrate content (54.18±0.23%) was the highest while crude fat was the least (0.85±0.02). Protein content was found to be 7.40±0.08, the ash content (4.51±0.06), moisture content (13.17±0.06) and crude fibre (19.90±0.24). The high value of carbohydrate indicates its possible use as energy giving food. The relative high value of the crude fibre further shows its nutritional potential as fibre rich food substances are good for lowering of serum cholesterol levels, risk of coronary heart disease, hypertension, diabetes, colon and breast cancer, management of diarrhea and detoxification of poisonous metals (Cohn and Cohn, 1996). Ash content of a plant based food is the function of the mineral elements present. Its presence shows that *Ruspolia hypocrateriformis* possesses mineral elements. Other important proximate available includes crude fat and crude protein which are in appreciable quantity. Their presence goes further to expose the nutritional benefit of the leaf.

The result of the vitamins evaluation is presented on Table (4). It revealed the presence of vitamins A, $B_1$, $B_2$, $B_9$, C, D and E. Vitamin C content (1220.735±486.855mg/100g) was the highest while vitamin $B_1$ was the least (0.422mg/100g).

Vitamins are reported to decrease the harm by free radicals and repress degenerative disease (Bartlett and Eperjesi (2008)[23]. In this study, vitamin E content was 28.47mg/100g. According to FAO (2002)[24], the RDA requirement for vitamin E is 10mg/day for normal healthy adult men between the ages of 19-65 years while that of adult non-pregnant women within same age range is 7.5mg/day. Vitamin E is a powerful antioxidant that helps to guard body cells from injury due to reactive oxygen species. It is very crucial for the production and normal function of erythrocytes and muscles [25].

Our result showed that vitamin C content was 1220.75mg/100g and is very high when compared to values for vitamin C reported by Blessing *et al.* (2011)Elfalleh *et al.*, (2009), Nkafamiya *et al.*, (2010), [25],[26],[27], for pumpkin accessions (3.47 to 4.39mg/100g), Misra and Misra (2014) for *Moringa oleifera* and *Ipommea aquatic* (2.17mg/100g and 0.34 mg/100g respectively) Igile *et al.* (2013)[22] for *Vernonia calvoana* (11.33mg/100g), and Acikgoz (2011) for kale (98.30mg/100g). The relatively high vitamin C content obtained here makes this plant a good source of vitamin C. Vitamin C is a powerful antioxidant that assists non-haeme iron transport and uptake at the intestinal mucosa, the reduction of intermediates of folic acid as well as the synthesis of cortisol. Its deficiency includes fragility of blood capillaries and its susceptibility to destruction, gum decay and scurvy ( Jacab and Sotoudeh (2002)[28]. Low ascorbic levels have been associated with fatigue and increased severity of respiratory tract infections (Lukaski, 2004)[29], while high intake of vitamin C from food had been shown to increase serum HDL-cholesterol and lower serum triacylglycerols concentration (Osse, 1970)[30]. Besides, it helps in the purification of blood (Igile *et al.,* 2013)[22]. From the result, this plant could be good source of vitamin C for meeting the recommended daily allowance of the vitamin among vulnerable groups such as children and pregnant women as well as in times of food shortage where micronutrient deficiencies become apparent. The recommended daily requirement for Vitamin C according to FAO (2001)[24] is between 45.83 - 68.50 mg/day for both male and female adults between the ages of 19 to 65 years.

Furthermore, our result showed that *R. hypocrateriformis* leaf is a good source of other antioxidants which are necessary for the maintenance of health and the prevention of stress and prostate cancer (Igile *et al.,* 2013)[22]. The values obtained for vitamins A, C and E suggest that the plant may be of help in reducing the prevailing micronutrient deficiency in poverty stricken communities especially in Sub-Sahara Africa. These anti-oxidants have been associated with prevention of nutritional related diseases such as cancer, diabetes mellitus, coronary heart diseases and obesity, [31].

The result also showed appreciable quantities of vitamin A in the leaves of *Ruspolia hypocrateriformis*. Vitamin A is vital for normal vision, gene expression, growth and immune function by its maintenance of epithelial cell functions (Lukaski, 2004)[29]. Adequate supply of dietary antioxidants may prevent or delay diabetes complications including renal and neural dysfunction by providing protection against oxidative stress [23].

The vitamins B are a group of water soluble vitamins that perform vital functions in cell metabolism. They include thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, cyanocobalamine and folic acid. The deficiency syndromes of some B vitamins are beriberi, peripheral neuropathies, pellagra, and oral and genital lesions. Many vitamins of the B-group act primarily as coenzymes in the metabolism of food to generate energy (Ball, 2006)[13]. The vitamin B9 level in *R. hypocrateriformis* leaves was 20.63mg/100g. It is a key factor in the synthesis of nuceic acid, vital for pregnant women and those trying to conceive, it aids in rapid cell division and growth, production of healthy red blood cells and enhances brain health. The recommended daily intake level of B9 is 400mg/day from food. B complex vitamins found in *Ruspolia hypocrateriformis* are essential for growth, development, and a variety of other bodily functions. They play a major role in the activities of enzymes.

The result of the mineral analysis is as shown on table 5. The sodium content (38.29mg/100g) was the highest and copper content (6.20mg/100g) was the least.

Iron and copper for are vital in the formation of blood and copper as well is associated with normal carbohydrate and lipid metabolism. Copper aids in the formation of bones, conversion of iron into hemoglobin. It is also reported that copper imbalance raises cholesterol by destroying proper HDL to LDL balance.

Zinc is a multifunctional nutrient involved in glucose and lipid metabolism, hormonal function and wound healing Kizito and Angela (2009)[32], and is also associated with proper hair growth (Misra and Misra, 2014)[33]. It works with vitamin C for the production of elastrin, necessary for the production of RNA, phospholipids, protein metabolism and ATP, help to convert tyrosine into pigment that color the skin and hair; it is also involved in the healing process, taste, healthy nervous and formation of collagen.

Potassium is the key cation in intracellular fluid and help in the maintenance of weight (Igile *et al*., 2013)[22], control of acid-base balance, conduction of nerve impulse, contraction of cardiac muscles, proper functioning of the cell membrane, regulation of the sodium-potassium ATPase system and the maintenance of fluid volume [6]. Besides, it performs an essential function in the transfer of phosphate from adenosine triphosphate to pyruvic acid. The RDA for potassium for both normal healthy males and non-pregnant

females between the ages of 19 and 50 years is 4700mg/day (Institute of Medicine, 2005). The level of potassium reported in this research (29.52mg/100g) showed that the *R. hypocrateriformis* leaf might be a poor source.

The result of sodium content was 38.29±3.12mg/100g. This value is high compared to the range of values reported by Iheanacho and Udebuani (Kizito and Angela, 2009) for *Amaranthus hybridus* and *Curcubita pepo* (17.50 and 24.80mg/100g respectively), the 1.08g/100g obtained by Igile *et al.* (2011)[22] for *Vernonia calvaona,* and Akpana *et al.*, (2017). Sodium maintains of body fluid composition, proper acid-base balance in addition to the transmission of nerve impulses. The RDA requirement for sodium is 1500mg and 2300mg/day for normal healthy male adults aged 19 to 50 years and female non-pregnant adults aged 19-50 years respectively (Institute of Medicine, 2005)[31]. Sodium and potassium are important for chemical reaction in the cells and regulates the transfer of nutrients to the cells. Sodium works in conjunction with potassium for extracellular fluid balances (Otitoju, *et al.*, 2014)[34].The level of sodium obtained for this plant in this study indicate that it can be consumed by hypertensive patients as it can keep body sodium levels low.

The result of calcium content of *R. hypocrateriformis* leaves is comparatively high compared to most of the values reported by Agbaire and Emoyan (2012)[6] for some local vegetables in Delta State, Nigeria, and the calcium content of *Solanum nigrum* (17.33mg/100g) obtained by Udo, *et al.* (2013)[35]. Conversely, the level for calcium in this work is much lower than the data reported by Nkafamiya *et al.* (2010)[27] for *F. asperifolia* and *Ficus Sycomorus* (428.65 and 390.78mg/100g respectively) and Vunchi *et al.*, (2011)[36]. Our result agrees with the submission of Osse (1970)[30] that leaves have high calcium concentration. Calcium is required in the development and sustenance of strong bones and teeth particularly in fetuses, infants, children, and the elderly, regulation of contraction of muscle and relaxation, nerve function and absorption of cyanocobalamin (vitamin B12) (Otitoju *et al.*, 2014)[34]. Calcium may therefore be of use in prevention of osteoporosis in the elderly (Dias, 2012)[18]. Further, it plays a vital role in blood coagulation as it activates the process leading to the conversion of prothrombin to thrombin. According to Vunchi *et al.,*(2011)[36], good food calcium content is greater than one and poor if less than 0.5. From the result, this implies that *R. hypocrateriformis* leaves, is a good source of calcium.

Phosphorus is concerned with many metabolic processes including those involving body fluid buffers, maintenance of normal kidney function as well as in the transfer of nerve impulses Vunchi *et al.,*(2011)[36], Besides, phosphorus performs a vital role in strengthening bones and teeth and in the maintenance of muscle growth (Udo *et al.*, 2013)[35]. For good calcium absorption to take place, calcium-phosphorus ratio must be 1:1 (Vunchi *et al.,* 2011)[36]. The calcium-phosphorus ratio for the leaf of *R. hypocrateriformis* evaluated in this study is 1.3:1. The RDA requirement for phosphorus in both adult males and non-pregnant females is 700mg/day (Institute of Medicine, 2005)[31]. Our results have shown that daily consumption of this leaf can help achieve the recommended dietary allowance (RDA) of mineral element which is necessary for improving health and well being.

In conclusion, our results clearly showed that *R. hypocrateriformis* leafcan serve both as food and important medicinal plant. It is rich in phenol, flavonoids, and saponins which have proven medicinal activities. Furthermore, *R. hypocrateriformis* leaf is a good source of some vital minerals and vitamins as it contains substantial quantities of macro nutrients like proteins, carbohydrates, fibre; minerals and vitamins, therefore may be included in diets to supplement daily dietary allowances needed by the body, thereby enhancing nutritional status and ameliorating the problem of protein energy malnutrition and micronutrient deficiency especially among the rural dwellers in developing countries like Nigeria. Finally, the plant could be exploited for novel drug development, and as raw plants treatment of several diseases like anemia, cancer, antioxidant; it can also be used for formulation of natural vitamin premix for livestock feed [36].

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