Communication in Physical Sciences, 2021, 7(4): 431-437

Proximate Analysis, Thin Layer Chromatography Profile and Haematinic Activity of Organic Extracts of *Brillantaisia Owariensis* Leaves

Onuchi. M. Mac-kalunta*, Ahamefula. A Ahuchaogu and*Johnbull O Echeme
Received: 16 November 2021/Accepted 03 December 2021/Published online:25 December 2021

Abstract*: In this work, the proximate analysis, concentration profile from thin-layer chromatography and haematinic activity in rats induced phenylhydrazine anemia were investigated using the n-hexane, chloroform, ethyl acetate and methanol extracts of Brillataisia owariensis leaves. The results we recorded indicated a significant presence of carbohydrates, fiber, protein and traces concentrations of fat. Analysis of the thin layer chromatogram gave evidence that the plant extract is rich in various phytochemicals. However, the activity of the plant extract based on the hematological parameters (red blood cell count (RBC), hemoglobin concentration (HB), white blood cell count (WBC) and hematocrit (PCV), showed that the ethyl acetate extract has the least activity, whereas, the crude methanol and chloroform extracts demonstrated the most significant activity*.

Keywords:*Brillantaisia owariensis, proximate and phytochemical analyses, biological activity, hematological assay*

Onuchi.M. Mac-kalunta*
*Department of Chemistry, College of Physical and Applied Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria
Email: **marygemkal@gmail.com**
Orcid id: **0000-0002-7895-9030**

Ahamefula. A. Ahuchaogu
Department of Pure and Industrial Chemistry, Abia State University, Uturu, Abia State, Nigeria
Email: ahuchaogua@gmail.com
Orcid id:0000-0002-6412-7487

Johnbull O .Echeme
Department of Chemistry, College of Physical and Applied Sciences, Michael Okpara University of Agriculture, Umudike, Abia State.
Email: jb.tbulle@gmail.com
Orcid id: 0000-0002-7754-7560

1.0 Introduction

The commonest and most significant medical challenges among humans are deficiency of hemoglobin, which is an iron-rich protein in the red blood cells. The function of the hemoglobin involves the transportation of oxygen to the cells, consequently, several symptoms have been reported for decreasing levels of hemoglobin in humans, such as body weakness, shortness of breath, headaches, anorexia, somnolence, reduced immunity, anemia, decreased quality of life (Lakshmanasamy, 2011). Severe drop in the level of hemoglobin can create a health condition known as anemia, which may be identified as classified as iron-deficiency anemia, pernicious anemia, aplastic anemia, hemolytic anemia, parasitic infection and drug toxicity (Saravanan and Manokaran 2012; Ong 1973). Anemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infections that tend to reduce the red blood count (Dacie and Lewis, 1994). In view of these conditions, several conventional and herbal remedies are recognised. Traditionally also, several medicinal plants have been reported to be

used in the management of anemia (Alada, 2000; Dina *et al*., 2006).

Brillantaisia owariensis is a perennial shrub, generally glandular and sticky; with stems up to 2 m tall. The leaf of the plant has petioles up to 14 -17cm long. The flowers are calyx dark purple with dense capitates glands, the corolla is pale to deep purple or blue to dark blue with a white neck 25- 53 mm long. The fruit is capsule-shaped 17-35 mm long and seeds 1.5 mm long. The plant grows in Nigeria, Togo, West Cameroon and across Uganda.

According to Ngbolua *et al* (2013) the leaves of B. owariensis are used traditionally in Congo for the treatment of anemia, rheumatism, menstrual pain, stomach ache and for their antiplasmodial and analgesic potentials (Asai *et al*., 2012; Makambila-Koubemba*et al*., 2011; Mbatchi*et al*., 2006). Also, local midwives or traditional birth attendants in some parts of Nigeria, use Brillantaisia owariensis leaves to control bleeding and to manage anemia. Scientific data on the potency of B.owariensis against anemic conditions is scanty. However reports from a few studies have indicated that alcoholic extract of B. owariensis leaf has antibacterial and antioxidant activities (Aluko *et al*., 2014; Faparusi*et al*., 2012). Akuru and Amadi (2018) also reported that the leaf of Brillantaisia owariensis is rich in amino acids with a high quantity of glycine which is considered essential for rapid growth and the biosynthesis of porphyrin components of hemoglobin. The present investigation aims to establish the haematinic properties of leaf extracts of **Brillantaisia owariensis** in Wistar albino strain rats induced with anemia

2.0 Materials and Methods

2.1 Sample collection and preparation

Leaves of Brillantaisia Owariensis were harvested from Ndioro Oboro, Ikwuano Local Government Area, Abia State, Nigeria. The plant material was identified and authenticated at the Plant Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The harvested leaves were briefly rinsed to remove debris, and air-dried under shade for 12 days. Dried leaves were milled with a blender into powder. The powder was weighed and transferred into an air-tight amber container

2.2 Extraction and partition

200 g of the powdered sample was weighed into a 2.5 L capacity glass jar containing 1.2 L of methanol. The mixture was agitated with a stirrer for 15 minutes, covered and left allowed to stand for 72 hours before filtration with a Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator. The crude extract obtained weighed 6.32 g. 4 g was partitioned by Kupchan *et al*. (1973) protocol to give hexane (2.02 g), chloroform (1.44 g) and ethyl acetate (1.0 g) extracts.

2.3 Proximate analysis.

The moisture, crude fat, crude protein, ash, fibre and total carbohydrate of B.owariensisair air-dried leaves were determined according to AOAC (2006).

2.4 Thin-layer chromatography

Pre-coated thin-layer chromatography (TLC) aluminum plates were used; hence, the plate was cut to a size of 4 X 10 cm and used for the TLC. The R_f values were appropriately calculated.

2.5 Experimental animals

Albino Wister rats (24) of both sexes weighing 100-120 g were used for the study. The animals were obtained from the laboratory of the Animal Production Unit, Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike. The animals were assigned to six groups of four animals each, kept in a cage and were allowed to acclimatize within two weeks before commencement of the experiment. The experiment was carried out following international standards and ethics as approved by the ethics committee of the College of Natural sciences, Michael Okpara University of Agriculture, Umudike, Nigeria. The different groups were treated according to the order below.

Group 1: Control
Group 2: Phenyl hydrazine (30mg/kg body weight)
Group 3: Crude Extract (500mg/kg + Phenyl hydrazine)
Group 4: n-Hexane (500mg/kg + Phenyl hydrazine)
Group 5: Chloroform (500mg/kg + Phenyl hydrazine)
Group 6: Ethyl Acetate (500mg/kg + Phenyl hydrazine)
Treatment was done via oral root and lasted for 10 days before animals were sacrificed by cervical dislocation and blood was collected by cardiac puncture in K3 EDTA bottles for hematological analysis.

2.6 Determination of hematological parameters

Hematological parameters include; red blood cells(RBC), packed cell volume(PCV), hemoglobin(HB), white blood cells(WBC), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH) and mean corpuscular hemoglobin concentration(MCHC) were determined for each blood sample using an automated hematology analyzer, model BC-2300, Mindray Medical Company, India. To achieve this, blood samples were aspirated into the equipment and allowed to run for 1 minute. Results of all the parameters were displayed on the screen of the analyzer.

3.0 Results and Discussion

Table 1 presents information on the proximate composition of .B. owariensis leaf including moisture, protein, fat, fibre, ash and carbohydrate contents. Also, the hematological parameters of the blood samples that were tested are presented in Table 2. The information presented in the listed Tables is also represented in Figs. 1 and 2.

Table 1: Proximate composition of air dried *B. owariensis* leaves

Moisture Content	Protein	Fat	Fiber	Ash	Carbohydrate
10.42	6.73	3.56	23.50	5.22	52.57
10.36	6.55	4.10	23.72	5.28	51.99
10.44	6.55	3.68	23.66	5.26	52.41

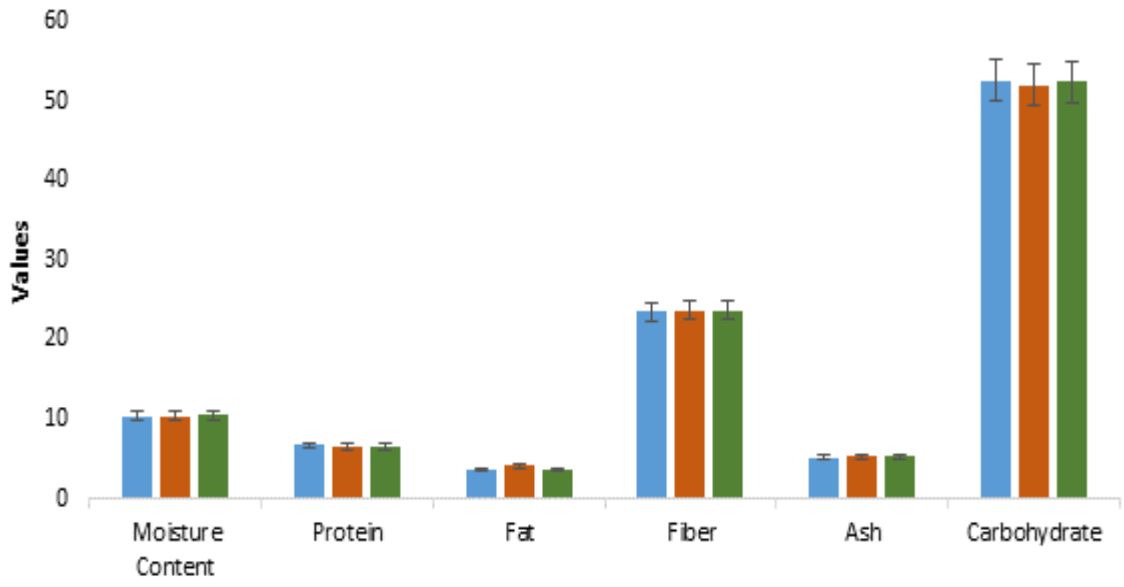

Fig. 1: Bar charts showing the profile for the proximate analysis of the crude extract of *B. owariensis* leaves

Table 2: Hematological parameters of the blood samples of test subjects

Groups	RBC	PCV	HB	WBC	MCV	MCH	MCHC
1	6.04	44.50	15.83	7.97	73.72	26.22	35.56
2	3.15	25.25	7.33	20.35	78.33	22.75	29.21
3	4.18	26.25	9.30	16.13	62.89	22.28	35.44
4	3.24	24.25	8.50	17.25	74.92	26.25	35.03
5	4.35	28.25	10.18	11.53	66.69	23.86	35.88
6	2.59	15.50	4.20	24.50	61.73	16.80	27.44

***Group 1**: Control ,**Group 2**: Phenyl Hydrazine (30 mg/kg body weight) , **Group 3**: Crude Extract (500 mg/kg + Phenyl Hydrazine), **Group 4:** n-Hexane (500 mg/kg + Phenyl Hydrazine), **Group 5**: Chloroform(500 mg/kg + Phenyl Hydrazine), **Group 6**: Ethyl Acetate (500 mg/kg + Phenyl Hydrazine). Red Blood Cell (**RBC**), Packed cell volume (**PCV**),Hemoglobin(**HB**), White blood cells(**WBC**), Mean corpuscular volume(**MCV**), Mean corpuscular hemoglobin(**MCH**) and Mean corpuscular hemoglobin concentration(**MCHC**). The **MCV**, **MCH** and **MCHC** are generally referred to as Red Cell Indices.

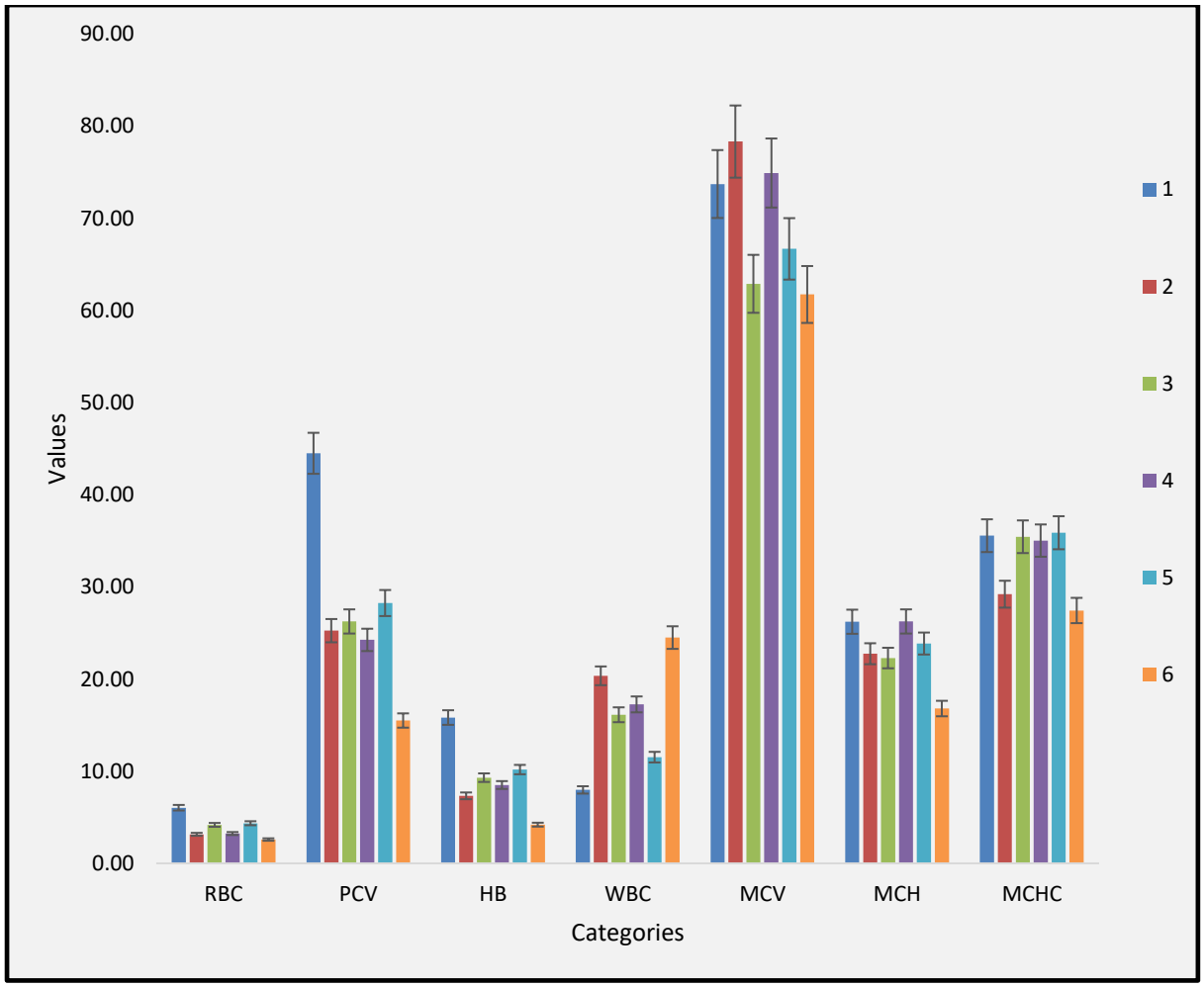

Fig. 2: bar chart showing the profile for hematological indices

Table 3: TLC profile of n-Hexane Extract of *Brillantaisia owariensis*

Solvent system (%)	Colour of spot	R_f value (cm)
C_6H_{14}(85):$CHCl_3$(15)	Green	0.07
	Yellow	0.19
	Yellow	0.3
	Yellow	0.43
	Yellow	0.93
$CHCl_3$(85): C_6H_{14}(15)	Light Green	0.08
	Green	0.13
	Yellow	0.19
	Green	0.29
	Green	0.38
	Pineapple	0.62
	Yellow	0.71
	Yellow	0.77
	Seaweed	0.81
	Yellow	0.87
	Yellow	0.92
	Yellow	0.98
C_6H_{14}(50):$CHCl_3$(50)	Yellow	0.14
$C_4H_8O_2$(85): C_6H_{14}(15)	Green	1

Table 4: TLC profile of Chloroform Extract of *Brillantaisia owariensis*

Solvent system (%)	Colour of spot	R_f value (cm)
CH_3OH(85):$CHCl_3$(15)	Yellow	0.57
	Pineapple	0.67
	Seaweed	0.72
	Green	0.78
	Yellow	0.83
	Yellow	0.93
$CHCl_3$(75): C_6H_{14}(25)	Yellow	0.16
	Green	0.28
	Green	0.34
	Green	0.41
	Seaweed	0.47
	Green	0.53
	Green	0.63
	Yellow	0.75
	pineapple	0.81
	Pineapple	0.88
	Green	0.91
	Green	0.94

The proximate composition of the air-dried *B. owariensis* leaf is shown in Table 1 while the chart representing the result is presented in Fig. 1. The relatively high carbohydrate and fibre content of *B. owariensis* leaf is closely related to the results obtained by Akuru *et al* *(*2018). Carbohydrates are hydrolyzed in the body to yield glucose which can be utilized immediately or stored as glycogen in the muscles and liver for future use (Raven *et al*., 1999; Okeke *et al*., 2008).

Carbohydrates are major sources of energy to

the body while fibres are parts of fruits, grains and vegetables that can neither be digested nor absorbed by the human system (Agarwal and Rastogi, 1974). Generally, dietary fibres operate to slow down the rate of glucose absorption in the blood and hence in the reduction of the risk associated with hyperglycemia (Bouttwell, 1998). Fibre can also facilitate the reduction of cholesterol in the plasma and the prevention of colon cancer and cardiovascular disease (Davidson *et al*., 1975). A high level of fibre is known as an anti-tumorigenic and hypochole-sterolemic agent (Okoro and Achuba, 2012)

The crude fibre content obtained from this study (which ranged from 23.50-23.72) suggests that *B. owariensis* leaves are a potential source of dietary fibre (roughages) and may be useful in the fulfillment of medicinal and nutritional roles ascribed to fibre (Kadiri and Fasidi, 1990; Chihara, 1993). Low crude fat recorded from this study in comparison to protein is quite permissible as fat tends to aid absorption of certain vitamins and also enhances cell growth, in excess can cause damages to the body: obesity, heart disease and high blood pressure.

Table 5: TLC profile of Ethyl acetate Extract of *Brillantaisia owariensis*

Solvent system (%)	Colour of spot	R_f value (cm)
CH_3OH(85):$CHCl_3$(15)	Green	0.71
	Green	0.79
	Yellow	0.83
	Yellow	0.91
$CHCl_3$(85): CH_3OH(15)	Green	No movement

The changes observed in the hematological parameters of the rats during the study are presented in Table 2 and Fig. 2 the RBC, HB, PCV, and MCH of rats that were administered with phenylhydrazine (PHZ) decreased significantly while the MCV and WBC increased. These findings are in accord with those reported by; Gabriel et al (2005). PHZ-induced anemia in rats was alleviated as there was an appreciable increase in the concentration of the hemoglobin (HB), red blood cells (RBC) and packed cell volume (PCV). The major function of the RBC is in the transportation of oxygen to the body. Therefore, any pathological or physiological condition that affects the RBC may alter this function and may create health challenges in the body (Gabriel et al., 2005).

Ayawa et al (2021) observed that the treatment of Trypanosoma brucei-induced infection in BALB/c mice with aqueous and methanol extracts of B. owariensis, led to the significant restoration of the hemolytic condition and an increase in the survival time in all the treated groups over the negative (non-treated) control group. Such observation may be attributed to the presence of secondary metabolites such as alkaloids, that have the potential to reverse hemolysis. It has also been documented that *B. owariensis* is rich in amino acids (Akuru and Amadi, 2018). Similarly, Brillantasia nitens is reported to have haematinic activity (Akahet al., 2009). An increase in the hematological indices observed with the crude and the chloroform fraction might not be unconnected with the chemical composition of the extracts. However, the same observation applies to the ethyl acetate extract which exhibited a decline in the hematological parameters and resulted in the death of one of the subjects.

The TLC profiling (Tables 3-5) revealed that the hexane fraction contained 12 spots with a solvent system of 85 % chloroform and 15 % hexane, while the chloroform fraction contained 13 spots with a solvent system of 75% chloroform and 25% hexane and the ethyl acetate fraction contained 4 spots with a solvent system of 85 % methanol and 15 % chloroform The solvent system is indicating the solvent polarity that may help in the selection of a particular solvent system for

further isolation of any compound from the plant extracts in techniques such as chromatographic and spectroscopic methods. (Biradar and Ranchetti, 2013). Lesser Rf value is indicative of polar constituents (spots), therefore, the lower the R_f values the more the attraction to the polar stationary phase, but the higher the R_f value the less polar is the constituent (spots).

4.0 Conclusion

The essence of this work was to ascertain the rational for the application of *Brillantaisia owariensis* leaf as a traditional hematinic and to establish relative activities of the different extracts (methanol, ethyl acetate, chloroform and hexane). Results obtained from this study showed that the methanol and chloroform extracts of *Brillantaisia owariensis* leaves possess heamatinic activity, while the ethyl acetate extract showed no significance. *A B. owariensis leaf is* a good source of nutrients and it constitutes diverse phytoconstituents as seen from the thin-layer chromatography profile studies.

5.0 References

Agarwal, S. K. &, Rastogi, R. P. (1974): Triterpenoid saponins and their genius. *Phytochemistry*, 13, pp. 2623-2645.

Akah, P. A, Okolo, C. E. &, Ezike, A.C. (2009): The haematinic activity of the methanol leaf extract of Brillantasia nitens Lindau (Acanthaceae) in rats. *African Journal of Biotechnology*, 8, 10, pp. 2389-2393**.**

Akuru, U. B. &, Amadi, B. A. (2018): Phytochemicals and antioxidant properties of some selected medicinal plants. *Journal of Pharmacognosy/Phytochemistry*. 7, 5, pp. 283–285.

Akuru, U. B. Amadi, B. A, &, Abbey B. W. (2018): Proximate, Amino Acid and Fatty Acid Composition *of* Eremomastax polysperma, Brillantaisia owariensis Leaves *and* Sorghum vulgare Leaf-Sheath. *Eur Exp.* 8, 5, pp 32-34

Alada, A. R. (2000): The hematological effect of Telfelriaoccidentalisdiet preparation. *African Journal of Biomedicals*. 3, 3, pp. 185-186.

Aluko, B. T., Alli Smith, Y. R. &, Omoyeni, O. A. (2014): Phytochemical analysis and antioxidant activities of ethanolic leaf extract of Brillantaisia patula. *World Journal of Pharm*. 3, pp. 4914–4924

AOAC (2006): International official methods of analysis of the AOAC. W. HorwitizEditi on, 18thedn. Washington D.C, U.S.A: AOAC international.

Asai, T., Hirayama, Y. &, Fujimoto, Y. (2012): Epi-α-bisabolol6-deoxy-β-D-gulopyranoside from the glandular trichome exudate of Brillantaisia owariensis. *Phytochemical Letters*. 5, 376–378

Ayawa, N. G., Ramon-Yusuf, S. B. & Wada, Y. A. (2021): study and anti-trypanosomal activities of aqueous and methanol whole plant extracts of Brillantaisia owariensis on *Trypanosoma brucei*-induced infection in BALB/c mice. *Clinical Phytoscience* **7,** 39

Biradar, R. S. &, Ranchetti D. B. (2013): Extraction of some secondary metabolites and TLC from different parts of centellaasiatica. *America Journal of Life Sciences* 1, 6, pp 243-247

Bouttwell, R. K. (1998): An Overview of the Role of Nutrition in Carcinogenesis. In: Nutrition Growth and Cancer, Harris, C.C. and P.A. Cerutti (Eds.). Alan R. Liss, London, pp. 387-418

Chihara, G. (1993): Medicinal aspects of Lentian Isolated from Lentinus edodes (Berk). Hong Kong, Chinese University Press. pp. 261-266

Criswell, K., Sulhanen A., Hochbaum, A. F. &, Bleavens, M. (2002): Effect of PHZ or phlebotomy on peripheral blood, bone marrow anderythropoietin in Wistar rats. *Journal of Applied Toxicology*, 20, pp. 25-29.

Dacie, I. V. & Lewis S. M. (1994): Practical Haematology, 8th edn. Churchill Livingstone, London, pp. 49-59.

Davidson, S., Brock, J. F. &, Truswell, A. S. (1975): Human Nutrition and Dietetics.

Churchill Livingstone, Edinburgh, UK., pp. 107-119, 221-224

Dina, O. A, Adedapo, A. A. Oyinloye, O. P. &, Saba A. B (2006): Effect of *Telfairia occidentalis* extract on experimentally induced anaemia in domesticrabbits. *African Journal Biomedical Research* 3, pp. 181-183.

Faparusi, F., Bello-Akinosho, M. M., Oyede, R. T., Adewole, A., Bankole, P. O. & Ali, F. F. (2012): Phytochemical screening and antibacterial activity of Brillantaisia patula leaf. *Research Journal of Phytochemistry,* 6. Pp. 9–16.

Gabriel, A. A., Julius E. O., &, Jeanne Y. N (2005): Haematinic activity of Hibiscus cannabinus. *African Journal of Biotechnology* 4, 8, pp. 833-837

Gbadamosi1, I. T., Moody, J. O. & Yekini, O. (2012): Nutritional Composition of Ten Ethnobotanicals Used for the Treatment of Anaemia in Southwest Nigeria. *European Journal of Medicinal Plants*, 2, 2, pp. 140-150.

Giffin, H. &, Allen, E. (1993): The control and complete remission of polycythemia vera following the prolonged administration of phenylhydrazine hydrochloride. *American Journal of Medical Sciences*, 8, pp. 185-186

Kadiri, M. and Fasidi, I. C. (1990): Studies on enzyme activities of Plerotus tuber regium Hein at various fruit body stages. Nahrung, 34, 8, pp. 695-999

Kupchan S. M., Tsou, G. & Sigel C. W. (1973): Datiscacin, a novel cytotoxic cucurbitacin 20-acetate from Datisca glomerata. Journal of Organic Chemistry 38, 7, pp. 1420-1421

Lakshmanasamy A. (2011): *Clinical pediatrics*. 3rd ed. New Delhi: Wolters Kluwer Pvt. Ltd.

Makambila-Koubemba, M. C., Mbatchi, B., Ardid, D., Gelot, A., Henrion, C., Janisson, R., Abena, A. A. &, Banzouzi, J. T. (2011): Pharmacological studies of ten medicinal plants used for analgesic purposes in Congo Brazzaville. *International Journal of Pharmacology*, 7, pp. 608–615.

Ngbolua, K. N., Benamambote, B.M., Muanda, D.M., Ekutsu, E., Tshibangu, D.S.., Gbolo, B.Z., Muanyishay, C.L., Basosila, N.B., Bongo, G.N. &, Robijaona, B. (2013): Ethno-botanical survey and ecological study of some medicinal plants species traditionally used in the district of bas-fleuve (Bas-Congo province, democratic republic of Congo). *Research Journal of Chemistry.* 1, pp. 1–10.

Okeke, C. U., Izundu A. I & Uzoechinda E. (2008): Phytochemical and proximate study of female pawpaw (*Carica papaya* L.) *Caricaecae*. Journal of Science and Engineering Technology 15, pp. 8207-8216.

Raven, P.M., Johnson, G.B &, Madison, W.I (1999): Biology. 2nd Edn., McGraw-Hill, London, pp: 1567

Okoro, I. O. &, Achuba, F. I. (2012): Proximate and mineral analysis of some wild edible mushrooms. *African Journal of Biotechnology*, 11, 30, pp. 7720-7724,

Ong, H.C. (1973): Anaemia in pregnancy in an Aborigine population. *Medical Journal of Malays.* 28, pp. 22-28

Saravanan, V. S & Manokaran S. (2012): Anti anaemic activity of some plants in Cucurbitaceae on phenylhydrazine induced anaemic rats. *Thailand Journal of Pharmaceutical Science*, 36, pp. 150-154.

Conflict of Interest

The authors declared no conflict of interest