EUROPEAN ACADEMIC RESEARCH
Vol. VIII, Issue 12/ March 2021

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Effect of Aqueous Extracts of Selected Medicinal Plants on Some Hematological Parameters in Phenylhydrazine Induced Anemic Rats

M.Sc. RASHEED-ADELEKE, A.A. (Lecturer II)
Department of Chemistry
The Polytechnic, Ibadan, Oyo State, Nigeria
M.Sc. SALAUDEEN, I.A. (Lecturer II)
Department of Chemistry
The Polytechnic, Ibadan, Oyo State, Nigeria
M.Phil. ANIFOWOSE, A.O. (Lecturer I)
Department of Chemistry
The Polytechnic, Ibadan, Oyo State, Nigeria
HND. RASAKI, I.A. (Student)
Department of Chemistry
The Polytechnic, Ibadan, Oyo State, Nigeria

Abstract

Medicinal plants have been proclaimed for improving haematological parameters such as packed cell volume, neutrophil count and the white blood cell count. This study was designed to investigate the presence of bioactive compounds and the effects of the aqueous leaf extract of Telfairia occidentalis, Parquetina nigrescens and Sorghum bicolor stem on packed cell volume, white blood cell count and neutrophil count of phenyl hydrazine induced anaemic rats.

Aqueous crude extracts of Sorghum bicolor stem, Parquetina nigrescens leaf and Telfairia occidentalis leaf were obtained by macerating the leaves and stem in distilled water for 24 hours, sieved, and dried using a rotary evaporator. 30 rats were obtained and separated into 6 groups. Rats in group 1-5 were administered 20mg/kg phenylhydrazine for 8 days to induce anaemia. After the eight days, blood samples were collected and haematological analysis was carried out. Anaemic rats were treated with 200mg/kg and 400mg/kg of the extracts, combination of the extracts and a named standard drug. Results revealed oral administration of 200mg/kg and 400mg/kg of

aqueous leaf extract of Telfairia occidentalis, Parquetina nigrescens and Sorghum bicolor stem and the co-administration of the extracts to the rats after inducing increased packed cell volume, white blood cell count and neutrophil count with the highest effect on packed cell volume observed in rats administered 200mg/kg of Parquetina nigrescens aqueous extract and 400mg/kg of Sorghum bicolor stem aqueous extract. The observation of this study revealed that administration of these extracts could cure haemolytic anaemia.

Keywords: Medicinal plant, Haematological parameters, Phenylhydrazine, Blood samples, Anaemic rats, Haemolytic anaemia

INTRODUCTION

The use of medicinal plants as food supplement and in the treatment of specific diseases dates back to the antiquities (Balunas and kinghorn, 2005). Medicinal plants are considered as a rich source of ingredient which can be used in drug development either pharmacopoeial, non pharmacopoeial or synthetic drugs. Although several synthetic drugs are available, attention is currently being focused on the use of plants and plant produce in preventon or correction of metabolic disorder or in the treatment of specific diseases because of several side effects associated with the use of synthetic drugs (Zhang 2005). Medicinal plants contain a wide variety of secondary metabolites or compounds such as tannins, terpenoids, alkaliods, flavonoids; that dictates the therapeutic potency of the plants (Evans,2002). These metabolites or compounds are known as phytochemicals. The phytochemicals with anti oxidative properties such as vitamin C,beta-carotene, flavoniods exhibit a wide range of pharmacological actions and medicinal properties including carcinogenic, anti inflammatory and protecting against degenerative diseases.

The reason for two-third of the world's population dependency on herbal medicine for primary health care is because of their better cultural acceptability, compatibility and adaptability with the human body and pose lesser side effects (Kumar *et al.*,2008). WHO estimated that 80% of people worldwide rely on herbal medicine for some aspect

of their primary health care needs. Some of these have been demonstrated to be of significant value in the treatment of anaemia and its complications.

Anaemia is the low level of haemoglobin in the blood, as evidenced by fewer numbers of functioning red blood cells. Haemoglobin in red blood cells is an oxygen carrying protein that binds oxygen to its iron carrying component. Haemoglobin transports oxygen to most cells in the body for the generation of energy. When haemoglobin levels are low,less oxygen reaches the cell to support the body's activities (Bates,2007). In Africa and other tropical developing countries, the great killer and disabler that results from malaria and other negleted tropical diseases coinfections is anaemia. Anaemia accounts for up to one half of malaria deaths in young children and is a leading contributor to the huge numbers of maternal deaths that result during pregnancy, as well as premature births (Brooker *et al.,*2006*). Telfairia occidentalis* commonly called fluted pumpkin occcurs in the forest zone of west and central Africa, most frequently in Benin, Nigeria and Cameroon. The plant produces luxuriant edible green leaves, which are rich in iron and vitamins. Studies have shown that *Telfairia occidentalis* leaf is rich in minerals such as iron, potassium, calcium, sodium, phosphorus and magnessium, antioxidants, vitamins such as thiamine, riboflavin, nicotinamide and ascorbic acid, phytochemicals such as phenols (Fasuyi, 2006).

The *Sorghum bicolor* stem is sweet to taste and is found to contain some sugar and minerals; this sugary nature makes it to be easily chewed in Africa and Asia and is used for the manufacture of syrup (FAO,1991). In the tropics, apart from the cereal being used as food (Ihekoronye and Ngoddy,1992), the mature black purple sheath is generally sold in small bundles and is used as colour additives in cooking meals and also taken as beverages when steeped or boiled in water in many homes in Nigeria. The stem has been used in the preparation of local medicine to treat anaemia and other related blood ailment. *Parquetina nigrescens* is a shrub found in equatorial West Africa and Central Africa and less common in East Africa which has been in use in traditional medicine for centuries (Burkett, 1968). The leaves of the plant have been used in the treatment of wonds,boils and cabuncles in Africa (Agyare *et al.*,2009). *Parquetina nigrescens* is also a constituent of a commercial herbal preparation (Jubi formula) in

Nigeria which is used in the treatment of sickle cell anaemia in humans. The herbal preparation was shown to restore decreased haematocrit and haemoglobin concentration in Trypanosoma brucei induced anaemia (Erah *et al.*,2003). The objective of this study is to carry out the qualitative analysis of the phytochemicals present in *Parquetina nigrescens* leaf, *Telfairia occidentalis* leaf and *Sorghum bicolor* stem, evaluate the effect of their aqueous extracts on some haematological parameters and compare the effects of each extracts of the selected medicinal plants on some haematological parameters.

MATERIALS AND METHODS

Sample collection and preparation

The fresh leaves of *Parquetina nigrescens* were collected from The polytechnic, Ibadan premises. *Telfairia occidentalis* and dried *Sorghum bicolor* stem were gotten from Bodija market in Ibadan. All samples were free of dirt by washing with distilled water. The leaves of *Telfairia occidentalis* and *Parquetina nigrescens* were squeezed and the extract was filtered using a muslin cloth into separate containers. The *Sorghum bicolor* stem was soaked in distilled water for three days for maximum extraction and the extract was filtered using a muslin cloth into a container. All extracts were then concentrated using a rotary evaporator to get the crude extract which is stored in airtight containers and kept refrigerated prior analysis.

Qualitative phytochemical screening of the aqueous extracts of *Telfairia occidentalis* leaf, *Parquetina nigrescens* leaf and *Sorghum bicolor* stem was carried out by testing for the absence or presence of the following plant constituents: flavonoids, saponnins, alkaloids, steroids, cardiac glycosides, tannins and phenols using standard methods.

In addition, thirty (30) male albino rats were gotten from the Department of Biochemistry, University of Ibadan and housed in cages in the animal house, The Polytechnic Ibadan under standard conditions. The animals were acclimatized for two weeks prior to on-set of experiment and fed with grower's feed and water.

The animals of group 1 to 5 received 20mg/kg body weight of phenylhydrazine for seven days, on the eight day, their blood samples were collected by ocular puncture of each rat for haematological

analysis. Rats with greater than or 30% reduction in packed cell volume were considered anaemic and used for this study. The anaemic group 1-4 received 200 and 400mg/kg body weight of extracts and group 5 received standard drug at 20ml/kg body weight. All drugs were administered for 8 consecutive days by oral feeding cannula.

The animals were grouped into five with six animals each in groups 1-4 and 3 each in group 5 and 6. Group1: 6 Phenyl hydrazine induced anaemic rats treated with 200 and 400mg/kg body weight of *Telfairia occidentalis* aqueous leaf extract. Group 2: 6 Phenyl hydrazine induced anaemic rats treated with 200 and 400mg /kg body weight of *Parquetina nigrescens* aqueous leaf extract.

Group 3: 6 Phenyl hydrazine induced anaemic rats treated with 200 and 400mg/kg body weight of *Sorghum bicolor* stem aqueous extract. Group 4: 6 Phenyl hydrazine induced anaemic rats treated with 200 and 400mg/kg body weight of combination of the three extracts. Group 5: 3 Phenyl hydrazine induced anaemic rats treated with 20ml/kg body weight of standard drug.Group 6: 3 Non-anaemic rats (control) fed only feed and water and administered normal saline. The animals were also anesthesized and blood samples were collected through ocular puncture into ethyldiaminetetraacetic acid (EDTA) bottles with heparinized capillary tubes and the animals were sacrificed. The packed cell volume (PCV), white blood cell count (WBC), neutrophil count, basophil count, eosinophil count and monocytes were determined.

RESULTS AND DISCUSSIONS

Qualitative phytochemicals analysis in aqueous extract

Qualitative analysis of phytochemicals in aqueous extract of *Telfairia occidentalis* leaf, *Parquetina nigrescens* leaf and *Sorghum bicolor* Stem were determined (Table 1), the presence of alkaloid, flavonoids, tannins, saponnins, cardiac glycosides and steroids with phenol undetected in the aqueous extract of *Parquetina nigrescens* leaf. This result agrees with Olatunbosun *et al*., (2018) who stated the presence of the above-mentioned secondary metabolites in aqueous leaf extract of *Telfairia occidentalis*.

Table 1: Qualitative analysis of phytochemicals in aqueous extracts

Phytochemical constituents	*Telfairia occidentalis*	*Parquetina nigrescens*	*Sorghum bicolor* stem
Alkaloid	+	+	+
Tannins	+	+	+
Saponnins	+	+	+
Phenols	+	-	+
Steroids	+	+	-
Flavonoids	+	+	+
Cardiac glycoside	+	+	_

Keys: + = present, - = not detected.

These phytochemicals have been reported to perform many functions in plants with different biochemical and pharmacological actions in animal species when ingested (Owolagba *et al*., 2009). Alkaloids have a wide range of pharmacological activities including antimalarial (e.g. Quinine), antibacterial (e.g. Chelerythrine), analgesic activities (e.g. Morphine) etc. The presence of Saponins has been reported to be capable of boosting immune system (Okwu, 2004). This is because saponins have been attributed to formation of foams in aqueous solutions, haemolytic activity to cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Okwu, 2004). Several functions of steroids in living organisms range from acting as haemolytic activity, cholesterol binding and anti-bacterial properties (Raguel, 2007).

Effect of Oral Adminstration of Phenylhydrazine on Packed cell Volume ofRrats

Daily administration of 20mg/kg body weight of phenyl hydrazine for 8 days via oral feeding canula to the rats caused a significant decrease in their packed cell volume. This could be as a result of breakdown of red blood cells caused by the phenyl hydrazine. The packed cell volume of the rats before induction and after induction with phenylhydrazine (Table 2). Group 1 showed 47% and 40% decrease, group 2 showed 44% and 43% decrease, group 3 had 47% and 52% decrease, group 4 had 47% and 49% decrease while group 5 had 38% decrease in mean packed cell volume. This result agrees with Toma *et al.,* (2015) who reported a decrease from 48% to 45% in packed cell volume of phenyl hydrazine induced anaemic rats. Damilola *et al*., (2017) reported a >30% reduction in packed cell volume of rats after oral administration of 20mg/kg phenyl hydrazine for eight days.

Table 2: Mean packed cell volume (PCV) of rats before induction and after induction with phenylhydrazine

GROUPS	Before Induction (%)	After Induction (%)	Percentage Decrease (%)
Group 1	50	30	40
	49	26	47
Group 2	50	26	44
	49	28	43
Group 3	47	25	47
	48	23	52
Group 4	47	25	47
	49	25	49
Group 5	47	29	38
Group 6	46	-	-

All values are in mean.

Effect of Aqueous Extracts and Standard Drug on Packed Cell Volume

The mean packed cell volume of rats before and after treatment with aqueous extracts and standard drugs (Table 3). Rats in group 1 were treated with 200 and 400mg/kg of *Telfairia occidentalis* aqueous leaf extract. Rats treated with 200mg/kg body weight of the extract showed 13.3% increase in packed cell volume while those fed with 400mg/kg showed 73.1% increase in packed cell volume. This result agreed with the work of Salman *et al*., (2008), who reported that there was a significant increase in haematological parameters of rats that were treated for two weeks with the aqueous leaf extract of *Telfairia occidentalis*. Some scientists have proposed the use of *Telfairia occidentalis* in treatment of anaemia, following studies which reported that extracts of *Telfairia occidentalis* helped to maintain blood level in subjects given its extracts (Hamlin and Latunde- Dada, 2011).

Table 3: Mean packed cell volume (PCV) of rats after induction and after treatment with extracts and standard drug

GROUPS	Before Treatment (%)	After Treatment (%)	Percentage Increment (%)
Group 1 (200mg/kg)	30	34	13.3
400mg/kg	26	45	73.1
Group 2 (200mg/kg)	26	51	96
400mg/kg	28	43	53.6
Group 3 (200mg/kg)	25	45	60
400mg/kg	23	50	96
Group 4 (200mg/kg)	25	41	64
400mg/kg	25	48	92
Group 5 (20ml/kg)	29	32	12

All values are in mean.

***Parquetina nigrescens* leaf**

Rats in group 2 were treated with 200 and 400mg/kg of *Parquetina nigrescens* aqueous leaf extract. Rats treated with 200mg/kg of the extract showed 93.3% increase in packed cell volume while those treated with 400mg/kg of the extract showed 53.6% in packed cell volume). Agbor and Odetola, (2011) reported an increase in erythrocyte indices after oral administration of aqueous leaf extract of *Parquetina nigescens.*

***Sorghum Bicolor* Stem**

Rats in group 3 were treated with 200 and 400mg/kg of aqueous extract of *Sorghum bicolor* stem. Rats treated with 200mg/kg of the extract showed 60% increase in packed cell volume while those treated with 400mg/kg of the extract showed 96% increase in packed cell volume, the highest increase observed in all extract and concentration. Ogunmike (2002) reported the hematopoietic effect of the aqueous extract of the sheath of *Sorghum bicolor* in albino rats. There was an increase in haematological parameters in a dose dependent manner.

Co-administration of all three aqueous extracts

Rats in group 4 were treated with 200 and 400mg/kg of the combination of the three extracts. Rats treated with 200mg/kg showed 64% increase in packed cell volume while those treated with 400mg/kg showed 92% increase in packed cell volume. Group 5 rats treated with 20ml/kg of the standard drug showed 12% increase in packed cell volume, the lowest increase observed on packed cell volume.

The increase in haematological parameters occurred in a dose dependent manner except in group 2 rats treated with *Parquetina nigrescens* aqueous extract. Those treated with 200mg/kg had 96% increase while those treated with 400mg/kg had 53.6% increase in packed cell volume (PCV).

Highest increase in packed cell volume was observed in group 2 rats treated with 200mg/kg of *Parquetina nigrescens* and group 3 rats treated with 400mg/kg of *Sorghum bicolor* stem extract.

Comparing the percentage increase in mean packed cell volume of all groups, it would be observed that rats treated with

extracts had higher increase in packed cell volume than the group treated with the standard drug.

White Blood Cell Count after Treatment

The mean white blood cell count after treatment with extracts and the standard drug (Table 4) revealed that Rats in group 1 had 15.6 and 18.5 count, group 2 showed 13.7 and 13.5 count, group 3 showed 18.3 and 12.6 count, group 4 showed 10.2 and 14.5 count, group 5 rats showed 15.7 count, while group 6, which is the control group, showed 9.3 count.

White blood cells form part of the immune system in animals working against invading pathogens. Significant increase in white blood cell count of rats after treatment when compared to that of the control rats could be due to the defense mechanism against the entrance of a foreign material in the body system of the rats. Group 1 rats administered 400mg/kg of aqueous leaf extract of *Telfairia occidentalis* had 18.5 (1000/mm^3) count, the highest in all groups.

Table 4: White blood cell count after treatment with extracts and standard drug.

GROUPS	WHITE BLOOD CELL COUNT (1000/mm^3)
Group 1	15.6 18.5
Group 2	13.7 13.5
Group 3	18.3 12.6
Group 4	10.2 14.5
Group 5	15.7
Group 6	9.3

All values are in mean

Neutrophils after Treatment

The mean neutrophil after treatment with extracts and the standard drug (Table 5) showed Rats in group 1 had 71% and 70% neutrophils, group 2 rats had 63% and 67% neutrophils, group 3 rats had 65% and 59% neutrophils, group 4 rats had 47% and 58% neutrophils while group 5 rats had 60% neutrophils. Neutrophils are a type of white blood cell that fights against infection. Table 5 show neutrophil count of rats after treatment and that of the control.

The results of this study indicated that administration of aqueous leaf extract of *Parquetina nigrescens*, *Telfairia occidentalis* and *Sorghum bicolor* stem is useful in alleviating anaemic condition and this could be due to the presence of bioactive compounds as well as its direct effect on the hematopoietic systems (Friday *et al*, 2010).

Table 5: Neutrophil count after treatment with extracts and standard drug

GROUPS	NEUTROPHILS (%)
Group 1	71 70
Group 2	63 67
Group 3	65 59
Group 4	47 58
Group 5	60
Group 6	64

All values are in mean.

CONCLUSION

This study was designed to carry out the qualitative phytochemical analysis, evaluate the effect on haematological parameters and compare the effect of each aqueous extract of *Parquetina nigrescens* leaf, *Telfairia occidentalis* leaf and *Sorghum bicolor* stem on phenyl hydrazine induced anaemic rats. Results show that 200 and 400mg/kg aqueous leaf extracts of *Telfairia occidentalis, Parquetina nigrescens* and *Sorghum bicolor* stem, combination of the three extracts and a standard drug could alleviate haematological parameters of phenyl hydrazine induced anaemic rats. Hence, administration of this extracts could cure haemolytic anaemia.

Isolation of active compounds in all extracts and elucidation of their mechanism of action would constitute further studies. Toxicity tests can also be carried out on the co-administration of the extracts to establish its safety.

ACKNOWLEDGEMENT
Authors are thankful to the management of The Polytechnic, Ibadan for allowing the use of their research facility in conducting this work and Dr Mrs Adegbola, R.A for her contribution.

REFERENCES

1. Agbor A.G., Odetola A.A. (2001). Haematological studies of *Parquetina nigrescens* on haemorrhagic anaemic rats.*African Journal of Medicine and Medical Sciences*,30:105-109.
2. Agyare C., Asase A., Lechtenberg M., Niehues M., Deter A., Hensel A. (2009). An Ethnopharmacological survey and in-vitro confirmational ethnopharmacological use of medicinal plants used for wound healing in Bosorntwi Atwima-Kwanwoma area, Ghana, *Journal of Ethnopharmacology,*125(3):393-403.
3. Balunas M.J.,Kinghorn A.D., (2005). Drug Discovery from Medicinal Plants,*Life Sciences*, 78;431-441.
4. Bates I., Mckew S., Sarkinfada F.(2007). Anaemia: A useful indicator of negleted disease burden and control. *PloS Med 4:e231.doi:10:1371/journal.pmed.0040231.*
5. Brooker S., Clements A.C., Hotez P.J., Hay S.I. and Tatem A.J.(2006). The co-distribution of *Plasmodium falciparum* and hookworm among African school children. *Malar J.*, 5:99.doi:10.1186/1475-2875-5-99.
6. Burkett H.M.,(1968). The results of plants of West Africa,*Royal Botanic Gardens*, Kews, England 1:603-604.
7. Hamlin and Latunde-Dada: Iron bioavalaibility from a tropical leafy vegetable in anaemic mice. *Nutrition and Metabolism* 2011 8.9.
8. Erah O.P., Asonye C.C. and Okhamafe O.A. (2003).Responses of Trypanosoma Brucei-induced anaemia to a commercial herbal preparation,*African Journal of Biotechnology,*2:307-311.
9. Evans, W.C. (2002). Trease and Evans Pharmacognosy, 15th edition. W.B. Sounders & co. London. 585pp
10. Fasuyi, A. O. (2006). Nutritional potential of some tropical vegetable leaf meals : Chemical characterization and functional properties. *Afr. J. Biotech*., 5: 49- 53.
11. FAO 1991, "Protein quality evaluation", report of Joint FAO/WHO Expert consultation, Food and Agriculture Organization of the United Nations.
12. Friday E.U, Iniobong, E.O, Moses B.E (2010). Effects of aqueous extract of Psidium guajava leaves on liver enzymes, histopathological integrity and haematological indices in rats. Gastroenterol. Res. Elmer Press.3(1):32-38. 28.
13. Ihekoronye, A.I and P.O Ngoddy (1985). Integrated Food Science and Technology for the Tropics, 1st Edn., Macmillan Publishers, London, UK., ISBN: 9780333388839, Pages:386.
14. Kumar M., Mondal P., Borah S. and Mahato K. (2013). physico-chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC

analysis of leaves of the plant *lasia spinosa* (lour) thwaites. International Journal of Pharmacy and Pharmaceutical Sciences. 5(2): 306-310.

15. Ogwumike O.O. Hemopoietic Effect of Aqueous Extract of the Leaf Sheath of *Sorghum Bicolor* In Albino Rats. AJBR. 2002;5(1-2):69-71.
16. Okwu D.E (2004). Phytochemicals and Vitamin content of indigenous species of South eastern Nigeria. J. Sustenance Agric. Environ. 6(1):30-37.
17. Ologundudu A, Ololade AL, Igara CE, Otuchristian G. Phytochemical screening and haematological studies of *Parquetina nigrescens* ethanol and chloroform leaves extract in albino rats. *Afr J Biochem Res*. 2015; 9(7):95-98.
18. Owolagba G.K, Avwioro O.G, Oduola T, Adeosun O.G, Enaowho T.N, Wilson J.I, (2009). Histological observation of 24 h oral administration of haematological parameters of the extract of *Sorghum bicolor* on albino rats . *J. Cell Anim. Biol*.,3(1):001-003.
19. Raguel F.E (2007). Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds. Biochim. Biophys. Acta 1768(10):2500-9.
20. Salman T.M., Olayaki., L.A., and Oyeyemi W.A., Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats. *Afr J. Biotechnol.* 2008:7:2299-303.
21. Sodipo O.A, Akiniyi J.A, Ogunbamosu J.U (2000). Studies on certain characteristics of extracts of bark of *Pansinystalia macroceras* (K Schemp) PicrreExbeille. *Glob J. Pure Appl. Sci.* 6;83-87.
22. Toma, Victory N.C, Kabir Y. The effect of aqueous leaf extract of fluted pumpkin on some haematological parameters and liver enzymes in 2,4-dinitrophenylhydrazine-induced anaemic rats. *Afr J Biochem Res*. 2015; 9(7):95-98.
23. World Health Organization (1999). Drug information. WHO, Geneva, 13(4):230-233.
24. Zhang J.,Yulong H, Tiande H. and Yunpu W (2005). Effect of Azadirachta indica leaf extract on serum lipid profile changes in normal and streptozotoc in induced diabetic rats. *African journal of biomedical research* 8:101-104.