Book Chapter

Evaluation of the Haematinic Activities of Extracts of *Justicia secunda* Vahl Leaves in Red Blood Cells of Laboratory Rats

Ahimsa Yamoah[1], Reimmel K Adosraku[1], Justice D Amenu[1,2], Michael K Baah[3] and Daniel A Abaye[2]*

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Ghana
[2]Department of Basic Sciences, School of Basic and Biomedical Sciences, University of Health and Allied Sciences, Ghana
[3]Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Ghana

***Corresponding Author:** Daniel A Abaye, Department of Basic Sciences, School of Basic and Biomedical Sciences, University of Health and Allied Sciences, Ho, Ghana

Published **April 15, 2020**

This Book Chapter is a republication of an article published by Daniel A Abaye, et al. at Journal of Biosciences and Medicines in March 2020. (Yamoah, A. , Adosraku, R. , Amenu, J. , Baah, M. and Abaye, D. (2020) Evaluation of the Haematinic Activities of Extracts of *Justicia secunda* Vahl Leaves in Red Blood Cells of Laboratory Rats. Journal of Biosciences and Medicines, 8, 48-57. doi: 10.4236/jbm.2020.83006.)

How to cite this book chapter: Ahimsa Yamoah, Reimmel K Adosraku, Justice D Amenu, Michael K Baah, Daniel A Abaye. Evaluation of the Haematinic Activities of Extracts of *Justicia secunda* Vahl Leaves in Red Blood Cells of Laboratory Rats. In: Malik Badshah, editor. Prime Archives in Biosciences. Hyderabad, India: Vide Leaf. 2020.

Acknowledgements: The authors are grateful to the following KNUST technicians who assisted in various roles: Fulgencios Somkang (Dept. of Pharmacology), Michael O. Affum (Dept. of Pharmaceutical Chemistry), Clifford Asare (Dept. of Pharmacognosy), Gariba Laarey (Hospital Laboratory). The authors also acknowledge the anonymous reviewers for their very helpful comments.

Conflicts of Interest: The authors report no conflicts of interest.

Authors' Contributions: The idea was developed by AY, RKA, JDA and DAA. Experiments were designed, performed, and data were collected by all authors. Data analysis was performed by DAA, AY, JDA and MKB. All authors contributed to manuscript writing which was led by AY and DAA. All authors approve the final manuscript.

Abstract

The use of plant parts (leaves, flowers, stems, barks, roots etc.) in traditional medicine is increasingly gaining ground in modern medicine, as plant sources have long been recognized as sources of secondary metabolites which can be used to treat a wide range of diseases. The effect of extracts of *Justicia secunda* leaves on red blood cells (RBC) count and haemoglobin (Hb) concentration was investigated in adult Sprague-Dawley rats to establish haematinic activity. Phenylhydrazine (PHZ)-induced anaemic rats were treated with water, methanol or ethyl acetate extracts at 200 mg/kg body weight. RBC counts and Hb concentration were analysed using a haematology analyser at 3-day intervals for 21 days. The extracts were compared with rats administered the haematinic tonic Feroglobin® and vehicle-treated (normal saline). Rats administered the water extract exhibited the most significant increase (P<0.001) in the number

of RBCs and Hb concentration compared with the vehicle-treated PHZ-induced anaemic rats. Rats administered the methanol extract followed with significant increase (P<0.01) in RBC counts and Hb concentration (<0.05). The RBC count of the water extract-treated rats recovered sufficiently to the original level by the end of the study. These findings indicate that the water extractable fraction of *J. secunda* leaves has excellent haematinic properties and this provides the pharmacological basis of its use in Ghanaian traditional medicine for the treatment of anaemia.

Keywords

Justicia Secunda; Haematinic Activity; Phenylhydrazine; Anaemia; RBC Count; Haemoglobin Concentration; Adult Sprague-Dawley Rats

Introduction

Anaemia is a condition in which the number of red blood cells (RBCs) or their oxygen-carrying capacity is insufficient to meet the body's physiological demands. In humans, it is characterised by haemoglobin (Hb) concentration < 12.0 g/dL in women and < 13.0 g/dL in men. Anaemia is a global public health concern especially in developing countries [1]. In Ghana, 66% of children within age 6-59 months and 42% of women within age 15-49 years have some levels of anaemia. In rural areas, 72% of children are prone to anaemia compared with children who live in urban areas (58%). Malaria, helminth infections and micronutrient deficiencies are the main causes or risk factors for anaemia [2].

Medicinal plants have found significant roles in the treatment of diseases and drug discovery [3]. *Justicia secunda* Vahl (family: Acanthaceae) commonly known as St. John's Bush is locally called *Yehowafo mogya duro* in the Akan language in Ghana which means 'Jehovah Witnesses' blood tonic'. Although this plant is a tropical herbaceous plant that originated from South America, it is currently cultivated in many African countries including Ghana. Traditionally, the fresh leaves of the plant are

boiled until a deep purple colour and drunk as a tonic or beverage.

In traditional medicine, extracts of the leaves are used in the management of diabetes, hypertension and sickle cell disease [4]. Research has demonstrated that the methanol extract of *Justicia secunda* leaves exhibit antioxidant, anti-inflammatory and antinociceptive activities [5]. Anthocyanins extracted from *J. secunda* leaves have demonstrated anti-sickling activity in sickle cell human erythrocytes during *in vitro* tests [6]. The total aqueous extract of *J. secunda* leaves has demonstrated antihypertensive effects in rats [7]. Other secondary metabolites, including the phenylalanine derivatives, auranamide and aurantiamide acetate, and the alkaloid, quindoline have been isolated from the dichloromethane extracts of the stems of *J. secunda* [8]. The major flavonoid compounds in the leaves are luteolin derivatives [9]. The *N*-arylpyrrolidones secundarellone A, B and C have been isolated from *J. secunda* leaves [10]. Thus, the presence of these secondary metabolites is largely responsible for the medicinal role of the plant for the treatment of various diseases.

In traditional Ghanaian medicine, the decoction of this plant is used in the management of anaemia. Though undocumented, people who drink this decoction anecdotally show improved health status. However, there is little scientific evidence on the use of the plant as a haematinic. This study, therefore, aims to evaluate the haematinic effects of extracts of *J. secunda* leaves. Specifically, the study was designed to study the effects of the leaf extracts of three solvents, methanol, ethyl acetate and water, on rat RBC counts and Hb concentration

Materials and Methods

Plant Samples and Extract Preparations

Fresh leaves of *Justicia secunda* Vahl (fam: Acanthaceae), collected from the botanical garden (Physic Garden) of the Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), were authenticated at the Department of Herbal Medicine. A voucher

specimen (KNUST/HM1/2018/L045) was deposited at the herbarium, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Ghana.

The fresh leaves were washed with water, cut into smaller pieces and dried under shade at room temperature for a week. The dried plant material was coarsely powdered using a hammer mill (Schutte, Buffalo, NY, USA). Sub-samples of the powdered plant material (200 g each) was cold macerated, separately, in 800 mL each of methanol and ethyl acetate for 72 hours with frequent agitation and the mixtures obtained filtered. The water extractable fraction was first heated on a water bath (temp 70-75^{O}C), with frequent agitation (72 hr) and filtered. It was then allowed to cool. This was done to be consistent with indigenous means of preparation. The extracts were separately concentrated using a rotary evaporator under reduced pressure.

Phytochemical Screening

The dried powdered leaves were screened for their constituent phytochemicals using the methods outlined by Khandelwal [11] and detailed in our previous work [12]. The presence of alkaloids, tannins (polyphenols), reducing sugars, sterols, flavonoids, coumarins and triterpenoids was investigated.

Adult Sprague-Dawley Rats

Adult Sprague-Dawley rats of either sex (wt. range 150-215 g), obtained from the animal house of the Department of Pharmacology, KNUST, were used. The animals were kept in well-ventilated cages under normal temperature, humidity and light, and fed on normal rat chow (obtained from the animal house) and water *ad libitum.* The methods and processes employed in the investigations agreed with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [13].

Drugs

A liquid tonic Feroglobin® (Vitabiotics Ltd, London, UK), containing iron 0.2%, zinc 0.06%, copper 0.02%, manganese 0.025% and vitamin B-complex 0.39% in a blend of honey and malt, was used as the reference haematinic. Phenylhydrazine ((PHZ); Fisons Ltd, Guildford, UK) was used to induce anaemia.

Determination of Haematinic Activity

RBC count and Hb concentration of blood were determined for Sprague-Dawley rats before induction of anaemia. Anaemia was induced using PHZ (20 mg/kg, intraperitoneal (i.p.), per day for 3 consecutive days). Blood samples were then taken and analysed before treatment the following day with the vehicle, Feroglobin® or solvent extracts of *J. secunda*. Anaemia was considered induced when RBC count and HB concentration of the blood decreased by about 30% [14]. The induced anaemic rats were randomly placed in five groups A-E, five per group and treated as follows; Group A: vehicle (normal saline)-treated, Group B: Feroglobin® 0.15 mg/kg, and for Groups C, D and E: 200 mg/kg each of *J. secunda* extracts of water, methanol and ethyl acetate, respectively, daily. These rates of administering the various treatments were guided by the work of Onoja *et al.* [5] and Koffuor *et al.* [14]. The RBC count and Hb concentration were determined using a KX-21 Haematology analyser (Sysmex, Kobe, Japan) in 3-day intervals for the next 18 days.

Data Analysis

For statistical analysis, GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was employed. Data were analysed by one-way ANOVA. It was followed by Bonferroni's multiple Comparison test (post-test); $P \leq 0.05$ was considered statistically significant for the analyses.

Results

Plant Extract Yields

The methanol and ethyl acetate extracted fractions yielded dark green extracts, while the water-extractable fraction generated a deep purple extract. Quantitatively, the yields (w/w) were: the methanol extract, 2.47%; ethyl acetate, 2.21% and water extract, 2.03%.

Phytochemical Screening

Results of phytochemical screening conducted on the powdered leaves of *Justicia secunda* are shown in **Table 1**.

Table 1: Results of phytochemical screening.

Test	Results
Alkaloids	+
Tannins	+
Glycosides	+
Flavonoids	+
Saponins	+
Coumarins	+
Sterols	+
Triterpenes	-

(+) indicates presence and (-) indicates absence of phytochemical

Haematinic Activity

Prior to inducing anaemia, the mean RBC counts and Hb concentration for the whole group rats were 7.54 x 10^6/µL and 15.58 g/dL of blood. Three days after induction of anaemia with PHZ, the mean number of RBCs decreased from 7.54 x 10^6/µL to 4.97 x 10^6/µL, a decrease of 34.1%. The mean Hb concentration similarly decreased from 15.58 g/dL to 10.01 g/dL, a decrease of 35.8%.

Compared with the vehicle-treated group, the RBC counts of three other groups except ethyl acetate, increased significantly (P<0.05). This is observed from Day 3 till the end of the

experiment (**Figure 1**). There was no significant difference (P>0.05) in the number of RBCs between the vehicle-treated group and ethyl acetate-treated group during the first nine days. The greatest increase in RBC counts was the water extract-treated which was most significant (P<0.001) compared with the vehicle-treated. The order of increase in RBC count was; water> methanol> Feroglobin®> ethyl acetate> vehicle-treated (**Figure 1**).

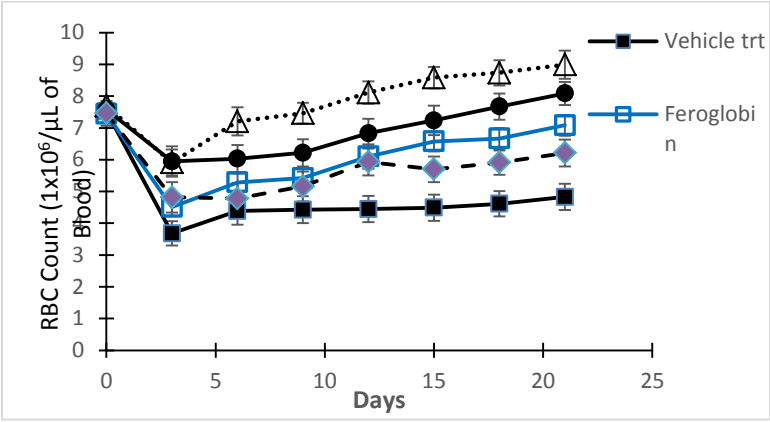

Figure 1: The relationship between the Red Blood Cells count and time for treated Sprague-Dawley rats. From Day 6, compared with Vehicle-treated, Water-treated (P<0.001), Methanol (P<0.01); From Day 12, Feroglobin and ethyl acetate-treated P<0.05) (n=5; 2-way ANOVA followed by Bonferroni's *post hoc* test).

The Hb concentration of the PHZ-induced anaemic rats showed a similar trend to RBC counts although this was less marked **(Figure 2**). From Day 9, the water-treated group showed a significant (P<0.01) increase in Hb concentration compared with the vehicle treated. The difference in Hb concentration increased over the period of the study. The methanol-treated showed a significant (P<0.05) increase in Hb concentration from Day 15 compared with the vehicle-treated. There were no significant (P>0.05) differences between the vehicle-treated and ethyl acetate-treated. The increase in Hb concentration was; water> methanol> Feroglobin> ethyl acetate = vehicle-treated (**Figure 2**).

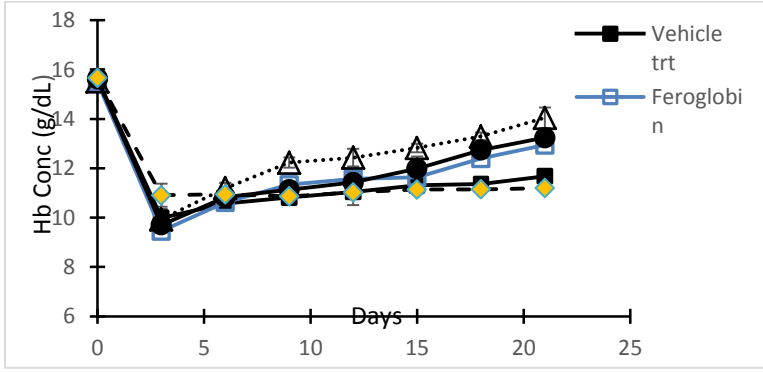

Figure 2: The relationship between the Hb concentration and time for treated Sprague-Dawley rats. Water-treated vs. Vehicle-treated ($P < 0.01$); Methanol, Feroglobin vs Vehicle ($P < 0.05$) (n=5; 2-way ANOVA followed by Bonferroni's *post hoc* test).

Area under the curve (AUC) values for the RBC counts and Hb concentration are as shown in **Table 2**. As the anaemic condition improves, the AUC value increases. Treatment of PHZ-induced anaemic rats with water extract (200 mg/kg) had the highest AUC value.

Table 2: AUC values obtained from curves for mean RBC count and mean Hb concentration against time (days) for different treatments groups.

Treatment groups	AUC Values for graphs obtained for	
	Mean Hb concentration	Mean RBC count
Vehicle-treated	197.4	79.91
Feroglobin® 0.15 mg/kg	206.3*	107.8*
Water extract 200 mg/kg	219.6***	142.8***
Methanol extract 200 mg/kg	208.8*	122.8**
Ethyl acetate extract 200 mg/kg	198.2	98.9

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with vehicle-treated group (One-way ANOVA followed by Newman-Keul's *post hoc* test).

Discussion

Phytochemical Screening

Phytochemical screening of the leaves showed the presence of the following groups of phytochemicals: alkaloids, tannins, flavonoids, coumarins, saponins and sterols. Our analysis did not show the presence of terpenoids which is similar to what was reported eleswhere [15]. We reasoned that, cultivation conditions in different geographical locations such as soil fertility and pH, water supply, climate and seasonal variations may account for the difference observed. Flavonoids are the largest group of plant phenols and are responsible for the flavour and colour present in fruits and vegetables [16]. This may be the reason for a deep purple colouration when *J. secunda* leaves are boiled although the leaves are green. The presence of these groups of phytochemicals may be responsible for the ethnomedicinal uses of *J.secunda.*

Haematinic Activity

The results of this study indicate that following the induction of anaemia in the rats, significant increase in RBC generation was observed in the groups of rats administered water and methanol (at 200 mg/kg body wt), and in the Feroglobin® treated group. By Day 15, the RBC counts of the water treated group had recovered sufficient to the level prior to anaemia induction. By the end of the experiment (Day 21), the RBC count had exceeded the original count. In contrast, although there were corresponding increases in Hb concentration of the extracts of water, methanol and Feroglobin® administered groups, the concentration did not attain the same level as the RBC counts. Our decision to use extracts of *J. secunda* at 200 mg/kg of body weight are in line with of the work of Onoja *et al.* [5] who performed toxicity tests on rats using 2 g/kg of the extracts. The LD_{50} of the extract was greater than 2 g/kg, therefore, 200 mg/kg of extracts was considered safe for our study. No deaths or signs of toxicity were observed after 48 hours in that report [5]. We also recorded no signs of toxicity or deaths in our work.

Our study indicated that there is a lag in Hb concentration behind an increase in RBC count. That is, while the RBC counts for rats

on water and methanol treatments returned to the original counts during the course of the study, Hb concentration did not. This may be due to reduced oxygen demands of tissues during that period of experiment, possibly a means of controlling the oxygen carrying capacity of the blood during the recovery period. Also, protein synthesis of some RBC components may have been reduced because of certain biochemical mechanisms that warrant further investigations. Tissue hypoxia which could be caused by a quantitative deficiency of circulating RBCs and Hb was not enough to elevate Hb concentration of the rats back to the original levels. Tissue hypoxia stimulates the erythropoietin-producing cells in the kidney to produce erythropoietin which is a hormone that regulates the proliferation and differentiation of hematopoietic progenitor cells in the bone marrow of all the anaemic rats [17]. This results in the correction of anaemia provided that the bone marrow response is not impaired by red-cell nutritional deficiency (especially iron deficiency).

Treatment of PHZ-induced anaemic rats with the reference hematinic, Feroglobin®, resulted in a significant increase ($P < 0.05$) in the number of RBCs and Hb concentration compared with the vehicle-treated PHZ-induced anaemic rats. Feroglobin® contains vitamins B_1, B_2, B_3, B_6 and B_{12}, zinc, folic acid and iron. Iron forms the nucleus of the iron-porphyrin haem ring and together with globin chains forms Hb. The B complex vitamins act as precursor in the synthesis of cofactors for haematopoiesis and protein synthesis [17]. This may have accounted for the increase in number of RBCs and Hb concentration shown by the rats fed Feroglobin®.

Treatment of PHZ-induced anaemic rats with water extracts showed the most significant increase ($P < 0.001$) in the number of RBCs and ($P<0.05$) Hb concentration with mean RBC count increasing from 5.91 x 10^6/µL to 8.91 x 10^6/µL, and mean Hb concentration increasing from 9.93 g/dL to 14.05 g/dL over the experimental period. The increase in RBC count could be attributed to phytochemicals in the plant extracts and thus could reverse the damaging effects of PHZ-induced anaemia. These results are consistent with reports on extracts of the plants *Solanum torvum* and *Tectona grandis* in PHZ-induced anaemia

in rats [14,18]. Another study revealed that *J. secunda* had an iron content of 26.6 mg/100 g [19]. In that same study, stem bark of *Khaya senegalensis* (Mahogany), a popular haematinic, had an iron content of 33.3 mg/100g. Iron forms the nucleus of the iron-porphyrin haem ring and together with globin chains forms Hb. This may account for the heamatinic properties exhibited by the extracts of *J. secunda* in the rats. The exact mechanisms by which the extracts exhibited the reported effect need further mechanistic investigations.

Conclusion

The study evaluated the haematinic properties of methanol, ethyl acetate and water extracts of the leaves of the plant *Justicia secunda* in PHZ-induced anaemic Sprague-Dawley adult rats. This set up was compared with rats administered with the haematinic tonic Feroglobin® and a vehicle (normal saline)-treated. The study was conducted over a 21-day period. The RBC count and Hb concentration were determined. The order of the RBC counts was water > methanol > Feroglobin® > vehicle > ethyl acetate. This was clearly observed from day 6 till the end of the study. The RBC counts of the rats fed the water extract recovered sufficiently to the original level. The Hb concentration of rats also mirrored that of the RBC counts and was observable from day 9. The increase in Hb concentration was; water> methanol> Feroglobin> ethyl acetate=vehicle-treated There was a lag in the Hb concentration as that did not recover to the original levels during the course of the experiment. The presence of some phytochemicals together with the iron content in the leaves of *Justicia secunda* Vahl may be responsible for the observed haematinic activity of the extracts. This provides scientific justification for its use in Ghanaian traditional medicine for the treatment of anaemia.

Footnotes: ****Special description of the title. (dispensable)**

References

1. De Benoist B, McLean E, Egli I, Cogswell M. Worldwide prevalence of anaemia 1993-2005. WHO Global Database on Anaemia. 2008. Availble Online at: https://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596657/en/
2. Ghana Statistical Service (GSS), Ghana Health Service (GHS), ICF International (2015) Ghana Demographic and Health Survey. 2014. Rockville, Maryland, USA. Availble Online at: http://www2.statsghana.gov.gh/docfiles/DHS_Report/Ghana_DHS_2014-KIR-21_May_2015.pdf
3. Houghton PJ. The role of plants in traditional medicine and current therapy. Journal of Alternative and Complementary Medicine. 1995; 1: 131–143.
4. Theiler BA, Istvanits S, Zehl M, Marcourt L, Urban E, et al. HPTLC bioautography guided isolation of α□glucosidase inhibiting compounds from Justicia secunda Vahl (Acanthaceae). Phytochemistry Analysis. 2017; 28: 87–92.
5. Onoja SO, Ezeja MI, Omeh NY, Onwukwe BC. Antioxidant, anti-inflammatory and antinociceptive activities of methanolic extract of Justicia secunda Vahl leaf. Alexandria Journal of Medicine. 2016; 53: 207-213.
6. Mpiana PT, Ngbolua KNN, Bokota MT, Kasonga TK, Atibu EK, et al. In vitro effects of anthocyanin extracts from Justicia secunda Vahl on the solubility of haemoglobin S and membrane stability of sickle erythrocytes. Blood Transfusion. 2010; 8: 248–254.
7. Manda P, Abrogoua DP, Bahi C, Dano DS, Gnahoui G, et al. Evaluation of the antihypertensive activity of total aqueous extract of Justicia secunda Vahl (Acanthaceae). African Journal of Pharmacy and. Pharmacology. 2011; 5: 1838–1845.
8. Calderón AI, Hodel A, Wolfender JL, Gupta MP, Correa M, et al. LC–DAD–MS□based metabolite profiling of three species of Justicia (Acanthaceae). Natural Product Research. 2013; 27: 1335–1342.
9. Koffi EN, Le Guerneve C, Lozanoa PR, Meudec E, Adje FA, et al. Polyphenol extraction and characterization of

Justicia secunda Vahl leaves for traditional medicinal uses. Industrial Crops and Production. 2013; 49: 682–689.
10. Theiler BA, Revoltella S, Zehl M, Dangl C, Caisa LOE, et al. Secundarellone A, B and C from the leaves of Justicia secunda Vahl. Phytochemistry Letters. 2014; 10: cxxxix-cxxxii.
11. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiment, 20th edn. Pune, India. 2010.
12. Amenu JD, Neglo D, Abaye DA. Comparative study of the antioxidant and antimicrobial activities of compounds isolated from solvent extracts of the roots of Securinega virosa. Journal of Biosciences and Medicines. 2019; 7: 27-41.
13. National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services Publication No. 83-23, 8th edn. 2011. Availble Online at: https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf).
14. Koffuor GA, Amoateng P, Andey TA. Immunomodulatory and erythropoietic effects of aqueous extract of the fruits of Solanum torvum Swartz (Solanaceae). Pharmacognosy Research. 2011; 3: 130-134.
15. Osioma E, Hamilton-Amachree A. Comparative study on the phytochemical and in vitro antioxidant properties of methanolic leaf extract of Justicia secunda Vahl. Nigerian Journal of Science and Environment. 2017; 15.
16. Tanwar B, Modgil R. Flavonoids: dietary occurrence and health benefits. A review. Spatula DD. 2012; 2: 59–68.
17. Craig CR, Stitzel RE. Modern Pharmacology. 3rd Ed. Boston: Little Brown and Company. 1990; 1074–1075.
18. Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokou K, et al. Effect of Tectona grandis on phenylhydrazine induced anaemia in rats. Fitoterapia. 2008; 79: 332–336.
19. Koné WM, Koffi AG, Bomisso EL, Tra Bi FH. Ethnomedical study and iron content of some medicinal herbs used in traditional medicine in Cote d'Ivoire for the treatment of anaemia. African Journal of Traditional and Complementary and Alternative Medicine, AJTCAM. 2012; 9: 81–87.