Nigerian Journal of Pharmaceutical and Applied Science Research
Vol. 13 (1): 1-7; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net; https://doi.org/10.60787/nijophasr-v13-i1-536

Antioxidant effect of bi-herbal formula of *Picralima nitida* and *Cymbopogon citratus* extracts in phenyl-hydrazine induced Anaemia in albino Wistar rats

***Progress A. Obazelu and Daniel E. Williams**

Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Nigeria.

ABSTRACT

***Background*:** Medicinal plants such as *Picralima nitida* and *Cymbopogon citratus* contain bioactive compounds with antioxidant properties, which may mitigate oxidative stress and protect against various diseases. Antioxidant enzymes like glutathione peroxidase and superoxide dismutase play critical roles in cellular defense mechanisms against oxidative damage. Therefore, the aim of this study was to determine the effect of bi-herbal formula of *Picralima nitida* and *Cymbopogon citratus* aqueous leaves extracts on Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) gene expressions in Phenyl Hydrazine-Induced Anaemia in Albino Wistar rats.

***Methods*:** Sixty adult male albino Wistar rats were divided into six groups; A, B, C, D, E and F (control), phenyl-hydrazine group, ferrous sulphate group, phenyl-hydrazine + 100mg/kg bi-herbal formulation of *Picralima nitida* and *Cymbopogon citratus*, phenyl-hydrazine + 200mg/kg bi-herbal formulation of *Picralima nitida* and *Cymbopogon citratus* and phenyl-hydrazine + 400mg/kg bi-herbal formulation of *Picralima nitida* and *Cymbopogon citratus* respectively. mRNA of GPx and SOD were determined using polymerase chain reaction.

***Results*:** There was a significant increase in the mRNA expression of GPx of group C, D and E when compared to groups A and B ($p<0.05$). mRNA expression of GPx of group D and E was significantly lower, compared to group C ($p<0.05$). There was a significant increase in the mRNA expression of SOD of groups C, D, E and F compared to groups A and B ($p<0.05$).

***Conclusion*:** This study concluded that the groups treated with ferrous sulfate and varying doses of the bi-herbal formulation exhibited increased mRNA expression of GPx and SOD.

***Keywords*:** *Cymbopogon citratus*, Glutathione peroxidase, Phenyl hydrazine, *Picralima nitida*, Superoxide dismutase

1. INTRODUCTION

Medicinal plants have been used for centuries in traditional medicine systems worldwide due to their diverse array of bioactive constituents with therapeutic properties [1]. These bioactive compounds, such as alkaloids, flavonoids, terpenoids, and polyphenols, exert various pharmacological effects, including anti-inflammatory, antioxidant, antimicrobial, and analgesic activities [2]. *Picralima nitida*, commonly known as *Akuamma*, is a tropical tree indigenous to West Africa, particularly found in countries like Ghana, Nigeria, and Ivory Coast [3]. *Picralima nitida* has demonstrated antioxidant activity, which may contribute to its cytoprotective effects against oxidative stress-induced damage [4]. *Cymbopogon citratus*, commonly known as Lemongrass, is a tropical plant revered for its culinary and medicinal uses. This versatile herb has been employed in traditional medicine systems for centuries, revered for its antimicrobial, anti-inflammatory, antifungal, and antioxidant effects. Its antioxidant constituents play a crucial role in scavenging free radicals and protecting cells from oxidative damage [5]. Antioxidants are a diverse group of compounds that play a crucial role in protecting cells from oxidative damage caused by free radicals and reactive oxygen species (ROS). Antioxidants neutralize free radicals by donating electrons, thereby stabilizing them and preventing further damage to cells [6]. Antioxidants may be vitamins, enzymes, or other organic compounds that

* **Corresponding author: Email: progress.obazelu@uniben.edu; Phone: +2348056733255**

can inhibit or delay the oxidation of molecules by neutralizing free radicals and preventing oxidative damage. Examples of vitamins that act as antioxidants include vitamins C and E. In addition to vitamins C and E, examples of antioxidant enzymes include superoxide dismutase (SOD), catalase, and glutathione peroxidase [7]. Glutathione peroxidase is a vital antioxidant enzyme that plays a central role in protecting cells from oxidative damage. Found in various tissues and cell types throughout the body, glutathione peroxidase functions by catalyzing the reduction of hydrogen peroxide and organic hydro-peroxides, using glutathione as a cofactor [8]. By neutralizing these harmful molecules, glutathione peroxidase helps to maintain cellular redox balance and protect biomolecules such as lipids, proteins, and DNA from oxidative damage. Superoxide dismutase (SOD) is another essential antioxidant enzyme that catalyzes the conversion of superoxide radicals into hydrogen peroxide and molecular oxygen, thereby preventing the accumulation of superoxide radicals and reducing oxidative stress [9]. SOD is found in various cellular compartments, including the cytoplasm, mitochondria, and extracellular space, where it plays a crucial role in neutralizing superoxide radicals and protecting cells from oxidative damage [10]. Phenyl hydrazine, a chemical compound commonly utilized to induce hemolytic anaemia in experimental animal models, serves as a valuable tool for studying the pathophysiology of anaemia and evaluating potential therapeutic interventions [11]. By inducing the formation of reactive oxygen species (ROS), phenyl hydrazine promotes oxidative stress and leads to the destruction of red blood cells. *Picralima nitida* and *Cymbopogon citratus* are traditional medicinal plants known for their antioxidant properties and potential health benefits. Both plants have been reported to contain bioactive compounds with antioxidant and cytoprotective effects, which could mitigate oxidative damage and improve red blood cell function in anemic conditions. By evaluating the effects of these plant extracts on the expression of key antioxidant enzymes such as glutathione peroxidase (GPx) and superoxide dismutase (SOD), this research seeks to elucidate the underlying molecular mechanisms involved in their therapeutic action. The findings from this study could contribute to the development of novel therapeutic interventions for managing anaemia and related hematological disorders, ultimately improving the health outcomes of affected individuals. The aim of this study therefore is to determine the effect of bi-herbal formula of *Picralima nitida* and *Cymbopogon citratus* aqueous leaf extract on Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) gene expressions in Phenyl Hydrazine-Induced Anaemia in Albino Wistar rats.

2. MATERIALS AND METHODS

2.1. Materials

2.2.1. Biological Materials

Cymbopogon citratus leaves, *Picralima nitida* leaves and albino Wistar rats.

2.1.2 Reagents and equipment

Eppendorf Containers, Sorvall biofuge, Germany eppendor mastercycler, Germany Labnet, Electrophoresis system, USA micro pipettes, Hisense Microwave, UV-visible, Trizol, Chloroform, ZymoDNA extraction kit, Loading dye, EZ-Vision, TBE buffer, Nuclease Free Water, Agarose [12].

2.2. Methods

2.2.1. Study Population

In this study, animal (rats) model was used. A total of sixty (60) of the Albino Wistar strain were purchased from the animal holdings of the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed at the animal housing wing of the Department of Anatomy, University of Benin.

2.2.2. Identification of Cymbopogon citratus and Picralima nitida Leaves

Cymbopogon citratus and Picralima nitida leaves were collected at Oluku community in Ovia North- East Local Government Area, Edo State on the 23rd of December 2023. The leaves were then identified and authenticated by Dr. A. O Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City.

2.2.3. Processing of Cymbopogon citratus and Picralima nitida Leaves

The procedure began by removing any unhealthy leaves from the sample. Subsequently, the leaves underwent a thorough washing process followed by drainage. To facilitate proper grinding, the leaves were air-dried under shade for duration of two weeks. Further drying was then carried out using a hot air oven at 50ºC for 24 hours. This ensured that the leaves were adequately dried and prepared for grinding. The grinding process itself was conducted using a high-speed grinding machine, specifically an industrial 1000A high-speed grinder. Finally, 250 grams of each leaf were precisely weighed for subsequent usage.

2.2.4. Preparation of Plants Extract
250 grams of ground powder were mixed with 2.5 liters of distilled water. Subsequently, the mixture was left to soak for duration of 24 hours under constant storage conditions. After the specified duration, the mixture underwent filtration using Whatman's (Nitro cellulose 45; 0.45µm pore size) filter paper, with the residue being discarded. Following filtration, the resulting filtrate was subjected to concentration in a Water bath maintained at 45°C until it reached a paste-like consistency. The paste obtained from this process was then accurately weighed and subsequently dissolved in distilled water to achieve the recommended concentrations for administration.

2.2.5. Animal Care
Animals were housed in a cross ventilated room in the animal holdings of the department of anatomy, University of Benin, Benin City. Animals were exposed to 12 hours dark and light cycles with access to feed and water *ad libitum*. The rats were acclimatized for a period of two (2) weeks before commencement of the experiment.

2.2.6. Ethical Consideration
Ethical approval was obtained from Research Ethics Committee on animal subjects from Edo State Ministry of Health, Benin City (Ref Number: HA/737/23/B.200600195 issued on 14th, December, 2023).

2.2.7. Preparation of Phenyl-hydrazine and Ferrous Sulphate Drug Solution
2.2.7.1. Phenyl-hydrazine Solution
Phenyl-hydrazine solution was prepared by combining phenyl-hydrazine (manufactured by Sigma-Aldrich, Batch Number: PHZ789001) with distilled water v/v and 2-propanol in a ratio of 1:5:5. This entailed mixing 1 part of phenyl-hydrazine with 5 parts of distilled water v/v and 5 parts of 2-propanol. Subsequently, 0.2ml of this phenyl-hydrazine solution was administered to each animal in the various test groups, with an average weight of 150g, every 48 hours for duration of 28 days.

2.2.7.2. Ferrous Sulphate Drug Solution
Ferrous sulphate Drug Solution was made by mixing 1000mg of the powdered drug in 50ml of distilled water. 0.3ml of this drug solution was administered orally to each animal in group C of an average weight of 150g every 48 hours for 28 days.

2.2.8. Research Design
Grouping of Animals: Sixty (60) Mature Wistar rats weighing 150-200g were randomly selected and divided into six groups (n = 10 per group). The Groups were the Group A, Group B, Group C, Group D, Group E and Group F.

Group A: This was the control group. Animals in this group received only standardized feed (Manufactured by KARMA AGRIC FEEDS AND FOOD LIMITED, Oyo State) and clean water *ad libitum.*

Group B: This group received only phenyl-hydrazine intraperitoneally.

Group C: Animals in this group were administered phenyl-hydrazine solution and treated with the standard drug solution (ferrous sulphate) intraperitoneally.

Group D: Animals in this group were administered phenyl-hydrazine solution intraperitoneally and treated with low dose of bi herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally.

Group E: Animals in this group were administered phenyl-hydrazine solution intraperitoneally and treated with a higher dose of bi herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally.

Group F: Animals in this group were administered phenyl-hydrazine solution intraperitoneally and treated with the highest dose of bi herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally.

2.2.9. Administered Doses of bi herbal formulation of Cymbopogon citratus and Picralima nitida Leaves Extract
Group A (control) received only standardized feed and clean water *ad libitum*. Group B (phenyl-hydrazine treated group) were administered 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days. Group C (ferrous sulphate drug solution treated group) were administered 0.2ml of phenyl-hydrazine solution intraperitoneally

every 48 hours for 28 days and treated with 0.3ml of 6mg/ml of ferrous sulphate 48 hourly for 28 days. Group D were administered with 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days and treated with 0.15ml of 100mg/kg body weight of bi herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally using a gavage tube every 24 hours for 28 days. Group E were administered with 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days and treated with 0.3ml of 200mg/kg body weight of bi herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally using a gavage tube every 24 hours for 28 days. Group F were administered with 0.2ml of phenyl-hydrazine solution intraperitoneally every 48 hours for 28 days and treated with 0.6ml of 400mg/kg body weight of bi herbal formulation of *Cymbopogon citratus* and *Picralima nitida* leaves extract orally using a gavage tube every 24 hours for 28 days.

2.2.10. Sacrifice of Animals and Collection of Samples

At the end of the experimental period, the animals were grossly observed for general physical characteristics. A midline incision was made through the ventral wall of the rats after anaesthetizing (using chloroform) and cervical dislocation. Bone marrow samples were also obtained from the rats by opening the femur longitudinally and exposing the marrow cavity. A sterile forceps was used to obtain the bone marrow from the cavity and placed in an Eppendorf container containing Trizol for molecular analysis.

2.2.11. Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) mRNA Assay

2.2.11.1. Isolation of Total RNA

Total RNA was isolated from whole rat samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNAse I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

2.2.11.2. cDNA conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65°C for 5 min, 42 °C for 1 h, and 80°C for 5 min [13].

2.2.11.3. PCR amplification and Agarose Gel Electrophoresis

Polymerase chain reaction (PCR) for the amplification of gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa): PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95ºC for 5 min, followed by 30 cycles of amplification (denaturation at 95ºC for 30 s, annealing for 30 s and extension at 72ºC for 60 s) and ending with final extension at 72ºC for 10 min. The amplicons were resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J" software [14].

2.2.11.4. Primers

GPx
Forward: TGAGAAGTGCGAGGTGAATG
Reverse: GAAAGCGCCTGGTGTATCT

SOD
Forward: AGGGCCTGTCCCATGATGTC
Reverse: AGAAACCCGTTTGCCTCTACTGAA

GAPDH
Forward: CTCCCTGGAGAAGAGCTATGA
Reverse: AGGAAGGAAGGCTGGAAGA

2.3. Statistical Analysis

Data obtained from this research was presented and analyzed using statistical package for social sciences (SPSS) version 21.0 (IBM Inc. USA). Bar chart was used to represent the mRNA gene expression patterns. A p value of ≤0.05 was considered statistically significant.

Nigerian Journal of Pharmaceutical and Applied Science Research
Vol. 13 (1): 1-7; March 2024 ISSN: 2971-737X (Print); ISSN: 2971-7388.
Available at www.nijophasr.net https://doi.org/10.60787/nijophasr-v13-i1-536

3. RESULTS

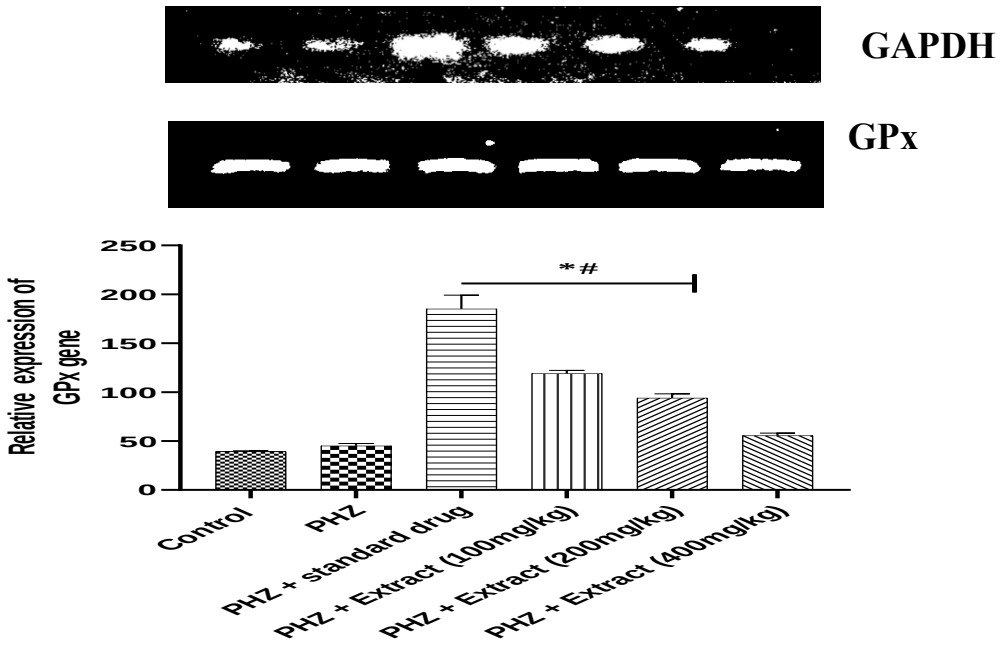

Figure 1: mRNA Expression of Glutathione peroxidase (GPx) of the Studied Groups.
* Represents statistical difference to control. # Represents statistical difference to phenyl-hydrazine induced group at p<0.05.
Key: PHZ=Phenyl-hydrazine, GAPDH=Glyceraldehyde-3-Phosphate Dehydrogenase

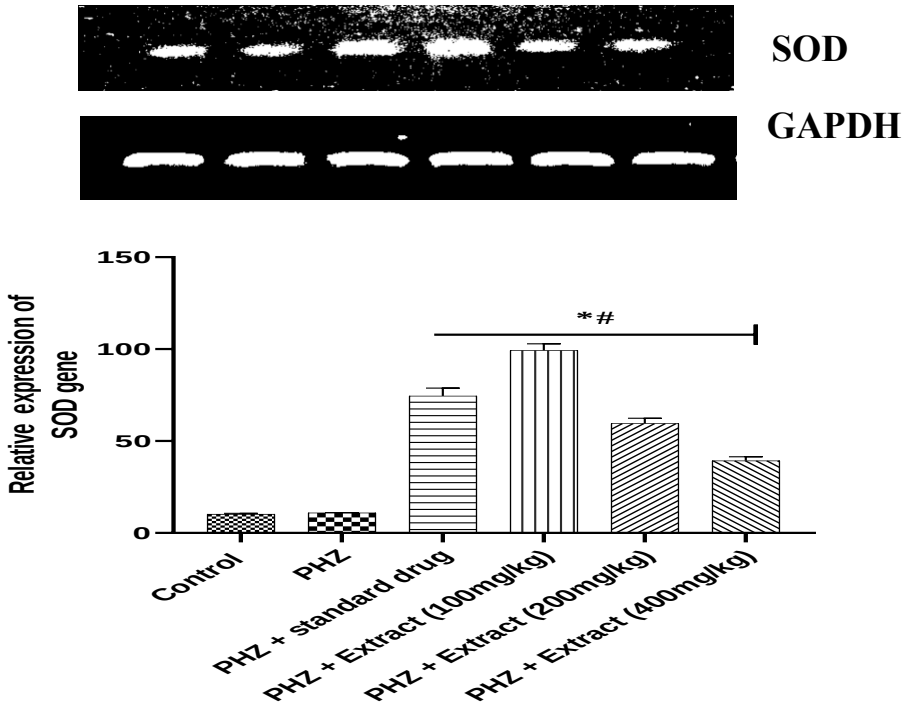

Figure 2: mRNA Expression of Superoxide dismutase (SOD) of the Studied Groups.
* Represents statistical difference to control. # Represents statistical difference to phenyl-hydrazine induced group at p<0.05.
Key: PHZ=Phenyl-hydrazine, GAPDH=Glyceraldehyde-3-Phosphate Dehydrogenase.

4. DISCUSSION

The investigation into the effect of a bi-herbal formula comprising *Picralima nitida* and *Cymbopogon citratus* aqueous leaves extract on glutathione peroxidase (GPx) and superoxide dismutase (SOD) in phenyl hydrazine-induced anaemia in Albino Wistar rats delves into a crucial aspect of herbal medicine and its potential therapeutic applications. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) generation and the body's antioxidant defense mechanisms, leading to cellular damage and dysfunction [15]. The utilization of *Picralima nitida* and *Cymbopogon citratus* highlights the rich potential of herbal remedies in addressing health disorders [16]. The rationale behind this research lies in exploring the ability of these plant extracts to ameliorate oxidative stress-induced damage in anaemia, specifically targeting the enzymatic antioxidants GPx and SOD. By evaluating the impact of the bi-herbal formula on these parameters, insights into its potential therapeutic efficacy can be gained, contributing to the development of novel treatments for anaemia and related conditions. This study showed that ferrous sulphate treated group exhibited a substantial increase in the mRNA of GPx compared to the control and phenyl-hydrazine-treated groups. This up-regulation of GPx mRNA expression in this group suggests a potential induction of antioxidant defense mechanisms in response to ferrous sulphate supplementation, emphasizing its role in mitigating oxidative stress associated with phenyl hydrazine-induced anaemia. Furthermore, the groups treated with different doses of the bi-herbal formulation, demonstrated significantly higher GPx mRNA expression levels compared to the control and phenyl-hydrazine-treated groups, mirroring the observations in the ferrous sulphate treated group. This indicates a dose-dependent effect of the bi-herbal formulation on GPx gene expression, further highlighting its potential antioxidant properties. However, it is noteworthy that the mRNA expression of GPx in the group administered 100 mg/kg and 200 mg/kg of the bi-herbal formulation was significantly lower than that of ferrous sulphate treated group, despite the observed increase compared to the control and phenyl-hydrazine-treated groups. This discrepancy may suggest differential effects of ferrous sulphate and the bi-herbal formulation on GPx expression levels. These findings align with the work of Feyisayo and Victor [17] who in their study reported the antioxidant nature of *Picralima nitida* and that of Vázquez- Briones et al [18] who showed the antioxidant properties of *Cymbopogon citratus*.. SOD expression in the group treated with ferrous sulphate, demonstrated a substantial increase compared to the control and phenyl-hydrazine-treated groups. This augmentation in SOD mRNA expression indicates a potential induction of antioxidant defense mechanisms in response to ferrous sulphate supplementation. The groups treated with different doses of the bi-herbal formulation, exhibit significantly higher SOD mRNA expression levels compared to the control and phenyl-hydrazine-treated groups. This dose-dependent effect of the bi-herbal formulation on SOD gene expression further showed its potential antioxidant properties. Notably, the increased SOD mRNA expression across these groups implied enhanced scavenging of superoxide radicals and subsequent reduction of oxidative damage, highlighting the therapeutic efficacy of the bi-herbal formulation in combating phenyl hydrazine-induced anaemia.

5. CONCLUSION

Data from this study concludes that the ferrous sulphate treated group and the groups treated with different doses of the bi-herbal formulation, demonstrated significantly higher GPx mRNA expression levels compared to the control and phenyl-hydrazine-treated group. Furthermore, the ferrous sulphate treated group and the groups treated with different doses of the bi-herbal formulation, exhibited significantly higher SOD mRNA expression levels compared to the control and phenyl-hydrazine-treated group.

Acknowledgement

We wish to acknowledge all staff of the Animal house, Department of Anatomy, University of Benin, Benin City for the space to house our study rats.

Conflict of Interest

The Authors declare no conflict of interest.

Contribution of the Authors

Progress A. Obazelu: conceptualization and manuscript writing.
Daniel E. Williams: Laboratory Analysis.

6. REFERENCES

[1] Ramawat, K. G., Dass, S., Mathur, M. (2009). The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. Herbal Drugs: Ethnomedicine to Modern Medicine, 1(1): 7-32.

[2] Mustafa, G., Arif, R., Atta, A., Sharif, S., Jamil, A. (2017). Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. Matrix Science Pharmaceuticals, 1(1): 17-26.

[3] Erharuyi, O., Falodun, A., Langer, P. (2014). Medicinal uses, phytochemistry and pharmacology of *Picralima nitida* (Apocynaceae) in tropical diseases: A review. Asian Pacific Journal of Tropical Medicine, 7(1): 1-8.

[4] Fakeye, T. O., Itiola, O. A., Odelola, H. A. (2000). Evaluation of the antimicrobial property of the stem bark of *Picralima nitida* (Apocynaceae). Phytotherapy Research, 14(5): 368-370.

[5] Lawrence, R., Lawrence, K., Srivastava, R., Gupta, D. (2015). Antioxidant activity of lemongrass essential oil (Cympopogon citratus) grown in North Indian plains. Journal of Scientific Temper, 4(1): 23-9.

[6] Sisein, E. A. (2014). Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences, 2(2): 110-118.

[7] Yadav, A., Kumari, R., Yadav, A., Mishra, J. P., Srivatva, S., Prabha, S. (2016). Antioxidants and its functions in human body-A Review. Research in Environmental and Life Science, 9(11): 1328-1331.

[8] Brigelius-Flohé, R., Maiorino, M. (2013). Glutathione peroxidases. Biochimica et Biophysica Acta (BBA)-General Subjects, 1830(5): 3289-3303.

[9] Bafana, A., Dutt, S., Kumar, A., Kumar, S., Ahuja, P. S. (2011). The Basic and Applied Aspects of Superoxide Dismutase. Journal of Molecular Catalysis B: Enzymatic, 68(2): 129-138.

[10] Fattman, C. L., Schaefer, L. M., Oury, T. D. (2003). Extracellular superoxide dismutase in biology and medicine. Free Radical Biology and Medicine, 35(3): 236-256.

[11] Berger, J. (2007). Phenyl hydrazine haematotoxicity. Journal of Applied Biomedicine, 5(3): 125-30.

[12] Obazelu, P. A., Wasa, F. E. (2024). Proinflammatory Effects of Launaea Taraxacifolia Aqueous Leaves Extract in Benzene Induced Haematotoxicity in Albino Male Wistar Rats. Nigerian Journal of Pharmaceutical and Applied Science Research, *12*(4): 34-40.

[13] Elekofehinti, O. O., Lawal, A. O., Ejelonu, O. C., Molehin, O. R., Famusiwa, C. D. (2020). Involvement of fat mass and obesity gene (FTO) in the anti-obesity action of Annona muricata Annonaceae: in silico and in vivo studies. Journal of Diabetes and Metabolic Disorders, 19: 197-204.

[14] Olumegbon, L. T., Lawal, A. O., Oluyede, D. M., Adebimpe, M. O., Elekofehinti, O. O., Umar, H. (2022). Hesperetin protects against diesel exhaust particles-induced cardiovascular oxidative stress and inflammation in Wistar rats. Environmental Science and Pollution Research, 29(35): 52574-52589.

[15] Ozougwu, J. C. (2016). The role of reactive oxygen species and antioxidants in oxidative stress. International Journal of Research, 1(8): 1-8.

[16] Tepa, A. G. N., Ambassa, P., Ayong, L. S., Biapa Nya, P. C., Pieme, C. A. (2022). The antiplasmodial potential of medicinal plants used in the Cameroonian pharmacopoeia: an updated systematic review and meta-analysis. Evidence-Based Complementary and Alternative Medicine, 2(1): 1-5.

[17] Feyisayo, A. K., Victor, A. C. (2019). Assessment of antioxidant and antidiabetic properties of Picralima nitida seed extracts. Journal of Medicinal Plants Research, 13(1): 9-17.

[18] Vázquez-Briones, M. D. C., Hernández, L. R., Guerrero-Beltrán, J. Á. (2015). Physicochemical and Antioxidant Properties of *Cymbopogon citratus* Essential Oil. Journal of Food Research, 4(3): 36-45.