

Phytochemical composition, hematinic activity, and *in silico* prediction of bioactive compounds in ethanolic leaf extracts of *Hibiscus acetosella*

Willy Nzulu Kisoi^{1,*}, Kenneth Ogila¹, Albert W. Nyongesa², Kagali Robert Nesta¹, Kinuthia Mwangi¹, Evans N. Nyaboga³

¹Department of Zoology, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000 – 00200, Nairobi, Kenya

²Department of Veterinary Anatomy and Physiology, University of Nairobi, P.O. Box 30197 – 00100, Nairobi, Kenya

³Department of Biochemistry, University of Nairobi, P.O. Box 30197 – 00100, Nairobi, Kenya

*Corresponding author

Willy Nzulu Kisoi
Department of Zoology, Jomo
Kenyatta University of Agriculture
and Technology, P.O. Box 62000 –
00200, Nairobi, Kenya
Email: willynzulu@gmail.com

Academic editor

Md. Abdul Hannan, PhD
Bangladesh Agricultural University,
Bangladesh

Article info

Received: 18 March 2026

Accepted: 18 June 2026

Published: 29 June 2026

Keywords

Anaemia, Bioactive compounds,
Haemolytic anaemia, *Hibiscus*
acetosella, Leaf extract

ABSTRACT

Anaemia affects nearly 1.74 billion people globally, with increased risk of mortality and severe morbidity in developing countries. Medicinal plants have the potential as alternative sources of therapeutic compounds for the management of anaemia, because they are accessible, affordable, and without side effects. *Hibiscus acetosella* is a medicinal plant that has been used traditionally to manage several ailments, including fever and even anaemia. However, scientific support for its efficacy remains limited. This study evaluated the phytochemical profile and anti-anaemic activities of ethanolic leaf extract of *H. acetosella* in 2,4-dinitrophenylhydrazine (2,4-DNPH)-induced anaemia in Wistar rats. *In silico* molecular docking analysis was performed to support the findings. Bioactive compounds in the ethanolic extracts were evaluated using gas chromatography-mass spectrometry (GC-MS) for chemical profiling. Rats were divided into 6 groups (n = 3), and anaemia was induced through oral administration of 2,4-DNPH (40 mg/kg b.w) for 7 days, except the control group. Thereafter, the ethanolic extract was administered orally (100, 200, 400 mg/kg b.w) for 28 days. GC-MS analysis revealed the presence of phytol, beta-amyrone, 2-methoxy-4-vinylphenol, and squalene. Ethanolic extract significantly (p < 0.05) increased red blood cell (RBC), haematocrit (HCT), and haemoglobin (HGB) in treatment groups following 28 days of exposure compared to the control group. The beta-amyrone exhibited lower binding energy values of -7.6 kcal/mol to -11.3 kcal/mol. The results point to the presence of anti-anaemic properties of *H. acetosella*.

INTRODUCTION

Anaemia is a pathological condition characterized by HGB levels of <13.5 g/dL and <12.5 g/dL in males and females, respectively [1]. It affects roughly 30 % of women of reproductive age and 40 % of children, and it can have serious health consequences [2]. According to the World Health Organization (WHO) data, it affects roughly 1.74 billion of the global population [3], and it has different causes and prevalence in different settings; hence, its real burden cannot be described [2]. Around 47.4 % of infants below 5 years are anaemic globally, with around 67.6 % of toddlers below 5 years in Africa being anaemic. Anaemic children have a greater risk of mortality than children without anaemia [4]. Moreover, about 40 % of cancer patients present with iron deficiency, and 39 % have anaemia at the time of diagnosis; of these, around a third have HGB concentrations < 12 g/dl [5].

The causes of anaemia can include blood loss due to bleeding because of injury, surgery, or inflammation of the gut, and decreased RBC production due to iron or



Copyright: © by the authors. This
article is an open access article
distributed under the terms and
conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)
[Attribution 4.0 \(CC BY 4.0\)](https://creativecommons.org/licenses/by/4.0/)
[International license](https://creativecommons.org/licenses/by/4.0/).

vitamin B₁₂ deficiency, bone marrow tumours, or thalassemia [6]. Furthermore, increased RBC breakdown due to infections (malaria), autoimmune diseases or genetic disorders (sickle cell anaemia) [6]. Management of anaemia through conventional medicine is quite expensive [7]. The management of iron deficiency anaemia (IDA) is through iron supplements, although one may experience side effects like nausea, dark, hard stools, constipation, and abdominal contraction [7]. Even more so, iron supplements are associated with gastrointestinal effects and worsen constipation, which is frequent in pregnancy because of high progesterone concentrations that lower bowel movement and enlarge the uterus, thus depressing the rectum [8]. Blood transfusion poses the risks of incompatibility or infection, whereas iron supplementation poses the disadvantage of accumulating in the body due to limited elimination mechanisms and can cause complications like cancer or neurogenic disorders [9]. Because of the side effects and high costs of conventional treatments, medicinal plants should be considered as alternatives in anaemia management [9]. Hence, there is a need to find alternative novel strategies for the management of anaemia.

H. acetosella Welw. Ex Heirn (Malvaceae), usually known as the African Rosemallow, is an amphidiploid plant, indigenous to Africa. The leaves are traditionally consumed as vegetables [10]. In Nigerian folk medicine, it is used in the treatment of haemorrhoids, headaches, anaemia, inflammation, fever, rheumatism, scurvy, conjunctivitis, and diarrhoea. It is also used as a blood-purifying tonic in Uganda and in the stimulation of lactogenesis in breastfeeding women [11-15]. In Nigeria, the leaves are used in the control of menstrual disorders, afterbirth complications, and the treatment of stomach aches and dysentery [16]. The qualitative screening of an aqueous leaf extract of *H. acetosella* revealed the presence of alkaloids, saponins, steroids, and coumarins, while flavonoids, phenols, terpenoids, anthocyanins, and anthraquinones (bound and unbound quinones) were not found [14].

Studies in Cameroon and Uganda have demonstrated the anti-anaemic effects of *H. acetosella* aqueous extract in anaemic rats [14, 17]. The anti-anaemic activity of *H. acetosella* is undoubtedly linked to its phytochemical compounds in the aqueous extracts. These studies did not explore the ethanolic leaf extracts of *H. acetosella* for hematinic activity or their phytochemical properties. In addition, considering the ecological heterogeneity and sensitivity of plant secondary metabolism to environmental factors, targeted phytochemical and hematinic activity in Kenya is required. Moreover, there is a lack of research on the phytochemical compounds of *H. acetosella* based on *in silico* molecular docking studies. Therefore, this research was performed to assess the phytochemical profiles and hematinic properties of *H. acetosella* ethanolic leaf extract through *in vivo* (using the model of 2,4-DNPH-induced anaemia in Wistar rats) and *in silico* molecular docking methods.

MATERIALS AND METHODS

H. acetosella collection, identification, and preparation

Fresh *H. acetosella* leaves (170.6 g) were collected from Oyugis, Homa Bay County, Kenya, with geographical coordinates: 0° 30' 0" S, 34° 43' 0" E. The identity of the plant was authenticated by a taxonomist in the East African Herbarium of the National Museums of Kenya (NMK), and a voucher specimen with reference number NMK/BOT/CTX/2/ID/20/2023 was archived. Following authentication, the leaves of *H. acetosella* were gently washed with distilled water to remove debris, chopped, and air-dried under shade at 27 ± 2 °C for 14 days. The leaves were pulverized into a powder

according to the procedure outlined by Kumar *et al.* [18] and the powder stored in airtight packages at -20 °C.

Crude extract preparation of *H. acetosella*

The ethanolic extract of *H. acetosella* was prepared according to the maceration method outlined by Kediso *et al.* [19]. About 150 g of the plant powder was cold-macerated in 1,500 mL of 70 % ethanol (Sigma-Aldrich, St. Louis, Missouri, USA), and the mixture was kept for 72 hours at 4 °C in the cold room. The mixture was filtered using Whatman Filter Papers No. 1, and the filtrate was collected, concentrated using a rotary evaporator (BÜCHI Labortechnik AG, CH-9230, Flawil, Switzerland), the resultant slurry/paste was weighed (Mettler Toledo model: PB1501-S/Fact, Mettler Toledo company, Greifensee, Switzerland), and frozen at -20 °C until further use. The formula provided by Dumrongphuttidecha *et al.* [20] was used to give the percentage yield of the extract.

$$\% \text{ Yield} = \frac{\text{Weight of Dry Extracts}}{\text{Weight of Taken Extraction}} \times 100$$

Qualitative phytochemical screening of the ethanolic leaf extracts of *H. acetosella*

The phytochemical compounds were determined following published procedures as follows: the presence of tannins was determined following the procedure outlined by Maheshwaran *et al.* [21]. Also, hydrolysable tannins and cardiac glycosides [22], steroids, glycosides, and terpenoids [23], quinones [24], anthraquinones [25], and anthocyanins [26] were identified as previously described.

GC-MS analysis of ethanolic leaf extracts of *H. acetosella*

This was done according to the procedure described by Olivia *et al.* [27], with slight modifications. About 1 g of the sample of ethanolic leaf extracts previously extracted was dried in a MIVAC DNA Concentrator (Genevac Ltd., Ipswich, United Kingdom) (37 °C, 40 minutes) and then reconstituted in acetone (Sigma-Aldrich, St. Louis, Missouri, USA) solvent (1 mL), shaken and filtered using NY 0.22 µm syringe filters into an autosampler vial and the filtrate run through the GC-MS.

This was done using GCMS-QP2010 SE (Shimadzu Corporation, Kyoto, Japan), equipped with a GC-2010 Plus gas chromatograph interfaced to a mass spectrometer. The system was operated under the following conditions: capillary column BPX5 (30.0 m × 0.25 mm i.d. × 0.25 µm film thickness); injection temperature of 250.0 °C; and the injection mode was split. The carrier gas was helium (99.999 %) at the flow control mode of linear velocity, pressure of 8.0 psi, total flow of 13.2 mL/min, a column flow of 0.92 mL/min, a linear velocity of 35.2 cm/sec, purge flow of 3.0 mL/min, and a split ratio of 10.1 (injection mode: split). The column oven temperature program was 70 °C (held for 0 minutes), increased at a 10 °C/min rate to 250 °C (held for 8 minutes), then increased at a 5 °C rate to 270 °C (held for 10 minutes); the total program time was 40.00 minutes. The MS parameters were: the ionisation voltage of 70 eV, the ionisation technique used was electron ionisation, the ion source temperature was 200 °C, the interface temperature was 250 °C, and the solvent cut time was 3.5 minutes. The detector was GC coupled with a Mass Spectrometer, the mobile phase was helium (carrier gas), and the stationary phase was BPX5. The sample inlet was GC, and the injection source was the CTC Analytics (Combi PAL) autosampler (CTC Analytics AG,

Zwingen, Switzerland). The injection volume was 1 µL (Scale 54 mm, ID 01), using a CTC Analytics (Combi PAL) autosampler.

The phytochemical analysis was carried out using GC-MS. Mass spectra in the database of the National Institute of Standards and Technology (NIST14.lib) were compared with the mass spectra of sample components in order to identify the compounds present in samples. Mass spectra of unknown compounds were matched with reference spectra in the NIST database to establish their structures, molecular weights and names. The phytochemical components in the *H. acetosella* leaf extracts were hence confirmed by GC-MS analysis.

Mineral analysis

Moisture-free samples of *H. acetosella* were prepared following the procedure outlined by Balarabe [28], with slight modifications. About 6 g of *H. acetosella* powder was weighed and divided into 3 pre-weighed crucibles. The samples were left in the oven at 105 °C for 16 hours. The samples were transferred into pre-weighed crucibles and then placed in a muffle furnace at 550 °C for 4 hours to obtain the ashes. The ash samples were then transferred into volumetric flasks. About 10 mL of 20 % HCl (hydrochloric acid) (Sigma-Aldrich, St. Louis, Missouri, USA) was added to each flask to digest the ash. The mixture was left for 1½ hours and then filtered into separate flasks using Whatman Filter Papers No.1. The filtrates in the flasks were then made to 50 mL using distilled water. An Atomic Absorption Spectrophotometer (AAS) (Model: AA-6300, Shimadzu Corporation, Kyoto, Japan) was used to measure iron, manganese, copper, zinc and magnesium. Flame Photometer Corning (Model 410, Sherwood Scientific, Cambridge, England, United Kingdom) was used to measure potassium, calcium and sodium.

Experimental animals and housing

Adult Wistar rats, aged 8 to 10 weeks and weighing between 150 and 210 g, were used for this study. The animals were bred and raised in standard cages in the animal house of the Department of Biochemistry, University of Nairobi, Kenya, under standard conditions of a 12-hour light/dark cycle and temperature (23 - 25 °C). The rats were fed standard mouse pellets and water *ad libitum*. Regular health monitoring was done by a qualified veterinarian, and only healthy animals were used for the research work. The research was conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). The research protocol was approved by the Jomo Kenyatta University of Agriculture and Technology Institutional Scientific and Ethics Review Committee (JKUAT ISERC), approval number JKU/ISERC/02317/1353.

Experimental design and induction of anaemia

Rats of both sexes were randomly assigned into 6 groups (n = 3 per group): Control, Ana (anaemic), Ana+FA (anaemic rats treated with folic acid), Ana+100 HA, Ana+200 HA, and Ana+400 HA (anaemic rats treated with 100, 200, and 400 mg/kg b.w./day of ethanolic extract of *H. acetosella*, respectively). The control group was not induced, while anemia was induced in the remaining groups using 2,4-DNPH (RANKEM, New Delhi, India) (40 mg/kg, orally administered (p.o), daily for 7 consecutive days) following the protocols of Ezeigwe *et al.* [29], with slight modifications. Rats were

considered anaemic when they had $\geq 30\%$ reduction in HCT or RBC count [30, 31]. Treatment with the extract began immediately after 2,4-DNPH-induction and continued daily for 28 days. The experimental groups were organized as shown in Table 1.

Table 1. Animal grouping, treatment, and anaemic induction.

Animal group	Treatment	Anaemia status
Con	Distilled water	Not induced
Ana	Distilled water	Induced
Ana+FA	75 $\mu\text{g/kg}$ b.w/day of folic acid	Induced
Ana+100 HA	100 mg/kg b.w/day of ethanolic extract	Induced
Ana+200 HA	200 mg/kg b.w/day of ethanolic extract	Induced
Ana+400 HA	400 mg/kg b.w/day of ethanolic extract	Induced

Here, b.w.= body weight, Con = control; Ana = anaemia-induced group; FA = folic acid; HA = *H. acetosella*

Blood collection for the determination of haematological parameters

Blood samples were collected in EDTA bottles through tail venipuncture at baseline (pre-induction, Day 1), post-induction/initiation of treatment (Day 8), during treatment (Day 22; 14 days after treatment initiation), and post-treatment (Day 36; 24 h after the final dose), and thereafter via cardiac puncture.

The blood samples were obtained for haematological analysis using a haematology analyser (Mindray Auto Haematology Analyser, Model: BC-10, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). The parameters measured were the HCT, MCV, MCHC, MCH level, HGB concentration, and RBC counts.

Molecular docking

Molecular docking was performed following the method of Talapatra *et al.* [32] with modifications. The proteins (PDB formats) downloaded from the RCSB Protein Data Bank (<https://www.rcsb.org/>) with the following Protein Data Bank Identifiers (PDB IDs): human prolyl hydroxylase domain-containing protein 2 (PHD2) (PDB ID: 2G19), human EpoR (PDB ID: 6MOE), rat HGB (PDB ID: 3DHT), prolyl hydroxylase domain-containing protein 2 (PHD2) (PDB ID: 3HQR), T-state HGB (PDB ID: 2D5Z), human erythropoietin bound to its receptor complex (PDB ID: 1EER), methemoglobin (PDB ID: 3HF4), ferritin (PDB ID: 1GWG), human hepcidin (1M4F) and transferrin (PDB ID: 6WB6) and human nuclear factor erythroid 2-related factor 2 (PDB ID: 7O7B). The ligands (3D conformers-SDF formats), which were compounds of *H. acetosella* extracts, were downloaded (<https://pubchem.ncbi.nlm.nih.gov/>). Proteins were prepared using AutoDockTools in MGLTools version 1.5.6 (Molecular Graphics Lab (MGL) of the Scripps Research Institute, La Jolla, California, USA). Protein PDB files were loaded, water molecules deleted, hydrogen atoms added to polar atoms, Kollman charges added, and saved as PDBQT format. The SDF formats of the ligands were converted to PDB formats using PyMOL (Schrödinger, Inc., New York City, United States) (<https://www.pymol.org/>), then prepared using AutoDockTools. The ligands PDB files were loaded to AutoDock Tools, gasteiger charges added, and saved as PDBQT files. Grid formations (proteins and ligands in PDBQT formats) were done using AutoDockTools. Molecular docking of ligands to proteins was performed using AutoDock Vina (Molecular Graphics Lab (MGL) of the Scripps Research Institute, La Jolla, California, USA) (<https://vina.scripps.edu/downloads/>). The docked ligand poses were visualized with PyMOL. Protein-ligand interactions were visualised using LigPlot+ version 2.3.1 (European Bioinformatics Institute (EMBL-EBI) located in Hinxton, Cambridgeshire, United Kingdom) (<https://www.ebi.ac.uk/thornton->

srv/software/LigPlus/). Drug feasibility tests for the ligands were performed using SwissADME (Molecular Modeling Group of the Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland) (<https://www.swissadme.ch/>).

Statistical analysis

All statistical analyses were performed using SigmaPlot (Version 16) (Grafiti LLC, Palo Alto, California, United States), and graphs were generated using GraphPad Prism 8.0.1 (GraphPad Software, San Diego, California, United States). Haematological data are presented as mean $\pm$ standard error (SE). Numeric data were subjected to one-way ANOVA followed by Tukey's HSD post hoc test and multiple comparison tests for significant differences in means among groups. Mineral analysis data were presented as mean $\pm$ SE, parts per million (ppm). The statistical significance was set at $p < 0.05$.

RESULTS

Quantitative and qualitative phytochemical components of *H. acetosella* leaves

The percentage yield for the ethanolic extract was 16.2 %. The phytochemical screening of ethanolic extracts of *H. acetosella* leaves revealed the presence of tannins, hydrolysable tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, glycosides, phenols, quinones, anthraquinones, and anthocyanins (Table 2).

Table 2. Phytochemical constituents in the ethanolic extract of *H. acetosella* leaves.

Components	Observation	Leaf extracts
Tannins	Blue-black colouration	+++
Hydrolysable tannins	Formation of emulsion	+++
Phlobatannins	Red precipitate	+++
Saponins	Stable foam	+++
Flavonoids	Green precipitate	+++
Steroids	Bilayer formation (greenish bottom layer and red top layer)	++
Terpenoids	Reddish-brown colouration	+++
Cardiac glycosides	Bluish black colour	+++
Glycosides	Yellowish green/yellow colour	+++
Phenols	Bluish black colour	+++
Quinones	Blue colour	+++
Anthraquinones	Red colour	+++
Anthocyanins	Blue colour	+++

Here, +++: Present in high amount; ++: Present in moderate amount; and +: Present in trace amount

Phytochemicals of ethanolic leaf extracts in *H. acetosella* leaves using GC-MS analysis

The GC-MS analysis indicated that 20 components were identified in the *H. acetosella* ethanolic extract (Figure 1 and Table 3). 24-norursa-3,12-diene had the highest peak value with an area of 26.99 % and a retention time of 31.217 minutes, followed by .beta.-amyrone (11.24 %), naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a. alpha.,7.alpha., 8a. beta.)]- (10.75 %), 4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one (6.4 %) and 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (5.79 %), the lowest peak area was exhibited by dibutyl phthalate (0.29 %). The component name, peak names,

retention times, molecular weights, and formulas, area, and area percentages of the constituents present in *H. acetosella* extract are presented in Table 3.

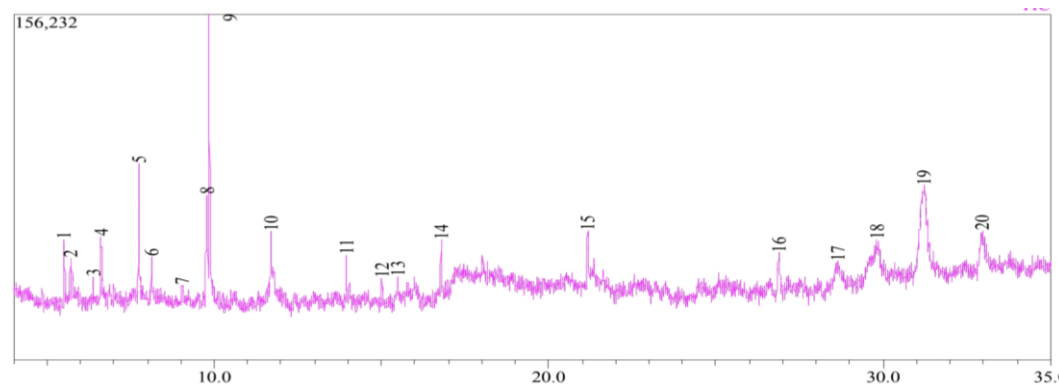


Figure 1. GC-MS chromatographic profile of ethanolic extract of *H. acetosella* leaves. TIC-Total Ion Chromatogram; Y-axis: total ion current (abundance or intensity) (measured in counts per second (cps) or arbitrary units (a.u.)); X-axis: retention time (in minutes); Peak: Different peaks correspond to individual compounds eluting from the GC column at particular retention times.

Table 3. List of GC-MS detected compounds in the ethanolic extract of *H. acetosella* leaves.

Peak#	RT	Area	Area %	Name	MF	MW	GC
1	5.501	71824	4.13	1,6-diazabicyclo(3.1.0)hexane-5-carboxylic acid-, methyl ester	C ₆ H ₁₀ N ₂ O ₂	142	Methyl ester
2	5.699	26158	1.51	2,6-dimethyl-6-nitro-2-hepten-4-one	C ₉ H ₁₅ NO ₃	185	Ketone
3	6.385	6592	0.38	5-ethyl-3-methylhept-1-en-4-ol	C ₁₀ H ₂₀ O	156	Alkenol
4	6.62	63438	3.65	2-pentenoic acid, 3-ethyl-, methyl ester	C ₈ H ₁₄ O ₂	142	Acid
5	7.746	97741	5.62	Benzeneethanamine, N-(1-methylethylidene)-	C ₁₁ H ₁₅ N	161	Amines
6	8.121	26781	1.54	2-methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	Phenol
7	9.04	9901	0.57	DL-proline, 5-oxo-, methyl ester	C ₆ H ₉ NO ₃	143	Flavonoid/alpha amino acid ester
8	9.788	100645	5.79	1,3-propanediol, 2-(hydroxymethyl)-2-nitro-	C ₄ H ₉ NO ₅	151	Nitro compound
9	9.847	62417	3.59	Benzaldehyde, 2-hydroxy-4-methyl-	C ₈ H ₈ O ₂	136	Aldehyde
10	11.698	64635	3.72	Tetradecanal	C ₁₄ H ₂₈ O	212	Aldehyde
11	13.954	26976	1.55	Octadecanal	C ₁₈ H ₃₆ O	268	Aldehyde
12	15.013	22029	1.27	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	276	α, β - unsaturated ketone/flavonoid
13	15.487	5032	0.29	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278	Phthalate
14	16.786	41796	2.4	Phytol	C ₂₀ H ₄₀ O	296	Diterpene
15	21.162	72405	4.17	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330	Acid
16	26.883	77194	4.44	Squalene	C ₃₀ H ₅₀	410	Terpenoids
17	28.646	111162	6.4	4,4,6a,6b,8a,11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	C ₃₀ H ₄₈ O	424	Triterpenoids
18	29.814	186868	10.75	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	C ₁₅ H ₂₄	204	Sesquiterpene
19	31.217	469037	26.99	24-norursa-3,12-diene	C ₂₉ H ₄₆	394	Pentacyclic triterpene
20	32.956	195298	11.24	beta.-Amyrone	C ₃₀ H ₄₈ O	424	Terpene
		1737929	100				

RT = retention time, MF = molecular formula, MW = molecular weight, GC = group of compounds

Concentration of selected minerals in powder from *H. acetosella* leaves

Mineral analysis of *H. acetosella* leaves in Table 4 reveals high calcium content (1055 ± 82 ppm) compared to iron (97.5 ± 8.0 ppm), magnesium (12.5 ± 1.0 ppm), copper (2.65 ± 0.25 ppm), zinc (0.35 ± 0.00 ppm), potassium (2495 ± 235 ppm), sodium (29.9 ± 2.5 ppm) and manganese (40.5 ± 9.0 ppm).

Table 4. Concentration of selected minerals in *H. acetosella* powdered leaves.

Minerals	Mineral Content (ppm)
Iron (Fe)	97.5 ± 8.0 ppm
Manganese (Mn)	40.5 ± 9.0 ppm
Copper (Cu)	2.65 ± 0.25 ppm
Zinc (Zn)	0.35 ± 0.00 ppm
Potassium (K)	2495 ± 235 ppm
Sodium (Na)	29.9 ± 2.5 ppm
Calcium (Ca)	1055 ± 82 ppm
Magnesium (Mg)	12.5 ± 1.0 ppm

The quantity of minerals presents in the *H. acetosella* powdered leaves sample. Results are expressed as mean values $\pm$ SE (ppm), n=3, ppm=parts per million

Effect of ethanolic leaf extracts of *H. acetosella* on RBC counts in anaemic rats

The effect of the ethanolic leaf extract of *H. acetosella* on RBC in anaemic Wistar rats is shown in Figure 2. No statistically significant differences were observed on Day 1 ($p = 0.862$). On Day 8, the control group was statistically significant ($p < 0.001$) when compared to the Ana and Ana+FA groups, as well as the Ana+100 HA, Ana+200 HA, and Ana+400 HA on this measure. On Day 22, the control group was not statistically significant ($p = 0.055$) to the Ana+100 HA group, but statistically significant ($p < 0.05$) when compared to the remaining groups. Moreover, Ana+100 HA was statistically significant ($p < 0.05$) when compared to the Ana, Ana+FA, Ana+200 HA, and Ana+400 HA groups. On Day 36, Ana+100 HA was statistically significant ($p = 0.043$) when compared to the Ana group.

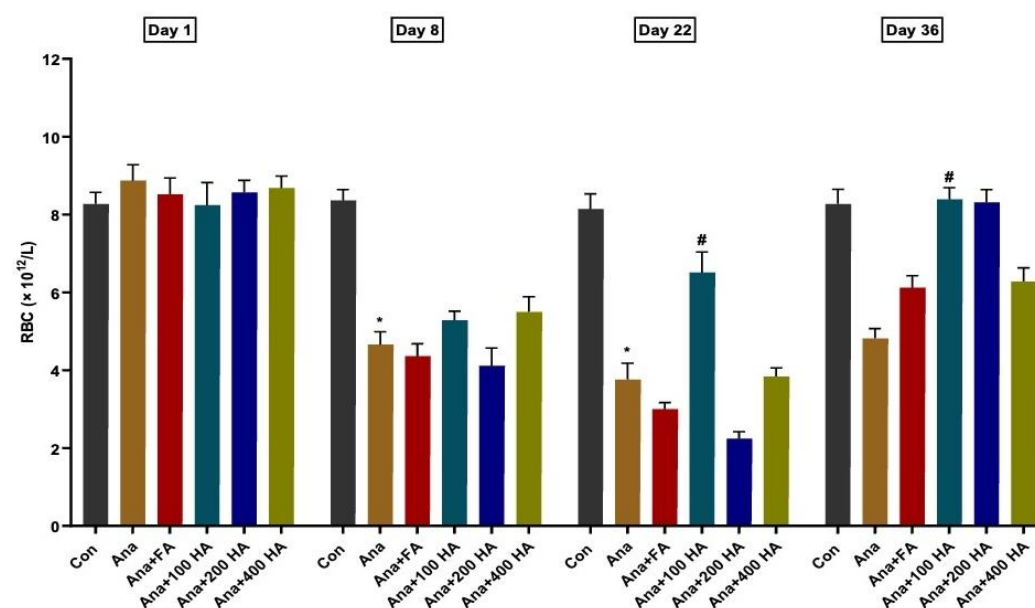


Figure 2. Effect of ethanolic leaf extracts of *H. acetosella* on RBC counts in 2,4-DNPH-induced anaemic Wistar rats. Here, Con = control; Ana = anaemia-induced group; FA = folic acid; HA = *H. acetosella*, and * indicates a significant difference between Con and Ana; # indicates a significant difference between Ana and treatment groups.

Effect of ethanolic leaf extracts of *H. acetosella* on HGB concentration in anaemic rats

The changes in HGB during treatment of anaemic Wistar rats with ethanolic leaf extract of *H. acetosella* are recorded in Figure 3. On Day 1, the Ana+100 HA group showed statistically significant difference ($p = 0.039$) in HGB concentrations when compared to the Ana+FA group. On Day 8, the control group was statistically significant ($p < 0.001$) when compared to the Ana and Ana+FA groups, as well as the Ana+100 HA, Ana+200 HA, and Ana+400 HA groups on the same measure. On Day 22, the control group was significantly different ($p < 0.001$) when compared to the Ana and Ana+FA as well as the Ana+100 HA, Ana+200 HA, and Ana+400 HA groups. Moreover, the Ana+100 HA group showed a statistically significant difference ($p < 0.001$) in HGB levels when compared to the Ana, Ana+FA, and the Ana+200 HA groups, but was not significantly different ($p = 0.869$) when compared to the Ana+400 HA group. On Day 36, the Ana+200 HA was statistically significantly different ($p = 0.013$) when compared to the Ana group.

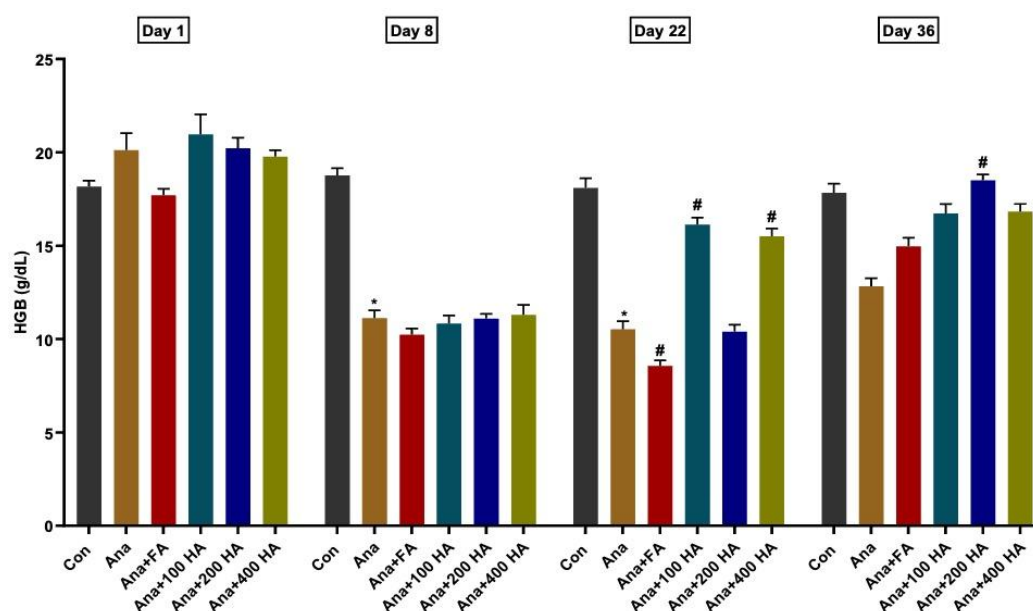


Figure 3. Effect of ethanolic leaf extract of *H. acetosella* on HGB concentration in 2,4-DNPH-induced anaemic Wistar rats. Here, Con = control; Ana = anaemia-induced group; FA = folic acid; HA = *H. acetosella*, and * indicates a significant difference between Con and Ana; # indicates a significant difference between Ana and treatment groups. g/dL = grams per decilitre.

Effect of ethanolic leaf extracts of *H. acetosella* on HCT in anaemic rats

The effect of *H. acetosella* extracts on HCT in anaemic Wistar rats is shown in Figure 4. On Day 1, the Ana group was statistically significantly different ($p < 0.001$) when compared to the Ana+FA and control groups, as well as Ana+100 HA and Ana+400 HA groups. However, the Ana group was not statistically significant ($p = 0.186$) on this measure when compared to the Ana+200 HA group. Moreover, Ana+200 HA showed a statistically significant difference ($p < 0.05$) when compared to Ana+100 HA and Ana+400 HA groups, as well as the control and Ana+FA groups on the HCT measure. On Day 8, the control group was statistically significantly different ($p = 0.010$) when compared to the other groups. On Day 22, the control group was statistically significantly different ($p = 0.008$) when compared to the Ana+200 HA group. On Day 36, the control group was statistically significantly different ($p = 0.021$) compared to the Ana+FA group.

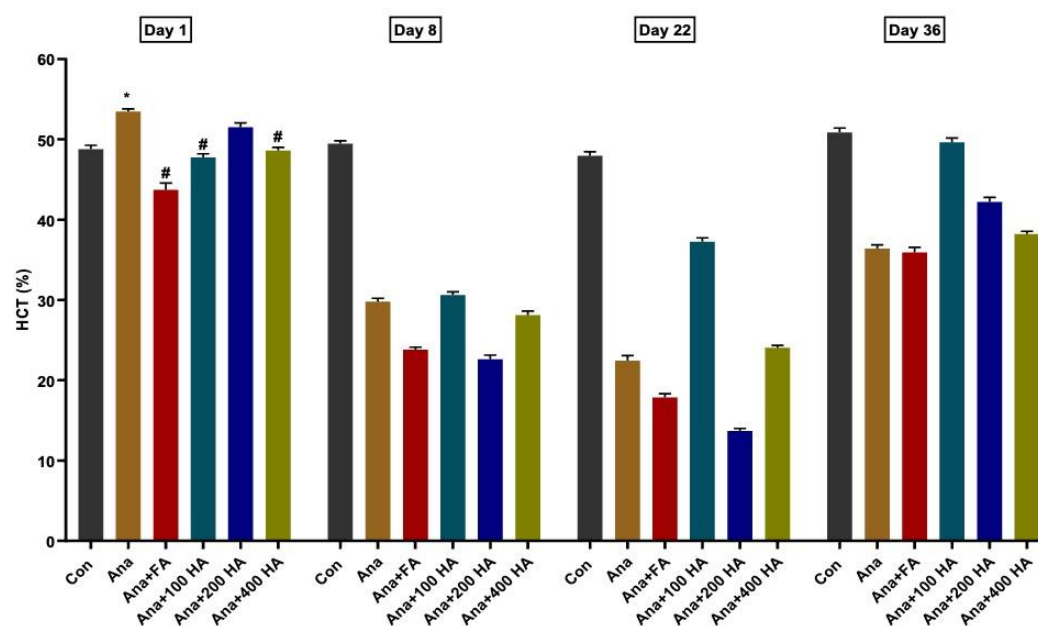


Figure 4. Effect of ethanolic leaf extract of *H. acetosella* on HCT in 2,4-DNPH-induced anaemic Wistar rats. Here, Con = control; Ana = anaemia-induced group; FA = folic acid; HA = *H. acetosella*, and * indicates a significant difference between Con and Ana; # indicates a significant difference between Ana and treatment groups.

Effect of ethanolic leaf extracts of *H. acetosella* on MCV in anaemic rats

Figure 5 shows the effect of the ethanolic leaf extract of *H. acetosella* on the MCV in anaemic Wistar rats. On Day 1, Ana+100 HA was statistically significantly different ($p < 0.001$) when compared to the other groups. On Day 8, the Ana+FA group was statistically significantly different ($p < 0.05$) when compared to the other groups. On Day 22, Ana+200 HA was statistically significant ($p < 0.05$) compared to the control and Ana+FA groups. Moreover, Ana+400 HA was statistically significantly different ($p < 0.05$) when compared to the control and Ana+FA groups, but not statistically significant when compared to the Ana+100 HA ($p = 1.000$) and Ana ($p = 0.999$) groups. On Day 36, Ana+100 HA was statistically significantly different ($p < 0.05$) when compared to the other groups except the Ana+400 HA group ($p = 0.982$). Ana+400 HA group was not statistically significantly different ($p = 0.093$) when compared to the Ana+200 HA group. Moreover, the Ana+FA was not statistically significant ($p = 0.586$), to the Ana group. All other pairwise combinations were statistically significant ($p < 0.05$) on Day 36.

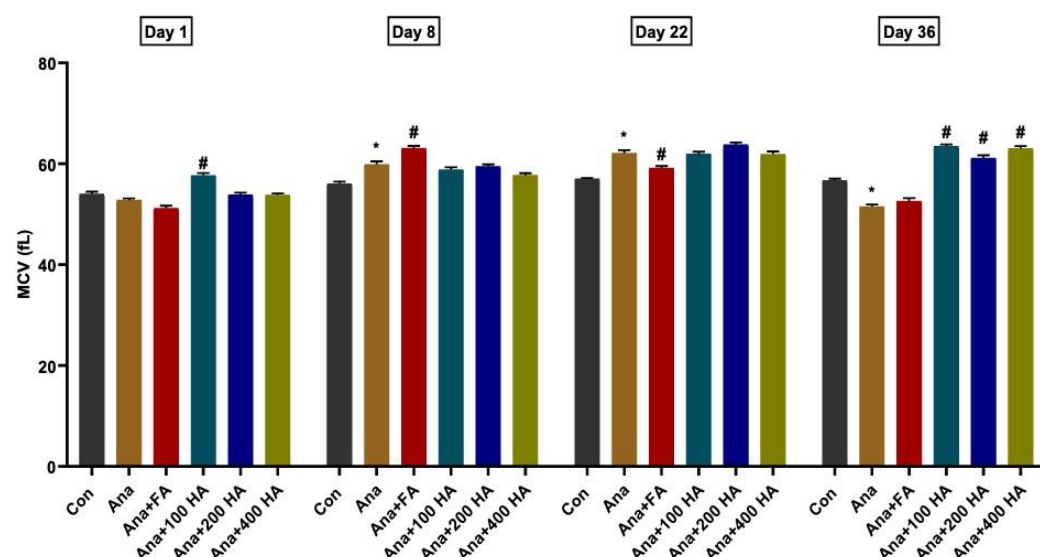


Figure 5. Effect of ethanolic leaf extract of *H. acetosella* on MCV in 2,4-DNPH-induced anaemic Wistar rats. Here, Con = control; Ana = anaemia-induced group; FA = folic acid; HA = *H. acetosella*, and * indicates a significant difference between Con and Ana; # indicates a significant difference between Ana and treatment groups. fL = femtoliters.

Effect of ethanolic leaf extracts of *H. acetosella* on MCHC in anaemic rats

Figure 6 shows the effect of *H. acetosella* extract on the MCHC of anaemic rats. On Day 1, the Ana+FA group was statistically significantly different ($p < 0.05$), when compared to Ana+400 HA, control, and Ana groups, but not significant to Ana+100 HA ($p = 0.792$) and Ana+200 HA ($p = 0.257$). On Day 8, the Ana+FA group was not statistically significantly different ($p = 0.059$) compared to the Ana group, but was significantly different ($p < 0.001$) when compared to the other groups. Additionally, the Ana group was statistically significant ($p < 0.001$) when compared to the other groups. Ana+400 HA group was not statistically significant ($p = 0.973$) compared to Ana+200 HA group. On Day 22, Ana+200 HA was statistically significantly different ($p < 0.001$) when compared to the other groups. Ana+400 HA group was statistically significantly different ($p < 0.001$) when compared to the other groups, except the control group ($p = 1.000$). On Day 36, the control group was statistically significant to Ana+400 HA group ($p = 0.031$) and Ana+200 HA group ($p = 0.033$). Moreover, Ana+FA was statistically significant ($p = 0.05$) compared to Ana+400 HA.

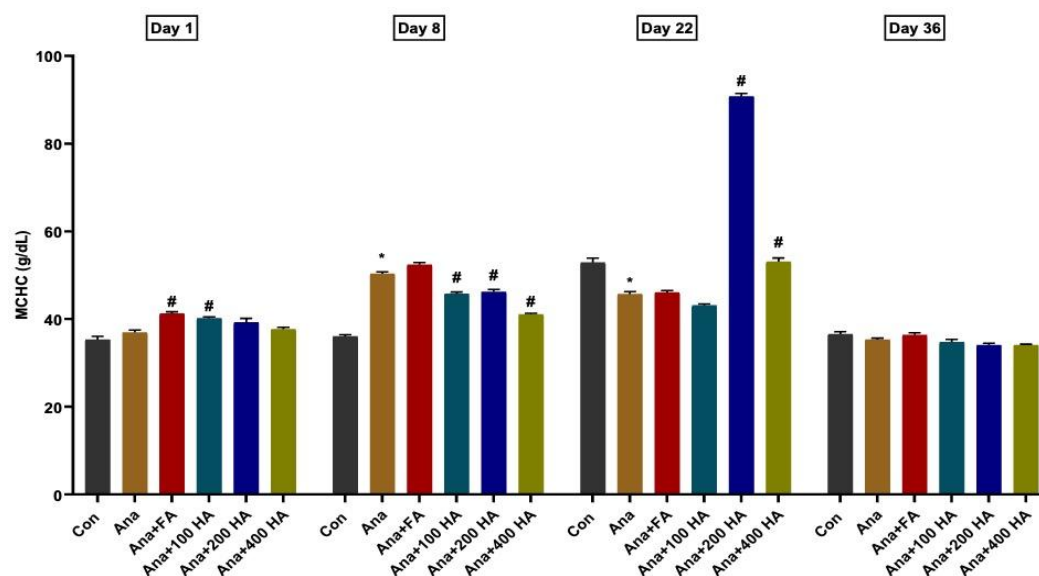


Figure 6. Effect of ethanolic leaf extract of *H. acetosella* on MCHC in 2,4-DNPH-induced anaemic Wistar rats. Here, Con = control; Ana = anaemia-induced group; FA = folic acid; HA = *H. acetosella*, and * indicates a significant difference between Con and Ana; # indicates a significant difference between Ana and treatment groups. g/dL = grams per decilitre.

Effect of ethanolic leaf extracts of *H. acetosella* on MCH in anaemic rats

The changes in the MCH values of anaemic Wistar rats under extract treatments are recorded in Figure 7. On Day 1, Ana+100 HA was statistically significantly different ($p < 0.05$), when compared to the Ana+200 HA, control, Ana and Ana+FA groups except for Ana+400 HA ($p = 0.281$). On Day 8, the Ana+FA group was statistically significantly different ($p = 0.032$) when compared to the Ana group and ($p < 0.001$) when compared to the other groups. The Ana group was statistically different ($p = 0.001$) when compared to Ana+200 HA and ($p < 0.001$) when compared to the other groups. On Day 22, Ana+200 HA group was statistically significant ($p < 0.001$) when compared to the other groups.

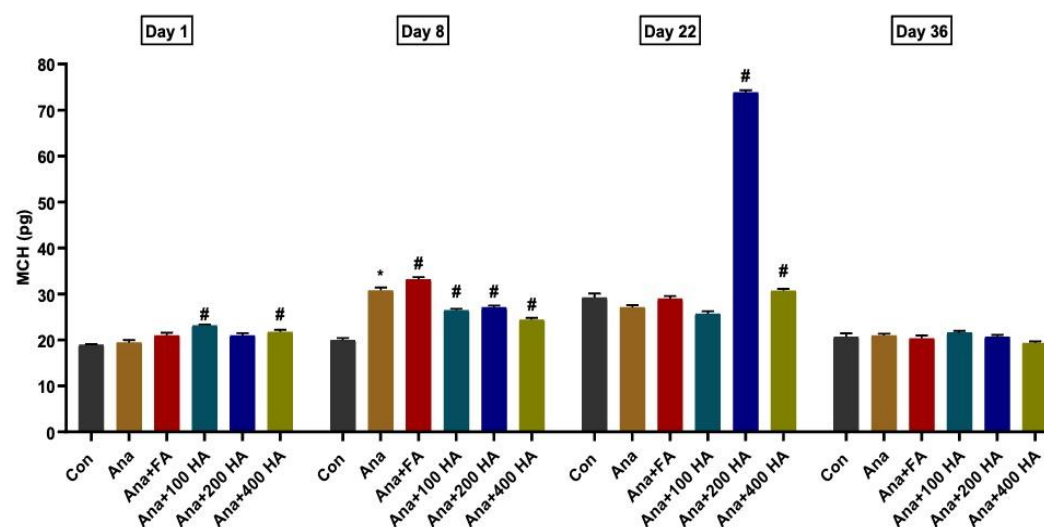


Figure 7. Effect of ethanolic leaf extract of *H. acetosella* on MCH in 2,4-DNPH-induced anaemic Wistar rats. Here, Con = control; Ana = anaemia-induced group; FA = folic acid; HA = *H. acetosella*, and * indicates a significant difference between Con and Ana; # indicates a significant difference between Ana and treatment groups. pg = picograms.

Molecular docking studies of target proteins

The binding affinities of compounds from *H. acetosella* ethanolic extract with selected controls (folic acid and quercetin) against several target proteins are shown in Table 5. The beta-amyrone mostly exhibited stronger interactions with the target proteins, with lower binding energy values ranging from -7.6 kcal/mol to -11.3 kcal/mol. The references exhibited binding energies that were consistent across the target proteins, ranging from -5.4 kcal/mol to -8.5 kcal/mol for folic acid and from -6.0 kcal/mol to -9.3 kcal/mol for quercetin.

Table 5. Binding affinity values of *H. acetosella* compounds.

Ligand	Target Protein											
	2G19	6MOE	3DHT	3HQR	6WB6	1EER	3HF4	1GWG	1M4F	2D5Z	7O7B	Mean
Folic acid (reference 1)	-6.8	-8.4	-7.1	-7.8	-7.7	-7.0	-8.5	-6.6	-5.4	-9.9	-6.2	-7.4
Quercetin (reference 2)	-7.2	-7.3	-7.4	-7.8	-7.9	-7.7	-7.9	-6.4	-6.0	-8.0	-6.2	-7.3
Tetradecanal	-3.3	-4.6	-3.7	-4.8	-4.3	-3.9	-4.4	-3.9	-2.7	-5.4	-3.2	-4.0
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	-4.0	-4.2	-4.3	-4.3	-4.5	-4.5	-4.6	-4.1	-3.9	-4.1	-3.0	-4.1
2-Pentenoic acid, 3-ethyl-, methyl ester	-4.1	-4.5	-4.1	-4.7	-4.4	-4.3	-4.4	-4.3	-3.5	-4.8	-3.7	-4.3
5-Ethyl-3-methylhept-1-en-4-ol	-4.4	-4.7	-4.4	-5.2	-4.2	-5.1	-4.3	-4.0	-3.8	-4.8	-3.9	-4.4
Phytol	-4.1	-4.2	-4.9	-4.9	-4.6	-4.8	-5.1	-4.1	-3.7	-4.9	-3.9	-4.5
DL-Proline, 5-oxo-, methyl ester	-4.4	-4.7	-4.6	-4.7	-5.0	-4.9	-4.7	-5.0	-3.9	-5.0	-3.8	-4.6
1,6-Diazabicyclo(3.1.0)hexane-5-carboxylic acid-, methyl ester	-4.4	-5.1	-4.8	-4.8	-4.9	-4.7	-4.8	-4.6	-4.3	-5.1	-3.6	-4.6
Benzaldehyde, 2-hydroxy-4-methyl-	-4.9	-5.5	-4.9	-5.5	-5.1	-5.3	-5.2	-4.7	-4.3	-6.2	-4.4	-5.1
2,6-Dimethyl-6-nitro-2-hepten-4-one	-4.5	-7.6	-4.8	-5.4	-5.2	-5.6	-5.1	-4.7	-4.1	-5.5	-3.7	-5.1
2-Methoxy-4-vinylphenol	-4.9	-5.6	-5.1	-5.6	-5.5	-5.1	-5.0	-4.9	-4.5	-6.3	-4.3	-5.2
Benzeneethanamine, N-(1-methylethylidene)-	-4.9	-5.9	-5.0	-6.3	-5.0	-5.6	-5.3	-5.4	-4.0	-5.9	-4.5	-5.3
Dibutyl phthalate	-5.1	-5.6	-5.4	-6.0	-5.3	-6.4	-5.3	-5.1	-4.1	-5.9	-4.3	-5.3
Squalene	-6.0	-4.8	-6.0	-6.1	-6.6	-5.8	-6.7	-5.8	-3.0	-6.4	-4.1	-5.6
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	-5.9	-5.8	-6.2	-7.1	-6.1	-6.0	-6.7	-5.9	-4.8	-7.0	-5.3	-6.1
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	-6.5	-6.5	-6.5	-6.8	-6.6	-6.2	-7.5	-6.2	-6.2	-7.0	-5.1	-6.5
Beta.-Amyrone	-7.8	-7.6	-8.6	-8.7	-9.2	-8.1	-11.3	-8.3	-6.0	-7.9	-6.6	-8.2

References included folic acid (reference 1) and quercetin (reference 2). Key: Binding energy values (kcal/mol) signify predicted interaction strength between the ligand and target proteins. Kilocalories per mole = kcal/mol, change in Gibbs free energy in binding = $\Delta G_{\text{binding}}$

Drug feasibility tests of *H. acetosella* compounds

The drug-likeness of *H. acetosella* compounds was evaluated based on Lipinski's rule (rule of five), which is the logarithm of the partition coefficient (log P), molecular weight (MW), and hydrogen-bond (H-bond) capacity. The majority of the *H. acetosella* compounds had molecular weights less than 500 g/mol, log P values of less than 5, hydrogen bond acceptors of less than 10, and hydrogen bond donors of less than 5 (Table 6).

Table 6. Lipinski's rule drug feasibility analyses of *H. acetosella* compounds.

Compound	MW < 500 (g/mol)	RB	H-bond acceptors < 10	H-bond donors < 5	MR	Log P < 5	Lipinski violations	BS
.beta.-Amyrone	424.7	0	1	0	133.92	4.51	1	0.55
7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	276.37	2	3	0	79.66	2.91	0	0.55
Naphthalene, decahydro-4a-methyl-1- -methylene-7-(1-methylethenyl)-, [4aR- (4a.alpha.,7.alpha.,8a.beta.)]-	204.35	1	0	0	68.78	3.31	1	0.55
Squalene	410.72	15	0	0	143.48	3.31	1	0.55
Dibutyl phthalate	278.34	10	4	0	77.84	2.97	0	0.55
2-methoxy-4-vinylphenol	150.17	2	2	1	45.05	2.14	0	0.55
2,6-dimethyl-6-nitro-2-hepten-4-one	185.22	4	3	0	53.25	1.75	0	0.55
1,6-diazabicyclo(3.1.0)hexane-5-carboxylic acid-, methyl ester	142.16	2	4	1	41.58	1.39	0	0.55
Benzaldehyde, 2-hydroxy-4-methyl-	136.15	1	2	1	38.82	1.29	0	0.55
Phytol	296.53	13	1	1	98.94	4.85	1	0.55
Benzeneethanamine, N-(1-methylethylidene)-	161.24	3	1	0	54.32	2.66	0	0.55
DL-proline, 5-oxo-, methyl ester	143.14	2	3	1	37.04	1.28	0	0.55
1,3-propanediol, 2-(hydroxymethyl)-2-nitro-	151.12	4	5	3	32.97	0.05	0	0.55
5-ethyl-3-methylhept-1-en-4-ol	156.27	5	1	1	50.87	2.82	0	0.55
2-pentenoic acid, 3-ethyl-, methyl ester	142.2	4	2	0	41.38	2.48	0	0.55
Tetradecanal	212.37	12	1	0	69.61	3.63	0	0.55
Folic acid (Reference 1)	441.4	10	9	6	111.92	0.04	2	0.11
Quercetin (Reference 2)	302.24	1	7	5	78.03	1.63	0	0.55

References included folic acid (reference 1) and quercetin (reference 2). Key: MW = molecular weight, H-bond = hydrogen bond, MR = molar refractivity, log p = logarithm of partition coefficient, BS = bioavailability score, RB = rotatable bonds

Molecular docking visualization

The visualization of molecular docking of beta-amyrone to the human erythropoietin receptor (1EER) (Figure 8). The docking of beta-amyrone (ligand) to 1EER (protein) revealed hydrophobic interactions between carbon and oxygen atoms of the ligand and the amino acids of the protein (LigPlot⁺ version 2.3.1). Hydrophobic amino acid residues of the protein included Thr152(C), Ser153(C), Thr149(C), Pro150(C), Leu17(A), Glu18(A), Lys97(A) and Glu13(A).

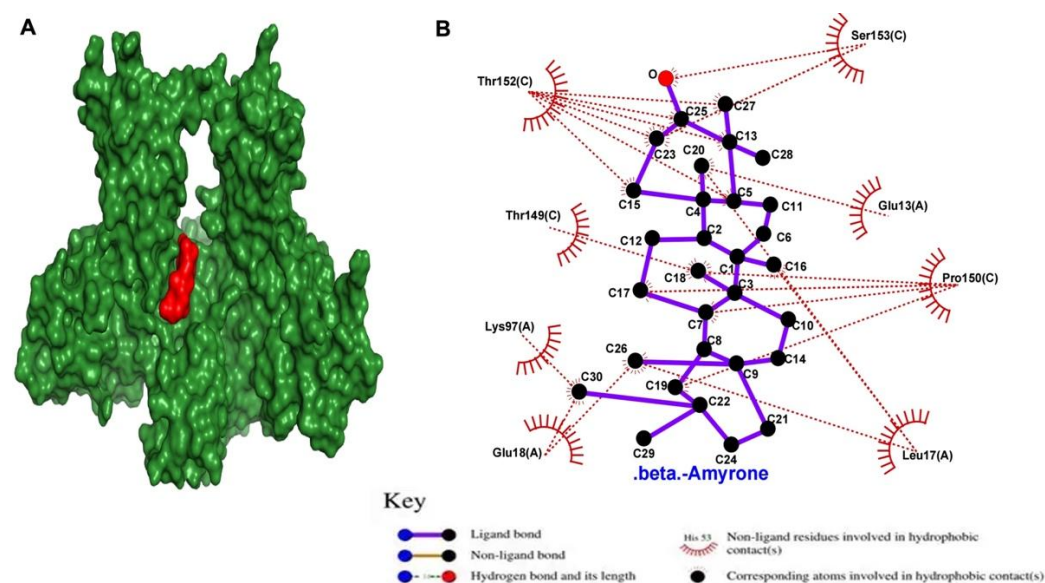


Figure 8. A) 3D molecular docking structure visualization of the beta-amyrone (red colour) that binds to the 1EER receptor protein (green). B) 2D visualization of the hydrophobic interactions that bind to the 1EER protein. Here, beta-Amyrone = ligand, red dot = oxygen atom of ligand, black dot = carbon atom of ligand, purple bond = ligand bonds, red lines = hydrophobic interaction.

DISCUSSION

The results of the present study on qualitative phytochemical screening revealed the presence of tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, glycosides, phenols, quinones, anthraquinones, and anthocyanins in ethanolic *H. acetosella* leaf extracts. This is similar to the findings reported by Makau *et al.* [33], who demonstrated the presence of similar phytochemicals but with the absence of quinone in *H. acetosella* leaf extracts prepared with 80 % ethanol containing 0.1 % HCl or 2 % acetic acid. Phytochemical screening of aqueous extracts of *H. acetosella* revealed varying results across studies: in Uganda, Abubakar [17] reported the presence of reducing sugars, saponins, tannins, steroids, flavonoids, cardiac glycosides, and diterpenes, although alkaloids, resins and anthraquinones were absent while in Nigeria, Junior *et al.* [14] reported the presence of saponins, alkaloids, coumarins, and steroids, while phenols, flavonoids, terpenoids, anthraquinones and anthocyanins were absent.

Phytochemicals like tannins, steroids, diterpenes, and flavonoids exhibit hematinic activities [17]. Moreover, they inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase, hence piling up cAMP. This leads to protein phosphorylation and protein synthesis stimulation, ultimately promoting erythropoiesis [17]. Anthocyanins have a possible hindrance against cadmium-mediated hematologic toxicity and potent antioxidant activity against oxidative stress [34]. Anthocyanins bind ROS and stabilize plasma membranes, hence protecting erythropoiesis precursor cells, and, as a result, cause an elevation in the content of mature RBCs [35]. The presence of anthocyanins in *H. acetosella* extracts in the present study may have contributed to elevated RBC levels.

Flavonols restrict the generation of free radicals through binding to metal ions. They can chelate metal ions such as Cu and Fe, thus preventing the stimulation of free radical generation through these ions [36]. The presence of phyto-compounds such as tannins, flavonoids, and terpenes in the ethanolic leaf extracts of *H. acetosella* may be responsible for the hematopoietic activities and protection of RBCs from oxidative damage [37]. Similarly, previous reports showed that aqueous leaf extracts of *H. acetosella* were efficient in increasing the erythrocytes in non-pregnant anaemic rats induced by phenylhydrazine (PHZ) [17]. Moreover, the presence of anthocyanins in the *H. acetosella* ethanolic leaf extract may be responsible for the haematopoietic-promoting effects. Anthocyanins (water-soluble flavonoids) act as antioxidants through the neutralization of free radicals through electron donation, thus preventing cell damage [38]. Phenolic compounds and flavonoids are presumed to be the primary compounds responsible for bone marrow protection against PHZ's harmful effects [39]. Moreover, flavonoids have antioxidant activity and can repair erythrocytes damaged due to PHZ oxidative damage [31].

Mineral analysis revealed important nutritional components: calcium, iron, copper, zinc, and magnesium. Deliza & Maibam [40] analysed minerals in *H. acetosella* and found Zn (0.12 ± 0.002 ppm), calcium (0.92 ± 0.011 ppm) and sodium (0.65 ± 0.082 ppm) and *H. sabdariffa* had K (1.83 ± 0.013 ppm), Fe (1.23 ± 0.27 ppm) and Mg (0.04 ± 0.009 ppm) which are similar in magnitude to those found in this study, reflecting natural variation arising from environmental factors, species differences and analytical methods. Elements such as zinc (HGB formation), iron (essential for the production of erythrocytes), copper, which promotes iron absorption, and manganese (essential for growth) are essential in anaemia management [41].

The GC-MS analysis of the ethanolic extract of *H. acetosella* revealed the presence of compounds such as squalene and phytol, which are in agreement with previous findings of Makau *et al.* [33], where the GC-MS of hexane extracts of *H. acetosella*

revealed the presence of squalene, toluene, and phytol. The compounds phytol (diterpene) and squalene (terpenoid) have been reported to have antioxidant activities [42-44]. 2-methoxy-4-vinylphenol has anti-inflammatory and antioxidant activities [45, 46]. DL-proline, 5-oxo-, methyl ester is a flavonoid reported to have antioxidant, anti-inflammatory, and antitumor activities [47, 48]. These compounds may have contributed to the hematinic effects of *H. acetosella* through their antioxidant activities, thereby protecting erythrocytes from oxidative damage caused by 2,4-DNPH.

In the present study, oral administration of 2,4-DNPH (40 mg/kg b.w) for 7 days led to a decrease in haematological parameters of RBC, HCT, and HGB. This finding agrees with studies of Junior *et al.* [14], who reported a reduction in HCT, RBC, and HGB in Wistar rats following intraperitoneal injection of 2,4-DNPH (40 mg/kg b.w) for 7 days. Amali *et al.* [30] reported that administration of PHZ in rats led to a decrease in packed cell volume (PCV) levels. Sheth *et al.* [31] reported that intraperitoneal injection of PHZ (40 mg/kg) for 2 days led to a significant decrease in HCT, HGB, and RBC. Administration of PHZ led to an elevation in the MCV and MCH, which are signs of macrocytosis, hence stating the anaemia as being macrocytic [49]. Results of the present study showed that 2,4-DNPH induction led to an increase in MCH, MCV, and MCHC in the treatment groups. This confirms findings of Junior *et al.* [14], who reported an increase in MCV (indicating haemolytic anaemia) but a decrease in MCH levels and MCHC in treatment groups. Meena *et al.* [50] reported that MCV was increased, whereas no significant change in MCHC, MCH in anaemic rats administered PHZ for 7 days, which signified the induction of haemolytic anaemia. This is in agreement with Rajendran *et al.* [51], who reported that treatment groups restored the MCHC levels to normal, signifying their therapeutic efficacy in alleviating normal erythrocyte phenotype and curing hemolytic anaemia.

The decrease in RBC levels in the 2,4-DNPH-treated groups was improved through treatment with *H. acetosella* extracts. Sheth *et al.* [31] reported that the administration of 2,4-DNPH caused a decrease in RBC count. The results of the present study on the enhancement of RBC levels in the treatment groups may be ascribed to the restorative potential of *H. acetosella* extracts to prevent oxidative stress and 2,4-DNPH-induced haemolysis. Gheith & El-Mahmoudy [52] stated that PHZ-induced erythropenia reduced HCT and HGB indices that continued for 8 to 12 days. This could explain the decrease in RBC, HGB, and HCT seen after 14 days despite the administration of *H. acetosella* in some treatment groups, although it remains speculative.

In brief, after anaemia induction, all groups, with the exception of the control group, exhibited a notable decrease in HCT, RBC, and HGB, validating successful anaemia induction [49]. In the negative control group, these haematological indices continued to decrease through Day 22, signifying continued anaemia and slightly improved after Day 36. The slight improvement in the negative control group may be attributed to the animals' natural recuperation from the damaging effects of anaemia caused by 2,4-DNPH, a phenomenon also supported by the findings of [53]. In contrast, groups treated with ethanolic *H. acetosella* extract showed progressive recovery, with the most pronounced recoveries observed at higher doses and at later time points. By Day 36, the HCT, HGB, and RBC indices in treatment groups approached or exceeded those of the control group, indicating effective hematinic activity. While an overall trend of improved haematological indices was observed with increasing extract doses, the response was not strictly dose-dependent across all time points; a pattern also reported elsewhere [54]. Moreover, in Ana+100 HA, Ana+200 HA, and Ana+400 HA groups, a general trend of improvement is seen from Day 8 to Day 36 in parameters such as RBC,

HGB, and HCT, some even reaching levels close to those of the control group or even a few surpassing initial levels seen on Day 1.

Molecular docking is used to evaluate the binding free energy and the conformations involved in protein-ligand interactions. The binding affinity pertains to the Gibbs free energy that is produced from the ligand-receptor interaction. The more negative the values of the binding affinity signify stronger binding affinities [55]. Moreover, the more negative binding affinity values indicate more stable interactions between the ligands and the target proteins [56]. Compounds can regulate or disrupt biological pathways, either through enzyme activity inhibition, receptor blocking, or disrupting the interactions between specific molecules in the biological systems [56]. Moreover, molecular docking is utilized to determine new ligands for protein structure and has a role in structure-based drug design [57]. In the present study, docking scores revealed compounds such as beta-amyrone to have lower binding affinities to the selected protein targets. Additionally, compounds such as phytol and squalene have been reported to have antioxidant activities [40].

In spite of the hematinic activities of *H. acetosella* extracts observed in this study, some limitations should be acknowledged. Although the study combined *in vivo* haematological assessments with *in silico* molecular docking analysis, it did not include *in vivo* molecular validation or histopathological assessments to confirm the predicted mechanisms of action. Moreover, the small sample size may impede the generalizability of these findings. Further studies incorporating histopathological evaluations, *in vivo* molecular evaluation, larger sample sizes, and long-term safety assessments are recommended to further validate and expand upon these findings.

CONCLUSIONS

The ethanolic leaf extracts of *H. acetosella* showed significant hematinic activities as demonstrated in the study. The presence of flavonoids, anthocyanins, and other phytochemicals present in the ethanolic leaf extracts of *H. acetosella* may have resulted in their hematinic activities seen in ameliorating anaemic parameters in rats. Minerals, especially those involved in blood formation and HGB synthesis, such as copper present in the leaf of *H. acetosella*, may have caused the anti-anaemic activities observed in the study.

ACKNOWLEDGEMENTS

None.

AUTHOR CONTRIBUTIONS

WNK: Conceptualization, methodology, investigation, data analysis, data curation, writing-original draft, writing-review & editing, visualization, validation. KO: Conceptualization, methodology, investigation, validation, writing - review & editing, supervision. AWN: Conceptualization, methodology, investigation, validation, writing -review & editing, supervision. KRN: Conceptualization, methodology, validation, writing - review & editing. KM: Conceptualization, methodology, validation, writing - review & editing. ENN: Conceptualization, methodology, investigation, validation, writing - review & editing, supervision. All authors have approved the final version.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

DATA AVAILABILITY

All data for this work are included within the article.

REFERENCES

- [1] Cappellini MD, Motta I. Anemia in clinical practice—definition and classification: Does hemoglobin change with aging? *Seminars in Hematology*. 2015;10:01-52.
- [2] Safiri S, Kolahi A-A, et al. Burden of anemia and its underlying causes in 204 countries and territories, 1990–2019: Results from the global burden of disease study 2019. *Journal of Hematology & Oncology*. 2021;14.
- [3] Gemechu K, Asmerom H, et al. Anemia prevalence and associated factors among school-children of kersa woreda in eastern ethiopia: A cross-sectional study. *PLOS ONE*. 2023;18:e0283421.
- [4] Saha BUF, Choumessi AT, et al. Nutritional quality of three iron-rich porridges blended with moringa oleifera, hibiscus sabdariffa, and solanum aethiopicum to combat iron deficiency anemia among children. *Journal of Food Quality*. 2022;2022:1-10.
- [5] Woo S, Krzyzanski W, et al. Pharmacodynamic model for chemotherapy-induced anemia in rats. *Cancer Chemotherapy and Pharmacology*. 2008;62:123-33.
- [6] Muñoz M, Gómez-Ramírez S, et al. Pre-operative anaemia: Prevalence, consequences and approaches to management. *Blood Transfusion*. 2015; 16:13.
- [7] Thomas M Musyoka NWD, Arika M Wycliffe, Kelvin K Juma, Mutua D Nzioka, David Maina, Stanely K Waithaka, Mathew P.Ngugi, George O Orinda, Geoffrey M Karau, Eliud NM Njagi. In vivo antianaemic effect and safety of aqueous extracts of erythrina abyssinica and zanthoxylum usambarensis in mice models. *Journal of Hematology & Thromboembolic Diseases*. 2015;4.
- [8] Juul E, Sandra, Derman J, Richard, et al. Perinatal iron deficiency: Implications for mothers and infants. *Neonatology*. 2019;115:269-74.
- [9] Marcelin KKA, Innocent KK, et al. Anti-anemic activity of aqueous root bark extract of morinda lucida benth against phenylhydrazine-induced hemolytic anemia in wistar rats. *Journal of Pharmaceutical Research International*. 2025;04:07-37.
- [10] Silva LYdS, Ramos AdS, et al. Hibiscus acetosella: An unconventional alternative edible flower rich in bioactive compounds. *Molecules*. 2023;16:28.
- [11] Ssegawa P, Kasenene JM. Medicinal plant diversity and uses in the sango bay area, southern uganda. *Journal of Ethnopharmacology*. 2007;09:25-113.
- [12] Kapepula PM, Ngombe NK, et al. Comparison of metabolic profiles and bioactivities of the leaves of three edible congolese hibiscus species - pubmed. *Natural product research*. 2017 31(24):2885-2892.
- [13] Ahomadegbe M, Toklo M, et al. Plants used against diarrheal diseases in traditional african medicine: Cross- referencing, pharmaco-chemical for a valorization pedagogical perspective. *Journal of Organic Chemistry Research*. 2021;6:89-110.
- [14] Mbock A, Nguemfo E, et al. Anti-anaemic effect of aqueous leaves extract of hibiscus acetosella welw. Ex hiern (malvaceae) on two experimental models of anaemia induced by 2, 4-dinitrophenyl-hydrazine and blood lose in wistar rat. 2021;8:146-54.
- [15] Omara T, Omara T. East african quintessential plants claimed to be used as blood purifiers, cleansers, detoxifiers and tonics: An appraisal of ethnobotanical reports and correlation with reported bioactivities. *Bulletin of the National Research Centre* 2021 45:1. 2021-10-18;45.
- [16] Olusola JA, Akintan OB, et al. Heavy metals and health risks associated with consumption of herbal plants sold in a major urban market in southwest, nigeria. *Journal of health & pollution*. 2021, 11(31):210915.
- [17] Abubakar A. Evaluation of hematinic effects of aqueous leaf extract of hibiscus acetosella welw. Ex hiern on phenyl hydrazine-induced anemia in wistar rats. *IDOSR journal of biochemistry, biotechnology and allied fields*. 2024, 9(2):22-33
- [18] Kumar SS, Manoj P, et al. Effect of different drying methods on chlorophyll, ascorbic acid and antioxidant compounds retention of leaves of hibiscus sabdariffa L. *Journal of the science of food and agriculture*. 2015, 95(9):1812-20
- [19] Ibrahim H, Shayoub ME, et al. Effect of ethanolic leaf extract of moringa oleifera on aluminum-induced anemia in white albino rats. *Jordan Journal of Biological Sciences*. 2012;5:255-60.
- [20] Dumrongphuttidecha T, Thungmungmee S, et al. Antioxidant and free radical scavenging activity of hibiscus acetosella leaves extracts. *International Journal of Applied Pharmaceutics*. 2019:86-9.

- [21] Edeoga H, Okwu DE, et al. Phytochemical constituents of some nigerian medicinal plants. *African Journal of Biotechnology*. 2005;4.
- [22] Singh V, Kumar R. Study of phytochemical analysis and antioxidant activity of *allium sativum* of bundelkhand region. *International Journal of Life-Sciences Scientific Research*. 2017;3:1451-8.
- [23] Dubale S, Kebebe D, et al. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in ethiopia. *Journal of experimental pharmacology*. 2023;2:8:15.
- [24] Kumar S, Venkateshwar C, et al. Phytochemical screening of some compounds from plant leaf extracts of *holoptelea integrifolia* (planch.) and *celestrus emarginata* (grah.) used by gondu tribes at adilabad district, andhrapradesh, india. *International Journal of Engineering Science Invention*. 2013;Volume 2:65-70.
- [25] María R, Shirley M, et al. Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from guayas province ecuador. *Journal of King Saud University – Science*. 2018/10/01;30.
- [26] Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*. 2020;8.
- [27] Olivia NU, Goodness UC, et al. Phytochemical profiling and gc-ms analysis of aqueous methanol fraction of *hibiscus asper* leaves. *Future Journal of Pharmaceutical Sciences* 2021 7:1. 2021-03-04;7.
- [28] Balarabe MA, Balarabe MA. Nutritional analysis of *hibiscus sabdariffa* l. (roselle) leaves and calyces. *Plant*. 2019;7: 62.
- [29] Ezeigwe OC, Nzekwe FA, et al. Effect of aqueous extract of *f. Capensis* leaves and its combination with *c. Aconitifolius* leaves on essential biochemical parameters of phenylhydrazine-induced anemic rats - pubmed. *Journal of experimental pharmacology*. 2020;7:2;12.
- [30] Muhammad A, Ibrahim R, et al. Activity of aqueous extract of *phyllanthus niruri* linn in 2,4-dinitrophenylhydrazine induced anaemic rats. *Journal of Pharmacy and Pharmacology Research*. 2020;04.
- [31] Sheth PA, Pawar AT, et al. Antianemic activity of polyherbal formulation, raktavardhak kadha, against phenylhydrazine-induced anemia in rats. *Journal of Ayurveda and Integrative Medicine*. 2021/04/01;12.
- [32] Talapatra SN, Talukdar P, et al. Interaction of t-state haemoglobin and phytochemicals of *hygrophila spinosa* t. Anders: An approach by molecular docking. *World Journal of Pharmaceutical Research*. 2016;5:1354-69.
- [33] Bernard W. Makau* BGO, Rose Tanui, Raja Kannan. Phytochemical analysis and characterisation of extract of african rosemallow (*hibiscus acetosella*) leaves from kisii county, kenya. *Journal of Natural Products and Resources*. 2024;09;26:9.
- [34] Orororo OC, Asagba SO. Treatment with *hibiscus sabdariffa* l anthocyanins improve hematological parameters in rats exposed to cadmium. *Journal of Exploratory Research in Pharmacology*. 2022;7:146-50.
- [35] Rybalkina OY, Fedorova EF, et al. Erythropoiesis-stimulating properties of anthocyanin-containing complexes in cytostatic anemic syndrome. *Bulletin of experimental biology and medicine*. 2021 Apr;170.
- [36] Dai F, Miao Q, et al. Protective effects of flavonols and their glycosides against free radical-induced oxidative hemolysis of red blood cells. *Life sciences*. 2006;4;18:78.
- [37] Jorum OH, Piero NM, et al. Haematological effects of dichloromethane-methanolic leaf extracts of *carissa edulis* (forssk.) vahl in normal rat models. *Journal of Hematology & Thromboembolic Diseases*. 2016;4.
- [38] Ologundudu A, Ololade I, et al. Effect of *hibiscus sabdariffa* anthocyanins on 2, 4-dinitrophenylhydrazine-induced hematotoxicity in rabbits. *International Journal of Physical Sciences*. 2009;4.
- [39] Ufearoh SM, Akoh OU, et al. Gc-ms characterization and anti-anaemic/haematological activity of ethanol extract of *solanum aethiopicum* leaves. *Communication in Physical Sciences*. 2024;12.
- [40] Deliza H, Maibam D. Evaluation of elemental and volatile compounds of three selected plants of genus *hibiscus*. *European Journal of Medicinal Plants*. 2022;02:09.
- [41] Shorinwa OA, Mbajorgu AC, et al. Trace element composition and anti-anaemic effects of the ethanol extract of *harungana madagascariensis* lam. Ex poiret. In phenyl hydrazine induced anaemia in albino rats. *Journal of Pharmaceutical Research International*. 2018;02:23.
- [42] Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem, and tuber parts of *solena amplexicaulis* (lam.) gandhi using gc-ms. *International Scholarly Research Notices*. 2014;14;2014.
- [43] Davoodbasha MA, Edachery B, et al. An evidence of c16 fatty acid methyl esters extracted from microalga for effective antimicrobial and antioxidant property. *Microbial pathogenesis*. 2018;115.
- [44] Nyalo PO, Omwenga GI, et al. Antibacterial properties and gc-ms analysis of ethyl acetate extracts of *xerophyta spekei* (baker) and *grewia tembensis* (fresen). *Heliyon*. 2023;3;11:9.
- [45] Rubab M, Chelliah R, et al. Bioactive potential of 2-methoxy-4-vinylphenol and benzofuran from *brassica oleracea* l. Var. *Capitata f, rubra* (red cabbage) on oxidative and microbiological stability of beef meat. *Foods*. 2020;4:9.

- [46] Asami E, Kitami M, et al. Anti-inflammatory activity of 2-methoxy-4-vinylphenol involves inhibition of lipopolysaccharide-induced inducible nitric oxidase synthase by heme oxygenase-1. *Immunopharmacology and immunotoxicology*. 2023 ;45.
- [47] Amala VE, Jeyaraj M. Determination of antibacterial, antifungal, bioactive constituents of triphala by ft-ir and gc-ms analysis. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2014;6:123-6.
- [48] Arroyave Murillo LM, Gómez Barrera M, et al. Determinación de la actividad antioxidante de *malvaviscus arboreus* cav. (*malvavisco*). *Revista Cubana de Plantas Medicinales*. 2018;23.
- [49] Chauhan SP, Sheth NR, et al. Hematinic effect of fruits of *opuntia elatior* mill. On phenylhydrazine-induced anemia in rats. *AYU (An International Quarterly Journal of Research in Ayurveda)*. 2015;36.
- [50] Agbor GA, Agbor GA, et al. Academic journals - african journal of biotechnology - haematinic activity of *hibiscus cannabinus*. *African Journal of Biotechnology*. 2005;08;31:4.
- [51] Rajendran K, Chellappan DR, et al. Therapeutic efficacy of punarnavadi mandura against phenylhydrazine-induced hemolytic anemia in rats. *J Tradit Complement Med*. 2025;15:93-104.
- [52] Gheith I, El-Mahmoudy A. Laboratory evidence for the hematopoietic potential of *beta vulgaris* leaf and stalk extract in a phenylhydrazine model of anemia. *Brazilian Journal of Medical and Biological Research*. 2018;11:51.
- [53] Nneoyi-Egbe AF, Onyenweaku E, et al. Haematinic and hepatoprotective properties of *telfairia occidentalis* fruit mesocarp on phenylhydrazine-induced anaemia in experimental rats. *Biochemistry Research International*. 2023;9;2023.
- [54] Nurhayati T, Fathoni MI, et al. Effect of *moringa oleifera* leaf powder on hematological profile of male wistar rats. *J Blood Med*. 2023;14:477-85.
- [55] Sururi AM, Rahayu DA, et al. Gc–ms and adme profile analysis of *carcinoscopus rotundicauda* bioactive compounds and their potential as covid-19 antiviral. *Future Journal of Pharmaceutical Sciences* 2023 9:1. 2023-12-11;9.
- [56] Fattara FP, Idami Z, et al. Molecular docking in red spinach plants (*amaranthus tricolor* l.) as an inhibitory agent for genetic anemia. *Science Midwifery*. 2024;08;31:12.
- [57] Ralte L, Khiantge L, et al. Gc-ms and molecular docking analyses of phytochemicals from the underutilized plant, *parkia timoriana* revealed candidate anti-cancerous and anti-inflammatory agents. *Sci Rep*. 2022;12:3395.

Disclaimer: The contents of all publications are solely those of the individual author(s) and contributor(s) and not of JABET and/or the editor(s). JABET and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any of the contents.