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Integration of In Vitro, In Vivo and In Silico Molecular Docking Approaches to Unravel the Antianaemic Properties of Aqueous Extracts of *Hibiscus acetosella* Leaves

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ABSTRACT

Introduction: Anaemia remains a major global health challenge, necessitating the exploration of alternative therapeutic agents from medicinal plants. *Hibiscus acetosella* has traditionally been used in the treatment of anaemia.

Aim: This study evaluated the phytochemical composition and antianaemic effects of the aqueous leaf extract of *H. acetosella* in 2,4-dinitrophenylhydrazine (2,4-DNPH)-induced anaemia model in Wistar rats.

Methods: Phytochemical screening was performed using qualitative assays and gas chromatography-mass spectrometry (GC-MS) followed by in silico molecular docking analyses of bioactive compounds. Following baseline haematological assessments, the rats were divided into six groups ($n = 3$) and anaemia was induced using 2,4-DNPH (40 mg/kg body weight [b.w.]) for 7 days. Subsequently, the aqueous extract was administered orally (100, 200, 400 mg/kg b.w.) for 28 days. Haematological data were analysed using two-way repeated measures analysis of variance (RM-ANOVA), with statistical significance set at $p < 0.05$.

Results: Qualitative analysis revealed the presence of anthocyanin, flavonoids, phenols, steroids and terpenoids. The GC-MS identified compounds including 2-methoxy-4-vinylphenol, hexadecanoic acid, methyl ester and 3-pentenoic acid, 3-ethyl-, methyl ester. Induction of anaemia resulted in a significant ($p < 0.05$) decrease in haematological parameters of haematocrit (HCT), red blood cell (RBC) and haemoglobin (HGB), accompanied by increases in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Aqueous extract significantly ($p < 0.05$) increased HGB, HCT and RBC in the treatment groups after 28 days. Molecular docking analyses revealed that N-[4-methoxy-3-methoxycarbonyl)benzoyloxy]succininide exhibited the strongest binding affinities (from −4.4 to −7.7 kcal/mol) with key protein targets.

Conclusion: These findings suggest that *H. acetosella* extract exhibits promising antianaemic activity in this study and warrants further investigation as a potential source of plant-based therapies for the management of anaemia.

1 | Introduction

Anaemia refers to a reduction in RBC, HCT or HGB levels [1]. It affects almost 40% of toddlers and 30% of reproductive females [2], thus making it a major global public health concern, specifically in developing countries [3]. Furthermore, in Kenya, the prevalence of anaemia in toddlers and iron deficiency anaemia (IDA) is 45.9% and 26.9%, respectively [4]. Anaemia is associated

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with the increased risk of chronic kidney disease (CKD) progression and contributes to reduced standards of living in patients with CKD [5]. Moreover, it is associated with increased mortality in patients with heart failure [6]. The pathophysiology of anaemia in patients with heart failure may include reduced iron absorption, release of stored iron, gastrointestinal (GI) blood loss and nutritional deficiencies [6].

Rapid growth, impaired iron absorption, gut bleeding and gestation commonly lead to IDA [7]. Haemolytic anaemia and IDA are the most common types of anaemia. Treatment of anaemia varies depending on the type and may include oral vitamin B_{12} or B_9 and iron, corticosteroids or immunosuppressants, blood transfusion, erythropoietin (Epo) injections or bone marrow transplants [8]. The absence of necessary equipment for blood transfusion in rural settings poses a challenge for the treatment of severe anaemia [9]. Furthermore, treatment of IDA involves using oral supplementation or treating intestinal bleeding [10]. The oral iron supplementation causes unfavourable side effects in patients with IDA, including nausea, diarrhoea, constipation and decreased appetite [10]. GI side effects have been attributed to unabsorbed iron reaching the intestines, which interacts with intestinal microbiota, causing changes in microbial composition, oxidative stress and inflammation [11]. Inhibitors of angiotensin-converting enzyme (ACE) and metformin may cause reduced RBC production, thus contributing to anaemia [12]. Management of anaemia through conventional medicine is quite expensive [13]. Additionally, because of the undesirable side effects of the conventional therapies, attention should be focused on the utilisation of medicinal plants for anaemia treatment [8].

Several *Hibiscus* species, such as *Hibiscus cannabinus,* have been investigated for their hematinic properties over the years [14]. The *Hibiscus acetosella* Welw. Ex Hiern (African rosemallow) is a member of the Malvaceae family, indigenous to Africa, and its leaves are traditionally consumed as vegetables [15]. In Uganda and the Democratic Republic of Congo, decoction drinks are made from the leaves and shoots of *H. acetosella* and then consumed for their antianaemia and antipyretic properties [16, 17]. Qualitative screening of *H. acetosella* aqueous leaf extract revealed the presence of alkaloids, saponins, steroids and coumarins [18].

Studies in Uganda and Cameroon have demonstrated hematinic effects of aqueous extract of *H. acetosella* leaves in anaemic Wistar rats [18, 19]. These studies, however, did not analyse the phytochemical compounds present in aqueous extracts of *H. acetosella* using GC-MS, while integrating these findings with the in silico molecular analysis. Moreover, biotic factors such as herbivores and pathogens, alongside abiotic factors encompassing salinity levels, drought susceptibility, irrigation patterns, temperature variations, soil fertility, altitude and light exposure, collectively influence the synthesis of secondary metabolites in medicinal plants [20]. Consequently, the composition of secondary metabolites and hematinic activity of Kenyan *H. acetosella* may vary from those reported in other geographical areas. Therefore, this study aimed is aimed at characterising the phytochemical composition of aqueous leaf extract of *H. acetosella* using GC-MS and evaluating its antianaemic effect in rats. Moreover, the study sought to investigate the molecular interactions of identified phytochemicals with selected anaemia-related targets by in silico molecular docking.

2 | Materials and Methods

2.1 | *H. acetosella* Preparation and Extraction

Fresh *H. acetosella* leaves were sourced from Oyugis, Homa Bay County, Kenya, at coordinates 0° 30′ 0″ S and 34° 43′ 0″ E. They were identified and authenticated by a taxonomist at the East African Herbarium of the National Museums of Kenya (Reference Number: NMK/BOT/CTX/2/ID/20/2023). The leaves were further rinsed with distilled water, cut into small pieces and air-dried in the shade at 27 ± 2°C for 14 days. They were ground into a powder and stored in airtight zip-lock bags at −20°C following the procedure outlined by Kumar et al. [21].

2.2 | Preparation of Aqueous Extract

The aqueous extract of *H. acetosella* was prepared according to the cold-maceration method described by Malomo et al. [22]. Approximately 194.28 g of powdered *Hibiscus* was cold-macerated in 1942.8 mL of distilled water for 72 h at 4°C in the cold room. The mixture was filtered to obtain the aqueous extract filtrate. The filtrate was freeze-dried using a freeze-dryer (FreeZone 4.5 L Benchtop Freeze Dryer, Labconco, Kansas City, United States) to obtain powdered aqueous extract. The extract was stored in an airtight container and in a freezer at −20°C until further use.

2.3 | Qualitative Screening of Phytochemical Constituents in the Aqueous Extract

The phytochemical compounds were determined following published procedures as follows: cardiac glycosides and hydrolysable tannins [23], flavonoids [24], saponins and phenols [25], phlobatannins [26], terpenoids, glycosides and steroids [27], anthraquinones [28], quinones [29] and anthocyanins [30]. Tannins' presence was determined following the procedure outlined by [31].

2.4 | GC-MS Analysis of Aqueous *H. acetosella* Leaf Extract

About 0.23 g of freeze-dried aqueous leaf extract of *H. acetosella* was mixed with 1.2 mL of absolute methanol following the procedure outlined by Rn et al. [32], with slight modifications. The mixture was then filtered through a 0.2 μm polypropylene filter to obtain the methanol filtrate in a vial, and the extract was stored in the freezer (−20°C) for GC-MS analysis. The extract was dried in a MIVAC DNA Concentrator (37°C, 40 min) and then reconstituted with methanol solvent (1 mL), shaken and filtered using NY 0.22 μm syringe filters into an autosampler vial and the filtrate was then run through the GC-MS equipment (GCMS-QP2010 SE, Shimadzu), equipped with a GC-2010 plus gas chromatograph interfaced to a mass spectrometer. The system was operated under the following conditions: capillary

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column BPX5 (30.0 m × 0.25 mm i. d. × 0.25μm film thickness); injection temperature of 250.0°C; and the injection mode was set to split. The carrier gas was helium (99.999%) at a flow control mode of linear velocity, pressure of 9.4 psi, total flow of 13.2 mL/min, a column flow of 0.93 mL/min, a linear velocity of 35.0 cm/sec, purge flow of 3.0 mL/min and a split ratio of 10.1 (injection mode: split). The column oven temperature programme was 50°C (held for 1 min), increased at a 4°C/min rate to 200°C (held for 2 min), then increased at a 10°C rate to 260°C (held for 10 min); the total programme time was 56.50 min. The MS parameters were: the ionisation voltage was 70 eV and the ionisation technique was set as electron ionisation; the ion source temperature was 200°C, the interface temperature was 280°C and the solvent cut time was 3.5 min. 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. The injection volume was 1 μL (Scale 54 mm, ID 01), using a CTC Analytics (Combi PAL) autosampler.

Mass spectra of unknown *H. acetosella* compounds were matched with reference spectra in the National Institute of Standards and Technology (NIST14.lib) database to establish their structures, molecular weights (MWs) and names.

2.5 | In-Vivo Evaluation of Antianaemic Activity of *H. acetosella* Aqueous Leaf Extract

2.5.1 | Laboratory Animals

The study used adult Wistar rats (both sexes), aged 8–10 weeks, weighing 150–210 g. The animals were bred and raised in standard cages in the animal house at the Department of Biochemistry, University of Nairobi, Kenya, under standard conditions of a 12-h light/dark cycle and temperature (25°C–27°C). The rats were fed standard rat pellets and water ad libitum.

2.5.2 | Experimental Design and Animal Treatment

Wistar rats were randomly allocated into six cages (three rats per cage) using a Microsoft Excel-generated randomisation procedure. The cages were labelled, and a second independent Microsoft Excel-generated randomisation was performed to assign each cage to one of the six experimental groups (normal control, negative control, positive control, aqueous extract 100 mg/kg b.w., 200 mg/kg b.w. and 400 mg/kg b.w.) as illustrated below (Table 1). Because of the limited solubility of 2,4-DNPH [33], it was dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solution. The final solution of 2,4-DNPH was prepared before each use, at a final concentration of 0.1% DMSO following a method described by Gabardo et al. [34], with slight modifications. Group 1 was assigned as the normal control and remained untreated throughout the study; this group did not receive 2,4-DNPH. The remaining rat groups were induced anaemia through oral administration of 2,4-DNPH (40 mg/kg b.w.), for 7 consecutive days) following the protocol described by Ezeigwe et al. [35] with slight modifications. Rats were considered anaemic when they had ≥ 30% reduction in RBC [36]. Oral administration (using oral gavages) of aqueous leaf extracts and folic acid began immediately on Day 8, after confirmation of the anaemic states of rats and continued daily for 28 consecutive days. Aqueous extracts and folic acid were both administered in a fixed volume of 1 mL of distilled water per rat according to allocated treatment groups.

TABLE 1 | Animal grouping.

Animal Group	Treatment	Anaemic/ not anaemic
Normal control	Distilled water	Not anaemic
Negative control	Distilled water	Anaemic
Positive control	75 μg/kg b.w/ day (folic acid)	Anaemic
Aqueous (100 mg/ kg b.w.)	100 mg/kg b.w./ day (aqueous extract)	Anaemic
Aqueous (200 mg/ kg b.w.)	200 mg/kg b.w./ day (aqueous extract)	Anaemic
Aqueous (400 mg/ kg b.w.)	400 mg/kg b.w./ day (aqueous extract)	Anaemic

Abbreviation: b.w., body weight.

The experimental duration was selected to allow monitoring of progressive haematological recovery during the treatment period. Although rat RBCs have an estimated lifespan of about 60 days [37], alterations in the circulating haematological parameters during treatment were assessed rather than the complete turnover of RBCs.

2.5.3 | Blood Collection and Haematological Analysis

Blood samples were collected at baseline (preinduction) (Day 1), postinduction (Day 8), then on Day 22 and Day 36. Blood samples (0.2–0.25 mL) were collected through tail bleeding during the study and later through cardiac puncture (3 mL) at the end of the experiment.

Whole blood was collected in EDTA-treated plastic tubes for haematological analysis using a haematology analyser (Mindray Auto Haematology Analyser, Model: BC-10, Shenzhen Mindray Bio-Medical Electronics Co. Ltd., China). The parameters measured were the HCT, mean corpuscular volume (MCV), RBC counts, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and HGB concentration.

2.6 | Molecular Docking Analysis

Molecular docking was done following the procedure outlined by Talapatra et al. [38]. The protein receptors (PDB formats) downloaded from the RCSB Protein Data Bank (https://www.rcsb.org/) with the following Protein Data Bank Identifiers (PDB IDs): human nuclear factor erythroid 2-related factor 2 (Nrf2) (PDB ID: 7O7B), rat HGB (PDB ID: 3DHT), human Epo receptor (EpoR)

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(PDB ID: 6MOE), prolyl hydroxylase domain-containing protein 2 (PHD2) (PDB ID: 3HQR), human Epo bound to its receptor complex (PDB ID: 1EER), T-state HGB (PDB ID: 2D5Z), methemoglobin (PDB ID: 3HF4), transferrin (PDB ID: 6WB6), human hepcidin (1M4F) and ferritin (PDB ID: 1GWG) and human PHD2 (PDB ID: 2G19). The test ligands (3D conformers-SDF formats), which were compounds of *H. acetosella* aqueous extract, were downloaded (https://pubchem.ncbi.nlm.nih.gov/). BIOVIA Discovery Studio Visualizer (v25.1.0.24284) was used to identify the grid coordinates of native ligands attached to the crystallised structures of the proteins (PDB formats). The native ligands and water molecules were then deleted from the proteins in BIOVIA Discovery Studio. AutoDockTools in MGLTools Version 1.5.6 (https://ccsb.scripps.edu/mgltools/downloads/) was used to prepare the proteins. Protein PDB files were loaded in AutoDockTools, hydrogen atoms added to polar atoms, Kollman charges added and saved in PDBQT format. The SDF formats of the ligands were converted to PDB formats using PyMOL (https://www.pymol.org/), then prepared using AutoDockTools. The Gasteiger charges were added to the ligands and files were saved as PDBQT files. Molecular docking of ligands to proteins was done utilising AutoDock Vina (https://vina.scripps.edu/downloads/). PyMOL was used to visualise the docked ligand poses and save the poses in PDB formats for visualisation in BIOVIA Discovery Studio. Protein-ligand 3D-interactions were visualised using BIOVIA Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download).

2.6.1 | Grid Coordinates and Docking Validation

The grid coordinates were as follows: 1EER (center_x = −15.593, center_y = 19.506, center_z = 102.288), 1GWG (center_x = 14.156000, center_y = 1.894000, center_z = 53.165000), 1M4F (center_x = 0.355, center_y = −0.308, center_z = −0.118), 2D5Z (center_x = −2.347480, center_y = −4.917960,center_z = 14.771640),2G19(center_x = 3.547364, center_y = 44.901409, center_z = 25.880455), 3DHT (center_x = 26.008767, center_y = −32.123372, center_z = 41.082000), 3HF4 (center_x = 16.197698, center_y = −13.175116, center_z = 16.936419), 3HQR (center_x = 19.218400, center_y = −6.234200, center_z = −4.652600), 6MOE (center_x = −12.508625, center_y = −5.256375, center_z = −14.978375), 6WB6 (center_x = −14.377000, center_y = −1.751000, center_z = 9.169000) and 7O7B (center_x = 1.176, center_y = 0.636, center_z = −1.618). AutoDock Vina parameters were as follows: grid box size (size_x = 15, size_y = 15, size_z = 15), energy_range = 4, num_modes = 9 (number of binding modes), exhaustiveness = 8 and seed = 12345.

To validate the molecular docking protocol, the cocrystallised native ligands were extracted from the corresponding receptor crystal structures using BIOVIA Discovery Studio. The native ligands were prepared using the same docking protocol applied to the test compounds. The prepared native ligands were subsequently redocked into their respective protein binding sites. The resulting docked poses were then compared with the prepared crystallographic ligand conformations and the root mean square deviation (RMSD) values were calculated based on the corresponding heavy atoms using BIOVIA Discovery Studio.

2.6.2 | Biological Relevance of Selected Protein Targets and Ligand References

The 2G19 and 3HQR are crystal structures of human PHD2 enzymes. PHD hydroxylates hypoxia-inducible factor-alpha (HIF-α), hence regulating the ubiquitination and degradation of HIF [39]. The inhibition of PHD2 enzymes by the ligands can stabilise HIF-1α and potentially enhance erythropoietic responses. 6MOE represents the crystal structure of human EpoR [40], which is relevant to Epo-mediated modulation of erythropoiesis and haematopoiesis. 1EER is a crystal structure of human Epo bound to the human EpoR. Epo is a haematopoietic growth factor that modulates erythropoiesis in the bone marrow [41]. 1EER and 6MOE were used since Epo signalling through EpoR modulates erythroid progenitor cell proliferation and differentiation.

3DHT represents the crystal structure of rat HGB; relevant to the study since the models used were rats. The 2D5Z represents the deoxy (T)-state of human HGB [38]. Moreover, this study is aimed at exploring the effects of different compounds of *H. acetosella* on 2D5Z as potential heterotropic effectors. 6WB6 is the crystal structure of transferrin [42]. Transferrin functions in the sequestration and transportation of iron by binding it tightly and reversibly [43]. 1GWG represents the crystal structure of human ferritin [42]. Ferritin modulates the sequestration, storage of ferrous (Fe^{2+}) iron and iron levels [44]. 1M4F represents human hepcidin [45]. Hepcidin production increases with the interaction of iron or inflammation and declines during erythropoiesis [45]. Hepcidin is a key regulator of iron homeostasis.

7O7B represents the Nrf2 protein structure [46]. Phenylhydrazine (PHZ) causes anaemia through oxidative stress induction in rats [9]. Nrf2 dissociates from Keap1 during oxidative stress, then translocates to the nucleus and combines with the antioxidant response element (ARE), activating downstream reactive oxygen species (ROS) scavenging enzymes (catalase/superoxide dismutase), exerting antioxidant effects [46]. This justifies the relevance of using Nrf2 in this study. The selected protein receptors represent molecular pathways involved in haematopoiesis, oxygen transportation by HGB, iron homeostasis and antioxidant defence mechanisms.

Moreover, folic acid is used in the management of anaemia [47]. Quercetin is known for its antioxidant activity [48]; thus, it was also used as a reference for comparison with the *H. acetosella* compounds.

2.7 | Drug-Likeness Profiling

Pharmacokinetic properties and drug-likeness of the identified phytochemicals (retrieved from PubChem) were evaluated using SwissADME (https://www.swissadme.ch/). Toxicity profiles (hepatotoxicity, nephrotoxicity and cytotoxicity) of the identified phytochemicals represented by their Simplified Molecular Input Line Entry System (SMILES) structures were predicted using ProTox-3.0 (https://tox.charite.de/protox3/). The GI, blood-brain barrier (BBB), permeability-glycoprotein substrate (P-gp) and cytochrome P450 3A4 (CYP3A4) of chemical compounds were predicted using SwissADME.

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2.8 | Statistical Analysis

The haematological data are represented as mean ± standard error of mean (SEM). Normality was assessed using the Shapiro–Wilk test, and equal variance was assessed using the Brown–Forsythe test. Moreover, the sample size was $n = 3$, the α level was set at 0.05 and the statistical tests were two-tailed. The data were analysed using two-way repeated measures analysis of variance (RM-ANOVA) with treatment as the between-subject factor and time (Day 1, Day 8, Day 22 and Day 36) as the within-subject factor. Following the detection of significant main interactions or effects, Tukey's honestly significant difference (HSD) pairwise multiple comparison post hoc test was conducted for statistically significant differences in the means among treatment groups and within treatment groups over time while controlling for Type I error. Graphs for haematological data were prepared using GraphPad Prism (Version 8.0.1). All statistical analyses were performed using SigmaPlot (Version 16), with statistical significance set at $p < 0.05$. Effect size was calculated following the formula outlined by Mordkoff [49] as follows: $n_p^2 = SSeffect / SSeffect + SSerror$, where n_p^2 = partial eta-squared; $SSeffect$ = sum of squares of effect and $SSerror$ = error sum of squares. Therefore, partial eta-squared = effect size, $SSeffect$ = sum of squares for the factor of interest (treatment, time or treatment with time interaction) and $SSerror$ = residual (error) sum of squares.

3 | Results

3.1 | Phytochemical Composition of Aqueous Leaf Extract of *H. acetosella*

The percentage yield for the *H. acetosella* aqueous extract was 19.05%.

3.1.1 | Qualitative Phytochemical Composition of Aqueous Leaf Extract of *H. acetosella*

The phytochemical screening of aqueous extract in *H. acetosella* leaves showed the presence of glycosides, phenols, hydrolysable tannins, quinones, steroids, anthocyanins, terpenoids, cardiac glycosides, flavonoids, phlobatannins, saponins, tannins and anthraquinones (Table 2).

TABLE 2 | Qualitative screening of phytochemical constituents in aqueous extract from *H. acetosella* leaves.

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

Note: +, present in trace amount; ++, present in moderate amount; +++, present in high amount; and –, absent.

3.1.2 | GC-MS Analyses of Aqueous Extract of *H. acetosella*

The GC-MS analysis of the *H. acetosella* aqueous extract revealed the presence of 17 compounds (Figure 1). 3-pentenoic acid, 3-ethyl-, methyl ester (36.17%), 5-iodo-4-oxohex-2-enoic acid, methyl ester (18.92%), benzaldehyde, 2-hydroxy-4-methyl- (16.29%), DL-proline, 5-oxo-, methyl ester (5.42%), among others, whereas the lowest peak was exhibited by trisiloxane, 1,1,3,3,5,5-hexamethyl- (0.66%). The component name, peak names, retention times, MWs and formulas, area and area percentages of the constituents present in *H. acetosella* extract are presented in Table 3. Eight compounds had a similarity index (SI) of 80 and above, such as DL-proline, 5-oxo-, methyl ester, 2-methoxy-4-vinylphenol and dibutyl phthalate. The SI and retention index (RI) of the constituents present in the extract are presented in Table 4.

Key: TIC-total ion chromatogram; *x*-axis: retention time (in minutes); *y*-axis: total ion current (intensity or abundance) (measured in counts per second [cps]); peak: different peaks correspond to individual phytochemical compounds eluting from the GC column at particular retention times.

3.2 | Antianaemic Activity of Aqueous Leaf Extract of *H. acetosella*

3.2.1 | Effect of Aqueous *H. acetosella* Leaf Extract on RBC Counts

The effect of the aqueous extract of *H. acetosella* on RBC counts in anaemic Wistar rats is shown in Figure 2. A two-way RM ANOVA revealed significant effects of treatment ($F(5, 12) = 27.299$, $p < 0.001$, $SS = 94.065$, $n^2p = 0.911$), time ($F(3, 36) = 198.896$, $p < 0.001$, $SS = 151.968$, $n^2p = 0.943$) and a significant treatment and time interaction ($F(15, 36) = 19.560$, $p < 0.001$, $SS = 74.726$, $n^2p = 0.891$), indicating that changes in the RBC counts over time differed among the treatment groups.

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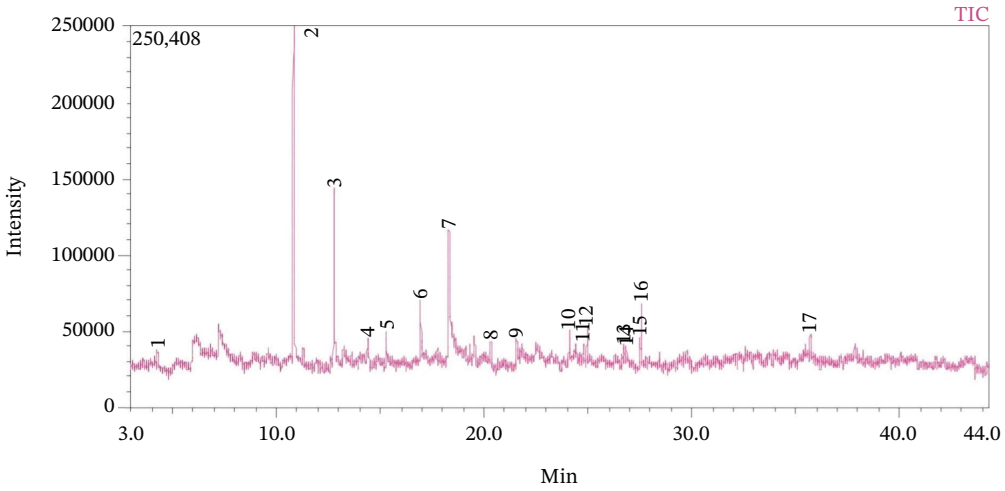

FIGURE 1 | GC-MS chromatographic profile of aqueous extract of *H. acetosella* leaves.

The residual sum of squares (SS) was 9.169. No significant differences in RBC counts were observed on Day 1 ($p > 0.050$), whereas on Day 8, RBC count in the normal control group was significantly higher ($p < 0.001$) than that in positive and negative controls and aqueous-treated groups. On Day 22, the RBC count in the normal control was not significantly higher ($p = 0.693$) than that in the aqueous (400 mg/kg b.w.) group, but was significantly higher ($p < 0.001$) than that in the remaining groups. Moreover, the RBC count in the aqueous (400 mg/kg b.w.) group was significantly higher ($p < 0.05$) than that in the negative and positive controls and the remaining aqueous-treated groups. On Day 36, RBC count in aqueous (400 mg/kg b.w.) was significantly higher ($p < 0.001$) than that in the negative and positive control, aqueous (100 mg/kg b.w.) and aqueous (200 mg/kg b.w.) groups except in normal control group ($p = 0.853$) The RBC count in the normal control group was significantly higher ($p < 0.001$) than in the negative control, aqueous (100 mg/kg b.w.), and ($p = 0.001$) in aqueous (200 mg/kg b.w.) and positive control group. All other pairwise multiple comparisons among RBC counts in different groups were not significant ($p > 0.05$).

3.2.2 | Effect of Aqueous *H. acetosella* Leaf Extracts on HGB Concentration

The changes in HGB concentration during treatment of anaemic Wistar rats with aqueous extract of *H. acetosella* are recorded in Figure 3. A two-way RM ANOVA revealed significant effects of treatment ($F(5, 12) = 91.822$, $p < 0.001$, $SS = 261.484$, $n^2p = 0.918$), time ($F(3, 36) = 305.331$, $p < 0.001$, $SS = 592.353$, $n^2p = 0.962$) and a significant treatment and time interaction ($F(15, 36) = 32.255$, $p < 0.001$, $SS = 312.876$, $n^2p = 0.931$), indicating that changes in the HGB concentrations over time differed among the treatment groups. The residual SS was 23.280. On Day 1, the aqueous (400 mg/kg b.w.) group had significantly higher ($p < 0.05$) HGB concentration than the normal, negative and positive controls and other aqueous-treated groups. Moreover, the negative control group had significantly higher HGB concentration than the positive control ($p = 0.006$), normal control ($p = 0.042$) and aqueous (200 mg/kg b.w.) ($p = 0.037$) groups, but this was not significantly higher ($p = 0.905$) than aqueous (100 mg/kg b.w.). On Day 8, HGB concentration in the normal control was significantly higher ($p < 0.001$) than that in the remaining groups. On Day 22, HGB concentration in the normal control was not significantly higher ($p = 0.571$) than that in aqueous (400 mg/kg b.w.). Although the normal control group had statistically significantly higher ($p < 0.001$) HGB concentration than the positive and negative controls, aqueous (100 mg/kg b.w.) and aqueous (200 mg/kg b.w.) ($p = 0.042$). Moreover, both aqueous (200 mg/kg b.w.) and aqueous (400 mg/kg b.w.) groups exhibited significantly higher ($p < 0.001$) HGB concentrations than aqueous (100 mg/kg b.w.), positive and negative control groups. On Day 36, the normal control exhibited significantly higher ($p < 0.05$) HGB concentration than the negative and positive controls and the aqueous (100 mg/kg b.w.) group. All aqueous-treated groups had significantly higher ($p < 0.001$) HGB concentrations than that in the negative control group.

3.2.3 | Effect of Aqueous *H. acetosella* Extract on HCT Levels

The effect of *H. acetosella* extract on HCT levels in anaemic Wistar rats is shown in Figure 4. A two-way RM ANOVA revealed significant effects of treatment ($F(5, 12) = 610.347$, $p < 0.001$, $SS = 3376.832$, $n^2p = 0.991$), time ($F(3, 36) = 1873.781$, $p < 0.001$, $SS = 4960.576$, $n^2p = 0.994$) and a significant treatment and time interaction ($F(15, 36) = 247.744$, $p < 0.001$, $SS = 3279.341$, $n^2p = 0.990$), indicating that changes in the HCT levels over time differed among the treatment groups. The residual SS was 31.768. On Day 1, the negative control exhibited significantly higher ($p < 0.001$) HCT levels than the normal and positive controls and aqueous-treated groups. On Day 8, the normal control group demonstrated statistically significantly higher ($p < 0.001$) HCT levels than the positive and negative controls and the aqueous-treated groups. On Day 22, the normal control exhibited significantly higher ($p < 0.05$) HCT levels than the negative and positive controls and the aqueous-treated groups. Moreover, aqueous (400 mg/kg b.w.) had significantly higher ($p < 0.001$) HCT levels than the negative and positive controls, aqueous (100 mg/kg b.w.) and aqueous (200 mg/kg b.w.) groups. On Day 36, HCT levels in aqueous (400 mg/kg b.w.) were not significantly higher ($p = 0.224$) than those in the normal control, but were significantly higher ($p < 0.001$) than those in

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TABLE 3 | List of GC-MS detected phytochemical compounds, their retention time, area (%), molecular weight and formula in the aqueous extract of *H. acetosella* leaves.

Peak#	RT	Area	Area %	Name	MF	MW	GC	BA
1	4.274	39270	2.24	2-pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116	Hydroxyketone	Antimicrobial [25]
2	10.816	633144	36.17	3-pentenoic acid, 3-ethyl-, methyl ester	$C_8H_{14}O_2$	142	Acid	Antioxidant [50]
3	12.761	331273	18.92	5-iodo-4-oxohex-2-enoic acid, methyl ester	$C_7H_9IO_3$	268	Alpha, beta-unsaturated ketone	Not found
4	14.39	23434	1.34	1,2,4,5-cyclohexanetetrol, (1.alpha.,2.alpha.,4.alpha.,5.beta.)-	$C_6H_{12}O_4$	148	Unknown	Not found
5	15.296	44926	2.57	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	Phenol	Antioxidant [51]
6	16.936	94855	5.42	DL-proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	Alpha-amino acid ester	Antioxidant and anti-inflammatory [52]
7	18.307	285165	16.29	Benzaldehyde, 2-hydroxy-4-methyl-	$C_8H_8O_2$	136	Aldehyde	Antioxidant possibility [53]
8	20.32	14631	0.84	Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl ester, (R)-	$C_8H_{14}O_5$	190	Dimethyl ester	Not found
9	21.576	37020	2.11	4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	$C_{13}H_{20}O_2$	208	Unknown	Not found
10	24.1	11536	0.66	Trisiloxane, 1,1,3,3,5,5-hexamethyl-	$C_6H_{20}O_2Si_3$	208	Linear siloxane	Not found
11	24.776	19129	1.09	N-[4-methoxy-3-methoxycarbonyl) benzoyloxy]succininide	$C_{14}H_{13}NO_7$	307	Unknown	Not found
12	24.957	54950	3.14	5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	$C_{12}H_{20}O$	180	Unknown	Not found
13	26.753	28441	1.62	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	Antimicrobial [54]
14	26.854	13575	0.78	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Fatty acid ester	Antibacterial [55], antifungal [56], anti-inflammatory [57]
15	27.511	15669	0.9	Tridecanoic acid	$C_{13}H_{26}O_2$	214	Acid	Antimicrobial [58]
16	27.573	65067	3.72	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	Phthalate	Induces peroxisome proliferation [59]
17	35.71	38573	2.2	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330	Monoacylglycerol	Antiviral [60]
		1750658	100					

Abbreviations: BA, biological activity; GC, group of compounds; MF, molecular formula; MW, molecular weight; RT, retention time.

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TABLE 4 | List of GC-MS detected phytochemical compounds, their similarity index and retention index in the aqueous extract of *H. acetosella* leaves.

Compound	SI	RI
2-pentanone, 4-hydroxy-4-methyl-	80.0	845.0
3-pentenoic acid, 3-ethyl-, methyl ester	79.0	969.0
5-iodo-4-oxohex-2-enoic acid, methyl ester	78.0	1379.0
1,2,4,5-cyclohexanetetrol, (1.alpha.,2. alpha.,4.alpha.,5.beta.)-	74.0	1472.0
2-methoxy-4-vinylphenol	85.0	1293.0
DL-proline, 5-oxo-, methyl ester	93.0	1091.0
Benzaldehyde, 2-hydroxy-4-methyl-	81.0	1316.0
Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl ester, (R)-	63.0	1292.0
4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	69.0	1457.0
Trisiloxane, 1,1,3,3,5,5-hexamethyl-	58.0	787.0
N-[4-methoxy-3-methoxycarbonyl) benzoyloxy]succininide	67.0	2501.0
5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	79.0	1309.0
7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	81.0	2081.0
Hexadecanoic acid, methyl ester	82.0	1878.0
Tridecanoic acid	81.0	1670.0
Dibutyl phthalate	94.0	2037.0
Glycerol 1-palmitate	78.0	2482.0

Abbreviations: RI, retention index; SI, similarity index.

the remaining groups. Normal control had significantly higher ($p < 0.001$) HCT levels than the remaining groups.

3.2.4 | Effect of Aqueous *H. acetosella* Extract on MCV Levels

Figure 5 shows the effect of the aqueous leaf extract of *H. acetosella* on the MCV levels in anaemic Wistar rats. A two-way RM ANOVA revealed significant effects of treatment ($F(5, 12) = 40.203$, $p < 0.001$, $SS = 184.389$, $n^2p = 0.899$), time ($F(3, 36) = 209.206$, $p < 0.001$, $SS = 362.116$, $n^2p = 0.946$) and a significant treatment and time interaction ($F(15, 36) = 51.037$, $p < 0.001$, $SS = 441.701$, $n^2p = 0.955$), indicating that changes in the MCV levels over time varied among the treatment groups. The residual SS was 20.771. On Day 1, aqueous (100 mg/kg b.w.) exhibited significantly higher ($p < 0.05$) MCV levels than the normal, negative and positive control groups. Moreover, aqueous (400 mg/kg b.w.) had significantly higher ($p < 0.001$) MCV levels than the positive and negative control groups. On Day 8, the positive control had significantly higher ($p < 0.001$) MCV levels than the normal and negative controls, aqueous (100 mg/kg b.w.) and aqueous (200 mg/kg b.w.) groups. Moreover, aqueous (400 mg/kg b.w.) had significantly higher ($p < 0.05$) MCV levels than normal and negative controls, aqueous (200 mg/kg b.w.) and aqueous (100 mg/kg b.w.) groups. On Day 22, MCV levels of aqueous (200 mg/kg b.w.) were not significantly different ($p = 0.411$) from those in the negative control group, but significantly higher ($p < 0.001$) than those in the remaining groups. Additionally, the negative control had significantly higher ($p < 0.05$) MCV levels than normal and positive controls, aqueous (100 mg/kg b.w.) and aqueous (400 mg/kg b.w.) groups. On Day 36, MCV levels of aqueous (200 mg/kg b.w.) were not significantly different ($p = 0.066$) from those in the aqueous (100 mg/kg b.w.), but were significantly higher ($p < 0.05$) than those in the aqueous (400 mg/kg b.w.) and the control groups. The MCV levels in aqueous (100 mg/kg b.w.) were not statistically significantly different ($p = 0.944$) from those in the aqueous (400 mg/kg b.w.) group, although aqueous (100 mg/kg b.w.) had significantly higher ($p < 0.001$) MCV levels than the control groups.

3.2.5 | Effect of Aqueous *H. acetosella* Extract on MCHC Levels

Figure 6 shows the effect of *H. acetosella* extract on the MCHC levels of anaemic rats. A two-way RM ANOVA revealed significant effects of treatment ($F(5, 12) = 343.527$, $p < 0.001$, $SS = 1232.283$, $n^2p = 0.976$), time ($F(3, 36) = 1769.002$, $p < 0.001$, $SS = 4518.694$, $n^2p = 0.993$) and a significant treatment and time interaction ($F(15, 36) = 315.992$, $p < 0.001$, $SS = 4035.811$, $n^2p = 0.992$), indicating that changes in the MCHC levels over time varied among the treatment groups. The residual SS was 30.653. On Day 1, MCHC levels in the positive control were significantly higher ($p = 0.05$) than those in the negative and normal controls, and the aqueous (200 mg/kg b.w.) group. Moreover, MCHC levels in the aqueous (100 mg/kg b.w.) and aqueous (4000 mg/kg b.w.) groups were significantly higher ($p < 0.001$) than those in the normal and negative control groups. On Day 8, the positive control had significantly higher ($p < 0.001$) MCHC levels than the normal control and aqueous-treated groups, but not significantly higher ($p = 0.075$) than the negative control group. Additionally, MCHC levels were significantly higher ($p < 0.001$) in the negative control than in the normal control and aqueous-treated groups. On Day 22, the aqueous (200 mg/kg b.w.) group had significantly higher ($p < 0.001$) MCHC levels than the remaining groups. Aqueous (100 mg/kg b.w.) had significantly ($p < 0.001$) higher MCHC levels than the remaining groups, except aqueous (200 mg/kg b.w.). On Day 36, aqueous (100 mg/kg b.w.) had significantly higher ($p < 0.005$) MCHC levels than the negative control, aqueous (400 mg/kg b.w.) and aqueous (200 mg/kg b.w.). All remaining pairwise comparisons among MCHC levels in different groups were not statistically significant ($p > 0.05$) on Day 36.

3.2.6 | Effect of Aqueous *H. acetosella* Extract on MCH Values

The changes in the MCH values of anaemic Wistar rats under extract treatments are recorded in Figure 7. A two-way RM ANOVA revealed significant effects of treatment ($F(5, 12) = 85.767$, $p < 0.001$, $SS = 488.021$, $n^2p = 0.946$), time ($F(3, 36) = 775.687$, $p < 0.001$, $SS = 1804.037$, $n^2p = 0.985$) and a

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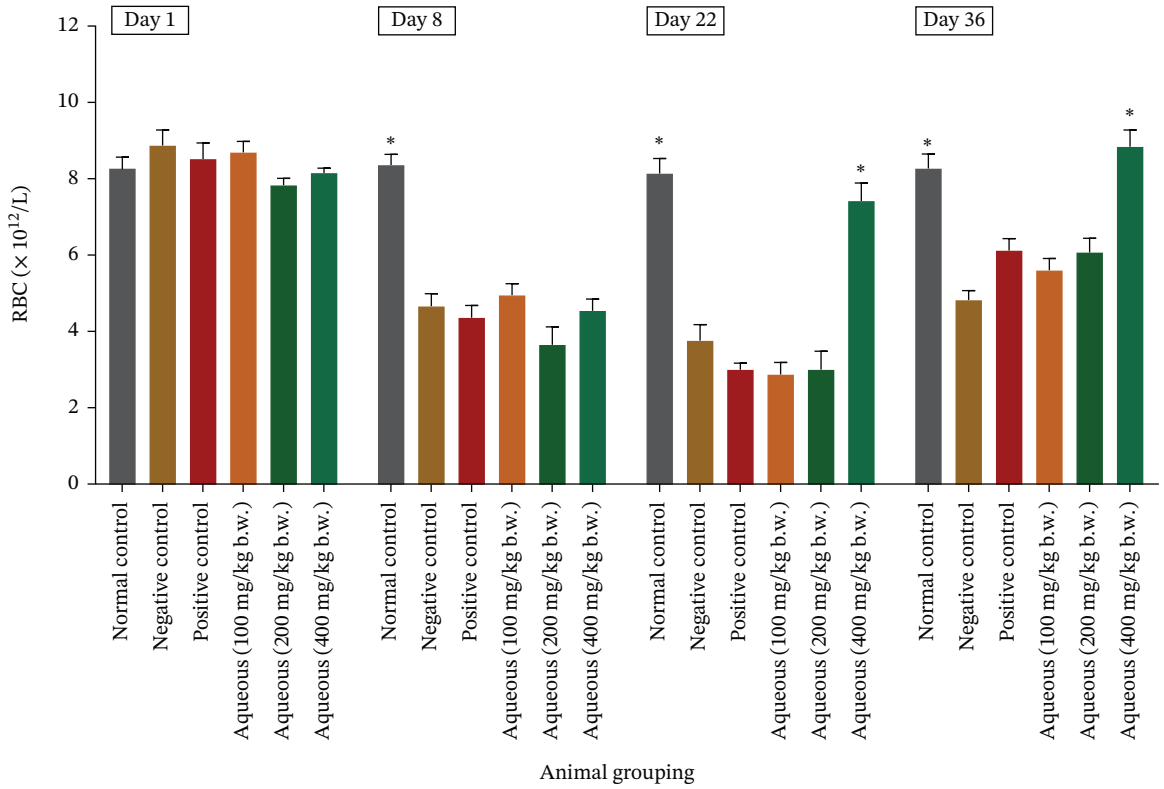

FIGURE 2 | Effect of aqueous leaf extract of *H. acetosella* on RBC counts in 2,4-DNPH-induced anaemic Wistar rats. Key: ∗ = statistically significant group. Aqueous extracts improved RBC counts, with the aqueous (400 mg/kg b.w.) group showing a higher RBC count by Day 36.

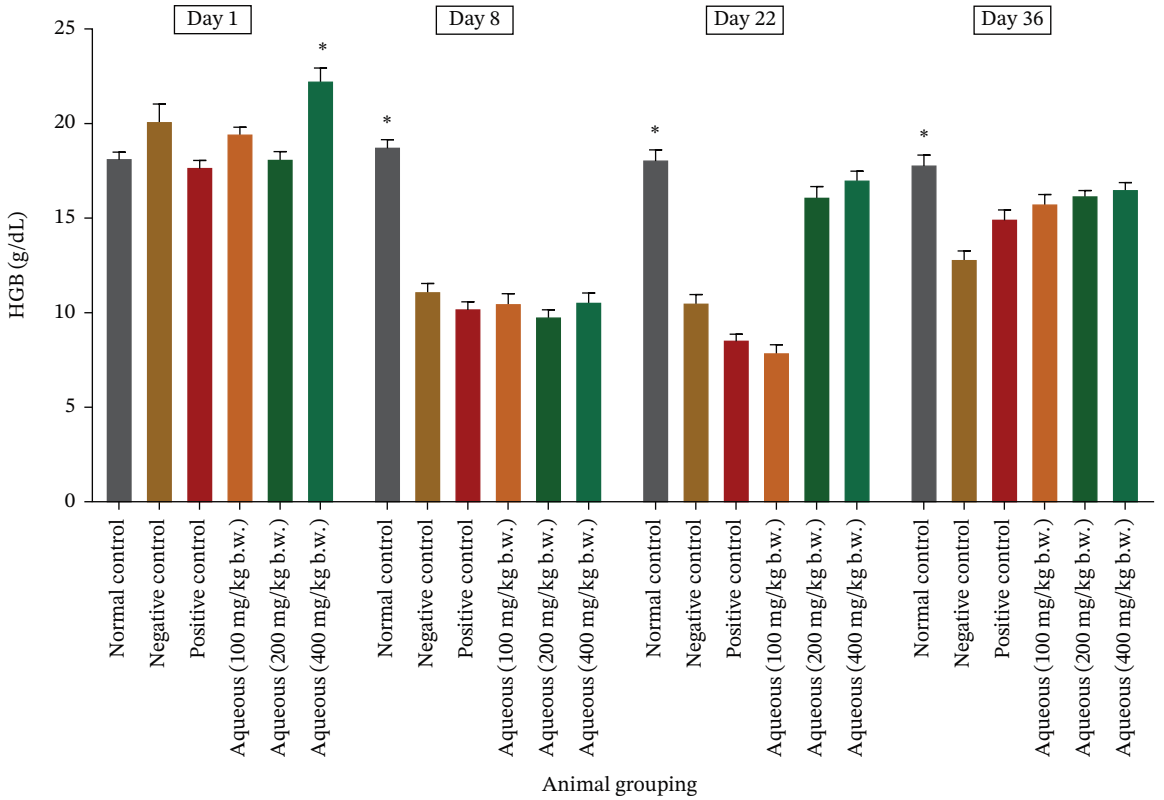

FIGURE 3 | Effect of aqueous leaf extract of *H. acetosella* on HGB concentration in 2,4-DNPH-induced anaemic Wistar rats. Key: g/dL = grammes per decilitre, ∗ = statistically significant group. Aqueous extracts improved HGB levels over time, with aqueous (400 mg/kg b.w.) having a higher HGB concentration by Day 36.

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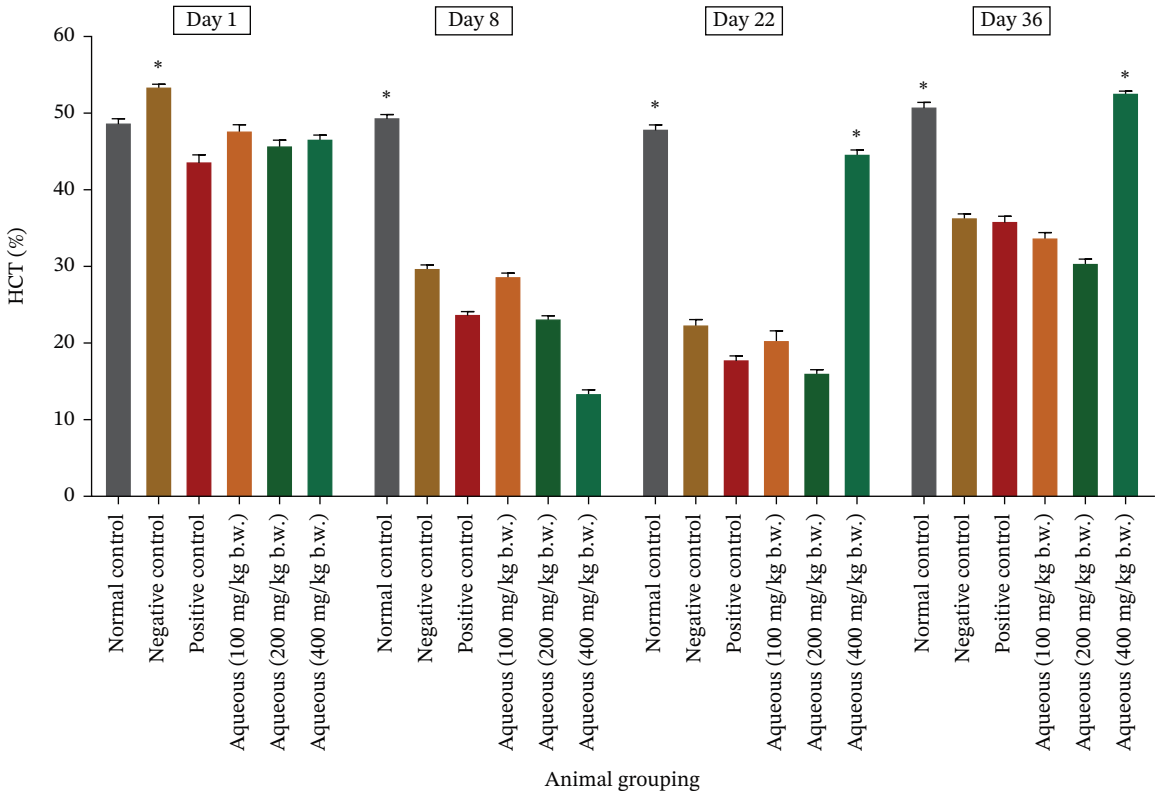

FIGURE 4 | Effect of aqueous leaf extract of *H. acetosella* on HCT levels in 2,4-DNPH-induced anaemic Wistar rats. Key: ∗ = statistically significant group. Aqueous extracts improved HCT levels over time, with aqueous (400 mg/kg b.w.) having higher HCT levels by Day 36.

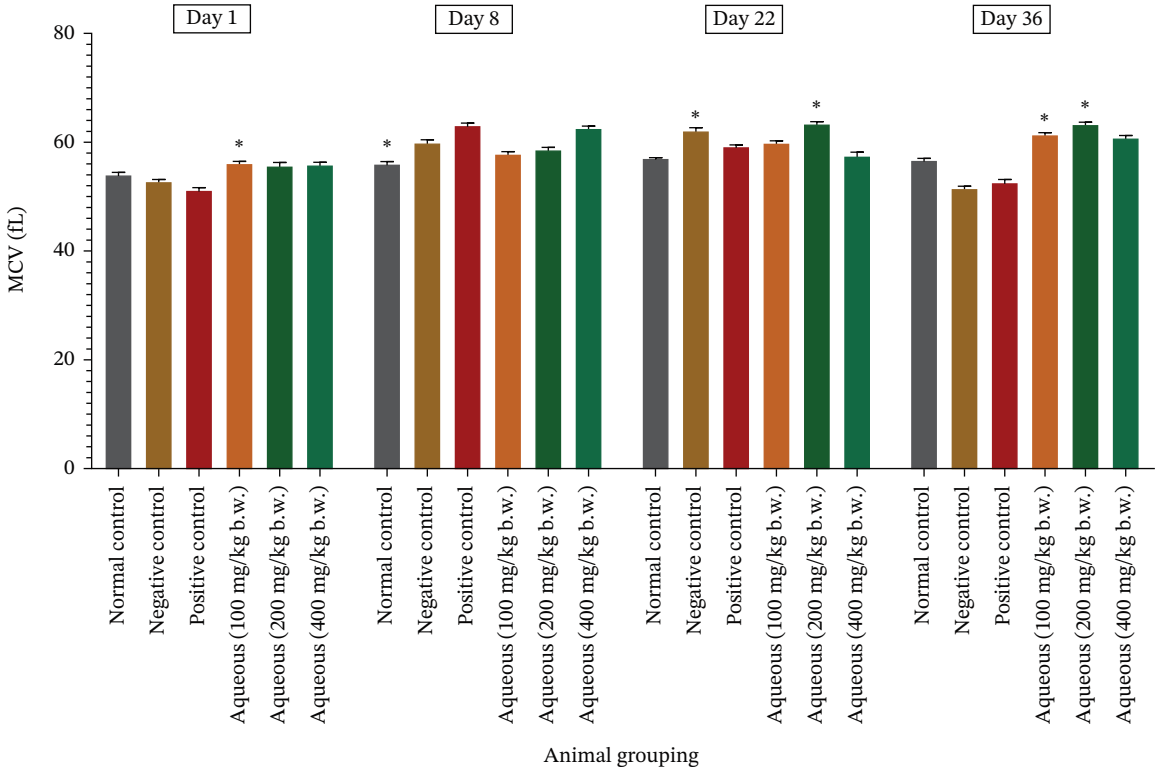

FIGURE 5 | Effect of aqueous leaf extract of *H. acetosella* on MCV levels in 2,4-DNPH-induced anaemic Wistar rats. Key: ∗ = statistically significant group, fL = femtoliters. Aqueous-treated groups had higher MCV levels than the control groups by Day 36.

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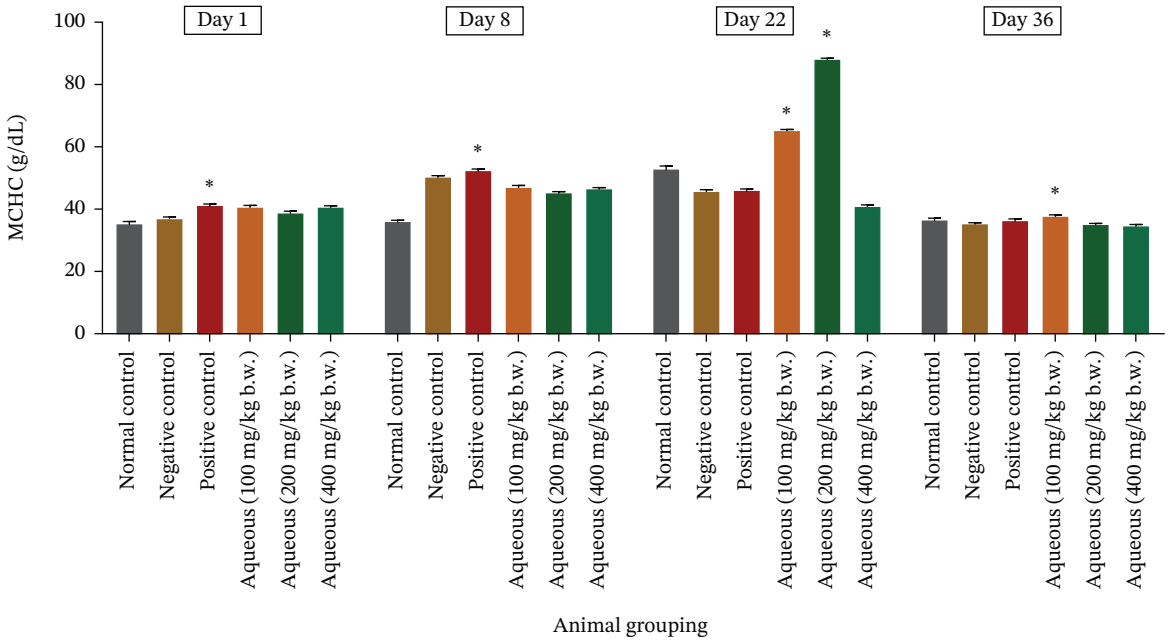

FIGURE 6 | Effect of aqueous leaf extract of *H. acetosella* on MCHC levels in 2,4-DNPH-induced anaemic Wistar rats. Key: g/dL = grammes per decilitre, * = statistically significant group.

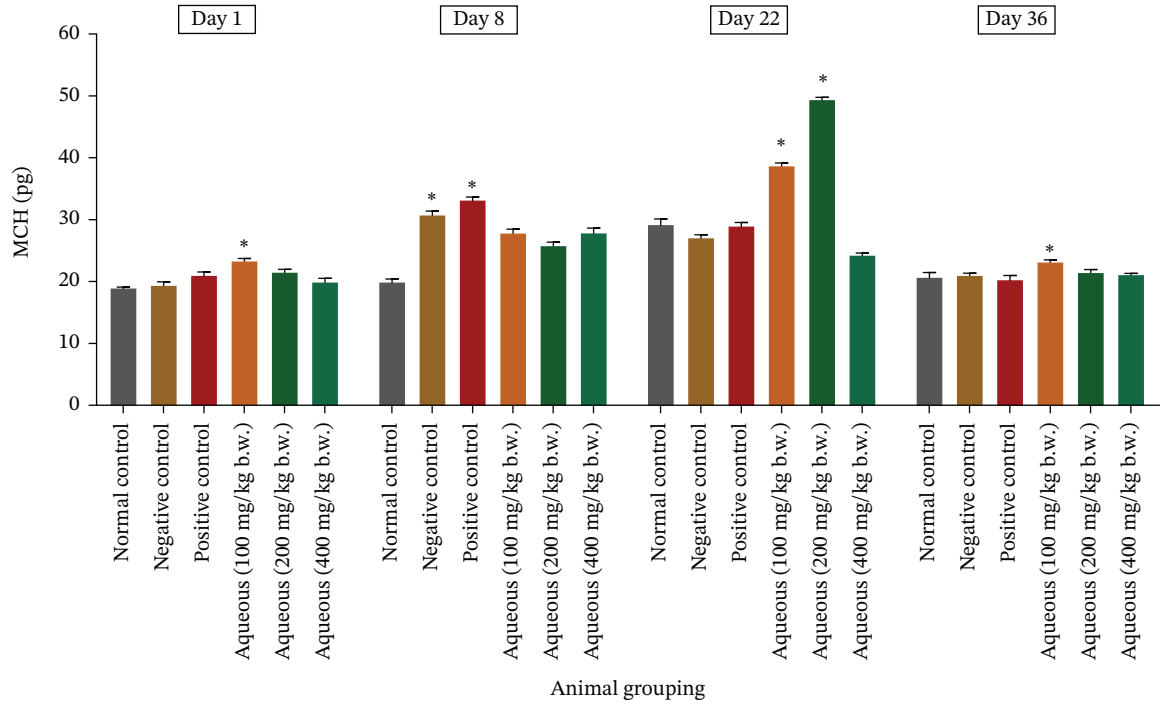

FIGURE 7 | Effect of aqueous leaf extract of *H. acetosella* on MCH in 2,4-DNPH-induced anaemic Wistar rats. Key: * = statistically significant group, pg = picograms. The MCH values of aqueous-treated groups decreased by Day 36.

significant treatment and time interaction ($F(15, 36) = 103.728$, $p < 0.001$, SS = 1206.220, $n^2p = 0.977$), indicating that changes in the MCH values over time varied among the treatment groups. The residual SS was 27.909. On Day 1, aqueous (100 mg/kg b.w.) had significantly higher ($p < 0.05$) MCH values than the normal, negative and positive controls and aqueous (400 mg/kg b.w.) groups. Although aqueous (100 mg/kg b.w.) showed no significantly higher ($p = 0.189$) MCH values than aqueous (200 mg/kg b.w.). Additionally, aqueous (200 mg/kg b.w.) exhibited significantly higher ($p = 0.018$) MCH values than those in the normal control group. All other pairwise comparisons amongst groups were not significant ($p > 0.05$). On Day 8, the positive control exhibited significantly higher ($p < 0.05$) MCH values than the negative and normal controls and the aqueous-treated groups. Additionally, the negative control group had significantly higher ($p < 0.05$) MCH values than the normal

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control and the aqueous-treated groups. The normal control exhibited significantly lower ($p < 0.001$) MCH values than those in the aqueous-treated groups. On Day 22, the aqueous (200 mg/kg b.w.) group had significantly higher ($p < 0.001$) MCH values than the controls, aqueous (100 mg/kg b.w.) and aqueous (400 mg/kg b.w.) groups. Aqueous (100 mg/kg b.w.) had significantly higher ($p < 0.001$) MCH values than the remaining groups, except aqueous (200 mg/kg b.w.). The aqueous (400 mg/kg b.w.) group had significantly lower ($p < 0.05$) MCH values than the control groups. On Day 36, the aqueous (100 mg/kg b.w.) group demonstrated significantly higher MCH values than the normal ($p = 0.025$) and positive control ($p = 0.006$) groups. All other pairwise comparisons of MCH values in different groups were not statistically significant ($p > 0.05$) on Day 36.

3.3 | Molecular Analysis

3.3.1 | Binding Affinity Values of Bioactive Compound against Target Proteins

The binding affinities of bioactive compounds from *H. acetosella* aqueous extract with selected references (quercetin and folic acid) against various protein targets are demonstrated in Table 5. N-[4-methoxy-3-methoxycarbonyl)benzoyloxy] succininide mostly exhibited stronger docking interactions to the protein targets with lower binding affinities spanning from −4.4 kcal/mol to −7.7 kcal/mol. The references exhibited binding affinities that were concordant across the protein targets, ranging between −0.0 kcal/mol and −8.0 kcal/mol for folic acid and between −4.5 kcal/mol and −9.0 kcal/mol for quercetin.

3.3.2 | Molecular Docking Validation

Molecular docking analyses of *H. acetosella* and reference compounds were validated following redocking of native ligands obtained from each target protein's original PDB structure (Table 6). The HEM docked to the rat HGB (3DHT) and rat methemoglobin (3HF4) with binding energies ranging from −11.5 to −10.8 kcal/mol, whereas L35 was bound to 2D5Z with a binding energy of −7.1 kcal/mol. The 4HG docked to PHD2 (2G19) with a binding energy of −7.9 kcal/mol. Moreover, OGA docked to PHD2 (3HQR) with a binding energy of −5.9 kcal/mol.

3.3.3 | Drug-Likeness Assessment of *H. acetosella* Compounds

The drug-likeness of aqueous extract compounds of *H. acetosella* was assessed based on Lipinski's rule, which is the MW, logarithm of partition coefficient (log P) and hydrogen bonding (H-bond) capacity. All of the aqueous compounds had MWs of < 500 g/mol, log P values of < 5, hydrogen bond acceptors of < 10 and hydrogen bond donors of < 5 (Table 7). The absorption, distribution, metabolism, excretion and toxicity (ADMET) of the compounds are presented in Table 8. The majority of the phytocompounds were predicted to have high GI absorption, whereas folic acid had low GI absorption. Only quercetin was predicted to have CYP3A4 inhibition. No compounds were predicted to be P-gp substrates. Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl ester, (R)-, DL-proline, 5-oxo-, methyl ester, N-[4-methoxy-3-methoxycarbonyl)benzoyloxy]succininide, folic acid and quercetin were predicted to be non-BBB permeant. All compounds were predicted to be inactive for hepatotoxicity and cytotoxicity. However, quercetin, folic acid, dibutyl phthalate, DL-proline, 5-oxo-, methyl ester and 5-iodo-4-oxohex-2-enoic acid, methyl ester were predicted to be active for nephrotoxicity.

3.3.4 | Molecular Docking Visualisation

The 3D visualisation of molecular docking interactions of 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione and N-[4-methoxy-3-methoxycarbonyl)benzoyloxy]succininide with the selected receptors (Figure 8). The docking of N-[4-methoxy-3-methoxycarbonyl)benzoyloxy]succininide to 2G19 protein revealed Asp254, Ile256, Met299, Ala385, Thr387, Ile327, His374, His313, Val376 and Try310 amino acid residues. Furthermore, N-[4-Methoxy-3-methoxycarbonyl)benzoyloxy] succininide docked to 3HQR revealed the following amino acid residues: Trp389, Arg322, Val314, His374, His313, Arg252, Tyr310, Val376, Try303, Ala385 and Ile327. 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione docked to 2D5Z revealed Ser102, Ser133 and Lys99 whereas docked to 1M4F revealed Lys24, Cys22 and Cys16.

4 | Discussion

The percentage yield for the *H. acetosella* aqueous extract was 19.05%, which reflects the amount of extractable material recovered. Extraction is affected by several factors such as temperature, time, solvent polarity and concentration of solvent. Moreover, depending on the chemical nature, several phytocompounds in plant materials are extracted in solvents of distinct polarity since no single solvent may be dependable to extract all phytocompounds present [61]. The SI quantitatively expresses the degree of similarity between the mass spectrum of an unknown chemical compound and the reference spectrum stored in a spectral library. Moreover, an SI of 100 means the patterns of two mass spectra are similar and an SI of 0 means the patterns are completely dissimilar [62]. The SI of 80% is usually considered acceptable for tentative identification of unknown chemical compounds [63]. The GC-MS analysis of the aqueous extract of *H. acetosella* revealed the presence of hexadecanoic acid, methyl ester, which is in agreement with the findings of Rassem et al. [64], who identified hexadecanoic acid, methyl ester in the *Hibiscus rosa-sinensis* methanol flower extract, reported to have antioxidant activities. Similarly, Sehim et al. [65] reported the presence of hexadecanoic acid, methyl ester from the methanol extract of *Hibiscus sabdariffa* flowers, whereas Nandagopalan et al. [66] reported the presence of hexadecanoic acid, methyl ester and 2-methoxy-4-vinylphenol from methanol extracts of *Hibiscus tiliaceus* leaves. 2-methoxy-4-vinylphenol and DL-proline, 5-oxo-, methyl ester have been reported to have antioxidant activities [51, 52]. The hematinic activity observed in the aqueous extract-treated rat groups may be attributed to the phytochemicals present with antioxidant activities, which protected RBCs from oxidative damage caused by ROS. Flavonoids

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TABLE 5 | Binding affinities of compounds from *H. acetosella* aqueous extract.

	Target protein										
Ligand	**2G19**	**6MOE**	**3DHT**	**3HQR**	**6WB6**	**1EER**	**3HF4**	**1GWG**	**1M4F**	**2D5Z**	**7O7B**
Folic acid (reference 1)	−7.7	−5.7	−7.4	−0.0	−6.9	−6.3	−7.3	−5.5	−6.0	−8.0	−4.1
Quercetin (reference 2)	−9.0	−5.5	−8.3	−5.8	−6.3	−7.7	−7.5	−5.6	−5.9	−7.5	−4.5
N-[4-methoxy-3-methoxycarbonyl) benzoyloxy]succininide	−7.7	−5.1	−7.3	−6.2	−6.5	−7.1	−7.0	−5.1	−5.2	−6.9	−4.4
7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	−6.6	−4.9	−7.6	−4.5	−5.8	−6.1	−6.9	−5.6	−5.7	−7.0	−4.1
Dibutyl phthalate	−6.6	−4.4	−6.6	−4.4	−4.9	−5.2	−6.6	−3.8	−4.1	−5.7	−4.1
4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.0(1,4)] dec-2-en-7-ol	−7.2	−4.5	−5.3	−3.5	−4.1	−5.7	−5.4	−3.4	−5.4	−5.7	−3.2
5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	−6.7	−4.3	−7.2	−5.5	−4.6	−5.2	−6.9	−3.9	−4.6	−5.3	−3.8
DL-proline, 5-oxo-, methyl ester	−5.5	−3.6	−4.5	−5.7	−4.3	−4.8	−4.9	−3.8	-3.9	−4.7	−3.6
2-methoxy-4-vinylphenol	−5.6	−3.9	−5.6	−5.7	−4.7	−5.0	−5.9	−4.4	-4.6	−4.8	−4.1
Benzaldehyde, 2-hydroxy-4-methyl-	−5.6	−3.7	−5.6	−6.0	−4.5	−5.3	−5.6	−4.8	−4.3	−4.7	−4.0
3-pentenoic acid, 3-ethyl-, methyl ester	−4.8	−3.4	−5.0	−5.2	−3.8	−4.4	−5.3	−4.2	−3.4	−4.3	−3.8
5-iodo-4-oxohex-2-enoic acid, methyl ester	−5.3	−3.4	−4.9	−5.5	−4.2	−4.3	−4.8	−4.0	−3.8	−4.6	−3.6
Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl ester, (R)-	−5.6	−3.9	−5.0	−5.8	−4.6	−4.7	−4.8	−3.5	−4.5	−4.9	−3.3
Tridecanoic acid	−5.1	−3.1	−5.2	−5.4	−4.3	−4.7	−5.1	−3.7	−3.8	−4.2	−3.2
Hexadecanoic acid, methyl ester	−5.2	−3.3	−5.6	−5.7	−4.5	−4.5	−6.0	−3.7	−3.3	−3.9	−3.6
2-pentanone, 4-hydroxy-4-methyl-	−4.5	−3.2	−4.3	−4.9	−4.0	−4.0	−4.4	−3.3	−4.0	−3.9	−3.5
Binding affinity for mode 1	kcal/mol or $\Delta G_{binding}$										

Note: Binding affinities (kcal/mol) signify predicted interaction strength between the ligand and target proteins.
Abbreviations: $\Delta G_{binding}$, change in Gibbs free energy in binding; kcal/mol, kilocalories per mole.

modulate gene expression linked to Epo and transferrin receptors [67]. Tannins and flavonoids have been reported to exhibit blood cell regeneration effects, thus could have indirectly stimulated the pituitary glands and hypothalamus, causing Epo synthesis in the kidneys, which travel to bone marrow to further stimulate the myeloerythroid cells [68].

The hydroxyl groups on the benzene rings of phenolic compounds contribute to the antioxidant activities [69]. Phenolic acids may exert their effects through the induction of enzymes, cell proliferation, DNA damage repair and scavenging of free radicals [70]. Flavonoids can activate glutathione-related enzymes [71]. Polyphenols chelate transitional metals via their OH groups and carbonyl moiety [72].

PHZ induces the production of hydrogen peroxide and superoxide radicals, leading to the lipid peroxidation of RBC membranes and Heinz body formation, hence causing the RBC count reduction and anaemia [9]. Results of the present study showed that oral administration of 2,4-DNPH (40 mg/kg b.w.)

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TABLE 6 | Redocking RMSD values of native ligands against their respective target proteins.

Native ligand	Target protein	kcal/mol	RMSD
4HG	2G19 (PHD2)	−7.9	1.097 Å
EPE	6MOE (EpoR)	−3.7	2.151 Å
L35	2D5Z (T-state HGB)	−7.1	1.750 Å
HEM	3DHT(rat HGB)	−11.5	0.397 Å
HEM	3HF4 (methemoglobin)	−10.8	0.199 Å
OGA	3HQR (PHD2)	−5.9	1.239 Å
IOD	1GWG (ferritin)	−0.9	0.574 Å

Abbreviations: Å, Ångström; $\Delta G_{binding}$, change in Gibbs free energy in binding; kcal/mol, kilocalories per mole; RMSD, root mean square deviation.

for 7 days led to a decrease in haematological parameters of HGB, HCT and RBC. This finding agrees with studies of Sheth et al. [36], who reported a significant decrease in HGB, HCT and RBC following administration of PHZ (40 mg/kg) for 2 days. Furthermore, the administration of PHZ caused an increase in the MCV and MCH, which characterise macrocytosis, thus macrocytic anaemia [73]. Administration of PHZ induced haemolytic stress in mice, followed by induction of reticulocytosis [74]. Furthermore, PHZ-treated mice generated high free heme concentrations and ROS in bone marrows, suggesting altered bone remodelling and dyserythropoiesis in haemolytic anaemia and signifying the probability of the liver or spleen being in charge of producing new RBCs [74]. In the present study, administration of 2,4-DNPH led to an increase in MCV levels and a decrease in RBC counts across the aqueous (400 mg/kg b.w.), negative and positive control groups. These shifts suggest the occurrence of dyserythropoiesis, characterised by the bone marrow generating RBCs that are subsequently damaged by the free heme and ROS before maturation.

The present study findings showed a significantly higher difference in the RBC counts between the negative control and the normal control group on Day 8 compared with Day 1, consistent with previous findings of Akhter et al. [75]. The decrease in RBC levels in the aqueous-treated groups was improved through treatment with *H. acetosella* extract. The results of the present study on the enhancement of RBC levels in treatment groups may be ascribed to the restorative potential of *H. acetosella* aqueous extract in the attempt to prevent oxidative stress and 2,4-DNPH-induced haemolysis. Gheith & El-Mahmoudy. [76] reported that PHZ-induced erythropenia reduced HCT and HGB parameters that continued for 8 to 12 days. This could explain the decrease in RBC, HGB and HCT observed after 14 days despite the administration of *H. acetosella* in some aqueous-treated groups, although it remains speculative. In brief, after anaemia induction, all groups, with the exception of the normal control, exhibited a notable decrease in RBC, HCT and HGB, validating successful anaemia induction, which agrees with the findings of Chauhan et al. [73]. In the negative control, these haematological indices continued to decrease through Day 22, signifying continued anaemia, although slightly improved by Day 36. The slight improvement in the negative control group may be attributed to animals' natural recuperation from the damaging effects of anaemia caused by 2,4-DNPH, a phenomenon also supported by the findings of Nneoyi-Egbe et al. [77]. Similarly, Ajugwo et al. [78] reported anaemic Wistar rats having HGB levels similar to the aqueous 200 mg/kg b.w group. By Day 36, the HCT, HGB and RBC indices in aqueous-treated groups approached or exceeded those of the normal control, indicating effective hematinic activity. Phenolic compounds, such as flavonoids, serve as electron donors to hydrogen peroxide, thereby scavenging it and neutralising it to water [9]. Moreover, they protect RBC membranes during oxidative stress, hence reducing methemoglobin formation, causing antianaemic effects that correct blood disorders [79]. Flavonoids, present in *H. acetosella,* may have contributed to the hematinic activity observed.

The normal control had significantly lower MCV levels on Day 22 compared with the aqueous (100 mg/kg b.w.), aqueous (200 mg/kg b.w.), negative and positive control groups. In contrast, Ousaaid et al. [79] reported a decrease in MCV after treatment with the extract for 14 days. Previous studies reported no significant differences in MCV among the groups [80, 81].

In the present study, *H. acetosella* extract-treated groups exhibited MCH and MCHC levels of similar magnitudes to the normal control on Day 36. This is in agreement with Rajendran et al. [82], who reported that punarnavadi mandura treatment groups restored the MCHC and MCH levels to normal, signifying their therapeutic efficacy in alleviating normal erythrocyte phenotype and curing PHZ-induced haemolytic anaemia. The presence of flavonoids, tannins and terpenes in the leaf extract of *H. acetosella* may be responsible for the haematopoietic activities and protection of RBCs from oxidative damage, a speculation earlier supported by Jorum et al. [83]. They protect RBCs from oxidative damage because of their free-radical scavenging and antioxidant activities [83].

The aqueous (100 mg/kg b.w.) and (200 mg/kg b.w.) groups did not consistently attain RBC, HGB and HCT levels comparable to the normal control group on Day 22 and Day 36. Despite these observations, both doses demonstrated improvement in haematological parameters by Day 36 from Day 8. This indicates that these doses were biologically active and contributed to recovery from anaemia; however, the magnitude or rate of recovery was less significant than that observed at the 400 mg/kg b.w. dose.

The RMSD values are classified as good when RMSD $\leq$ 2.0 Å, acceptable when RMSD is within 2.0 Å and 3.0 Å and bad when RMSD $\geq$ 3.0 Å [84]. In the current study, docking validation generated RMSD values $\leq$ 3.0 Å. Mechanisms of action of polyherbal formulations may include modulating iron-regulatory proteins, stimulation of Epo pathways or antioxidant effects [85]. The EpoR has an essential role in the erythropoiesis pathway, resulting in RBC production [82]. The high binding affinities for some phytocompounds, as compared with the reference compounds, support their potential as phytocompound agents in anaemia management [85]. The PHZ causes the deregulation of EpoR, which impacts the JAK/STAT and Ras/MAPK intracellular signalling pathways; it

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TABLE 7 | Lipinski's rule drug-likeness 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
N-[4-methoxy-3-methoxycarbonyl) benzoyloxy]succininide	307.26	6	7	0	75.01	2.3	0	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
Dibutyl phthalate	278.34	10	4	0	77.84	2.97	0	0.55
4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.0(1,4)] dec-2-en-7-ol	208.3	0	2	1	59.59	2.55	0	0.55
5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	180.29	0	1	0	55.96	2.73	0	0.55
DL-proline, 5-oxo-, methyl ester	143.14	2	3	1	37.04	1.28	0	0.55
2-methoxy-4-vinylphenol	150.17	2	2	1	45.05	2.14	0	0.55
Benzaldehyde, 2-hydroxy-4-methyl-	136.15	1	2	1	38.82	1.29	0	0.55
3-pentenoic acid, 3-ethyl-, methyl ester	142.2	4	2	0	41.38	2.44	0	0.55
5-iodo-4-oxohex-2-enoic acid, methyl ester	268.05	4	3	0	49.74	1.97	0	0.55
Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl ester, (R)-	190.19	5	5	1	44.04	1.96	0	0.55
Tridecanoic acid	214.34	11	2	1	66.38	3.21	0	0.85
Hexadecanoic acid, methyl ester	270.45	15	2	0	85.12	4.41	1	0.55
2-pentanone, 4-hydroxy-4-methyl-	116.16	2	2	1	32.36	1.52	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

Abbreviations: BS, bioavailability score; H-bond, hydrogen bond; log P, logarithm of partition coefficient; MR, molar refractivity; MW, molecular weight; RB, rotatable bonds.

inhibits RBC production and gene transcription. Moreover, PHZ induces RBC destruction either through the JAK/STAT signalling pathway inhibition or the induction of oxidative stress [82]. Bharati et al. [85] and Puspita et al. [40] reported binding energies to 6MOE (human EpoR): rutin (flavonoid) (−6.4 kcal/mol), alizarin (−6.3 kcal/mol), rubiadin (−6.3 kcal/mol), kaempferol (−6.2 kcal/mol), ellagic acid (−6.2 kcal/mol), iron (−4.8 kcal/mol), native ligands EPE (−5.0 kcal/mol) and ACI (−1.35 kcal/mol). Notably, the 6MOE and 1EER represent human EpoR, enabling comparability of the binding energies across them. The compounds may act via several mechanisms such as modulation of Epo pathways stimulation, modulation of iron-regulatory proteins and antioxidant effects as proposed by Bharati et al. [85].

Flavonoids such as quercetin have a crucial role in systemic iron metabolism through the control of hepcidin expression to mitigate pathological conditions brought about by excess iron in the system. Furthermore, quercetin can inhibit or promote hepcidin synthesis [45]. Khan et al. [45] reported binding energies to human hepcidin

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TABLE 8 | ADMET analyses of *H. acetosella* compounds.

Compound	GI absorption	BBB permeant	P-gp substrate	CYP3A4 Inhibitor	Cytotoxicity	Hepatotoxicity	Nephrotoxicity
Folic acid (reference 1)	Low	No	No	No	Inactive (0.72)	Inactive (0.97)	Active (0.52)
Quercetin (reference 2)	High	No	No	Yes	Inactive (0.99)	Inactive (0.69)	Active (0.62)
N-[4-methoxy-3-methoxycarbonyl) benzoyloxy]succininide	High	No	No	No	Inactive (0.63)	Inactive (0.58)	Inactive (0.70)
7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	High	Yes	No	No	Inactive (0.87)	Inactive (0.59)	Inactive (0.58)
Dibutyl phthalate	High	Yes	No	No	Inactive (0.89)	Inactive (0.71)	Active (0.56)
4,6,10,10-tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	High	Yes	No	No	Inactive (0.86)	Inactive (0.70)	Inactive (0.81)
5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2H-chromene	High	Yes	No	No	Inactive (0.72)	Inactive (0.81)	Inactive (0.87)
DL-proline, 5-oxo-, methyl ester	High	No	No	No	Inactive (0.74)	Inactive (0.77)	Active (0.51)
2-methoxy-4-vinylphenol	High	Yes	No	No	Inactive (0.88)	Inactive (0.66)	Inactive (0.63)
Benzaldehyde, 2-hydroxy-4-methyl-	High	Yes	No	No	Inactive (0.79)	Inactive (0.53)	Inactive (0.56)
3-pentenoic acid, 3-ethyl-, methyl ester	High	Yes	No	No	Inactive (0.75)	Inactive (0.61)	Inactive (0.58)
5-iodo-4-oxohex-2-enoic acid, methyl ester	High	Yes	No	No	Inactive (0.76)	Inactive (0.78)	Active (0.57)
Butanedioic acid, 3-hydroxy-2,2-dimethyl-, dimethyl ester, (R)-	High	No	No	No	Inactive (0.70)	Inactive (0.82)	Inactive (0.57)
Tridecanoic acid	High	Yes	No	No	Inactive (0.74)	Inactive (0.52)	Inactive (0.53)
Hexadecanoic acid, methyl ester	High	Yes	No	No	Inactive (0.73)	Inactive (0.58)	Inactive (0.59)
2-pentanone, 4-hydroxy-4-methyl-	High	Yes	No	No	Inactive (0.83)	Inactive (0.69)	Inactive (0.75)

Note: 'High' and 'low' indicate the predicted GI absorption, whereas 'yes' and 'no' signify the predicted presence or absence of the specified ADMET property, respectively. Toxicity predictions are represented as 'active' or 'inactive', with values in parentheses representing the prediction probability.

Abbreviations: BBB, blood-brain barrier; CYP3A4, cytochrome P450 3A4; GI, gastrointestinal; P-gp, P-glycoprotein.

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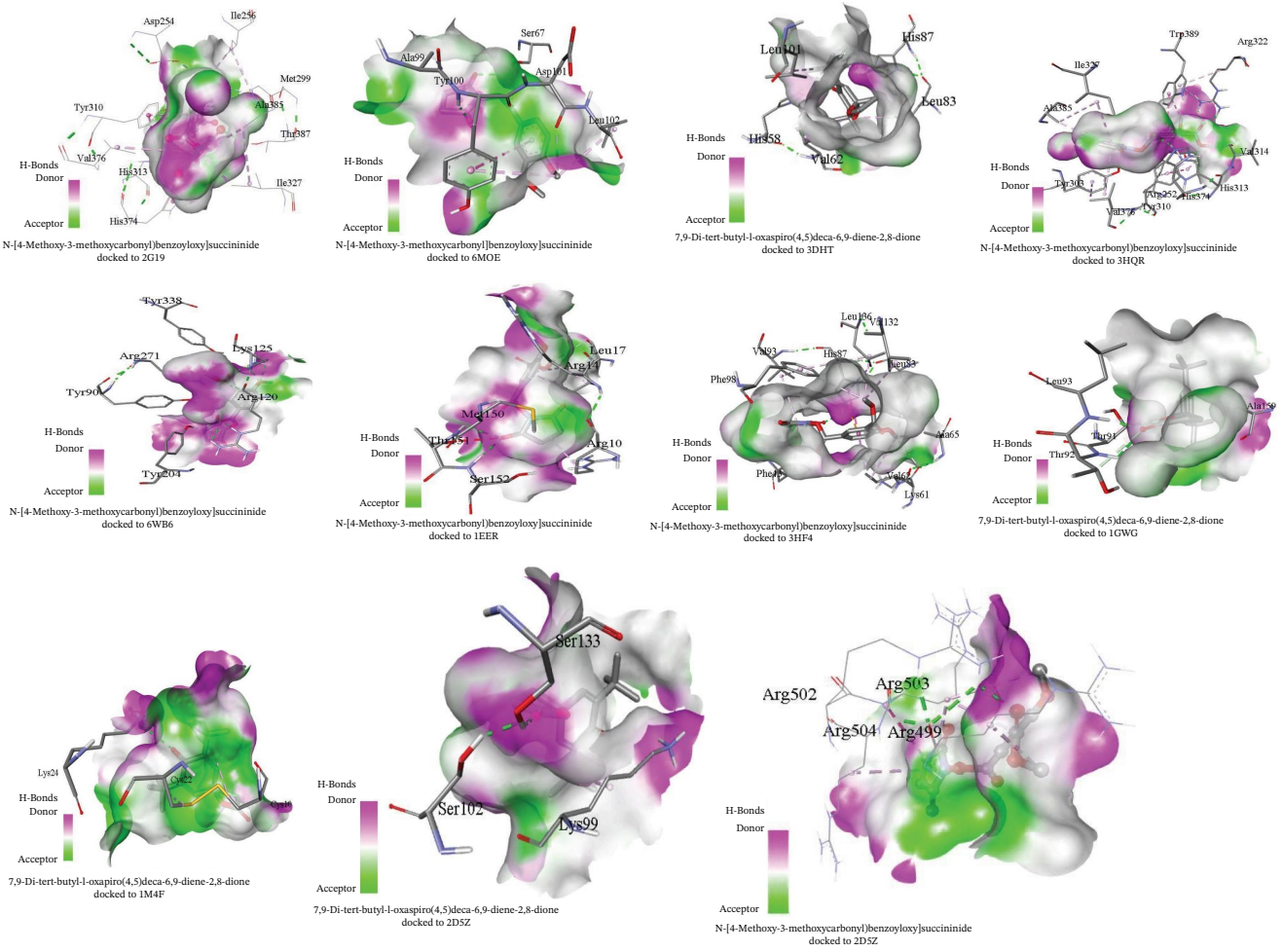

FIGURE 8 | 3D-visualisation of complexes using BIOVIA Studio. Key: dotted green lines represent the hydrogen bonds.

(1M4F): catechin (flavonoid) (−7.1 kcal/mol) and chlorogenic acid (phenolic compound) (−7 kcal/mol). By binding to 1M4F, compounds may modulate or inhibit the function of hepcidin, potentially restoring iron metabolism to normal, a mechanism proposed by Khan et al. [45].

The compounds of *H. acetosella* demonstrated high binding affinities to PHD2 (2G19 and 3HQR). These binding energies were higher in magnitude than those reported by Siswanto et al. [86] for PHD enzyme (3HQU) binding to ascorbic acid (−5.65 kcal/mol) and histidine (−4.25 kcal/mol). The binding energy produced from each ligand is brought about by the contribution of each energy interaction that emerges in the ligand and protein complex [86]. These results suggest *H. acetosella* compounds may potentially act as potent inhibitors of PHD enzymes, stabilising HIF-1α, hence modulating or stimulating Epo synthesis. However, this proposed mechanism requires further experimental validation. Obtaining PHD agonists/inhibitors provides a means to modulate the oxygen homeostasis pathway in vivo, offering potential for treating diseases linked with dysregulated oxygen sensing [39].

Normal human HGB has the possibility of binding to drug compounds or exogenous and endogenous molecules. Hence, HGB can improve the bioavailability of flavonoids or drug compounds through binding to them and facilitating their distribution through the bloodstream [87]. Talapatra et al. [35] reported findings for *Hygrophila spinosa* compounds (flavonoids and other phytochemicals) binding energies to T-state HGB (PDB ID: 2D5Z): lupenone (−10.4 kcal/mol), luteolin-7-rutinoside (−10.2 kcal/mol), β-carotene (−9.7 kcal/mol) and lupeol (−9.6 kcal/mol). Terpenoids, phenols and flavonoids have been reported to exhibit potent haematopoietic activity observed in IDA induced by haloperidol in rats. Moreover, phytochemicals may bind to the 2D5Z, thus acting as potential erythropoiesis-inducing agents (EIAs), which are used in anaemia management, a proposed mechanism by Talapatra et al. [38].

Methemoglobinemia is a disorder characterised by impaired oxygen delivery to tissues, since iron in HGB is oxidised from its ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}). Ferric HGB cannot bind oxygen, leading to tissue hypoxia and anaemia [88]. The PHZ destroys HGB through the formation of hydrazine free radicals and oxidised derivatives. The free radicals cause the formation of methemoglobin [89]. Ascorbic acid is used as a treatment for methemoglobinemia, where it penetrates the plasma membranes of RBCs, reducing methemoglobin formation and also stabilising their membranes, thereby hindering haemolysis [90]. In the present study, the phytochemicals of *H. acetosella* revealed binding energies to rat HGB (PDB ID: 3DHT) and rat methemoglobin (PDB ID: 3HF4). The *H. acetosella* compound 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, which

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has previously been reported to have antioxidant properties [55], may contribute to reducing methemoglobin formation and protecting erythrocytes against oxidative damage, thereby potentially limiting haemolysis. However, this proposed mechanism remains speculative and requires experimental validation. The *H. acetosella* compounds, such as 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, with antioxidant properties [55], may have penetrated cell membranes of RBCs, thus lowering the formation of methemoglobin and stabilising the erythrocytes, potentially preventing further haemolysis. These results complement the human-targeted HGB (2D5Z), highlighting the cross-species potency of *H. acetosella* phytochemicals in anaemia management. Furthermore, these observed findings suggest that bioactive compounds may have potential future applications in the transport of HGB-bound flavonoids or drugs, as proposed by Kaya [87].

Ferritin is the iron-storing molecule found in living organisms [42]. These researchers reported binding energies to ferritin (PDB ID: 1GWG): folic acid (−7.6 kcal/mol) and quercetin (−7.5 kcal/mol). The results of binding energies in the present study suggest that the phytocompounds of *H. acetosella* may have inhibited PHD2 enzymes, thereby inducing Epo synthesis and modulating hepcidin. Furthermore, while binding to ferritin directly, the phytochemicals may have orchestrated mobilisation of iron from the ferritin, enabling the restoration of iron homeostasis during anaemia recovery. However, this proposed mechanism remains speculative until further evaluation.

Transferrin prevents the formation of ROS and chelates the free toxic iron in circulation, thus acting as a protective scavenger [91]. Free iron is a potent neurotoxin that may cause oxidative stress by generating ROS [92]. Yaseen et al. [42] reported binding energies to transferrin (PDB ID: 6WB6): quercetin (−8.4 kcal/mol), rutin (−9.2 kcal/mol) and folic acid (−8.7 kcal/mol). Alrouji et al. [92] reported that ellagic acid (phenolic antioxidant) binding to human transferrin induced minimal structural changes in the structure of transferrin, thus forming a stable human transferrin-ellagic acid complex. The molecular docking results suggest that *H. acetosella* phytochemicals may interact with transferrin (PDB ID: 6WB6), potentially modulating its biological function in iron transport and homeostasis. Such interactions could contribute to improved iron delivery to the bone marrow and reduced iron toxicity. However, this proposed mechanism remains speculative and requires experimental validation. In the present study, *H. acetosella* phytochemicals may have formed stable complexes with transferrin (PDB ID: 6WB6), causing minimal structural alterations to the transferrin, hence regulating iron delivery to the bone marrow of rats and mitigating iron toxicity. However, further experimentation is required to validate this speculated mechanism.

Cells use several mechanisms to manage ROS levels, such as physical defences, repair, prevention and antioxidant actions [93]. Compounds of *H. acetosella* exhibited binding energies to the Nrf2 protein (PDB ID: 7O7B). Flavonoids have been reported to regulate iron transporters such as transferrin receptors, hence promoting iron absorption and systemic utilisation. Moreover, they promote activation of Nrf2, hence upregulating protective enzymes in haematopoietic tissues from oxidative injury [67]. Docking scores with more negative binding energy values than −5.0 kcal/mol signify robust ligand binding to the target protein, while more negative binding scores indicate stronger interactions with the active site of the target protein [87]. The activation of Nrf2 has been reported to promote the storage of iron, decrease cellular iron, inhibit ROS generation and suppress ferroptosis [46].

H. acetosella compounds such as 3-pentenoic acid, 3-ethyl-, methyl ester; 2-methoxy-4-vinylphenol; DL-proline, 5-oxo-, methyl ester and benzaldehyde, 2-hydroxy-4-methyl- may have contributed to the antianaemic activity observed in this study because of their reported antioxidant properties. 2-methoxy-4-vinylphenol has been reported to induce the degradation of Keap1 and the translocation of Nrf2 into the nucleus [94]. PHZ causes haemolytic anaemia through the oxidation of HGB, which results in the formation of hemichrome, precipitation to form Heinz bodies and later haemolysis [95]. Antioxidants protect the phospholipids from oxidation, deactivate ROS, hence preventing damage to lipids and proteins crucial for RBC function [95].

Lipinski's rules filter out compounds with a high likelihood of being drug candidates [96]. The bioavailability score of 0.55 implies that the identified compounds have a probability of 55% of being bioavailable and adhere to Lipinski's rules [96]. As stated by Lipinski's rules, compounds with a partition coefficient (logP) ≤ 5, MW ≤ 500 g/mol, number of H-donors ≤ 5 and H-bond acceptor ≤ 10 are regarded to have drug-likeness properties [97]. Compounds are more likely to have poor absorption when ≥ 2 of the above physicochemical conditions are not met [97]. The compounds identified in *H. acetosella* extract had only 0–1 Lipinski violations, whereas drugs like folic acid had 2 violations. However, the overreliance on Lipinski's rules of five constitutes the risks of losing opportunities for possible drug candidates and redundant failures [98]. Lipinski's rules should hence be regarded as a guideline for evaluating physicochemical properties linked with oral drug discovery rather than as a definitive determinant of the therapeutic potential of drugs. Similarly, *H. acetosella* compounds such as flavonoids, terpenoids and phenolics warrant further investigation for their hematinic effects, offering natural alternatives for anaemia management.

The ADMET prediction suggested that most of the identified phytochemicals present in *H. acetosella* had high GI absorption. Absorption of oral drugs is hindered by several factors within the enteric epithelium, such as enzymatic degradation and efflux transporters [79]. Moreover, limiting factors linked to the oral drugs are lipophilicity or hydrophilicity, substrates of efflux transporters and high MW [99]. The P-gp's major role is to protect the central nervous system from xenobiotics [100]. Moreover, P-gp, through hydrolysis of adenosine triphosphate (ATP), obtains energy to transport substrates or drugs out of the cell [101]. Cytochrome P450 (CYP) has a role in drug elimination via metabolic biotransformations [100]. Most drug lipophilic centres are transformed to hydrophilic centres during metabolic biotransformation that can elevate their water solubility to allow elimination in bile or urine. The CYP-mediated drug metabolism transforms lipophilic products into hydrophilic products to enable elimination [98]. The P-gp and CYP have been

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suggested to be capable of processing molecules synergistically to improve the protection of organisms and tissues [100]. The identified phytochemicals in *H. acetosella* extract were predicted to be non-P-gp substrates and noninhibitors of CYP3A4. Furthermore, the inhibition of CYP isoenzymes is a cause of pharmacokinetics-linked drug-drug interaction resulting in toxicity or other adverse effects because of the accumulation and decreased clearance of the drug or its metabolites [100].

A major limitation of this study is the relatively small sample size, which was chosen in line with the 3Rs (refinement, reduction and replacement) principles to minimise the number of rats used. This may have reduced the statistical power and increased the risk of committing Type II errors, possibly obscuring biologically relevant effects. Thus, the results should be interpreted with caution and validated in future studies with larger sample sizes following power analysis calculations, in accordance with the ARRIVE 2.0 recommendations. The identified compounds by GC-MS should be regarded as tentative since identification of compounds was done through matching the mass spectra of the compounds with reference spectra in the NIST14.lib. The present study did not assess biomarkers of iron metabolism such as serum iron, total iron-binding capacity (TIBC), ferritin, transferrin and hepcidin. Hence, the precise mechanisms involving iron metabolism and homeostasis could not be fully clarified. Future studies should assess these biomarkers to provide a more comprehensive understanding of the mechanisms by which *H. acetosella* extracts may exert their hematinic effect. The quantification of phytochemicals such as phenolic content, flavonoids and anthocyanins is recommended. Toxicity studies, biochemical analyses of liver and kidney function, histopathological examinations and safety assessments to establish the toxicological profile of the extract before therapeutic application are recommended.

5 | Conclusion

The findings of this study suggest that aqueous leaf extract of *H. acetosella* possesses promising antianaemic activity. The observed effects may be attributed to the presence of terpenoids, flavonoids, anthocyanins and other phytocompounds in the aqueous extract. The current findings reinforce the need for further investigation of the extract as a possible plant-derived intervention for anaemia management, including comprehensive safety and toxicity evaluations before any therapeutic application.

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Ethics Statement

The research protocol was approved by the Jomo Kenyatta University of Agriculture and Technology Institutional Scientific and Ethics Review Committee (Approval Number: JKU/ISERC/02317/1353).

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. J. Minigh, S. J. Enna, and D. Bylund, *Erythropoietin. in xPharm: The Comprehensive Pharmacology Reference* (Elsevier, 2008), https://doi.org/10.1016/B978-008055232-3.61704-0.

2. S. Safiri, A. A. Kolahi, M. Noori, 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* 14, no. 1 (2021): 1–16, https://doi.org/10.1186/s13045-021-01202-2.

3. A. Kumar, E. Sharma, A. Marley, M. A. Samaan, and M. J. Brookes, "Iron Deficiency Anaemia: Pathophysiology, Assessment Practical Management," *BMJ Open Gastroenterology* 9, no. 1 (2022): e000759, https://doi.org/10.1136/bmjgast-2021-000759.

4. E. Oyungu, A. W. Roose, A. R. Ombitsa, Z. Yang, R. C. Vreeman, and M. S. McHenry, "Anemia and Iron-Deficiency Anemia in Children Born to Mothers With HIV in Western Kenya," *Global Pediatric Health* 8 (2021): 2333794X21991035-2333794X21991035, https://doi.org/10.1177/2333794X21991035.

5. E. Palaka, S. Grandy, H. Van Haalen, P. McEwan, and O. Darlington, "The Impact of CKD Anaemia on Patients: Incidence, Risk Factors, and Clinical Outcomes—A Systematic Literature Review," *International Journal of Nephrology* 2020 (2020): 217692376, https://doi.org/10.1155/2020/7692376.

6. V. K. Chopra, and S. D. Anker, "Anaemia, Iron Deficiency and Heart Failure in 2020: Facts and Numbers," *ESC Heart Failure* 7, no. 5 (2020): 2007–2011, https://doi.org/10.1002/ehf2.12797.

7. N. Soundarya, and P. Suganthi, "A Review on Anaemia—Types, Causes, Symptoms and Their Treatments," *Journal of Scientific and Technological Innovations* 1 (2016): 10–17.

8. K. Marcelin, K. K. Innocent, M. A. C. Samuel, S. Duni, N. J. David, and D. A. Joseph, "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* 37, no. 4 (2025): 105–115, https://doi.org/10.9734/jpri/2025/v37i47682.

9. Z. A. Suleiman, Y. Salahuddeen, and U. A. Sabiu, "Effect Of Methanolic Leaves Extract of *Telfairia occidentalis* on 2,4-Dinitrophenylhydrazine Induced Anaemic Rats," *UMYU Journal of Microbiology Research* 7 (2022): 66–69.

10. M. J. Warner, and M. T. Kamran, *Iron Deficiency Anemia* (StatPearls (StatPearls Publishing, Treasure Island (FL), 2025).

11. C. Ciont, R. M. Pop, L. Pop, et al., "Impact of Side Effects on Anemia Therapy Compliance," *Nutrients* 17, no. 9 (2025): 1485, https://doi.org/10.3390/nu17091485.

12. M. Muñoz, S. Gómez-Ramírez, A. Campos, J. Ruiz, and G. M. Liumbruno, "Pre-Operative Anaemia: Prevalence, Consequences and Approaches to Management," *Blood Transfusion* 13, no. 3 (2015): 370–379, https://doi.org/10.2450/2015.0014-15.

13. T. Musyoka, D. W. Nyamai, W. M. Arika, et al., "In Vivo Antianaemic Effect and Safety of Aqueous Extracts of *Erythrina abyssinica* and *Zanthoxylum usambarensis* in Mice Models," *Journal of Hematology & Thromboembolic Diseases* 4 (2016): 242–242.

8086, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1155/tswj/5949952 by EBMG ACCESS - KENYA, Wiley Online Library on [23/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14. M. Thusiyanthan, T. Kalaichelvi, and T. Njq, "A Review on Hematinic Activity of *Hibiscus cannabinus* Leave in Anemia Management," *Journal of Biomedical Science and Research* 1 (2019): 37–40.

15. L. Y. dos Santos Silva, A. da Silva Ramos, D. Cavalcante, et al., "*Hibiscus acetosella*: An Unconventional Alternative Edible Flower Rich in Bioactive Compounds," *Molecules* 28, no. 12 (2023): 4819, https://doi.org/10.3390/molecules28124819.

16. P. M. Kapepula, N. Kabamba Ngombe, P. Tshisekedi Tshibangu, et al., "Comparison of Metabolic Profiles and Bioactivities of the Leaves of Three Edible Congolese *Hibiscus* Species," *Natural Product Research* 31, no. 24 (2017): 2885–2892, https://doi.org/10.1080/14786419.2017.1305382.

17. P. Ssegawa, and J. M. Kasenene, "Medicinal Plant Diversity and Uses in the Sango bay area, Southern Uganda," *Journal Of Ethnopharmacology* 113, no. 3 (2007): 521–540, https://doi.org/10.1016/j.jep.2007.07.014.

18. M. A. Junior, N. E. Laure, B. Z. Calvin, and M. F. Annie, "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," *International Journal of Pharmacognosy and Pharmaceutical Research* 8 (2021): 146–154.

19. A. Abubakar, "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* 9, no. 2 (2024): 22–33, https://doi.org/10.59298/IDOSR/JBBAF/24/92.223310000.

20. M. M. Alami, S. Guo, Z. Mei, G. Yang, and X. Wang, "Environmental Factors on Secondary Metabolism in Medicinal Plants: Exploring Accelerating Factors," *Medicinal Plant Biology* 3, no. 1 (2024): 0–11, https://doi.org/10.48130/mpb-0024-0016.

21. S. S. Kumar, P. Manoj, N. P. Shetty, and P. Giridhar, "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* 95, no. 9 (2015): 1812–1820, https://doi.org/10.1002/jsfa.6879.

22. S. O. Malomo, A. Ore, and M. T. Yakubu, "In Vitro and In Vivo Antioxidant Activities of the Aqueous Extract of *Celosia argentea* Leaves," *Indian Journal Of Pharmacology* 43, no. 3 (2011): 278–285, https://doi.org/10.4103/0253-7613.81519.

23. V. Singh, and R. Kumar, "Study of Phytochemical Analysis and Antioxidant Activity of *Allium sativum* of Bundelkhand Region," *International Journal of Life-Sciences Scientific Research* 3, no. 6 (2017): 1451–1458, https://doi.org/10.21276/ijlssr.2017.3.6.4.

24. S. A. Audu, I. Mohammed, and H. A. Kaita, "Phytochemical Screening of the Leaves of *Lophira lanceolata* (Ochanaceae)," *Life Science Journal* 4 (2007): 75–79.

25. P. Tiwari, M. Kaur, and H. Kaur, "Phytochemical screening and Extraction A Review," *Internationale Pharmaceutica Sciencia* 1, no. 1 (2011): 98–106.

26. M. S. Auwal, S. Saka, I. A. Mairiga, K. A. Sanda, A. Shuaibu, and A. Ibrahim, "Preliminary Phytochemical and Elemental Analysis of Aqueous and Fractionated Pod Extracts of *Acacia nilotica* (Thorn Mimosa)," *Veterinary Research Forum: An International Quarterly Journal* 5, no. 2 (2014): 95–100.

27. S. Dubale, D. Kebebe, A. Zeynudin, N. Abdissa, and S. Suleman, "Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia," *Journal of Experimental Pharmacology* 15 (2023): 51–62, https://doi.org/10.2147/JEP.S379805.

28. R. María, M. Shirley, C. Xavier, 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* 30, no. 4 (2018): 500–505, https://doi.org/10.1016/j.jksus.2017.03.009.

29. S. Kumar, C. Venkateshwar, G. Samuel, and G. Rao, "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* 2 (2013): 65–70.

30. J. R. Shaikh, and M. K. Patil, "Qualitative Tests for Preliminary Phytochemical Screening: An Overview," *International Journal Of Chemical Studies* 8, no. 2 (2020): 603–608, https://doi.org/10.22271/chemi.2020.v8.i2i.8834.

31. H. O. Edeoga, D. E. Okwu, and B. O. Mbaebie, "Phytochemical Constituents of Some Nigerian Medicinal Plants," *African Journal of Biotechnology* 4, no. 7 (2005): 685–688, https://doi.org/10.5897/AJB2005.000-3127.

32. A. Rn, S. K. Bai, K. Prasanna, and K. Geetha, "GC-MS Analysis of Aqueous and Ethanolic Extract in Different Plant Parts of *Alternanthera philoxeroides* (Mart.) Griseb," *Pharmaceutica Analytica Acta* 11 (2022): 108–117.

33. H. S. Ateeq, F. Iqbal, and A. Bano, "2,4-Dinitrophenylhydrazine," in *Encyclopedia of Reagents for Organic Synthesis* (John Wiley & Sons, Ltd, 2001), https://doi.org/10.1002/047084289X.rd403.

34. T. Gabardo, M. R. Frusciante, J. Pereira Marinho, et al., "In Vitro Antioxidant Activity of Purple Grape Juice (*Vitis labrusca*) Against Temozolamide Treatment in Rat Brains," *Beverages* 4, no. 4 (2018): 99, https://doi.org/10.3390/beverages4040099.

35. O. C. Ezeigwe, F. A. Nzekwe, O. F. Nworji, C. F. Ezennaya, E. L. Iloanya, and K. K. Asogwa, "Effect of Aqueous Extract of *F. capensis* Leaves and Its Combination With *C. aconitifolius* Leaves on Essential Biochemical Parameters of Phenylhydrazine-Induced Anemic Rats," *Journal of Experimental Pharmacology* 12 (2020): 191–201, https://doi.org/10.2147/JEP.S254003.

36. P. A. Sheth, A. T. Pawar, C. S. Mote, and C. More, "Antianemic Activity of Polyherbal Formulation, Raktavardhak Kadha, Against phenylhydrazine-Induced Anemia in Rats," *Journal of Ayurveda and Integrative Medicine* 12, no. 2 (2021): 340–345, https://doi.org/10.1016/j.jaim.2021.02.009.

37. C. M. Carter, "Alterations in Blood Components," *Comprehensive Toxicology* 27 (2018): 249–293, https://doi.org/10.1016/B978-0-12-801238-3.64251-4.

38. S. N. Talapatra, P. Talukdar, U. Pal, N. C. Maiti, and S. Swarnakar, "Interaction of T-State Haemoglobin and Phytochemicals of *Hygrophila spinosa* T. Anders: An Approach by Molecular Docking," *World Journal of Pharmaceutical Research* 5 (2016): 1354–1369.

39. A. M. Sayaf, K. Kousar, M. Suleman, et al., "Molecular Exploration of Natural and Synthetic Compounds Databases for Promising Hypoxia Inducible Factor (HIF) Prolyl-4- hydroxylase Domain (PHD) Inhibitors Using Molecular Simulation and Free Energy Calculations," *BMC Chemistry* 18, no. 1 (2024): 236, https://doi.org/10.1186/s13065-024-01347-4.

40. L. Puspita, B. Purwanto, B. Wasita, Y. L. Dewi, V. Widyaningsih, and A. Suhendi, "Antianaemic Potential of Flavonoids From Ajwa Date Fruits: An In Silico Study," *Tropical Journal of Natural Product Research* 8 (2024): 6832–6839.

41. N. Thahsin, K. I. Khan, A. N. M. R. Sarwar, M. N. Sakib, and M. S. Rahman, "Unveiling Novel Hyperglycosylated Analog of Human Erythropoietin: A Comprehensive Computational Exploration," *Computational and Structural Biotechnology Reports* 2 (2025): 100029, https://doi.org/10.1016/j.csbr.2024.100029.

42. C. M. Yaseen, C. B. Hamsa, K. Bhavani, A. Muthukumar, N. Srivastava, and P. M. Paarakh, "Insilico Docking Studies of Various Compounds of *Euphorbia hirta* L., Plant on Ferritin, Oxyhemoglobin, Hepcidin, and Transferrin Receptors," *Journal Of Microbiology, Biotechnology And Food Sciences* 14 (2025): e11047.

8086, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1155/tswj/5949952 by EBMG ACCESS - KENYA, Wiley Online Library on [23/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

43. J. J. Weber, M. M. Kashipathy, K. P. Battaile, et al., "Structural Insight Into the Novel Iron-Coordination and Domain Interactions of Transferrin-1 From a Model Insect, *Manduca sexta*," *Protein Science* 30, no. 2 (2021): 408–422, https://doi.org/10.1002/pro.3999.

44. S. Kumaraswamy, G. Arumugam, A. K. Pandurangan, V. S. Prabhakaran, and R. Narayanaswamy, "Molecular Docking Analysis of Organic Acids (OA) From Honey as Modulators of Human Ferritin, Transferrin, and Hepcidin," *Journal of Microbiology, Biotechnology And Food Sciences* 12, no. 5 (2023): e5743, https://doi.org/10.55251/jmbfs.5743.

45. N. Khan, S. Johri, and N. Paul, "Inhibitory Activity of Polyphenolic Compound Extracted From *P. betel* and *T. aestivum* With Hepcidin for Iron Deficiency Anemia: An In Silico Approach," *Indian Journal of Biochemistry and Biophysics* 60 (2023): 448–454, https://doi.org/10.56042/ijbb.v60i6.147.

46. S. Guan, R. Zhang, Y. Zhao, Z. Meng, and J. Lu, "1,3-Dichloro-2-Propanol Induced Ferroptosis Through Nrf2/ARE Signaling Pathway in Hepatocytes," *Environmental Toxicology* 37, no. 10 (2022): 2515–2528, https://doi.org/10.1002/tox.23615.

47. H. B. Castellanos-Sinco, C. O. Ramos-Peñafiel, A. Santoyo-Sánchez, et al., "Megaloblastic Anaemia: Folic Acid and Vitamin B12 Metabolism," *Revista Médica Del Hospital General De México* 78, no. 3 (2015): 135–143, https://doi.org/10.1016/j.hgmx.2015.07.001.

48. D. Xu, M.-J. Hu, Y.-Q. Wang, and Y.-L. Cui, "Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application," *Molecules* 24, no. 6 (2019): 1123, https://doi.org/10.3390/molecules24061123.

49. J. T. Mordkoff, "A Simple Method for Removing Bias From a Popular Measure of Standardized Effect Size: Adjusted Partial Eta Squared," *Advances in Methods and Practices in Psychological Science* 2, no. 3 (2019): 228–232, https://doi.org/10.1177/2515245919855053.

50. N. A. H. A. Nasir, N. A. L. Roslly, N. M. Rosli, and Z. Razali, N. Z. Alias, and R. Yusof, "Phytochemical Screening and Potential DPPH Radical Scavenging Activity of Banana Peel Extract," in *Charting the Sustainable Future of ASEAN in Science and Technology* (Springer Singapore, 2020), 249–258.

51. M. Rubab, R. Chelliah, K. Saravanakumar, et al., "Bioactive Potential of 2-Methoxy-4-Vinylphenol and Benzofuran from *Brassica oleracea* L. var. capitate f, rubra (Red Cabbage) on Oxidative and Microbiological Stability of Beef Meat," *Foods* 9, no. 5 (2020): 568, https://doi.org/10.3390/foods9050568.

52. I. Bashir, S. Ijaz, R. Shahzad, et al., "Screening of Biologically Active Compounds From *Megacarpaea polyandra* Benth Root: A Traditional Medicinal Plant of the Himalayas," *Journal of the Chilean Chemical Society* 70, no. 1 (2025): 6268–6279, https://doi.org/10.4067/s0717-97072025000106268.

53. S. Uthirapathy, and D. J. Ahamad, "Chemically Standardised Extract of *Withania somnifera (*Ashwagandha) Inhibits Tumour Growth, Promotes Apoptosis and Cell Cycle Arrest," *Indian Journal of Natural Products and Resources* 16 (2025): 236–245, https://doi.org/10.56042/ijnpr.v16i2.14135.

54. T. M. ALRaddadi, S. O. Bahaffi, L. A. Alkhateeb, and M. W. Sadaka, "Analysis of Bioactive Compounds Present in *Boerhavia* Elegans Seeds by GC-MS," *Open Chemistry* 22, no. 1 (2024): , https://doi.org/10.1515/chem-2024-0068.

55. V. Gupta, S. Tyagi, and R. Tripathi, "Hexadecanoic Acid Methyl Ester, a Potent Hepatoprotective Compound in Leaves of *Pistia stratiotes* L," *Applied Biology & Chemistry Journal* 4 (2023): 118–120, https://doi.org/10.52679/tabcj.2023.0012.

56. M. N. Abubacker, and T. Deepalakshmi, "In Vitro Antifungal Potentials of Bioactive Compound Methyl Ester of Hexadecanoic Acid Isolated From *Annona muricata* Linn. (Annonaceae) Leaves," *Biosciences Biotechnology Research Asia* 10, no. 2 (2013): 879–884, https://doi.org/10.13005/bbra/1211.

57. S. Vats, and T. Gupta, "Evaluation of Bioactive Compounds and Antioxidant Potential of Hydroethanolic Extract of *Moringa oleifera* Lam. From Rajasthan, India," *Physiology And Molecular Biology Of Plants* 23, no. 1 (2017): 239–248, https://doi.org/10.1007/s12298-016-0407-6.

58. S. K. Chowdhury, T. Dutta, A. P. Chattopadhyay, N. N. Ghosh, S. Chowdhury, and V. Mandal, "Isolation of Antimicrobial Tridecanoic Acid From *Bacillus* sp. LBF-01 and Its Potentialization Through Silver Nanoparticles Synthesis: A Combined Experimental and Theoretical Studies," *Journal of Nanostructure in Chemistry* 11, no. 4 (2021): 573–587, https://doi.org/10.1007/s40097-020-00385-3.

59. L. Cheng, J. Li, J. Cheng, and Z. Wu, "Dibutyl Phthalate-Induced Activation of ROS and ERK1/2 Causes Hepatic and Renal Damage in Kunming mice," *Human & Experimental Toxicology* 38, no. 8 (2019): 938–950, https://doi.org/10.1177/0960327119843583.

60. E. J. de Sousa Ferrão, and E. A. Janeque, "Anti-Viral Compounds From *Jatropha curcas* Seed Extract With Anti-HIV-1 and Anti-SARS-CoV-2 Action," *African Journal of Pharmacy and Pharmacology* 17 (2023): 1–9.

61. A. Shamsi, M. Shahwan, M. S. Khan, et al., "Elucidating the Interaction of Human Ferritin With Quercetin and Naringenin: Implication of Natural Products in Neurodegenerative Diseases: Molecular Docking and Dynamics Simulation Insight," *ACS Omega* 6, no. 11 (2021): 7922–7930, https://doi.org/10.1021/acsomega.1c00527.

62. X. Zhou, Y. Wang, Y. Yun, et al., "A Potential Tool for Diagnosis of Male Infertility: Plasma Metabolomics Based on GC–MS," *Talanta* 147 (2016): 82–89, https://doi.org/10.1016/j.talanta.2015.09.040.

63. Leema, S. Garg, H. Punetha, S. Sharma, S. Thakur, and D. Gupta, "Metabolite Profiling and Enhanced Antimicrobial and Nematicidal Activities of Zinc Oxide Nanoparticle-Biofortified *Pleurotus* mushrooms," *Scientific Reports* 16, no. 1 (2026): , https://doi.org/10.1038/s41598-026-59270-8.

64. H. Rassem, A. Nour, and R. Yunus, "GC-MS Analysis of Bioactive Constituents of Hibiscus Flower," *American-Eurasian Network for Scientific Information* 11 (2017): 91–97.

65. A. E. Sehim, B. H. Amin, M. Yosri, et al., "GC-MS Analysis, Antibacterial, and Anticancer Activities of *Hibiscus sabdariffa* L. Methanolic Extract: In Vitro and In Silico Studies," *Microorganisms* 11, no. 6 (2023): 1601, https://doi.org/10.3390/microorganisms11061601.

66. V. Nandagopalan, M. J. Gritto, and A. Doss, "GC-MS Analysis of Bioactive Components of the Methanol Extract of *Hibiscus tiliaceus* Linn," *Asian Journal of Plant Science & Research* 5 (2015): 6–10.

67. T. N. Ceaser, "Phytochemicals in Hematopoiesis: Unraveling the Role of Herbal Extracts in Nutritional and Inflammatory Anaemias," *INOSR Applied Sciences* 13 (2025): 77–81, https://doi.org/10.59298/INOSRAS/2025/13.2.7781.

68. M. Idu, M. O. Alugeh, M. O. Alugeh, and B. O. Gabriel, "Phytochemical Screening, Antioxidant Study and Hematinic Property of Mojeaga Herbal Remedy Using Animal Model," *Beni-Suef University Journal of Basic and Applied Sciences* 11, no. 1 (2022): 27, https://doi.org/10.1186/s43088-022-00207-1.

69. A. Zeb, "Concept, Mechanism, and Applications of Phenolic Antioxidants in Foods," *Journal of Food Biochemistry* 44, no. 9 (2020): e13394, https://doi.org/10.1111/jfbc.13394.

70. S. Kiokias, C. Proestos, and V. Oreopoulou, "Phenolic Acids of Plant Origin—A Review on Their Antioxidant Activity In Vitro (O/W Emulsion Systems) Along With Their in Vivo Health Biochemical Properties," *Foods* 9, no. 4 (2020): 534, https://doi.org/10.3390/foods9040534.

71. M. Olszowy, "What Is Responsible for Antioxidant Properties of Polyphenolic Compounds From Plants?," *Plant Physiology and Biochemistry* 144 (2019): 135–143, https://doi.org/10.1016/j.plaphy.2019.09.039.

72. A.-N. Li, S. Li, Y. J. Zhang, X. R. Xu, Y. M. Chen, and H. B. Li, "Resources and Biological Activities of Natural Polyphenols,"

8086, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1155/tswj/5949952 by EBMG ACCESS - KENYA, Wiley Online Library on [23/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Nutrients 6, no. 12 (2014): 6020–6047, https://doi.org/10.3390/nu6126020.

73. S. P. Chauhan, N. R. Sheth, and B. N. Suhagia, "Hematinic Effect of Fruits of *Opuntia elatior* Mill. on Phenylhydrazine-Induced Anemia in Rats," *Ayu* 36, no. 2 (2015): 208–213, https://doi.org/10.4103/0974-8520.175549.

74. R. Moreau, D. Tshikudi Malu, M. Dumais, et al., "Alterations in Bone and Erythropoiesis in Hemolytic Anemia: Comparative Study in Bled Phenylhydrazine-Treated and Plasmodium-Infected Mice," *Plos One* 7, no. 9 (2012): e46101, https://doi.org/10.1371/journal.pone.0046101.

75. S. Akhter, D. Begum, I. Jahan, A. H. Sharna, S. Khan, and I. Azmin, "Effects of Aloe Vera Gel on Red Blood Cell-Parameters in Phenylhydrazine-Induced Anaemic Wistar Albino Rats," *Ibrahim Cardiac Medical Journal* 13 (2023): 40–46.

76. I. Gheith, and A. El-Mahmoudy, "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* 51, no. 11 (2018): e7722–e7722, https://doi.org/10.1590/1414-431x20187722.

77. A. F. Nneoyi-Egbe, E. Onyenweaku, A. Akpanukoh, and P. Ebai, "Haematinic and Hepatoprotective Properties of Telfairia Occidentalis Fruit Mesocarp on Phenylhydrazine-Induced Anaemia in Experimental Rats," *Biochemistry Research International* 2023 (2023): 8838481, https://doi.org/10.1155/2023/8838481.

78. A. O. Ajugwo, P. E. Mounbegna, T. S. Kemajou, and V. C. Ofokansi, "Effects of *Moringa oleifera* Leaves Extract on Haematological Parameters of Phenylhydrazine Anaemia Induced Wistar Rats," *International Journal of Public Health and Safety* 2 (2017): 1–3.

79. D. Ousaaid, A. E. Ghouizi, H. Laaroussi, et al., "Anti-Anemic Effect of Antioxidant-Rich Apple Vinegar Against Phenylhydrazine-Induced Hemolytic Anemia in Rats," *Life* 12, no. 2 (2022): 239, https://doi.org/10.3390/life12020239.

80. E. Ani, A. Ime, A. Udefa, and U. Okon, "The Role of Aloe Vera on Haematological Parameters Following Phenylhydrazine (PHZ)-Induced Anaemia in Wistar Rats," *European Journal of Pharmaceutical and Medical Research* 5 (2018): 140–143.

81. O. E. Ofem, E. J. Ani, A. N. Archibong, and R. E. John, "Effect of Masfon Aloe Vera Gel on Some Blood Parameters in High Salt Loaded Rats," *Der Pharmacia Lettre* 7 (2015): 26–34.

82. K. Rajendran, D. R. Chellappan, V. Ramakrishnan, and U. M. Krishnan, "Therapeutic Efficacy of Punarnavadi Mandura Against Phenylhydrazine-Induced Hemolytic Anemia in Rats," *Journal of Traditional and Complementary Medicine* 15, no. 1 (2025): 93–104, https://doi.org/10.1016/j.jtcme.2024.03.017.

83. O. H. Jorum, N. M. Piero, and A. K. Machocho, "Haematological Effects of Dichloromethane-Methanolic Leaf Extracts of *Carissa edulis* (Forssk.) Vahl in Normal Rat Models," *Journal of Hematology & Thromboembolic Diseases* 4, no. 1 (2016): 1–8, https://doi.org/10.4172/2329-8790.1000232.

84. D. Ramírez, and J. Caballero, "Is It Reliable to Take the Molecular Docking Top Scoring Position as the Best Solution Without Considering Available Structural Data?," *Molecules* 23, no. 5 (2018): 1038, https://doi.org/10.3390/molecules23051038.

85. D. Bharati, S. Nirhali, A. Puri, P. Mohite, and S. Singh, "Development, Molecular Docking, and Anti-Anemia Potential of Polyherbal Formulation," *Biology* 14, no. 8 (2025): 1052, https://doi.org/10.3390/biology14081052.

86. S. Siswanto, H. Rasyid, N. A. Ramadhani, N. N. Caesar, N. Sunusi, and Z. D. Zainuddin, "Average Linkage Clustering Method AND Molecular Docking Study on Date Palm (*Phoenix Dactylifera* L.) as Potential Anti-Anemia Agent," *BAREKENG: Jurnal Ilmu Matematika dan Terapan* 18, no. 4 (2024): 2459–2470, https://doi.org/10.30598/barekengvol18iss4pp2459-2470.

87. Ş. Kaya, "Investigation of the Interaction of Phytochemical Compounds in *Vitis vinifera* L. With Human Hemoglobin Protein by Molecular Docking Method," *GİBTÜ Sağlık Ve Biyolojik Bilimler Dergisi* 1 (2025): 9–21, https://doi.org/10.65308/gjobhs.2025.001.

88. R. J. Chen, and T. M. Nappe, "Methemoglobinemia," (StatPearls (StatPearls Publishing, Treasure Island (FL), 2025).

89. C. Aloke, C. Uche Emelike, N. Ajuka Obasi, et al., "HPLC Profiling and Studies on *Copaifera salikounda* Methanol Leaf Extract on Phenylhydrazine-Induced Hematotoxicity and Oxidative Stress in Rats," *Arabian Journal of Chemistry* 14, no. 12 (2021): 103428, https://doi.org/10.1016/j.arabjc.2021.103428.

90. V. R. Sudheer, R. Krishnapriya, N. V. Kumar, and R. Umarani, "Management of Herbicide Induced Methemoglobinemia," *Journal of Drug Metabolism & Toxicology* 9, no. 1 (2018): , https://doi.org/10.4172/2157-7609.1000235.

91. A. S. Ogun, and A. B. Adeyinka, "Transferrin," in *StatPearls* (StatPearls Publishing, 2025).

92. M. Alrouji, F. A. Alhumaydhi, M. Furkan, et al., "Evaluation of the Binding Mechanism of Dietary Phytochemical, Ellagic Acid, with Human Transferrin: Spectroscopic, Calorimetric, and Computational Approaches Targeting Neurodegenerative Diseases," *ACS Omega* 9, no. 14 (2024): 16089–16096, https://doi.org/10.1021/acsomega.3c09792.

93. S. De Vita, E. González-Burgos, S. Terracciano, M. G. Chini, M. P. Gómez-Serranillos, and G. Bifulco, "Discovery of New Antioxidant Molecules Enhancing the Nrf2-Mediated Pathway: Docking Studies and Biological Evaluation," *International Journal of Molecular Sciences* 27, no. 4 (2026): 1862, https://doi.org/10.3390/ijms27041862.

94. E. Asami, M. Kitami, T. Ida, T. Kobayashi, and M. Saeki, "Anti-Inflammatory Activity of 2-Methoxy-4-Vinylphenol Involves Inhibition of Lipopolysaccharide-Induced Inducible Nitric Oxidase Synthase by Heme Oxygenase-1," *Immunopharmacology and Immunotoxicology* 45, no. 5 (2023): 589–596, https://doi.org/10.1080/08923973.2023.2197141.

95. F. Orrico, S. Laurance, A. C. Lopez, et al., "Oxidative Stress in Healthy and Pathological Red Blood Cells," *Biomolecules* 13, no. 8 (2023): 1262, https://doi.org/10.3390/biom13081262.

96. R. Saravanan, K. Raja, and D. Shanthi, "Correction to: GC-MS Analysis, Molecular Docking and Pharmocokinetic Properties of Phytocompounds From *Solanum torvum* Unripe Fruits and Its Effect on Breast Cancer Target Protein," *Applied Biochemistry and Biotechnology* 194, no. 4 (2022): 1851–1855, https://doi.org/10.1007/s12010-021-03749-9.

97. N. Basumatary, N. Basumatary, D. Baruah, P. K. Sarma, and J. Sarmah, "Structural Analysis of Glucose-6-Phosphate Dehydrogenase (G6PD) Variants From Northeast India and Natural Antioxidants as a Pharmacological Agent to Alleviate G6PD Deficiency Associated Challenges—An in Silico Approach," *Indian Journal of Biochemistry and Biophysics* 62 (2025): 25–37.

98. M. Zhao, J. Ma, M. Li, et al., "Cytochrome P450 Enzymes and Drug Metabolism in Humans," *International Journal Of Molecular Sciences* 22, no. 23 (2021): 12808, https://doi.org/10.3390/ijms222312808.

99. M. K. Lonare, A. Jahan, M. Sharma, and S. Ramanarayanan, A. Pathak, and S. P. Singh, "Drug Delivery to the Gastrointestinal Tract: Challenges and Opportunities," in *Next-Generation Drug Delivery Systems* (Springer), 285–301, https://doi.org/10.1007/978-1-0716-4554-3_13.

100. A. Daina, O. Michielin, and V. Zoete, "SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules," *Scientific Reports* 7, no. 1 (2017): 42717, https://doi.org/10.1038/srep42717.

101. Y. Kim, and J. Chen, "Molecular Structure of Human P-Glycoprotein in the ATP-Bound Outward-Facing Conformation," *Science* 359, no. 6378 (2018): 915–919, https://doi.org/10.1126/science.aar7389.

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