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GC-MS Characterization and Anti-Anaemic/Haematological Activity of Ethanol Extract of *Solanum Aethiopicum* Leaves

Stella Mbanyeaku Ufearoh*, Onyinyechi Uloma Akoh, Christian Odih, Felicia Uchechukwu Okwunodulu

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Abstract: *We aimed to investigate the GC-MS analysis of the ethanolic leaf extract of Solanum aethiopicum and assess the effect of the extract on haematological parameters in PHZ-induced anaemia in rats. The rats used in the study were divided into five groups. Group 1 served as normal control and received food and distilled water throughout the experiment, group II stood for anaemia-induced rats serving as a negative control, group III represented anaemia-induced rats that received 5 mg/kg of the standard drug (ferrous sulphate), groups IV and V were anaemia-induced rats that were administered 200 and 400 mg/kg of S. aethiopicum ethanolic leaf extract respectively. GC-MS analyses of the ethanol leaf extract revealed 15 bioactive compounds, most of which have antioxidant and other biological potentials. PHZ reduced the level of the haematological parameters: packed cell volume (PCV), red blood cell (RBC) count indices, haemoglobin (Hb) level, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) except for white blood cell count (WBC). The ethanolic leaf extract of S. aethiopicum extract though dose-dependent reversed the decrease in the haematological parameters indicating that the plant has anti-anaemic/haemolytic properties.*

Keywords: *Solanum aethopicum, anaemia, phenylhydrazine, toxicity, haematology, GC-MS*

Stella Mbanyeaku Ufearoh
Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia state, Nigeria. P.M.B. 7267, Umuahia, Abia State.
Email: akuchukwu57@gmail.com
Orcid id: 0000-0001-6713-5522

Onyinyechi Uloma Akoh
Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia state, Nigeria. P.M.B. 7267, Umuahia, Abia State.
Email: onyilac@gmail.com
Orcid id: 0000-0002-4357-5010

Christian Odih
Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia state, Nigeria. P.M.B. 7267, Umuahia, Abia State.
Email: ojemeriette@gmail.com
Orcid id: 0009-0003-1624-339X

Felicia Uchechukwu Okwunodulu
Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia state, Nigeria. P.M.B. 7267, Umuahia, Abia State.
Email: okwunodulufelicia@gmail.com

1.0 Introduction

Several investigations have established the beneficial role of most plants and their extracts, especially in medicinal/pharmaceutical and other applications. Anaemia is a condition that is characterized by a reduced number of red blood cells (RBCs) or decreased haemoglobin

concentration and poses a significant global health challenge *(Loechl,* et al*., 2023).* Some reported impact of anaemia *is that i*t leads to reduced oxygen transport capacity, resulting in fatigue, weakness, and a range of other health issues. Common causes of anaemia include nutritional deficiencies, chronic diseases, and genetic disorders (*Kajoba* et *al., 2024*). Although some conventional therapies exist, they are confronted with limitations such as side effects, high costs, and limited accessibility, particularly in low-resource settings. As a result, there is growing interest in exploring natural remedies that offer safer, cost-effective, and more accessible alternatives (*Zhu* et *al., 2021*).

Solanum aethiopicum, commonly known as the African eggplant or garden egg, is a widely cultivated plant in many African countries**,** including Nigeria. The plant has traditionally been used in folk medicine for the treatment of various ailments, including anaemia, due to its rich nutritional and medicinal properties (Osei Bonsu et al., 2021). Previous studies have identified *Solanum aethiopicum* as a source of bioactive compounds, including alkaloids, flavonoids, saponins, and phenolic compounds, which are known for their therapeutic potential (*Han* et *al., 2021*). These compounds have been associated with a range of pharmacological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Chaachouay and Zidane, 2024). However, the specific bioactive constituents responsible for the anti-anaemic properties of *Solanum aethiopicum* leaves and their modes of action have not been fully elucidated. However, most of the reported works on the medicinal applications of this plant are reported without detailed knowledge of its chemical composition.

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique widely used for the identification and characterization of complex mixtures of phytochemicals in plant extracts *(Eddy* et al*.,* 2011)**.** GC-MS allows for the separation, detection, and structural elucidation of volatile and semi-volatile compounds, providing valuable insights into the phytochemical profiles of medicinal plants (*Ma* et *al., 2024*). By employing GC-MS, researchers can identify the key bioactive compounds in *Solanum aethiopicum* leaves, which may contribute to its anti-anaemic and haematological effects *(Eddy* et al*.,* 2010**).**

This study aims to characterize the phytochemical constituents of ethanol extracts from *Solanum aethiopicum* leaves using GC-MS and to evaluate their anti-anaemic and haematological activities. By exploring the specific bioactive compounds present in the extracts and their potential mechanisms of action, this research seeks to provide scientific validation for the traditional use of *Solanum aethiopicum* in managing anaemia. Moreover, understanding the haematological effects of this plant could lead to the development of novel, natural therapies for anaemia and other blood-related disorders, offering a promising avenue for improving health outcomes, especially in regions with limited access to conventional medical treatments (Eze, 2023).

To meet the aim of this study, *Solanum aethiopicum* leaves were extracted using different solvents and different studies including anti-haemolytic studies but to the best of our knowledge, absolute ethanol has not been used, hence this study was aimed at using GC-MS to determine the active ingredients in the *S. aethiopicum* ethanolic leaf extract and studying the effect of the extract on phenylhydrazine-induced anaemia and haematology indices in Wistar rats.

2.0 Naterials and Methods

2.1 Sample Collection and Identification

Leaf samples of *solanum aethiopicum* were obtained from Orie Ugba market, Umuahia, Abia state, and were identified by Mr. Udo of the forestry department, Micheal Okpara University of Agriculture, Umudike. The

leaves were rinsed and cleaned. The leaves were then air-dried for about seven days and were well-blended to a powdery state. The blended leaves were then kept in an air-tight container ready for extraction.

Fig.1: *Solanum aethiopicum* growing plant

2.2 Sample preparation

The dried blended leaves were soaked in 96 % ethanol in an air-tight container (to avoid evaporation of the ethanol and contamination) for 48 hours and then filtered by the use of muslin cloth followed by filter paper. The filtrate was concentrated using a rotary evaporator at 37 – 40 ºC. The concentrate was then allowed to stay in a beaker covered with perforated foil for the remaining solvent to evaporate completely. The extract was kept in a sample bottle in the refrigerator for analysis.

2.3 Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis of the organic crude extract isolated from leaves of *S. aethiopicum* was performed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500, USA). 2µl of the sample extract was injected into the GC column for analysis and the relative percentage of the chemical constituents in crude extracts from *S. aethiopicum* leaves was expressed as a percentage by peak area normalization.

2.4 Identification of chemical constituents

Interpretation of the mass spectrum of GC-MS was conducted using the database of the National Institute of Standards and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, structure and biological activities of the components of the test materials were ascertained.

2.5 Experimental animal studies

Healthy Wistar rats of both sexes were used in the experiment. The rats were housed for acclimatization in the animal house of the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, under normal standard environmental conditions of temperature (25–28 °C), humidity (35–60%) and 12 h light/12 h dark condition. The rats were allowed free access to feed and water *ad libitum*. Experimental procedures and animal handling were approved by the Board of the Department of Biochemistry, which was in line with the National Institute of Health's guidelines for the care and use of Laboratory animals (NIH, 1978, publ. no. 8023; NRC, 1985). Ethical principles of the World Health Organization for Good Laboratory Practice regulations of 1998 and United States guidelines for experimental animals (NRC, 2010) were strictly adhered to throughout the study.

2.6 Experimental design

Twenty adult Wistar rats were randomly divided into five (5) groups. The haematological parameters of the rats under study were estimated. Apart from the rats serving as control, all other rats were induced with a single dose of 50 mg/kg phenylhydrazine (PHZ) via intraperitoneal (I.P) injection route for 2 consecutive days after an overnight fast. The PHZ administration was according to Ekweogu *et al*., (2019) 'when red blood cell (RBC) level as well as haemoglobin (Hb) concentration of the blood reduced to 30% or less'. The rats were then observed for 48 hrs during which they were fed with food and water *ad libitum* using a rat oral cannula,

after which the PCV of the rats was determined according to the procedures described by Ochei and Kolhatkar (2008). The rats with PCV lower than 50 % were considered anaemic and suitable for this study. The ethanolic leaf extract of *S. aethiopicum* was then administered orally to the rats for another 28 days. The rats were weighed daily throughout the experiment.

Group 1 stands for non-induced rats which served as normal control and received food and distilled water throughout the experiment, group II stands for anaemia-induced rats serving as negative control (i.e. without treatment), group III represents anaemia-induced rats that received 5 mg/kg of the standard drug (ferrous sulphate) purchased from Blessed Pharmacy, Umuahia, Nigeria), group IV are anaemia-induced rats that were administered 200 mg/kg of *S. aethiopicum* ethanolic leaf extract and group V are anaemia-induced rats that received 400 mg/kg of *S. aethiopicum* ethanolic leaf extract daily for 28 days. The method adopted was similar to the method of Oyeyemi *et al*., (2008) and Ogunka-Nnoka *et al*., (2018).

2.7 Acute toxicity studies

Ethanolic leaf extract of *S. aethiopicum* was studied for acute oral toxicity following the Organization for Economic Cooperation and Development (OECD) guideline 423 with little modifications. The extract was given in doses up to 5,000 mg/kg by oral route.

2.7.1 Blood sample collection

Exactly 28 days post-treatment with ethanolic leaf extract of *S. aethiopicum*, the rats were fasted overnight, anaesthetized by exposure to chloroform vapour for three minutes and sacrificed. Blood samples were collected by cardiac puncture and dispensed into EDTA (Ethylenediaminetetraacetic acid) containers for haematological tests. Haematology parameters investigated include: Packed Cell Volume (PCV), Red Blood Cell (RBC) count indices and white blood cell count (WBC), haemoglobin (Hb) level, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), according to the methods described by Bain *et al* (2016).

2.7.2 Statistical analysis

Data are given as mean ± standard error of the mean. Data were analyzed with analysis of variance (ANOVA) in which the negative control group was compared with the rest of the groups. Significance was set at $p < 0.05$.

3.0 Results and Discussion

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the ethanolic extract of *Solanum aethiopicum* leaves identified several compounds with varying retention times, molecular weights, and peak areas. These compounds include a range of bioactive molecules with potential therapeutic applications.

3.1 GC-MS analysis

While the GC-MS Chromatogram of the ethanol leaf extract of *S. aethiopicum* is shown in Figure 2, the signal peaks of the identified compounds are presented in Table 1, the physical properties and the biological effects of the bioactive compound in the extract are shown in Table 2 and 3 respectively. The GC-MS analysis of the ethanolic leaf extract of *S. aethiopicum* showed the presence of 15 bioactive compounds of known biological activities. This is in line with the findings of Zabbey et al., (2022). The GC-MS screening revealed that anthiaergostan-5,7,9,22-tetraen-3-ol also known as ergosterol has the least retention time of 3.113 mins while methaqualone has the highest retention time of 19.225 mins. Based on their structural features, Anthiaergostan-5,7,9,22-tetraen-3-ol has anti-inflammatory, antioxidant, anticancer, antimicrobial, cardioprotective, neuroproective and skin health functions while Methaqualone is a sedative-hypnotic drug that was popular in

the 1960s and 1970s. It has several biological activities and unfortunately, an abuse potential as well due to its sedative and euphoric effects. Due to its high potential for abuse, WHO (1980), classified it as a Schedule I controlled substance in many countries. Methaqualone acts as a sedative-hypnotic drug which like other drugs in that category can result in the depression of the central nervous system (CNS depression) (Jeffcoat et al.,1989). It helps in muscle relaxation, has anticonvulsant activity, and anxiolytic activity (anti-anxiety) but causes respiratory depression in overdose situations (Rubin et al.,1972). The biological activities of *Solanum aethiopicum* ethanol leaf extract as x-rayed in Table 3 are very crucial health desires hence *Solanum aethiopicum* leaves should be a household vegetable. The biological activities found out in this research work is in line with the findings of Zabbey et. al., (2022). Also, the antimicrobial activity of the *S. aethiopicum* leaf extract agrees with the findings of Adeyinka et. al., (2021) with another species of solanum called *Solanum sect. melongena*. Anosike et al., (2012) in their research with methanol as the extraction medium concluded that "methanol extract of *Solanum aethiopicum* has anti-inflammatory properties and can reduce inflammatory injury and tissue damage. Anosike et al., (2012) in another research also observed that the fruits of garden egg (*S. aethiopicum*) have anti-inflammatory activity when they studied the anti-inflammatory activities of this plant fruit using experimentally induced inflammatory models in rats.

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the ethanolic extract of *Solanum aethiopicum* leaves identified several compounds with varying retention times, molecular weights, and peak areas. These compounds include a range of bioactive molecules with potential therapeutic applications.

For example, anthiaergostan-5,7,9,22-tetraen-3-ol, with a retention time of 3.113 minutes and a molecular weight of 394 g/mol, showed an abundance of 5.89%. This compound is known for its anti-inflammatory, antioxidant, anticancer, and antimicrobial activities, and it may also have cardioprotective and neuroprotective effects. Benzoic acid, 4-(5,5-dimethyl-1,3-dioxan-2-yl)-, methyl ester, observed at a retention time of 3.799 minutes and with a molecular weight of 250 g/mol, had an abundance of 4.12%. It exhibits antibacterial, anti-inflammatory, antimicrobial, antifungal, antiviral, and antioxidant properties, with additional potential for neuroprotection and analgesia.

1H-1,2,4-Triazole, 3-methyl-5-(methylthio), with a retention time of 4.216 minutes and a molecular weight of 129 g/mol, had a peak area of 9.98%. It is noted for antimicrobial, antioxidant, anticancer, and anti-inflammatory activities. Dihexyl monoselenide, identified at a retention time of 7.731 minutes and a molecular weight of 250 g/mol, showed an abundance of 4.17%. This compound is recognized for its antimicrobial, antioxidant, anticancer, anti-inflammatory, immunomodulatory, and neuroprotective properties. Similarly, di-n-hexyl-diselenide, with a retention time of 9.023 minutes and a molecular weight of 330 g/mol, had an abundance of 11.54% and shares these properties: antimicrobial, antioxidant, anticancer, anti-inflammatory, immunomodulatory and neuroprotective activities

Hexane, 1,1-oxybis-, observed at a retention time of 10.177 minutes and with a molecular weight of 186 g/mol, had a peak area of 11.34%, though its biological effects are less documented. The compound 2,1,3-benzoselenadiazole, 5,6-dichloro, had a retention time of 10.920 minutes and a molecular weight of 252 g/mol, with a peak area of 6.85%. This compound exhibits anticancer, antioxidant, anti-inflammatory, antimicrobial, and neuroprotective activities. Mercury, chloromethyl, with a retention time

of 11.189 minutes and a molecular weight of 252 g/mol, had an abundance of 4.12%, but its biological effects are not well-characterized.

N-[3-Aminophenyl]-1-piperidinecarbothioamide, observed at a retention time of 12.384 minutes and with a molecular weight of 235 g/mol, showed a peak area of 4.30%.

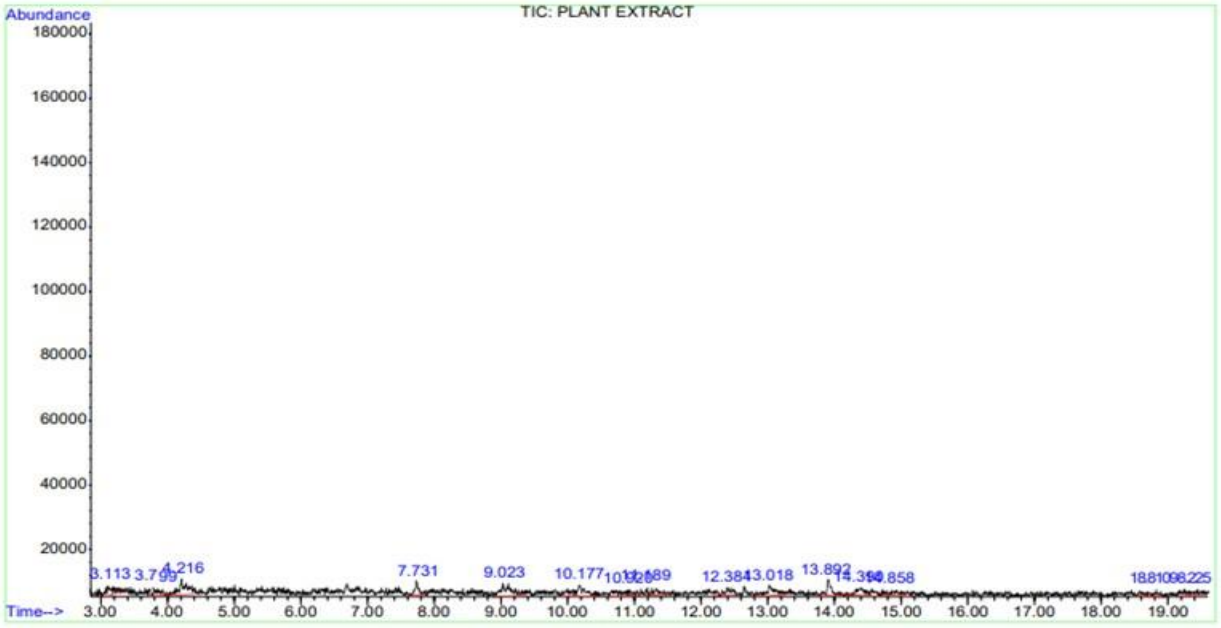


Fig 2: Chromatogram obtained from GC-MS screening of the ethanolic leaf extract of *S.aethiopicum.*

Table 1: Signal peaks of the identified compound

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.113	78	93	136	rBB	2842	20607	51.08%	5.892%
2	3.799	199	213	253	rBB	2388	14396	35.68%	4.116%
3	4.216	259	286	325	rBB	4548	34904	86.52%	9.981%
4	7.731	885	901	928	rBB	4132	14572	36.12%	4.167%
5	9.023	1104	1127	1189	rBB	3812	40343	100.00%	11.536%
6	10.177	1279	1329	1378	rBB	3111	39674	98.34%	11.345%
7	10.920	1404	1459	1486	rBB	1693	23943	59.35%	6.846%
8	11.189	1500	1506	1558	rBB	2076	14415	35.73%	4.122%
9	12.384	1675	1715	1738	rBB	2041	15045	37.29%	4.302%
10	13.018	1785	1826	1882	rBB	2946	30446	75.47%	8.706%
11	13.892	1955	1979	2035	rBB	4586	22475	55.71%	6.427%
12	14.390	2035	2066	2117	rBB	2198	25151	62.34%	7.192%
13	14.858	2125	2148	2197	rBB	1432	16343	40.51%	4.673%
14	18.808	2791	2839	2859	rBB	2120	18773	46.53%	5.368%
15	19.225	2892	2912	2962	rBB	1939	18631	46.18%	5.327%

Sum of corrected areas: 349718

Table 2: **Physical properties of bioactive compounds in the ethanolic leaf extract of *S.aethiopicum*.**

S/N	Name/Molecular Formula of Compound	Retention time (RT) (minutes)	Molecular weight (g/mol)	Peak Area/Abundance (%)
1	Anthiaergostan-5,7,9,22-tetraen-3-ol , $C_{28}H_{42}O$	3.113	394	5.89
2	Benzoic acid, 4-(5,5-dimethyl-1,3-dioxan-2-yl)-, methyl ester or Methyl 4-(5,5-dimethyl-1,3-dioxan-2-yl) benzoate/, $C_{14}H_{18}O_4$	3.799	250	4.12
3	1H-1,2,4-Triazole,3-methyl-5-(methylthio)- $C_4H_7N_3S$	4.216	129	9.98
4	Dihexyl monoselenide, $C_{12}H_{26}Se$	7.731	250	4.17
5	Di-n-hexyl-diselenide, $C_{12}H_{26}Se_2$	9.023	330	11.54
6	Hexane,1,1-oxybis- $C_{12}H_{26}O$	10.177	186	11.34
7	2,1,3-benzoselenadiazole,5,6-dichloro/5,6-Dichlorobenzoselenadiazole, $C_6H_2Cl_2N_2Se$	10.920	252	6.85
8	Mercury, chloromethyl, CH_3ClHg	11.189	252	4.12
9	N-[3-Aminophenyl]-1-piperidinecarbothioamide, $C_{12}H_{17}N_3S$	12.384	235	4.30
10	Methaqualone, $C_{16}H_{14}N_2O$	13.018	250	8.71
11	1-Methyl-2,5-dichloro-1,6-diazaphenalene, $C_{12}H_8Cl_2N_2$	13.892	250	6.43
12	3-(2-Hydroxy-6-methylphenyl)-2-methyl-4(3H)-quinazolinone $C_{16}H_{14}N_2O_2$	14.390	266	7.19
13	1-Methyl-2,5-dichloro-1,6-diazaphenalene, $C_{12}H_8Cl_2N_2$	14.858	250	4.67
14	4(3H)-Quinazolinone, 3-(3-hydroxy-2-methylphenyl)-2-methyl $C_{16}H_{14}N_2O_2$	18.808	266	5.37
15	Methaqualone, $C_{16}H_{14}N_2O$	19.225	250	5.33

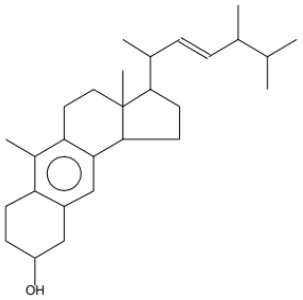
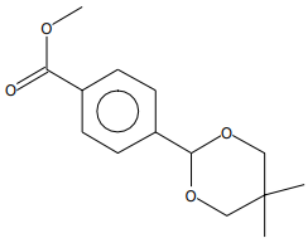
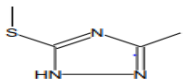
It is known for its antimicrobial, anticancer, analgesic, and antioxidant activities. Methaqualone, with a retention time of 13.018 minutes and a molecular weight of 250 g/mol, had an abundance of 8.71%. This compound is recognized for its sedative, hypnotic, muscle relaxant, anticonvulsant, and anxiolytic effects. Another compound, 1-Methyl-2,5-dichloro-1,6-diazaphenalene, with a retention time of 13.892 minutes and a molecular weight of 250 g/mol, had a peak area of 6.43% and was associated with antimicrobial activity.

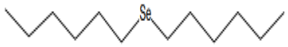
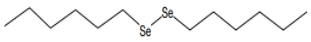
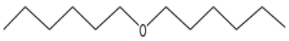
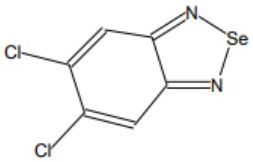
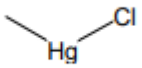
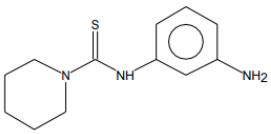
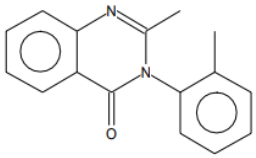
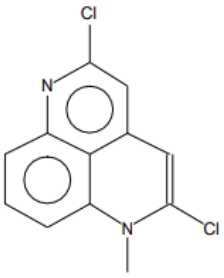
3-(2-Hydroxy-6-methylphenyl)-2-methyl-4(3H)-quinazolinone, identified at a retention time of 14.390 minutes and with a molecular weight of 266 g/mol, had an abundance of 7.19%. This compound is known for its antimicrobial, anticancer, anti-inflammatory, and neuroprotective effects. The same compound was identified again at a retention time of 14.858 minutes with a peak area of 4.67%.

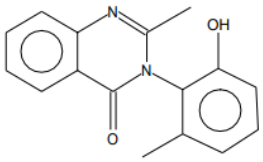
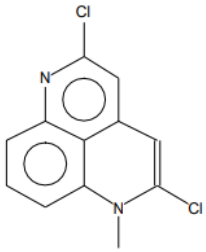
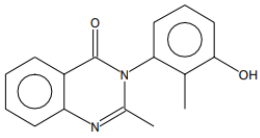
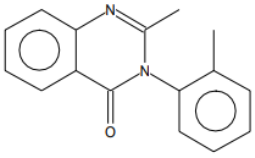
Lastly, 4(3H)-Quinazolinone, 3-(3-hydroxy-2-methylphenyl)-2-methyl, with a retention time of 18.808 minutes and a molecular weight of 266 g/mol, showed a peak area of 5.37%. It exhibits antimicrobial, anticancer, anti-inflammatory, and neuroprotective activities.

The GC-MS analysis results highlight the presence of several bioactive compounds in the ethanolic leaf extract of *Solanum aethiopicum*. These findings suggest significant potential for therapeutic applications, including anti-inflammatory, antioxidant, anticancer, antimicrobial, and neuroprotective effects. The diverse range of identified compounds underlines the plant's value as a source of bioactive molecules with potential health benefits. Further research could elucidate the specific mechanisms through which these compounds exert their effects and explore their potential for drug development or therapeutic use.

Table 3: Possible biological effects of some identified compounds in the ethanolic leaf extract of *S. aethiopicum.*

S/N	Structure	Possible Biological Effects
1		Anti-inflammatory activity (Kadian and Punia, 2019). Antioxidant activity, anticancer potential, antimicrobial effect (K. Patel and P. Patel, 2016) Cardioprotective effect, neuroprotective activity, skin health function.
2		Anti-bacterial, anti-inflammatory, antimicrobial (Williams and Barry,1983), antifungal, antiviral, anti-oxidant (Schewe and Yuen,1982), anti-cancer activities as well as neuroprotective and analgesic potentials.
3		Antimicrobial, antioxidant (Shah *et al*., 2011), anticancer, anti-inflammatory activities

4		Antimicrobial (Schomburg & Schweizer, 2009), antioxidant (Rayman, 2000), anticancer (Hatfield & Gladyshev, 2002), anti-inflammatory (Brigelius-Flohé, & Maiorino, 2013), immunomodulatory (Avery & Hoffmann, 2018) and neuroprotective activities (Cardoso et al., (2018).
5		Antimicrobial (Schomburg & Schweizer, 2009), antioxidant (Rayman, 2000), anticancer (Hatfield & Gladyshev, 2002), anti-inflammatory (Brigelius-Flohé & Maiorino, 2013), immunomodulatory (Avery & Hoffmann, 2018) and neuroprotective activities (Cardoso *et al*., (2018).
6		
7		Anticancer (Pan *et al*., 2019), antioxidant (Zhang & Taylor, 2005), anti-inflammatory (Brigelius-Flohé & Maiorino,2013), antimicrobial (Spallholz & Hoffman, 2002) and neuroprotective (Papp *et al*.,2007) activities.
8		
9		Antimicrobial(Gupta *et al*.,2010), anticancer (Chen *et al*., 2012), analgesic (Singh *et al*., 2002) and antioxidant activities.
10		Sedative and hypnotic effects, muscle relaxant activity, anticonvulsant activity and anxiolytic activity.
11		Antimicrobial activity.

12		Antimicrobial activity, anticancer activity, anti-inflammatory activity and neuroprotective.
13		Antimicrobial
14		Antimicrobial activity, anticancer activity, anti-inflammatory activity and Neuroprotective.
15		Sedative and hypnotic effects, m**uscle relaxant activity,** a**nticonvulsant activity** *and a*nxiolytic activity

3.2 Anti-anaemic Activity

3.2.1 Acute Toxicity Result

The acute toxicity test of the ethanol leaf extract of *s. aethiopicum* leaves on rats (Tables 4 and 5) showed no form of aggressiveness, pile erection, decreased motor activity, tremors, convulsions, muscle spasm, diarrhoea or mortality deaths amongst the rats, even at 5000 mg/kg, but touch response, respiration and alertness were observed. There were no significant behavioural or neurological changes. Thus, no symptoms of toxicity were observed during the investigation and hence the leaves could be safe for both human and animal consumption.

Table 4: Stage 1 Acute toxicity (LD_{50}) evaluation of the extract

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	200	0/3	0.00	No mortality was observed, instead, animals remained active and physically stable.
2	500	0/3	0.00	No mortality was observed, instead, animals remained active and physically stable.
3	1000	0/3	0.00	No mortality was observed, instead, animals remained active and physically stable.

Table 5: Stage 2 Acute toxicity (LD_{50}) evaluation of the extract

Group	Dose (mg/kg)	No. of Deaths	Percentage of mortality	Observations
1	2000	0/3	0.00	No mortality was observed, instead, animals remained active and physically stable.
2	3000	0/3	0.00	No mortality was observed, instead, animals remained active and physically stable.
3	5000	0/3	0.00	No mortality was observed, instead, animals remained active and physically stable.

3.3 Haematological Parameters

The results of the effect of the plant leaf extract on the studied haematological indices are presented in Table 6. The table showed that induction of anaemia caused a significant ($p < 0.05$) decrease in the levels of all the haematologic parameters except WBC. A significant ($p < 0.05$) decrease was observed in the anaemic control group (group 2) for all parameters except for WBC. No significant difference was observed between group 1(the control) and group III treated with the standard drug. Whereas the group treated with the standard drug generally performed better than the group treated with 200 mg/kg of the extract, those treated with an increased dose of the extract (400 mg/kg) showed better performance than those treated with the standard drug and their performances can be compared to those in the normal group that were not induced with anaemia. This indicates that there was a gradual reversal of anaemic condition when extracts of *S.aethiopicum* were administered to the rats and the rise in these factors was observed to be dose-dependent. Our studies revealed that phenylhydrazine reduced the level of the haematological parameters but the *S. aethiopicum* extract reversed them, especially at higher doses The increase in these blood parameters could be a result of the body's response to the effect of the PHZ resulting in the production of blood cells in order not to deprive the animal of oxygen in circulation.

Table 6: Result of ethanolic extract of *S.aethiopicum* on haematology

Treatment Group	Normal	PHZ control	Standard Control (5 mg/kg Ferrous sulphate)	S. aethiopicum 200 mg/kg/bw	S. aethiopicum 400 mg/kg/bw
RBC (x10^6)	6.95±0.07	2.78±0.12	7.65±0.05	4.83±0.11	7.85±0.18
PCV (%)	45.20±0.37	24.80±0.86	45.37±1.06	44.60±0.40	50.60±0.60
HB (g/dl)	15.52±0.19	8.32±0.19	15.45±0.19	12.04±0.19	15.51±0.18
WBC (x10^3)	8.79±0.11	10.71±0.33	10.82±0.35	9.85±0.31	11.40±0.10
MCV (fl)	65.10±0.91	63.31±1.30	65.31±0.09	62.08±1.36	67.05±2.73
MCH (pg)	22.35±0.31	18.05±0.95	23.31±0.61	22.27±0.53	27.95±1.00
MCHC (g/dl)	34.34±0.26	30.62±0.58	34.40±0.28	32.81±0.33	35.06±0.14

RBC: Red blood cell, PCV: Packed cell volume, Hb: Hemoglobin, WBC: White blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, Values are expressed as mean ± standard error mean.

The longer the treatment with the extract, the more significant ($P< 0.05$) increase there was with the higher dosage compared to the standard drug and the negative/PHZ control. This is in agreement with the findings of Ramu *et al.,* (2019) who evaluated the haematinic activity of *Tamarindus indica L.* leaf extract. Our findings except on WBC are also concordant with the findings of Aduwamai *et al.,* (2018), who studied the effect of *Solanum nigrum* methanol leaf extract on phenylhydrazine-induced anaemia in rats. In line with this, Ogunka-Nnoka *et al*., (2018) observed that the ethanolic stalk extracts of *S. aethiopicum* showed a significant increase in PCV, Hb and RBC levels in albino-treated rats, Onyeabo *et al*., (2017) also observed that the PCV and RBC count in PHZ anaemia-induced albino rats increased significantly after 28 days of treatment with *J. carnea* extract. The findings of this work also aligned with the result obtained by Ekweogu *et al*., (2019) though they observed no significant changes in MCHC relative to the negative control.

Our studies in addition are in agreement with the observations of Pandey *et al*, (2016), Aduwamai *et al*., (2017), Ramu *et al*., (2019). Our findings showed that the extract treatment increased the WBC level beyond the rise caused by PHZ, this is in contrast to the findings of Aduwamai *et al*., (2018), Onyeabo *et al*., (2017) and Ekweogo *et al*., (2019). They observed that the WBC increases on induction of phenylhydrazine and that treatment with both the extracts doses and the standard drug could not ameliorate the situation and the WBC levels decreased even as the extract concentration increased. They attributed the elevated level of total WBC count in the rats induced with anaemia without treatment to the injury caused by PHZ. Okonkwo *et al*., (2015) thought that the rat's immune system may assume that the cause of anaemia could be a result of infection or disease and hence an increase in the production of white blood cells to fight such infections. WBC counts increase rapidly following exposure to foreign attacks on the system by toxins and pathogens. It is expected that there should be a significant increase in the WBC of the extract-treated rats to reverse the anaemic condition induced by the PHZ and boost the body's immune system. Meanwhile, a system will boost its defence mechanism which is the WBC on suspecting of an attack (Eyong et al., 2004). A decrease in the haemoglobin (Hb) level in the blood (generally less than 13.5 g/dL in men and 12.5 g/dL in women) is an indication of the onset of anaemia. Iron deficiency, vitamin B(B12), or folate deficiencies decrease the production of Hb or may result in a defect in the structure of Hb (Rhodes and Varacallo, 2020) and a decrease in the number of RBCs. Phenylhydrazine (PHZ), though useful in the treatment of polycythemia vera and fever (Berger, 2007) has a toxic effect on red blood cells due to its activation of reactive oxygen species production hence it has been linked to oxidative stress (Berger, 2007) which induces haemolysis (Fibach and Rachmilewitz, 2008), Lim *et al.,* (2000), and Fujii *et al*., (2021). This PHZ anaemic can be reversed by phytochemicals and the antioxidant vitamins present in plants. Antioxidants counteract reactive oxygen species (ROS). *S. eathiopicum* contains phytochemicals and rich antioxidant vitamins hence its ability to exhibit anti-anaemic properties. Zabbey VZ *et. al*., (2022) stated that *S. aethiopicum* leaves contained alkaloids, phenols, and flavonoids which are rich in antioxidants, tannins among other phytochemicals. Sheth *et al*., (2021) stated that among phenolic compounds, flavonoids seem to be the main substances responsible for bone marrow protection against the deleterious effects of phenylhydrazine. For the prevention and treatment of anaemia, iron which is the main constituent of haemoglobin is of great importance in the body. Deficiency of iron in the body causes severe disorders of which iron deficiency anaemia is the most important (Lieu *et al*., 2001). Saha *et al*., (2022) in their

research found that *S. aethiopicum* leaf powder among *Moringa oleifera* and *Hibiscus sabdariffa*, had the highest iron content (6.15 mg/100 g DW) which is low compared to (36.86 mg/100g DW) recorded by Achikanu *et al*., (2013) and that *S. aethiopicum* leaf has the potential to address iron deficiency anaemia among children aged 6 to 23 months. Uzu *et al*., (2017) in their research detected the presence of Ca, Mg, K, Mn, Fe, Cu, Zn and P in both ripe and unripe *Solanum aethiopicum L*. fruits with Fe having the highest percentage increase in concentration. Copper functions in haemoglobin synthesis and aids in the transport of iron to cells (Turnlund, 1998). Eggplant constitutes a high level of minerals ($Fe^{2+,}$ Zn^{2+} and Cu^{2+}), vitamins (A, C, E, B) and phytochemicals such as polyphenols and flavonoids which are potent antioxidants, Anosike *et al*., (2011). The fruits and stalk of *S. aethipicum* possess ascorbic acid and flavonoids which have high antioxidant potential (Diatta *et al*., 2020). Phytochemical studies of another species of *Solanum,* '*S. macrocarpon'* leaves showed that the leaves contained appreciable quantities of flavonoids among other polyphenols (Komlaga *et al*., 2014), and these flavonoids have strong antioxidant properties. These phytochemicals and secondary metabolites that have antioxidant activity have the potency that can protect the body's cells from oxidative stress and free radical damage (Chansiw *et al*., 2018) which causes hemolytic anaemia. According to Aduwamai *et al*. (2018), these antioxidants may have moped up free radicals and inhibited the anaemia induced by PHZ. According to Trivedi and Pandey (2020), some phytochemicals or herbs directly induce the resolution of anaemia while others act pleiotropically through their antioxidant activity either by increasing oxidative stress resistance or by triggering cellular mechanisms, like autophagy. Other phytochemicals and herbs resolve anaemia in the elderly via their antioxidant activity by targeting inflammation and subsequently reducing the anaemia associated with chronic inflammation (Macciò and Madeddu, 2012). It has been reported that the important protective antioxidant effects exhibited by apple vinegar were related to the phenolic and flavonoid contents of apple vinegar (Ousaaid *et al*., 2022) and that the synergistic interaction between bioactive ingredients of apple vinegar provides its ability to counteract phenylhydrazine-induced haemolytic anaemia (Ousaaid *et al*., 2022).

4.0 Conclusion:

Compounds with good antioxidant activity can protect and prevent organ injuries induced by PHZ and other toxic agents by quenching the activities of free radicals. The findings of this study have been able to reveal that beyond the use of the ethanolic leaf extract of *S. aethiopicum* in ameliorating phenylhydrazine induced-anaemia due to its rich antioxidant content, it also has anti-inflammatory, anti-cancer, anti-microbial, anticonvulsant activities. In addition, it possesses analgesic, immunodulatory, cardioprotective, neuroprotective, muscle relaxant, anxiolytic and skin health functions. The plant has the potential phytoconstituents and minerals that can help to improve the overall health benefits of those that feed on it.

Based on the results and findings of the study, it is hereby recommended that *S. aethiopicum* leaves, fruit and even stalks should be consciously consumed either as snacks or as vegetables eaten as it is or included in daily diets for persons of all ages. It is also recommended that research be geared towards the development of industrial processes for the large-scale extraction of polyphenol and secondary metabolites concentrates of *S. aethiopicum* plant (and other species of *Solanum*) due to their numerous biological/ pharmacological potentials to serve as main, alternative, or adjuvant therapies in many diseases including various types of anaemia. In addition, this work lends voice to (Komlaga *et*

al., 2014) who suggested that there is an urgent need for a rapid increase of natural antioxidants in the food industry instead of synthetic antioxidants which have potential toxicity and carcinogenic effects.

5.0 References

Abdo, E.M.; Shaltout, O.E.-S.; El-Sohaimy, S.; Abdalla, A.E. & Zeitoun, A.M. (2021). Effect of Functional Beetroot Pomace Biscuit on Phenylhydrazine Induced Anemia in Albino Rats: Hematological and Blood Biochemical Analysis. *J. Funct. Foods*, 78, pp. 104385.

Achikanu, C. E.; Eze-Steven, P. E.; Ude, C. M. & Ugwuokolie, O. C. (2013). Determination of the vitamin and mineral composition of common leafy vegetables in southeastern Nigeria. *International Journal of Current Microbiology and Applied Sciences*, 2, pp. 347–353.

Adeyinka, O. A., Ayo, E. F., Abiola, G. F, ; Ayanronke, S. A. & Felicia, A. D. (2021). Comparative antimicrobial, phytochem - ical, nutritional and GCMS profiling of methanolic extracts of *Solanum Sect. Melongena. International Journal of Agriculture and Biological Sciences*, pp. 5, 2, pp.82-91.

Aduwamai, U. H., Abimbola, M. M. & Ahmed, Z. H. (2018). Effect of *Solanum nigrum* Methanol leaf extract on phenylhydrazine induced anemia in rats. *Jordan Journal of Biological Sciences*, 11,1, pp. 65 –67.

Anosike C.A.; Obidoa, O. & Ezeanyika, L.U. (2012). The anti-infammatory activity of garden egg (*Solanum aethiopicum*) on egg albumin-induced oedema and granuloma tissue formation in rats. *Asian Pacific Journal of Tropical Medicine,* 5, pp. 62–66.

Anosike, C.A., Abonyi, O. &Ubaka, C. (2011). Does the African garden egg offer protection against experimentally induced ulcers? *Asian Pacific Journal of Tropical Medicine,* 4, pp.163–166.

Anosike, C.A.; Obidoa, O. & Ezeanyika, L. U. (2012). Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). *DARU Journal of Pharmaceutical Sciences*, 20, 76. http://www.darujps.com/content/20/1/76

Avery, J. C. & Hoffmann, P. R. (2018). Selenium, selenoproteins, and immunity. *Nutrients*, 10, 9, pp. 1203.

Bain, B.J.; Bates, I. & Lafan, M.A. (2016). *Dacie and Lewis practical haematology*. *Elsevier*, Amsterdam.

Berger, J. (2007). Phenylhydrazine Haemotoxicity. *Journal of Applied Biomedicine*, 5, pp. 125–130.

Brigelius-Flohé, R. & Maiorino, M. (2013). Glutathione peroxidases. *Biochimica et Biophysica Acta (BBA)*-General Subjects, 1830, 5, pp. 3289-3303.

Cardoso, B. R.; Hare, D. J. & Bush, A. I. (2018). Selenium, selenoproteins and neurodegenerative diseases. *Metallomics*, 10, 10, pp. 1585-1587.

Chaachouay, N., Zidane, L. (2024). Plant-Derived Natural Products: A Source for Drug Discovery and Development. *Drugs and Drug Candidates*, 3, 1, pp. 184-207. https://doi.org/10.3390/ddc3010011.

Chansiw, N.; Paradee, N.; Chotinantakul, K. & Srichairattanakool, S. (2018). Anti-hemolytic, antibacterial and anti-cancer activities of methanolic extracts from leaves and stems of *Polygonum odoratum*. *Asian Pacific Journal of Tropical Biomedicine,* 8,12, doi: 10.4103/2221-1691.248094

Diatta, K., Diatta, W., Fall, A. D.; Ibra, S., Dieng, M., Mbaye, A. I., Sarr, A. & Adegbindin, M. A. (2020). Evaluation of the Antioxidant Activity of Stalk and Fruit of *Solanum aethiopicum L. (Solanaceae*). *Asian Journal of Research in Biochemistry,* 6, pp. 6-12, doi: 10.4274/tjps.77598

Ekweogu, C. N., Ude, V. C., Nwankpa, P., Emmanuel, O. & Ugbog, E.A. (2019).

Ameliorative effect of aqueous leaf extract of *Solanum aethiopicum* on phenylhydrazine-induced anaemia and toxicity in rats. *Toxicological Research*. https://doi.org/10.1007/s43188-019-00021-5

Eyong, E. U., Umoh, I. B., Ebong, P. E., Eteng, M. U.; Antai, A. B. & Akoa, A. O. (2004). Heamotoxic effects following ingestion of Nigerian crude oil and crude oil polluted shellfish by rats. *Nigerian Journal of Physiological Sciences*, 19, 1-2,, pp. 1–6.

Eze, U.A., Nwankwuo, I. U., Apeh, O. G., Ezeh, G. C. & Onoja, S. O. (2023). Antioxidant, antibacterial and antibiofilm activities of ethanol extract of Zapotecca portoricensis roots. Journal of Sustainable Veterinary and Allied Sciences, *5*, 2, pp. 105-111

Fibach, E. & Rachmilewitz, E. (2008). The Role of Oxidative Stress in Hemolytic Anemia. *Curr. Mol. Med*., 8, pp. 609–619.

Fujii, J., Homma, T., Kobayashi, S., Warang, P., Madkaikar, M. & Mukherjee, M. B. (2021). Erythrocytes as a Preferential Target of Oxidative Stress in Blood. *Free. Radic. Res.*, 55, pp. 781–799.

Hatfield, D. L. & Gladyshev, V. N. (2002). How selenium has altered our understanding of the genetic code. *Molecular and Cellular Biology*, 22. 11, pp. 3565-3576.

Jeffcoat, A. R., Perez-Reyes, M. & Hill, J. M. (1989). Pharmacokinetics and pharmacodynamics of methaqualone in humans. *Clinical Pharmacology and Therapeutics,* 46, 5, pp. 536-544.

Kadian, R. & Punia, S. (2019) Steroids: A Journey from Physiology to Therapeutics. *Journal of Applied Pharmaceutical Science,* 9, 6, pp.154-160.

Kajoba, D., Egesa, W. I., Muyombya, S., Ortiz Y. A., Nduwimana, M. & Ndeezi, G. (2024). Prevalence and Factors Associated with Iron Deficiency Anaemia among Children Aged 6-23 Months in Southwestern Uganda. *Hindawi International Journal of Pediatrics,*2024. https://doi.org/10.1155/2024/666377

Komlaga, G., Sam, G. H., Dickson, R. A., Mensah, M. L. K. & Fleischer. T. C., (2014). Pharmacognostic studies and antioxidant properties of the leaves of *Solanum macrocarpon. J Pharm Sci Res,* 6, pp. 1-4. .

Lieu, P. T., Heiskala, M., Peterson, P. A. & Yang. Y. (2001). The roles of iron in health and disease. *Molecular Aspects of Medicine*, 22, pp. 1-87.

Lim, Y. K., Jenner, A., Ali, A. B. ,Wang, Y., Hsu, S. I.. H., Chong, S. M, Baumman, H., Halliwell, B. & Lim, S. K. (2000). Haptoglobin reduces renal oxidative dna and tissue damage during Phenylhydrazine-Induced Hemolysis. *Kidney Int*., 58, pp. 1033–1044.

Loechl, C. U., Datta-Mitra, A., Fenlason, L., Green, R, ; Hackl, L., Itzkowitz. L., Koso-Thomas, M., Moorthy, D., Owino, V. O., Pachon, H., Stoffel, N., Zimmerman, M. B., Raiten, D. J. (2023). Approaches to address the anemia challenge. *The Journal of Nutrition,* 153, pp. S42-S59, https://doi.org/10.1016/j.tjnut.2023.07.017

Macciò, A. & Madeddu, C. (2012). Management of anemia of inflammation in the elderly. Anemia, 2012;pp. 2012:563251. doi: 10.1155/2012/563251

National Research Council (NRC) (2010) *Guide for the care and use of laboratory animals*. National Academies Press, Washington.

Ochei, J. & Kolhatkar, A. (2008). *Medical laboratory sciences: Theory and practice*. pp. 663–665. New York: Tata McGraw Hill. Retrieved from https://www.researchgate. net/publication/290158410.

Ogunka-Nnoka C. U.; Onyegeme-Okerenta, B. M. & Omeje, H. C. (2018). Effects of ethanol extracts of *S. aethiopicum* stalks

on lipid profile and haematological parameters of Wistar Albino Rats. *International Journal of Science and Research methodology*, 10, 3, pp. 215-229.

Okonkwo, C. C., Agu, C. V., Njoku, O. U., Abonyi, U., Apeh, V., Anaduaka, E. G. & Odo, C. E. (2015). Hypoglycaemic and haematinic properties of ethanol leaf extract of *Artocarpus heterophyllus* in alloxan induced diabetic rats. *Afr. J. Tradit. Comp. Altern. Med.*, 12, 2, pp. 144–148. http://dx.doi.org/10.4314/ajtcam.v12i2.21

Onyeabo, C., Achi, N. K, Ekeleme-Egedigwe, C. A., Ebere, C. U. & Okoro, C .K. (2017). Haematological and biochemical studies on J*usticia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats. *Acta Sci. Pol. Technol Aliment*, 16, 2, pp. 217-230. doi: 10.17306/J.AF - S.0492.

Organization for Economic Cooperation and Development (OECD) (2001) *Guideline 423: acute oral toxicity—acute toxic class method*. 470 adopted by the council on 17th, December 2001.

Ousaaid, D., Ghouizi, A. E., Laaroussi, H., Bakour, M.; Mechchate, H.; Es-safi, I. , Kamaly, O. A., Saleh, A., Conte, R., Lyoussi, B. & El Arabi, I. (2022). Anti-anemic effect of antioxidant-rich apple vinegar against phenylhydrazine-induced hemolytic anaemia in rats. *Life*, 12, 239, https://doi.org/10.3390/ life12020239.

Ousaaid, D.; Laaroussi, H.; Bakour, M.; El Ghouizi, A.; El Menyiy, N.; Lyoussi, B.; El Arabi, I. (2022). Effect of a Combination of *Rosa Canina* Fruits and Apple Cider Vinegar against Hydrogen Peroxide-Induced Toxicity in Experimental Animal Models. *J. Food Qual*. **https://doi.org/10.1155/2022/7381378**

Pandey, S., Ganeshpurkar, A., Bansal, D. & Dubey, N. (2016). Hematopoietic Effect of *Amaranthus cruentus* Extract on Phenylhydrazine-Induced Toxicity in Rats. *Journal of Dietary Supplements*, doi: 10.3109/19390211.2016.1155685.

Papp, L. V.; Lu, J.; Holmgren, A. & Khanna, K. K. (2007). From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxidants & Redox Signaling*, 9, 7, pp. 775-806.

Pan, J., Ke, S., Guo, H., Yang, M. & Wang, X. (2019). Synthesis and biological activity of benzisoselenazol-3(2H)-ones and benzisoselenazoles. *Heterocycles*, 98,1, pp. 415-427.

Patel, D. K. & Patel, P. (2016). In vitro antimicrobial activity of sapogenin extract of *Acacia nilotica (Linn.)* leaves. *International Journal of Pharmaceutical Sciences and Research,* 7, 2, pp.707-711.

Ramu, S., Murthy, S., K.; Krishna, S., Somashekaraiah, A. L.., Nandihalli, V. B. & Muzammil, K.M. (2019). Preclinical Evaluation of the Haematinic Activity of an Oral Indiffusible Mixture of *Tamarindus indica L*. Leaf Extract. *Turk J Pharm Sci.,*16, 1, pp.27-31.

Rayman, M. P. (2000). The importance of selenium to human health. *The Lancet*, 356,9225, pp. 233-241.

Rhodes, C. E. & Varacallo, M. (2020). *Physiology, oxygen transport. In StatPearls; StatPearls Publishing: Treasure Island,* FL, USA. Available online: https://www.ncbi.nlm.nih.gov/books/NBK538336/

Rubin, R. B.; Neugarten, J. & King, J. C. (1972). Respiratory effects of methaqualone and its interaction with ethanol. *Clinical Pharmacology & Therapeutics*, 13, 5, pp. 777-783.

Sarah, K., Meriem, K. & Mustapha, T. (2022). Antihemolytic activity of hydroalcoholic leaves and bark extracts from *Rhamnus alaternus* against AAPH 724induced hemolysis on human erythrocytes.

International Journal of plant-based pharmaceuticals, 2, 2, pp. 210-219.

Schewe, K. A. & Yuen, H. K. (1982). Antioxidant properties of alkyl esters of benzoic acid. *Journal of Agricultural and Food Chemistry*, 30, 4, pp. 722- 724.

Shah, A. M., Patel, A. P., Patel, H. B. *et al*. (2011). Synthesis and evaluation of some new triazole derivatives for antioxidant activity. *International Journal of Research in Pharmaceutical Sciences*, 2, 4, pp. 693-697.

Schomburg, L. & Schweizer, U. (2009). Hierarchical regulation of selenoprotein expression and sex-specific effects of selenium. *Biochimica et Biophysica Acta (BBA)*-General Subjects, 1790, 11, pp. 1453-1462.

Sheth, P. A., Pawar, A. T., Mote, C.S. & More, C. (2021). Antianemic Activity of Polyherbal Formulation, *Raktavardhak Kadha*, against Phenylhydrazine-Induced Anemia in Rats. *J. Ayurveda Integr. Med*., 12, pp. 340–345.

Siraki, A. G., Klotz, L.O. & Kehrer, J. P. (2018). Free radicals and reactive oxygen species. *Compr Toxicol.*; 1, pp. 262–294.

Spallholz, J. E. & Hoffman, D. J. (2002). Selenium toxicity: Cause and effects in aquatic birds. *Aquatic Toxicology*, 57, 1-2, pp. 27-37.

Trivedi, S. & Pandey, R. (2020). 50 -Hydroxy-6, 7, 8, 30, 40 -pentamethoxyflavone extends longevity mediated by DR-induced autophagy and oxidative stress resistance in C. elegans. Geroscience.

Turnlund, J. R. (1998). Human whole-body copper metabolism. *American Journal of Clinical Nutrition*, 67, 5, pp. 960-4.

Uzu, M. Adaramola, B., Aleshinloye, A., Jegede, D., Shokunbi, O., Adewumi, A.,
Ogunnowo, A. & Onigbinde, A. (2017). Assessment of variation in mineral content of ripe and unripe African eggplant fruit (*Solanum aethiopicum L.*) Exocarps. *Journal of Pharmacognosy and Phytochemistry*, 6, 5, pp. 2548-2551.

Vethanarayanan, P., Unnikannan, P., Baskaran, L. & Sundaramoorthy, P. (2011). Survey on traditional medicinal plants used by the village peoples of Cuddalore district, Tamilnadu, India. *Asian J Biochem Pharm Res*, 1, 3, pp. 351-361.

WHO (1980). Expert Committee on Drug Dependence. "Methaqualone: a review of the evidence of its dependence potential." *World Health Organization Technical Report Series*, 648, pp. 35-46.

Williams, A. A. & Barry, K. D. (1983). Evaluation of methyl and propyl paraben as preservatives in two pharmaceutical syrup formulations. *Journal of Pharmaceutical Sciences,* 72, 6, pp. 676-678.

Zabbey, V. Z. 1., Adienbo, O. M., Ojeka, S. O. & Dapper, D. V. (2022). Phytochemical screening and gc–ms analysis of bioactive compounds present in hydroethanolic leaf extract of S*olanum aethiopicum* (*L). International Journal of Biological and Medical Sciences, 5, 3, pp. 1-9.*

Zhang, J. & Taylor, E. W. (2005). Selenium and selenoproteins in the brain and brain diseases. *Journal of Neurochemistry*, 88, 3, pp. 580-593.

Zhu, Z., Sudfeld, C. R., Cheng, Y. *et al.* (2021). Anemia and associated factors among adolescent girls and boys at 10–14 years in rural western China. *BMC Public Health* **21**, 218. https://doi.org/10.1186/s12889-021-10268-z.

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Not Applicable

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