THE MELTING POT
ISSN 2504 – 8880 Journal of the School of General and Basic Studies
Volume 8 Number 1 March, 2023
themeltingpotj@gmail.com

PHYTOCHEMICAL AND NUTRITIONAL COMPOSITIONS OF *RUELLIA BREVIFOLIA* (CHRISTMAS PRIDE) LEAF

[1*]Nkama, O.J., [2]Igwenyi, I.O., [3]Esonwune, A. A., [4]Ndukwe M. K., [5]Ekuma, E. T. and [6]Atiaetuk, I. E.

[1*,3,4&6]Department of Science Laboratory Technology, School of Science, Akanu Ibiam Federal Polytechnic, Unwana-Afikpo, Ebonyi State.
[2]Department of Biochemistry, Faculty of Science, Ebonyi State University, Abakaliki.
Corresponding Author: Onochiejeff@gmail.com

Abstract

Genus Ruellia are dicotyledonous plants that are primarily called Ruellias or wild petunias. Ruellia brevifolia is one of the known species of Ruellias that is used in traditional medicine. Phytochemical and nutritional compositions of Ruellia brevifolia leaf were investigated. The chemical constituents (phytochemicals, proximate and minerals) were determined using standard laboratory procedures. The proximate analysis was performed on the whole leaf while the phytochemical analysis and mineral elements contents were evaluated on the aqueous leaf extract. The proximate analysis of the plant sample revealed the percentage of moisture, crude protein, carbohydrate and ash in the descending order. The extract showed the concentrations of phenols > flavonoids > alkaloids > glycosides > saponins > tannins > terpenoids > hydrogen cyanide and sterols as phyto constituents. The extract also revealed the concentrations of Mg > Ca > Na > Zn > Fe > P > Mn > NO^- and Cu as mineral compositions. The results show that R. brevifolia has bioactive compounds that could be pharmacologically useful in the treatment and management of various diseases. From the results, the plant leaf is also nutritionally rich and as such could be used as food or supplement for body nourishment and disease prevention or treatment. It is recommended that further research should be conducted to determine its anti-nutrient compositions and its pharmacological effects on certain disease conditions. The locals are encouraged to use R. brevifolia leaves as medicinal herbs and food supplements.

Key words: *Ruellia brevifolia*, phytochemicals, proximate, mineral elements, pharmacology, nutrition.

Introduction

Genus Ruellia are dicotyledonous plants that are primarily called *Ruellias* or wild petunias. They belong to *Acanthaeceae* (Acanthus family) containing about 1,250 genera with 2,500 species. Many of them are herbs, shrubs or twining vines while others grow as epiphytes. They are found in both temperate and tropical areas (regions) (Afzal *et al.,* 2015). *Ruellias* are found in Africa, Brazil, Indonesia, Malaysia, Pakistan and Central America. Some of them are used for medicinal purposes because many species have antioxidant, anti-inflammatory, analgesic, antiulcer, antidiabetes, etc. as their phytochemical constituents include glycosides, flavonoids, triterpenoids, alkaloids, etc. effects (Roopa *et al.,* 2011).

Ruellia brevifolia is one of the known species of *Ruellias* that is used in traditional medicine. It is ornitothophilous (bird pollinated), herbaceous plant and about 1.0m tall (Abreu & Vieira, 2004). In Vicosa, *R. brevifolia* is found in the forest understay, in shady to partially shaded locations, year round flowering and fruiting (Lima & Vieira, 2006). In Nigeria, especially in the Eastern part, *R. brevifolia* is planted as ornament and as herb. It is used in traditional medicine to treat/manage many diseases. The genus *Ruellia* and its isolated compounds have been shown to possess pharmacological properties which include healing of wound, antihyperglycemic, antioxidant, antimicrobial, antibacterial, anticancer, anti-inflammatory, cytotoxic and gastroprotective properties, purgative and angiotensin-converting enzyme inhibitory effect and estrogenic activity (Mamdouh *et al*., 2015).

Genus *Ruellia* (the family Acanthaceae) is a large plant family, a taxon of dicotyledonous plants that contain about 250 genera and 2500 species. It is named after a French herbalist, Jean de Ruelle (1474 – 1537). *Ruellia* mostly called *Ruellias* or wild petunias is commonly found in tropical, subtropical and temperate areas. They can also be found in Mediterranean regions, Australia and USA (Trease, 2002). Many species of *Ruellia* are employed in traditional medicine for the treatment of many diseases. They are also used as foods and spices. *Ruellia brevifolia* is one of the species of the genus *Ruellia* with medicinal properties. It is ornithophilous (bird pollinated) herbaceous plant that is very useful in disease treatment (Abreu & Vierra, 2004). *R. brevifolia* is called red Christmas pride in English and ogwu obara in Igbo.

Figure 1: *R. brevifolia* Plant. Height: 4160, Width: 3210, 4.86MB, Focal length: 3.5mm.

Source: Photographed by Nkama, Onochie Jeff

Materials and Methods

Chemicals/Reagents and Equipment

All chemicals and reagents used in this research were of high analytical grade. The chemicals were the products of May and Baker (MB), England; Merck, Germany, Sigma-Aldrich, Germany; British Drug House (BDH), UK and Kieselgel GmbH, Germany. The Reagents for the assays were

commercial test kits and products of Randox, UK; Biovendor, Czech Republic; TECO Diagnostic, USA and Centronic GmbH, Germany.
All equipment and instruments used were optimally functional and they included rotary evaporator, spectrophotometer, glass column chromatography, water bath, chemical balance and refrigerator.

Collection/Preparation of plant material
The *R. brevifolia* leaves were harvested from a garden at Okposi Street, Nkaliki, Abakaliki and were identified and authenticated by a Botanist, Dr. (Mrs.) Stella E. Obasi of Biology Research Unit, Science Laboratory Technology Department, Akanu Ibiam Federal Polytechnic, Unwana.
The harvested *R. brevifolia* leaves (500g) were washed with running tap water and macerated in 2.0 L of warm water for 72 hrs. After extraction, the extract was filtered and evaporated using a rotary evaporator to get a concentrated extract.

Proximate analysis
Proximate compositions of fresh *R. brevifolia* leaf were evaluated using the methods described by AOAC (1980). Proximate analysis was designed to produce top level, very wide arrangement of food components. The system comprises the determination of %moisture, %ash, %crude fat, %crude protein, %fibre and %carbohydrate using analytical procedures.

Moisture content: Two gram of *R. brevifolia* leaf was weighed and placed in the oven to dry at 105^0 C to a persistent weight. The weights of both the dish and sample were recorded after they were allowed to cool. The percentage moisture was then calculated using the equation: %Moisture = Wt2 - Wt3/Wt1×100
Where Wt1= weight of sample taken, Wt2 = weight of sample + dish before drying, and Wt3 = weight of sample + dish after drying.

Ash content: Exactly 2 g of *R. brevifolia* leaf was weighed into a platinum crucible and put into muffle furnace at $400\text{-}600^0$ C for 4 hours to obtain whitish-grey ash. A desiccator was used to cool the crucible and its weight determined.
%Ash = ash weight/sample weight × 100

Fat content: A flask was filled with 150 ml anhydrous diethyl ether whose boiling point is between 40 and 60^0 C. Exactly 2 g of *R. brevifolia* leaf was weighed into a thimble using cotton wool as a plug, placed into the flask and allowed to heat. As the diethyl ether vapor approached the condenser via the extractor`s side arm, liquid condensation took place and went into the thimble, the soluble substances got dissolved and moved into solution back to the flask. The extraction lasted for 4 hrs. The thimble was brought out as the solvent almost got distilled. There was disconnection of the flask as it was placed in the oven at 65^0 C for 4 hrs. It was allowed to cool using a desiccator and weighed.
% Fat = flask weight + extract – flask weight/sample weight × 100

Crude fibre: Exactly 2 g of the defatted *R. brevifolia* leaf was put into 500 ml flask, 200 ml of pre-heated 1.25 % H_2SO_4 added and mildly boiled for 30 minutes. The constant volume of th acid was maintained by adding hot water. Residue was washed thrice using hot water and brought back to the beaker. Then 200 ml of 1.25 % NaOH was added and boiled for another 30 minutes. This was filtered under suction and washed very well using hot water and with ethanol two times. The residue was allowed to dry at 65^0 C for 24 hrs and its weight taken, transferred into a crucible and placed in muffle furnace ($400\text{-}600^0$ C). It was allowed to ash for 4 hrs., cooled in a desiccator and its weight recorded.

% Fibre = Wt2 – Wt3/WtI × 100

Where W1 is the sample weight, W2 is the dry residue weight before boiling and W3 is the weight of residue weight after ashing.

Protein content

Digestion: Two gram of *R. brevifolia* leaf was weighed into kjeldahl digestion flask and 25 ml conc. tetraoxosulphate (vi) acid, 0.5 g copper(ii) tetraoxosulphate(vi) and a tiny spot of selenium tablet were added. Fume cupboard was used with applied heat slowly at the beginning to stop needless frothing. Digestion lasted for 45 minutes as the digest turned to clear pale green. It was allowed to cool, then 100 ml distilled water was added and the flask was rinsed three times with the rinsing added to the bulk.

Distillation: Markham distillation apparatus was employed. The apparatus was heated, 10 ml of the digest was added using a funnel and allowed to boil. 10 ml of 50 % sodium hydroxide was added to prevent loss of ammonia. Distillation was done into 50 ml of 2 % boric acid with screened methyl red indicator.

Titration: The formed ammonium borate (alkaline) was used to titrate directly with 0.01N hydrochloric acid. The volume of acid (titre value) used was taken.

% Protein = titre × 0.01N HCl × 14.01 (At. unN) × 100 ×50/1000 × 0.5 × 10 × 100

Carbohydrate: Percentage carbohydrate was evaluated using simple calculation. It was done by the subtraction of the sum of percentages of moisture, ash, lipids and protein from 100.
%Carbohydrate = 100- (%Moisture + %Ash + %Fat + %Protein + %Fibre)

Mineral contents determination

The mineral contents of the extract were determined according to the methods by A.O.A.C, (2010). Two gram of *R. brevifolia* extract was weighed into porcelain crucible, heated to ash at 450-500º C and then cooled in a desiccator to 25^0C. Digestion of the ash was done with 5 mL HNO_3, $HClO_4$ and HCl mixture in the ratio of 3:1:1. The resulting solution was transferred into a 100 mL volumetric flask and distilled water was used to make up the mark. The minerals contents (Na, K, Mg, Fe, Zn, Fe, P and Mn) were determined using Atomic Absorption Spectrophotometer (AAS). Individual elements were analyzed from the sample solutions because there is a specific cathode discharge lamp for each element. At a wavelength specific to each element, discharge lamp emits a radiation. This specificity can only be achieved from a pure sample of the element that is electrically excited to provide an arc spectrum on the element.

Quantitative phytochemical analysis

Tannins concentration of *Ruellia brevifolia* was assessed using Folin Denis colometric method of Nwaokonkwo (2009). The methods described by El-Olemyl *et al.* (1994) were used to determine flavonoids, alkaloids, saponins, hydrogen cyanide, phenol, terpenoid, steroids and glycosides.

Tannins: Exactly 1 g of *R. brevifolia* extract was macerated in 20 ml distilled water and filtered. Five (5) ml of the filtrate was dropped into a test tube with the addition of 0.3 ml 0.1N ferric chloride + 0.1N HCl and 0.3 ml of 0.0008M potassium ferricyanide. The contents were thoroughly mixed and measurement of absorbance done at 720nm.
Tannin = Absorbance-blank/slope × solvent volume/sample weight

Flavonoids: Exactly 1 g *R. brevifolia* extract was macerated in 20 ml ethyl acetate and filtered. Five (5) ml of the filtrate was pipetted into a test tube adding 5 ml dilute ammonia. The upper layer was collected and measured at 490nm absorbance.
Flavonoid = Absorbance - blank/slope × solvent volume/sample weight

Alkaloids: Exactly 1 g *R. brevifolia* extract was macerated in 20 ml ethanol, 20 % tetraoxosulphate (vi) acid in the ratio of 1:1 and filtered. One (1) ml of the filtrate was mixed with 60 % of H_2SO_4 and stood for 3 hrs. Absorbance measurement was done at 490nm.
Alkaloid = Absorbance - blank/slope × solvent volume/sample weight

Phenol: Exactly 1 g of *R. brevifolia* extract was macerated in 20 ml 80 % ethanol and filtered. Five (5) ml of the filtrate was put into a test tube with the addition of 0.5 ml folinciocaltu`s reagent. After 30 minutes, 2 ml of 20 % sodium trioxocarbonate (iv) was added and absorbance measurement was done at 650nm.
Phenol = Absorbance - blank/slope × solvent volume/sample weight

Saponins: Exactly 1 g *R. brevifolia* extract was macerated in 20 ml petroleum ether, decanted into a beaker and rewashed using 10 ml petroleum ether. The filtrate was combined and allowed to evaporate to dryness. Six (6) ml ethanol was used to dissolve the residue, 2 ml transferred into a test tube with the addition of 2 ml chromogen solution, allowed to stand for 30 minutes and absorbance measurement done at 550nm.
Saponin = Absorbance - blank/slope × solvent volume/sample weight

Hydrogen cyanide: Exactly 1 g of *R. brevifolia* extract was macerated in 50 ml distilled water and filtered. To 1 ml of the filtrate in a test tube, 4 ml alkaline picrate was introduced, boiled for 5 minutes, cooled and the absorbance measurement was taken at 490nm.
Hydrogen cyanide = Absorbance -blank/slope × solvent volume/sample weight

Terpernoid: Exactly 0.5 g of the extract was macerated with 20 ml ethanol and filtered. Exactly 2.5 ml of the filtrate was dropped into a test tube and 1ml of 5 % phosphomolybdic acid solution and 1 ml H_2SO_4 were added and mixed. It was kept to stay for 30 minutes and the absorbance measurement was taken at 700nm.

Terpenoid = Absorbance – blank/slope × solvent volume/sample weight
Steroids: Two gram of the extract was macerated in 20 ml ethanol and filtered. Into a test tube, 2ml of the filtrate and 2 ml ethanol were mixed. Two (2) ml of color reagent was put in both the sample test tube and blank test tube, allowed standing for 30 minutes and the absorbance measurement was taken at 550nm.
Steroids = Absorbance – blank/slope × solvent volume/sample weight

Glycosides: Exactly 1 g of the extract was macerated in 20 ml distilled water and filtered. About 2.5 ml of 15 % lead acetate was put in and filtered. There was addition of 2.5 ml chloroform with vigorous shaking. the upper layer was collected and allowed to evaporate to dryness. Three (3) ml acetic acid was used to dissolve the residue and 0.1 ml of 5 % ferric chloride introduced, after which 0.25 ml

tetraoxosulphate (vi) acid was added and kept in a dark area for 2 hrs. The absorbance measurement was taken at 530nm.
Glycoside = Absorbance – blank/slope × solvent volume/sample weight

Results

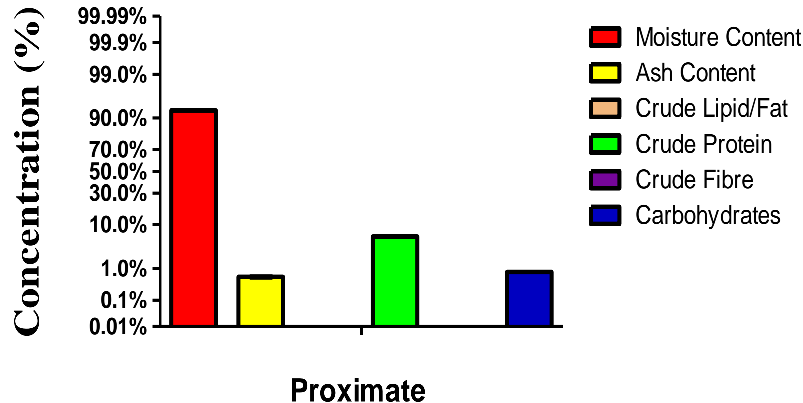

Figure 1: Proximate Compositions of *Ruellia brevifolia* Leaves. Data are shown as mean ± S.D (n=3)

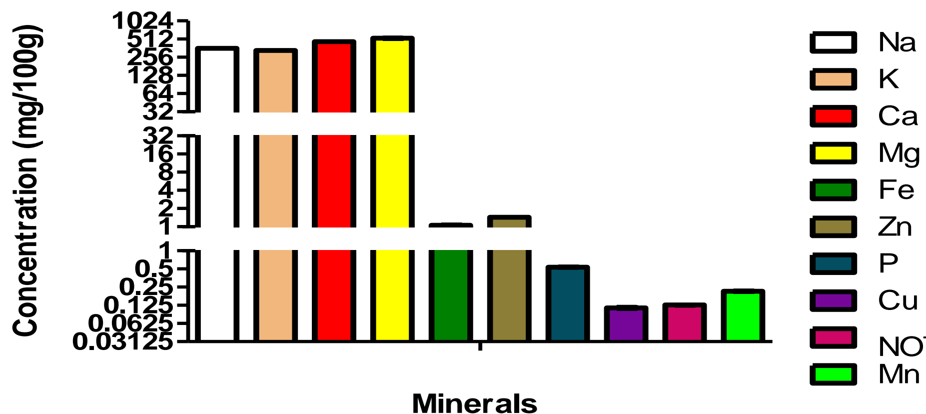

Figure 2: Mineral Compositions of Aqueous Leaf-Extract of *Ruellia brevifolia.* Data are shown as mean ± S.D (n=3)

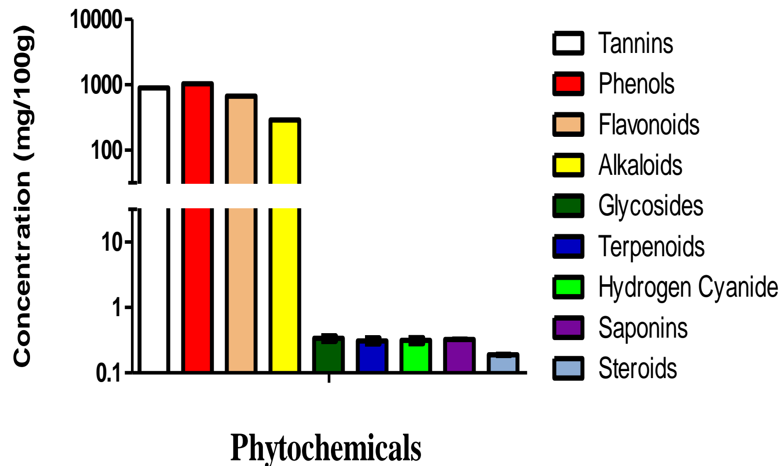

Figure 3: Phytochemical Compositions of Aqueous Leaf-Extract of *Ruellia brevifolia.* Data are shown as mean ± S.D (n=3)

Discussion

In this study, the proximate analysis of *R. brevifolia* revealed the contents of moisture, protein, carbohydrates and ash. This nutritional value was determined because plants have been used in disease treatment/management and as a nutrient source since the stone age, though their usage has been on the rise as the years go (Kolassani *et al*., 2011). Moisture content is the measure of water activity, proteins and carbohydrates are macromolecules that the body requires for energy metabolism and growth. Ash contains inorganic plant components as every organic material is removed during ashing. Plants with medicinal values have these classes of food which are important requirements for the body`s health and they are needed to perform different activities involving physiology, metabolism and morphology (Radha *et al.,* 2021). The leaf as a vegetable may be taken as a food plant, food supplements, or used for various preparations with meat or fish. Some locals mascerate the leaves in warm or cold water and drink it when they feel weak or have a disease. The result is within the range of the works of Kolassani *et al.* (2011) and Radha *et al.* (2021). Any marked difference may be as a result of several conditions such as season, location, maturity stage and whether (Radha *et al.,* 2021; Choudhury and Garg, 2007).

The results of the mineral contents of *R. brevifolia* extract showed the concentrations of Mg, Ca, K, Na, Zn, Fe, P, Mn, NO- and Cu in descending order. These mineral elements are essential in the management of various diseases. According to other findings, medicinal plants have relatively high and moderate mineral contents (Radha *et al*., 2021). *Ocimum gratissimum, Prosopis africana, Verninia amygladina*, and *Ruellia tuberosa* extracts contain these mineral elements (Mohamed *et al*., 2016; Ugwu *et al*., 2018; Ugwu *et al*., 2019). The overall study has indicated that *R. brevifolia* is an important source of mineral elements. These minerals contribute a little amount of the total composition of many plants and of total body weight. For this reason, they are very important, mostly during metabolic ativities and disease control. For instance, Ca is needed during the synthesis of vitamin D and Fe as a component of haemoglobin. Many researchers have been able to find mineral contents in plants extracts that are being used as herbs to supplement medicine or medicine itself. These elements are present in various quantities in different plants parts, mostly leaves, seeds or roots

(Radha *et al*., 2021). Macro and micro elements affect biochemical processes in the body since a good number of them influence metabolism. Some of them such as Ca and Zn are chelated with organic ligans to make sure they are available to the body (Mohanta *et al*., 2003). Determination of mineral elements is very essential since their concentrations and types affect the quality of many food items. It is not just to assess the absolute quantity of mineral, but also these minerals must be available for the organism (Prasad, 1993) as living things need mineral elements for the prevention of diseases.

All mineral elements play one physiological/biochemical role or the other. Calcium is one of the elements involved in fruit storage quality. It is the most plentiful element in the bones and is needed to maintain teeth, healthy bones and blood (Igwenyi *et al*., 2014). Iron is linked to haemoglobin and involved in the transfer of oxygen from the lungs to tissue cells (Kruezek, 2005). Iron deficiency is a common dietary shortfall that lead to anemia.

The outcome of phytochemical analysis showed the concentrations of tannins, phenols, flavonoid, alkaloids, glycosides, saponin, terpenoids, hydrogen cyanide and steroids in descending order. These phytochemicals possess properties of antioxidant, antimicrobial, antinutrigenomic, anti-stress, etc which make them interesting to be used as growth promoters in animal management (Yagi *et al*., 2013). In animal studies, flavonoids are observed to have antioxidant, anti-inflammatory, anti-carcinogenic, anti-bacterial, anti-viral, anti-thrombotic, anti-allergic and hepatoprotective properties (Radha *et al*., 2021). The study of Radha *et al*. (2021) had shown that plants with abundance of saponins supplemented with polyphenol enhanced weight gain relative to the ones on antibiotics and growth promoters, suggesting that saponin mix may serve as a choice to antibiotics and growth promoters in livestock farming. Many species of *Ruellia* have been used in herbal remedy to treat several diseases (Luciane *et al*., 2003). *R. tuberosa* is widely used as antioxidant, anti-diabetic, diuretic, analgesic, gastroprotective, antipyretic and antihypertensive agent (Roopa *et al*., 2011*). R. asperula* is used to treat fever, asthma, bronchitis, uterus inflammation and flu (Agra *et al.,* 2008). *Ruellia prostrate* has been used to treat eczema, cephalgia, chronic rheumatism and facial paralysis (Rajan *et al*., 2012). *Ruellia hygrophila* has analgesic and antispasmodic activities while *R. brittoniana* and *R. patula* are employed in treating cardiovascular disease (Ahmad *et al*., 1993). The presence of these phytochemicals in *R. brevifolia* could be part of its healing properties, which agrees with the reports of Wiart *et al*. (2005) and Afzal *et al*. (2015).

Conclusion

The *Ruellia brevifolia* leaf has significant percentage moisture, crude protein, carbohydrate and ash. It also has relatively high concentrations of magnesium, calcium, potassium and sodium as well as phytochemicals such as phenols, flavonoids and alkaloids, glycosides, saponins, terpenoids and HCN. The proximate composition is the reason why the locals use it as therapeutics and nutritional supplements. Mineral elements are very important in metabolism and disease management. The phytochemicals are pharmacological agents that used to treat or prevent diseases. *Ruellia brevifolia* is a medicinal plant that may be used in the treatment/management of many diseases especially those caused by inflammation and oxidative stress.

References

Abreu, C. R. M. & Vieira, M. F. (2004). Os beija-flores e seus recursors florais em um frangmento florestal de Vicosa, Sudeste brasileiro. *Lundiana*, **5**:129-134.

Afzal, K., Uzair, M., Chandhary, B. A., Ahmad, A., Afzal, S. & Saadullah, M. (2015). Genus Ruellia: Pharmacological and Phytochemical Importance in Ethnopharmacology. *Acta Poloniae Pharmacological-Drug Research*, **72**(5): 821-827.

Agra, M. D. F., Silva, K. N., Basilio, I. J. L. D., Freitas, P. F. D. & Barbosa-Filho, J. M. (2008). Survey of medicinal plants used in the Northeast of Brazil. *Brazilian Journal of Pharmacognosy,* **18**:472.

Ahmad, M., Akhtar, M. F., Miyase, T., Ueno, A., Rashid, S. & Usmanghani, K. (1993). Studies on the medicinal herb *Ruellia patula*. *Pharmaceutical Biology*, **31**:121-129.

A.O.A.C (2010). Official Methods of Analysis, (18th ed.). *Association of Official Analytical Chemists,* Washington D. C. Pp 428-435.

A.O.A.C. (1980). Official Methods of Analysis, (13th ed.). *Association of Official Analytical Chemists,* Washington D. C. Pp 376-384.

Choudhury, R. P. & Garg, A. (2007). Variation in essential, trace and toxic elemental contents in Murraya koenigii—A spice and medicinal herb from different Indian states. *Food Chemistry,* **104**:1454–1463.

El-Olemyl, M. M., Al-Muhtadi, F. J. & Afifi, A. F. A. (1994). *Experimental phytochemistry: A laboratory manual.* King Sand University Press, Saudi Arabia. Pp 21-27.

Igwenyi, I. O., Agwor, A. S., Nwigboji, I. U., Agbafor, K. N. & Offor, C. E. (2014). Proximate Analysis, Mineral and Phytochemical Composition of *Euphorbia Hyssopifolia*. *IOSR Journal of Dental and Medical Sciences,* **13**:41–43.

Kolasani, A., Xu, H. & Millikan, M. (2011). Evaluation of mineral content of Chinese medicinal herbs used to improve kidney function with chemometrics. *Food Chemistry*, **127**:1465–1471.

Kruczek, A. (2005). Effect of row fertilization with different kinds of fertilizers on the maize yield. *Acta Scientiarum Polonorum Agricultura*, **4**:37–46.

Lima, N. S. A. & Vieira, M. F. (2006). Fenologia de floracao e sistema reprodutivo de tres especies de *Ruellia* (Acanthaceae) em fragment florestal de Vicosa, Sudeste brasileiro. *Revista Brasileira de Botanica*, **29**:681-687.

Luciane, F. B., Felipe, T. G., Sidnei, B. O. F. & Fabio, S. M. (2003). Dirhamnosy flavonoid and other constituents from Brillantaisia palisatti. *Quimica Nova*, **26**:922-923.

Mamdouh, N. S., Sachiko, S., Katsuyoshi, M., Hideaki, O. & Mohammed, S. K. (2015). Chemical constituents and biological activities of genus *Ruellia*. *International Journal of Pharmacology*, 2(6): 270-279.

Mohamed, D. A., Rashed, M. M., Shallan, M., Fouda, K. & Hanna, L. M. (2016). Amelioration of Benign Prostate Hyperplasia in Rats through Plant Foods. *International Journal of Pharmacology and Phytochemical Research*, **8**(12): 2063-2070.

Mohanta, B., Chakraborty, A., Sudarshan, M., Dutta, R. K. & Baruah, M. (2003). Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. *Journal of Radioanalytical and Nuclear Chemistry*, **258**:175–179.

Nwaokonkwo, D. C. (2009). Phytovhemical analysis of the seed of Napolena imprerialis. *Journal of Chemical Society of Nigeria*, **34**(2):174-176.

Prasad, A. S. (1993). *Essential and Toxic Elements in Human Health and Disease: An Update*. Wiley-Liss, New York, USA.

Radha, K. M., Puri, S., Pundir, A., Bangar, S. P., Changan, S., Choudhary, P., Parameswari, E., Alhariri, A., Samota, M. K., Damale, R. D., Singh, S., Berwal, M. K., Dhumal, S., Bhoite, A. G., Senapathy, M., Sharma, A., Bhushan, B. & Mekhemar, M. (2021). Evaluation of 1Nutritional, Phytochemical, and Mineral Composition of Selected Medicinal Plants for Therapeutic Uses from Cold Desert of Western Himalaya. *Plants*, **10**:1429.

Rajan, M., Kumar, P. V. K., Kumar, S., Swathi, K. R. & Haritha, S. J. (2012). Antidiabetic, antihyperlipidaemic and hepatoprotective activity of methanolic extract of *Ruellia tuberosa* Linn leaves in normal and alloxan induced diabetic rats. *Journal of Chemical and Pharmaceutical Research*, **4**(6):2860-2868.

Roopa, K. A. N., Borar, S. & Thakral, J. (2011). Phytochemical constituents in Ruellia. *International Journal of Pharmaceutical Sciences Review and Research*, **2**:1015-1021.

Trease, G. E. Evans (2002). *Pharmacognosy* (5th ed.). Baillere and Tindall Press, London. Pp 64.

Ugwu, N. U., Asuk, A. A., Eteng, M. U. & Emuru, E. O. (2019). Evaluation of Impact of Some Selected Plants on Haematological Parameters of Testosterone and Estradiol Induced Benign Prostatic Hyperplasia in Adult Male Rats. *International Blood Research and Reviews*, **10**(1): 1-15.

Wiart, C., Hannah, M., Yassim, M., Hamimah, H. & Sulaiman, M. (2005). Antimicrobial activity of *Ruellia tuberosa L. American Journal of Chinese Medicine*, **33**(4), 683-689.

Yagi, S., Rahman, A. E., ELhassan, G. O. & Mohammed, A. M. (2013). Elemental analysis of ten Sudanese medicinal plants using X-ray fluorescence. *Journal of Applied and Industrial Sciences,* **1**:49–53.