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RESEARCH ARTICLE

HAEMOPOIETIC ACTIVITY OF *HIBISCUS SABDARIFFA* CALYX EXTRACT

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ABSTRACT

The controlled proliferation and differentiation of blood cells occurs in a highly organized system Haemopoiesis results in the formation of the cellular components of blood. *Hibiscus sabdariffa* is a common garden plant found in the tropics. Hibiscus tea is generally consumed by the general population as a pro-health drink. This research investigated the haemopoietic activity of fractionated *Hibiscus sabdariffa* calyx and identified chemical compounds present. The study adopted the experimental research design. Thin-layer and column chromatograph was used to purify and separate the extracts into fractions while LC-MS was used to identify the chemical constituents of the fractions. Male albino rats (64) aged 16-18weeks was used for the study. The extracts yielded seven fractions. Anaemia was induced by oral administration of 10mg/kg body weight phenylhydrazine for 3 days and 1ml of 0.5mg/kg body weight of each fraction was given to treatment groups. Blood sample (2.0ml) was collected on days 8, 15 and 22 from the retro-orbital plexus. Interleukin 3 (IL-3) and erythropoetin (EPO) were assayed were using the ELISA technique and haematological parameters by the electrical impedance method. Phytochemical screening revealed that the aqueous extracts is rich in flavonoids, terpenoids and anthocyanins. On day 8, there was a significant increase (P<0.05) in IL-3 EPO and Retics within the groups of rats treated with fractions 6 (36.55±18.74) and 7(68.50±2.26) when compared with positive control group (52.70±1.13) On day 15, there was a significant increase (P<0.05) in IL-3 and EPO within the groups of rats treated with fraction 1(54.85±1.91; 16.50±0.99), fraction 5(43.95±2.05; 13.25±0.35), fraction 6(42.40±0.85; 52.80±1.98) and fraction 7(62.85±3.32; 77.50±4.10) when compared with positive control group (54.00±1.98; 12.75±0.07). On day 22, there was no significant difference (P>0.05) in IL-3, EPO and Retics within the groups of rats. Also there was no significant difference in the mean body weight (P>0.05). The red cells, white cells and platelets values were not significantly altered on day 22. The Bone marrow was normocellular with normal myeloid/erythroid ratio. The study revealed that fractions 6 and 7 promotes haemopoietic activity in anaemic rats however the haemopoietic activity induced by fraction 7 was fastest.

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INTRODUCTION

The controlled proliferation and differentiation of blood cells occurs in a highly organized system (Birbair and Frenette, 2016). Haemopoiesis results in the formation of the blood cells from the haemopoietic stem cells which reside in the bone marrow. In a healthy adult approximately 1 x 10^{11} to 1 x10^{12} blood cells are renewed daily. Growth factors, cytokines and many regulators have been identified as some of the factors that control these events (Thomas *et al*., 2004). The haemopoietic system consists of the bone marrow and the cells it produces, including leucocytes, erythrocytes and platelets. Cellular proliferation, differentiation, maturation and lineage commitment in the bone marrow is influence by haematopoietic cytokines. Interleukin-3 (IL-3) is a multipotent haematopoietic growth factor. It is produced by activated T-cells, monocytes/macrophages and stroma cells. It enhances the regeneration of cells of the myeloid series (Hurret, 2017). Erythropoietin a glycoprotein cytokine, is produced by the peritubular cells of the kidney in response to hypoxia (Schodel and Radcliffe, 2019). It is essential for definitive erythropoiesis. It acts on the erythroid progenitors and precursors promoting their survival by protecting them from apoptosis. *Hibiscus sabdariffa* species are used as ornamental plants. The plant parts-the seed, the leaves, roots and fruits are used in food preparation and herbal medicine, among them the fleshy red calyces is the most popular. The calyces are used fresh or dried in food industries for making Hibiscus tea (sour tea), wine, jam, jellies syrups, ice cream and flavors. Hibiscus tea is generally consumed by the general population as a pro-health drink. *Hibiscus sabdariffa* has been reported to be rich in polyphenols – anthocyanins and flavonoids (Ebenezer, *et al.,* 2019). Perez-Torres and his colleagues *(*2019)

evaluated its antioxidant properties and found that it posseses enzymatic and non-enzymatic activities thus protecting the cardiac function from damage. Numerous studies exist regarding its multipotential in treating various diseases. It was reported to reduce serum cholesterol in males and females (Garcia *et al*., 2019). It was proven effective for the control of mild to moderate hypertension (Jalalyazdi *et al*., 2019). It also it has been revealed to renew pancreatic *B-cells* (Adeyemi and Adewole, 2019). Thus it is effective in the management of type I diabetics and metabolic syndrome. Gheller and his colleagues (2017) demonstrated that it possess some antimutagenic effects using animal models and postulated that they may be effective in the management of cancer. Normal red cell production is influenced by the presence of erythropoietin (Kumar *et al.,* 2019). Increased red blood cell destruction, decreased production or excessive blood loss could lead to anaemia if there is no adequate compensation. The causes of anaemia are either due to acquired or inherited factors. Nutritional deficiency is the major cause of anaemia in the developing countries. Conventional measures in the management is aimed at increasing the red cell mass and haemoglobin concentration by the administration of haematinics, erythropoietin or blood transfusion but these practiced have produce some adverse effects. Herbal remedies is being utilized traditionally for the management of anaemia, some of their effects has been proven scientifically. Herbal medicine is general preferred because it is more affordable, easier to obtain than most prescription drugs, cost effective and it stabilizes hormones and metabolism (Welz *et al.,* 2018).*Hibiscus sabdariffa* is valued for its mild laxative and diuretic effects. It is believed to have beneficial effects on the cardiovascular system. *Hibiscus sabdariffa* tea often taken as a cooling drink to provide relief in hot weather is believed to be rich in nutritionally important chemicals. Studies have shown that *Hibiscus sabdariffa* is effective in the management of hypertension and hyperlipidemia. The study proposes that the use of *Hibiscus sabdariffa* may have clinical efficacy in the management of anaemia by stimulating haemopoietic activity in the stem cells.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials: The study adopted the experimental research design. The red variety of *Hibiscus sabdariffa calyx* was obtained from Dankiri Farms in Babura local government area, Jigawa state, Nigeria. It was authenticated by a taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The calyx was air-dried and milled to coarse powder. One thousand grams was boiled in 3.0 litres of distilled water for 7minutes. On cooling, it was filtered using Whatman No.1 filter paper and vaporized a semi-solid concentrated crude extract. The aqueous extract was separated from the glycosides by liquid – liquid extraction method using water (mobile phase) and ethyl-acetate (stationary phase). Thin Layer chromatography was used to identify the number of chemical compounds that make up *Hibiscus sabdariffa* calyx extract fractions. Column chromatography was used to separate calyx extracts into fractions. LC – MSwas used to identify chemical constituents of the separated *Hibiscus sabdariffa* fractions.

Experimental Animals: Male albino rats (64) aged 16weeks and weighing approximately 170 – 220g was used for the study.They were kept in clean steel metal cages under a 12/12 hour dark/light cycle, they were fed with standard pellet-Guinea feed, Nigeria plc and clean water for the period of acclimatization (2 weeks) and throughout the duration of the study. The procedure for handling the animals was in agreement with the Ethics Committee of the College of Medicine, University of Nigeria, Enugu campus. The LD_{50} (median lethal dose) of the extract was determined using twelve (12) rats. They were divided into four (4) groups of three (3) each as described by Lorke (1983).The number of deaths in 24hour was recorded. The LD_{50} was calculated as the geometric mean of the highest non-lethal and lowest lethal doses.

Administration of the extracts fractions: The number of thin layer chromatography (TLC) fractions is 7, based on that the experimental animals were assigned to nine treatment group.

The groups were made up of 5 rats each and labeled (experimental and control animals). The animals were induced for anaemia with 10 mg/kg bodyweight of phenyl hydrazine administered orally for 3days. Oral administration of 1ml single dose (0.5mg/ml) of the fractions of *Hibiscus sabdariffa* were given the rats daily for 21 days. A group of anaemic rats were fed with Astymin 0.1ml/kg body weight-positive control (Group C). Negative controls animals (group B) were treated with phenylhydrazine, they were neither given the extracts nor astymin.

Assessment of Haemopoietic activity: Blood sample (2.0 ml) was collected on days 8, 15 and 22 from each rat through the retro-orbital plexus of the median canthus and 2.0 ml was dispensed into ethylene diamine tetra acetic acid container for full blood count (FBC), interleukin 3 and erythropoietin assay. On day 22, bone marrow was collected to assess for marrow cellularity. Erythropoietin assay and Interleukin-3 were assayed using the quantitative direct Enzyme Linked Immunosorbent Assay .FBC and reticulocytes was analysed using a haematology auto analyzer-Mindray BC-2800 Haematology Analyzer. A Romanowsky-stained thin blood film was be examined microscopically using the x40 and subsequently x100 objective lens by battlement method. A thin film made from bone marrow specimen was air-dried and stained with leishman stain. This was examined microscopically by the longitudinal method using x40 and x100 objective lens. The erythropoietic activity was assessed by calculating the myeloid/erythroid ratio. A total of 500 cells were counted.

Statisical analysis: Values were expressed as mean and standard deviation (Mean ± 2SD) and was analyzed using two-way analysis of variance (ANOVA) Probability value $\leq$ 0.05 was considered significant.

RESULTS

The aqueous extraction of 1000g of Hibiscus sabdariffa L. calyx yielded 41.5g of thick extract (8.2% yield). The liquid-liquid extraction yielded 20.35g of ethyl-acetate extract. Fractionation of the extract by column chromatography yielded 7 fractions. Liquid chromatography-mass spectrometry (LC-MS) was able to eluscidate the phytochemical compositons of the fractions (Table 4.1).

The mean differences of IL-3 on the 8th day of the experiment (Table 2) was statistically significant (P<0.05). The mean of IL-3 in the treatment Group G (with fraction 4) and treatment Group I (with fraction 6) was significantly decreased when compared to the positive control (Astymin treated). The mean EPO in the positive control was significantly higher when compared with group H (treatment with fraction 5) but however significantly lower when compared with groups I and J (treatments with fraction 6 and 7). The mean level of Retics in the positive control (C) was slightly higher than that in group H (treatment with fraction 5) and was much higher than the mean level of Retics in groups I and J (treatments with fractions 6 and 7). The Retics in groups I and J were significantly increased when compared with other groups treated with fractions of *Hibiscus sabdariffa*: D, E, F, G and H. On the 15th day of the experiment (Table 4.3) the IL-3 levels was significantly increased in groups D, E, H, I, and J (treatment with *Hibiscus sabdariffa* fractions) when compared with group C (positive control). The mean EPO in groups D, I and J (with fractions 1, 6 and 7) were significantly increased (P<0.05).when compared with group A (normal control). Also, the mean EPO in groups G, I and J were significantly increased when compared with the negative control (untreated) group B. Groups I and J (treatment with fraction 6 and 7) were significantly increased when compared with the mean EPO of rats in groups D, E, F, G, H and I. On the 22nd day of the experiments (Table 4.4) the mean IL-3 of the all the experimental groups were significantly decreased when compared with that of group A (normal control). The mean level of IL-3 in groups treated with Astymin i.e positive control group (C) was significantly higher when compared with that of Groups F, H, I and J (treatments with fractions 3, 5, 6 and 7). The comparison of mean EPO down the group on the 22nd day of the experiment was statistically insignificant (P>0.05). The mean comparison of Retics

was statistically significant (P<0.05). Red cell values on the day 8 of the experiment (Table 4.5) showed that the mean Hb values in Groups I and J (fractions 6 and 7) was significantly increase when compared with the mean of Hb in groups C (Positive control), also mean PCV level in Group I and J (fractions 6 and 7 treatment) was significantly higher when compared with that of the positive control. Mean PCV level were significantly increased in group I and J when compared with that of groups E, F, G, and H (fractions 2, 3, 4 and 5 treated). The mean levels of RBC in the rats treated with fractions of *Hibiscus sabdariffa* in groups E, G and H (i.e. treatment with fractions 2, 4 and 5) were significantly lower when compared with Astymin treated rats (positive control C). However, fractions 6 and 7 (Groups I and J) caused a very significant increase in the RBC value than that of the positive control. On the 15th day of the experiment (Table 4.6) the mean Hb in group G and H (fractions 4 and 5) were significantly increased when compared with the normal control (group A). Also mean Hb in Groups I and J (fractions 6 and 7) was significantly increased when compared that of the negative control group (B). The mean values of PCV values were significantly increased in Groups I and J when compared to the negative control group (B) but was significantly reduced in Group F (fraction 3 treated). The mean values of PCV in rats treated with *Hibiscus sabdariffa* fractions 5 (Group H), 6 (Group I) and 7 (Group J) were significantly increased when compared with the mean PCV values in the positive control (Astymin treated) group. The mean RBC of rats in the negative control (B) was significantly decreased when compared to that of the normal control (A). Fractions 1 (Group D), 3 (Group F), 4 (Group G). Fractions 6 (Group I), and 7 (Group J) respectively caused a significant increase in the mean levels of RBC when compared with that of the negative control group (Group B).

Red cells assesment on 22 showed that there was a significant decrease in the mean Hb in group in Group H (treatment with fraction 5) when compared with the positive control group (C). The mean PCV levels of rats in Group J (fraction 7 treated) was also significantly increased when compared to group H (fraction 5 treated). On day 22, The mean values of TWBC, MXD and platelet counts down the table were not statistically significant (P>0.05), However the increase in the mean values of neutrophils and lymphocytes down the group was statistically significant (P<0.05).

DISCUSSION

Anaemia is the most common haematological disorder seen in clinical practice, it is most prevalent in the less developed countries of the world. To investigate anaemia, the red cell count (RBC), packed cell volume or haemoglobin concentration is measured (Brawasten, 2022). The values obtained are compared to set threshold based on age, sex and physiological status of pregnancy and lactation. Public health approaches to the management of anaemia have demonstrated varying degress of effectiveness (Appiah *et al*., 2020). Future research efforts is aimed at dietary consideration for combating micronutrient deficiency and investigating phytochemical constituents, this is believed to hold promise for reducing the incidence of anaemia in low and middle income countries (WHO, 2014). In the group of rats fed with fraction 1, there was significant increase in interleukin 3 (IL-3) whereas the erythropoietin increase was not significant. It was observed that the red cell count, PCV and Haemoglobin returned to the baseline at day 15 of the experiment. The total white cells were not affected. Fraction 1 contains 3-tert-Butyl-4-methoxyphenol, Nigakilactone H and Stigmastone-3,6-dione. The phenolic antioxidant 3-tert-Butyl-4-methoxyphenol is an aromatic ether commonly used as food additive, it has been observed to regulate intracellular signaling pathways. Exposure leads to increased cellular proliferative through phosphoinositide 3-kinase (P13k)/AKT and mitogen activated protein kinase (MAPK) signaling pathway (Javad and Hermann, 2021). Nigakilactone H is bioactive tertepenoid, it has been shown to have antiproliferative activity (Yang *et al.,* 2022). This it does by down regulating prosurvival-Heat Shock Protein 70 (HSP70) and Survivin protein expression and activating multiple cell death pathways (Bathold and Liu, 2022).

Table 4.1. LC-MS analysis of *Hibiscus. sabdariffa* calyx fractions

No	Component name	bserved m/z	RT (minutes)
	Fraction 1		
1	3-tert-Butyl-4-methoxyphenol	181.1221	7.82
2	Nigakilactone H	425.2153	9.55
3	Stigmastane-3,6-dione	429.3720	10.38
4	Candidate mass $C_{45}H_{84}O_{15}$	887.5691	10.85
5	Candidate mass $C_{45}H_{84}O_{16}$	903.5645	10.77
	Fraction 2		
1	2-(2-Phenylethyl)chromone	251.1063	8.58
2	Coumarin	147.0435	5.06
3	3-tert-Butyl-4-methoxyphenol	181.1219	8.12
4	5,7-Dihydroxy-3-(4′-hydroxybenzyl)chromone	285.0756	8.02
5	Candidate mass $C_{35}H_{42}O_9$	607.2914	9.64
	Fraction 3		
1	Coumarin	147.0437	4.77
2	3-Hydroxy-7-methoxy baicalein	301.0704	6.74
3	5.7.2′,5′-Tetrahydroxy-flavone	287.0550	6.09
4	Digitopurpone	271.0599	6.64
5	Undecanoic acid	209.1531	6.35
	Fraction 4		
1	3-Hydroxy-7-methoxy baicalein	301.0702	6.75
2	5.7.2′,5′-Tetrahydroxy-flavone	287.0548	6.10
3	Artemisinin I	207.1376	4.95
4	Azedarachin C	609.2719	9.51
5	Digitopurpone	271.0599	6.63
	Fraction 5		
1	5.7.2′,5′-Tetrahydroxy-flavone	287.0548	6.06
2	5,7-Dihydroxychromone	179.0335	3.85
3	9,12-Octadecadienoic acid (Z,Z)-(2,2-dimethyl-1,dioxolan-4-yl) methyl ester	417.2967	9.68
4	Imperanene	331.1534	5.87
5	Candidate mass $C_{25}H_{12}O_8$	441.0602	7.53
	Fraction 6		
1	5.7.2′,5′-Tetrahydroxy-flavone	287.0549	6.05
2	5,7-Dihydroxychromone	449.1077	4.75
3	Kaempferol-3-o-β-D-glucopyranoside	179.0337	3.86
4	Salvianolic acid A	495.1288	4.66
5	Wedelolactone	337.0341	8.53
	Fraction 7		
1	Kaempferide-3-O-α-L-rhamnosyl-7-O-α-L-rhamnoside	593.1862	5.86
2	Kaempferol-3-O-rutinoside	595.1662	4.57
3	Kaempferol-3-O-β-D-glucopyranoside	449.1075	4.75
4	Kaempferol-7-O-α-L-rhamnoside	433.1124	5.20
5	Methyl gallate	185.0441	3.65

Stigmastone-3,6-dione a natural occurring sterol has been shown to possess anti-inflammatory activity causing the increase of neutrophils (Yuliastri *et al*., 2021). Serum IL-3 level of the group of rats administered with fraction 2 was increased on day 15. The red cells, PCV and Hb were seen to return to normal on day 22. Fraction 2 contains 3-tert-Butyl-4methoxyphenol as well as 2-(2-phenylethyl) chromone, coumarin and 5, 7-dihydroxy-3-(4-hydroxy benzyl) chromone. The aromatic compound 2-(2-phenylethyl) chromone is found in flavonoids. It has been observed to have antioxidant properties They has been shown to posses antiviral properties inhibiting the proteins essential for viral entry, replication and infection (Mishra *et al.*, 2019). It inhibits synthesis of vitamin K invivo. The possible molecular mechanism is proposed to be by regulation of AKT, NF-KB and antioxidative pathway NrF-2 pathway (Di Stoli, 2021). 5, 7-dihydroxy-3-(4hydroxy benzyl) chromone inhibits innate and adaptive immunity via GATA 1, STATI/3 and NF-KB signaling pathway. It is a flavonoid commonly known as genistein. It has been shown to induce G-2 phase arrest in human and murine cell lines (Javad and Hermann, 2021).

Table 4.2. Haemopoietic Activity of *Hibiscus sabdariffa* calyx (Day 8)

Groups	IL-3	EPO	RETICS
Group A (Normal Control)	21.85±2.05	14.20±0.28	0.90±0.14
Group B (Negative Control)	35.00±0.57[a]	33.10±0.42[a]	2.10±0.28[a]
Group C (Positive Control)	52.70±1.13[a]	19.15±1.34[ab]	3.90±0.14[a]
Group D (Fraction 1)	41.85±1.34[a]	21.15±1.91[ab]	4.05±0.21[a]
Group E (Fraction 2)	54.95±0.92[a]	33.20±1.41[ad]	4.30±0.14[a]
Group F (Fraction 3)	57.65±1.77[a]	19.70±0.14[abce]	3.80±0.28[a]
Group G (Fraction 4)	32.70±2.97[acdf]	21.30±1.56[abe]	2.44±0.14[a]
Group H (Fraction 5)	56.45±2.62[a]	13.55±0.35bcdefg	3.10±0.14abcdefg
Group I (Fraction 6)	36.55±18.74abcdefh	55.95±5.02abcdefgh	5.30±0.57abcdefgh
Group J (Fraction 7)	68.50±2.26abcgi	96.10±2.40abcdefgh	6.15±0.49abcdefgh

Significant at the level of p<0.05

Table 4.3. Haemopoietic Activity of *Hibiscus sabdariffa* Calyx (Day 15)

	IL-3	EPO	Retics
Group A (Normal Control)	22.65±3.46	12.55±1.91	0.60±3.39
Group B (Negative Control)	41.55±2.33[a]	16.00±0.42	2.90±0.14
Group C (Positive Control)	54.00±1.98[ab]	12.75±0.07	3.45±0.07
Group D (Fraction 1)	54.85±1.91[abc]	16.50±0.99[a]	4.20±0.14
Group E (Fraction 2)	41.80±1.56[acd]	15.95±0.64	3.95±0.35
Group F (Fraction 3)	34.20±0.99[abde]	12.55±0.49[d]	3.60±0.14
Group G (Fraction 4)	36.20±0.99[abde]	10.80±0.85[bcde]	3.35±0.21
Group H (Fraction 5)	43.95±2.05acdfg	13.25±0.35	3.10±0.14
Group I (Fraction 6)	42.40±0.85acdfg	52.80±1.98abcdefgh	5.90±0.14
Group J (Fraction 7)	62.85±3.32abcefghi	77.50±4.10abcdefgh	5.25±0.21

Significant at the level of p<0.05

Table 4.4. Haemopoietic Activity of *Hibiscus sabdariffa* Calyx (Day 22)

	IL-3	EPO	Retics
Group A (Normal Control)	20.75±0.21	11.90±1.27	0.90±1.27
Group B (Negative Control)	13.95±0.07 [a]	13.55±0.35	3.5±0.35
Group C (Positive Control)	15.75±1.48 [a]	10.10±6.22	3.35±6.22
Group D (Fraction 1)	13.75±0.64[a]	13.95±0.35	3.55±0.35
Group E (Fraction 2)	13.95±0.21 [a]	13.50±0.14	3.25±0.14
Group F (Fraction 3)	12.80±0.42 [ac]	12.95±0.21	2.95±0.21
Group G (Fraction 4)	13.45±0.35[a]	13.90±1.27	3.65±1.27
Group H (Fraction 5)	13.80±0.42 [ac]	14.20±0.71	3.20±0.71
Group I (Fraction 6)	13.55±0.21 [ac]	14.05±0.21	3.30±0.21
Group J (Fraction 7)	13.65±0.49[ac]	13.95±1.20	3.55±1.20

Significant at the level of p<0.05

Table 4.5. Effect of extract of *Hibiscus sabdariffa* calyx fractions on thered cells (Day 8)

	Hb	PCV	RBC
Group A (Normal Control)	14.75±0.35	45.50±0.71	6.45±0.07
Group B (Negative Control)	10.30±0.14[a]	30.50±0.71[a]	3.85±0.35[a]
Group C (Positive Control)	12.10±0.28[ab]	37.50±2.12[a]	5.55±0.21[ab]
Group D (Fraction 1)	12.30±0.14[ab]	34.5±0.07[abc]	5.50±0.14[ab]
Group E (Fraction 2)	11.90±0.42[ad]	36.00±1.41[ad]	3.85±0.07acde
Group F (Fraction 3)	12.20±0.28[abc]	37.00±2.83[ad]	5.45±0.21[ab]
Group G (Fraction 4)	11.80±0.28[adf]	35.00±1.41[ad]	3.75±0.07acdf
Group H (Fraction 5)	11.35±0.49[acdf]	32.50±0.71[ad]	3.90±0.14acdf
Group I (Fraction 6)	13.15±0.64bcdefgh	42.00±2.83bcdefgh	6.00±0.14abcdefgh
Group J (Fraction 7)	13.40±0.14bcegh	46.50±.71bcdefgh	7.20±0.28abcdefghi

Significant at the level of p<0.05

Table 4.6. Effect of extract of *Hibiscus sabdariffa* calyx fractions on the red cells (Day 15)

Groups	Hb	PCV	RBC
Group A (Normal Control)	15.25±0.92	47.50±0.71	6.85±0.92
Group B (Negative Control)	12.70±0.57[a]	35.70±2.12[a]	5.15±0.21[a]
Group C (Positive Control)	13.85±0.21	39.50±0.71[a]	6.55±0.21
Group D (Fraction 1)	13.85±0.07	39.00±1.41[a]	7.05±0.35[b]
Group E (Fraction 2)	13.60±0.28	39.00±1.41[a]	6.20±0.14
Group F (Fraction 3)	13.95±0.21	40.0±0.14abcd	6.65±0.21[b]
Group G (Fraction 4)	13.40±0.57[a]	37.50±0.71[af]	5.25±0.07[b]
Group H (Fraction 5)	12.80±0.28[a]	33.50±2.12[acf]	5.85±0.21
Group I (Fraction 6)	14.60±0.14[bh]	42.50±2.12bcdefgh	7.25±0.49[beh]
Group J (Fraction 7)	14.90±0.14[bh]	47.00±1.41bcdefgh	7.45±0.07[b]

Significant at the level of p<0.05

Table 4.7. Effect of extract of *Hibiscus sabdariffa* calyx fractions on the red cells (Day 22)

Groups	Hb	PCV	RBC
Group A (Normal Control)	15.15±0.21	46.00±1.41	6.10±0.85
Group B (Negative Control)	13.65±0.49	39.50±0.71	6.25±0.35
Group C (Positive Control)	14.80±0.28	44.50±1.41	7.30±0.14
Group D (Fraction 1)	14.45±0.21	45.50±2.12	7.35±0.64
Group E (Fraction 2)	14.35±0.07	44.50±2.12	7.30±0.42
Group F (Fraction 3)	14.45±0.21	43.50±2.12	7.20±0.14
Group G (Fraction 4)	14.15±0.35	43.00±2.83	7.10±0.28
Group H (Fraction 5)	13.45±0.21[cdf]	38.00±2.83acdefg	6.35±0.07
Group I (Fraction 6)	14.65±0.28	43.50±2.12	7.25±0.14
Group J (Fraction 7)	15.20±0.14[h]	46.00±2.12[h]	7.55±0.35

Significant at the level of $p<0.05$

Table 4.8. Effect of extract of *Hibiscus sabdariffa* calyx fractions on the white blood cells and platelets count (Day 22)

Groups	Total WBC	Neutrophils	Lymphocytes	MXD	Platelet counts
Group A (Normal Control)	.05±0.21	38.50±0.71	57.50±0.71	4.00±0.00	838.00±12.73
GroupB(Negative Control)	7.90±0.42	31.50±2.12	64.50±0.71[a]	4.50±0.71	818.50±0.71
Group C (Positive Control)	7.85±0.49	29.00±1.41[a]	67.50±0.71[a]	3.50±0.71	826.50±13.44
Group D (Fraction 1)	8.10±0.14	35.50±0.71	61.00±1.41	3.50±2.12	820.50±20.51
Group E (Fraction 2)	7.15±0.35	31.00±1.41	65.50±0.71[a]	3.50±0.71	822.00±14.14
Group F (Fraction 3)	7.45±0.64	33.00±1.41	63.50±0.71	3.50±0.71	833.00±16.97
Group G (Fraction 4)	7.75±0.49	33.50±2.12	62.50±0.71	4.00±1.41	849.00±2.83
Group H (Fraction 5)	7.65±0.49	33.00±2.83	63.00±4.24	4.00±1.41	844.00±42.43
Group I (Fraction 6)	7.85±0.49	34.00±2.83	63.00±1.41	3.00±1.41	840.00±22.63
Group J (Fraction 7)	7.45±0.78	31.00±2.83	65.50±0.71[a]	3.50±2.12	834.00±2.83

Significant at the level of $p<0.05$

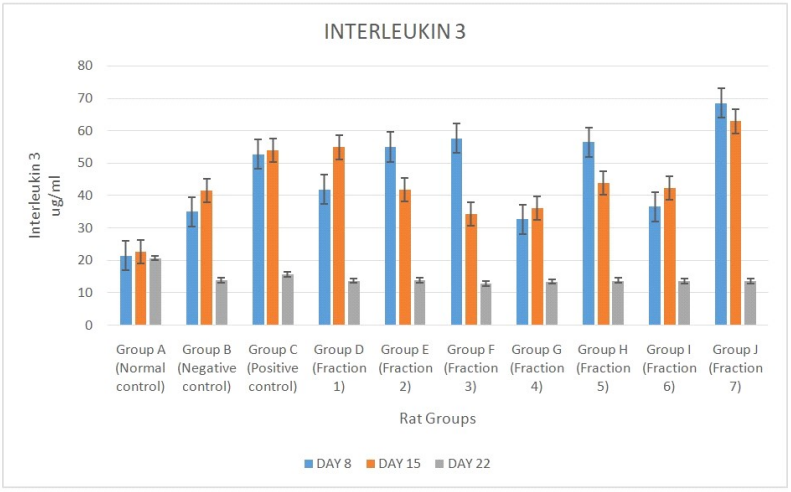

Figure 1. Effect of Hibiscus *sabdariffa* fractions on interleukin 3

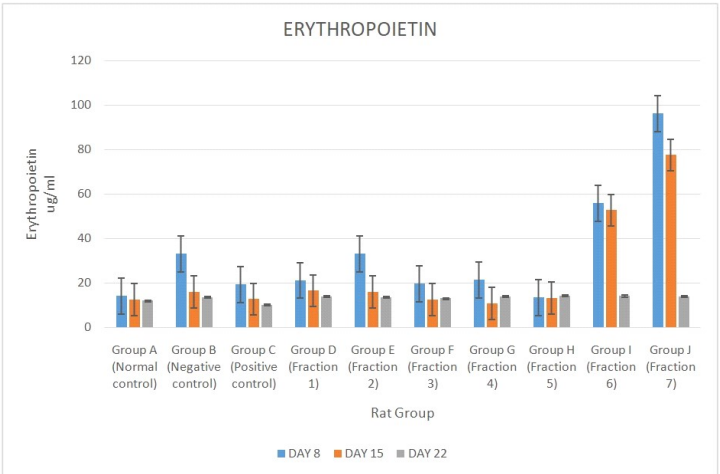

Figure 2 Effect of Hibiscus *sabdariffa* fractions on Erythropoietin

Group of rats fed on fraction 3 had an increase in IL-3 and EPO, the red cell count, PCV, Hb was increased on day 15. But this was not significant. The total white cells were significantly increased on day 15. The red cells, PCV, Hb returned to baseline level on day 22. Fraction 3 contains Digitopurpone and Undecanoic acid as well as coumarin. Digitopurpone is an anthroquinone. It possess strong antioxidant and anticancer activity (Prayogo *et al*, 2021). Undecanoic acid is a carboxylic acid with antifungal effect exerted by modulation of fungal metabolism, it also possess antibacteria effect (Jinx *et al.*, 2021). Administration of fraction 4 to group rats caused a reduction in IL-3 but the EPO level was not altered. The red cell count, PCV and Hb showed not significant difference from the astymin group on day 22. Artemisinin and Azedarachin C are present in fraction 4. Artemisinin is a sesquiterpene lactone commonly used as an antimalarial agent. It depletes erythrocytes by activating the apoptotic pathway thereby inducing programmed cell death. (Liu *et al*., 2021). Artemisinin alters the S-phase of cell cycle thereby inhibiting the differentiation of the red cells, it targets proerythroblasts and basophilic erythroblasts., Artemisin solubility in water is reportedly low (Dogan *et al*., 2012). Azedarachin C is a limonoid antifeedant. It is a highly oxygenated triterpenoid. Limonoids have been observed to reduce blood cholesterol and the incidence of several form of cancer. (Passos *et al*., 2021).

Administration of fraction 5 did not affect the serum IL-3 level significantly but the EPO was significantly decreased on day 15. The red cell count, PCV, Hb were significantly reduced at day 22 when compared with the positive control group. Fraction 5 contains 9, 12octadecanoic acid (Z, Z) – (2, 2-chimethyl-1, 3-dioxan – 4-yl) methyl ester and Imperanene. 9, 12-octadecanoic acid (Z, Z) – (2, 2chimethyl-1, 3-dioxan – 4-yl) methyl ester possess antifungal properties. It inhibits cell wall, nucleic acid and protein synthesis (Jabeen *et al*., 2018). Imperanene is a phenolic compound. Phenolic compounds inhibits erythroid differentiation by interfering in the transcription of erythroid specific gene especially genes responsible for haem synthesis (Tang *et al.*, 2016). Administration of fraction 6 of the extract of Hibiscus sabdariffa resulted in increase in IL-3 and EPO, this was significant on day 8 and 15. However, IL-3 was significantly reduced on day 22. Subsequently the red cell count, PCV, Hb were significantly increased on day 8 and 15. These returned to baseline on day 22. The retics count increased significantly on day 8, 15 and was significantly reduced on day 22. The white cells were not affected. Fraction 6 contains salvianolic acid and Wedelolactone. Salvianolic acid are water soluble compounds. It activates the expression of VEGF through the activation of the JAK2/STAT3 pathway leading to increased EPO/EPOR expression (Yu *et al*., 2017). Thus it is cardioprotective. Wedelolactone is a polyphenol. It has antioxidant property. It inhibits oxidative stress and is hepatoprotective. It has anticancer, anti-inflammatory, anti-diabetic effect. The pharmacological actions includes increased antioxidant and insulin secretions, suppression of NF-KB activity, decrease level of IL-1b, TNF-α, TGF-*B*I, increased expression of RUNX2 increased P21 protein expression, and inhibition of MU3 cell proliferation, invasion and migration (Vingagam *et al.,* 2020).

Administration of fraction 7 caused to a significant increase in IL-3 and EPO on day 8 and 15. The RBC, PCV and Hb increased significantly on day 8 and 15 when compared with the astymin group. It returned to the baseline on day 22. The retics count increased significantly on day 8, 15 and decreased significantly on day 22. Kaempferol, Kaempferide and methyl gallate are present in fraction 7. Kaempferol is a flavonoid with a ketone group. It modulates several proinflammatory signaling pathways such as NF-KB, P38 MAPK, AKT and B-catein cascade. (Santos et al 2021). thus it possess antiinflammation, anti-oxidant and anticancer properties. Kaempferide is a monomethoxy flavone that is the 4' 0-methyl derivative of kaempferol. It has antihypertensive properties, antiviral, antioxidant anti-bacterial and anti-tumor properties, it stimulates EPO production and MAPK14 resulting in increased expression of VEGF mRNA (Zhou et al., 2022). It possess a cardioprotective effect. Methyl gallate is a rare natural polyphenol. It is an antioxidant and has been shown to be cardioprotective (Ahmed et al., 2021). It modulates erythropoiesis (Ahmed et al., 2021) and was demonstrated to relieve the effect of doxorubicin induced cytopaenia in experimental animals. (Ahmed et al., 2021). It possess anti-oxidant and anti-inflammation by inhibiting NF-KB transcriptional activity (Correa et al., 2020).

Bone marrow studies are useful in the establishent of etiology of anaemia. Myeloid/Eythroid is useful as an index of total erythropoietic activity in the bone marrow with normal number of granulocytes precursors (Welz and Tvedten, 2012). Knowledge of bone peripheral blood findings are critical for bone marrow assessment interpretation (Reagen *et al*., 2021). The use of inbred mouse line have been essential in biomedical research because they are genetically equal and as such allow for observation of reproducibility in experiments (Foreman *et al*., 2015).

Hibiscus sabdariffa calyx fractions demonstrated many compounds that exhibit haemopoietic potential. The presence of these phytochemical compounds is remarkable. It was seen that the haemopoietic activity of treatment groups except the groups treated with fraction 5 were able to alter Hb, PCV and RBC by day 15. The haemopoietic activity of fractions 1, 2, 6 and 7 were significantly different from the positive control group by day 22. This suggests that these fractions have a better haemopoietic activity than astymin, the haemopoietic activity induced by factor 7 was fastest. The erythropoietic activity observed could have resulted from the synergistic effects of the compounds.

CONCLUSION

Hibiscus sabdariffa calyx fractions 6 and 7 were selected as compounds that possesses strong eryrthropoietic activity. Fraction 7 is the most promising. The identification of the compounds in the fractions demonstrated the domination of flavonoids. These findings reveals the significance of *Hibiscus sabdariffa* in the management of anaemia.

List of Abbreviations

ALT = Alanine transaminase
AST = Aspartate transaminase
EPO = Erythropoietin
FBC = Full blood count
Hb = Haemoglobin
IL-3 = Interleukin 3
PCV = Packed cell volume
WBC = White blood cells

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