Communication in Physical Sciences, 2025, 12(3) 834-845

Antioxidant Properties and Reproductive Health Benefits of *Opa eyin* Herbal Concoction: *In vitro* and *In vivo* Evaluation

Uduak Irene Aletan*, Abraham Gana Yisa, Sunday Adenekan and Abiodun Emmanuel Adams

Received: 26 December 2024/Accepted: 03 March 2025/Published: 13 March 2025
https://dx.doi.org/10.4314/cps.v12i3.13

Abstract: *There is an increased demand and consumption of aphrodisiac and reproductive-beneficial herbal mixtures in Nigeria. The empirical data to validate the perceived therapeutic properties of herbal concoctions like Opa eyin remains undocumented. Therefore, this study investigated the phytochemical composition and antioxidant properties of Opa eyin, a traditional herbal concoction used in South Western Nigeria. A total of 20 healthy male Wistar rats were assigned to 4 groups (n=5). The animals received graded doses of Opa in (0.20, 0.40, and 0.80 ml/kg body weight respectively) for 28 days. The animals were then sacrificed and the antioxidant evaluation of Opa eyin was carried out on the liver, kidney, and testes according to standard protocols. The data generated was subjected to statistical analysis in R statistical programming version 4.3.0. Phytochemical analysis revealed high levels of anthocyanins (109.91 mg/100 g), flavonoids (98.82 mg/100 g), steroids (58.39 mg/100 g), and alkaloids (56.55 mg/100 g), which are associated with antioxidant, anti-inflammatory, and antimicrobial effects. The concoction demonstrated significant ($p < 0.05$) in vitro antioxidant activity through DPPH radical scavenging, reducing power, and nitric oxide scavenging assays, exhibiting a dose-dependent response. In vivo experiments on Wistar rats revealed dose-dependent antioxidant effects in the liver, kidney, and testes. Opa eyin improved the reproductive health of the exposed animals, evidenced by reduced oxidative stress in the testes, suggesting potential benefits for fertility. These findings underscore its therapeutic potential and validate its traditional use, although further studies are needed to elucidate its long-term safety and efficacy.*

Keywords: Opa eyin, Oxidative stress, Antioxidants, Reproductive health

Uduak Irene Aletan*
Department of Biological Sciences, Faculty of Science, National Open University of Nigeria, Abuja, Nigeria
Email: ualetan@noun.edu.ng
Orcid id: https://orcid.org/0000-0003-0071-9632

Abraham Gana Yisa
Department of Biological Sciences, Faculty of Science, National Open University of Nigeria, Abuja, Nigeria
Email: ayisa@noun.edu.ng
Orcid id: https://orcid.org/0000-0002-9936-5418

Sunday Adenekan
Department of Biochemistry, University of Lagos, Lagos, Nigeria
Email: sundayadenekan2@gmail.com

Abiodun Emmanuel Adams
Department of Biological Sciences, Faculty of Science, National Open University of Nigeria, Abuja, Nigeria
Email: aadams@noun.edu.ng
Orcid id: https://orcid.org/0009-0006-6598-5172

1.0 Introduction

Oxidative stress occurs when there is an imbalance between free radicals and antioxidants in the body. These free radicals which are indispensable molecules in any biochemical process (Phaniendra *et al.*, 2015) are highly unstable and attack easily accessible biomolecules like lipids, protein, and nucleic acids. This imbalance has been

implicated in the etiology of several common diseases (Muscolo, 2024). Antioxidants however have been known to react with these free radicals acting as oxygen scavengers. Antioxidants can be enzymatic or non-enzymatic in biological systems. The enzymatic antioxidants include catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) while Vitamin C, Vitamin E (alpha-tocopherol) and some phytochemicals for example, flavonoids also have antioxidant properties (Jomova *et al*.,2024). These antioxidants are known to be present in plants used in the production of traditional herbal medicine (Ulewicz-Magulska & Wesolowski ,2023).

Traditional herbal medicine has been a significant source of health for man for many centuries. In the present-day world, traditional herbal medicines are still relevant in health care delivery (WHO,2023). This is especially true in developing countries with inadequate access to health services and basic medicines. A growing fraction of the population has continued to become dependent on traditional herbal medicine for reasons which include among other things cost (Tumuhaise *et al*.,2021), and presumed absence of toxicity among other things (Van Wyka & Prinsloo,2020). *Opa eyin* is one of such herbal medicine.

Opa eyin is a formulated herbal concoction mostly used to manage erectile dysfunction in South Western Nigeria. *Opa eyin* has great popularity among artisans and traders in the Lagos metropolis and it is used to enhance sexual performance. According to Zaid *et al*. (2020) *Opa eyin* consists of the following herbs: *Senna fistula, Chasmathera deperdens, Carpolobia lutea, Lecaniodiscus cupanioides, Crossandra puberula, Aristolochia indica.* An earlier study suggested that prolonged use of *Opa eyin* especially higher doses resulted in haematological abnormalities and hepato-renal toxicity (Aletan *et al.*,2022) in Wistar rats.

Furthermore, there is a dearth of information regarding the *in vitro* antioxidant activity of *Opa eyin* as well as the *in vivo* effect on oxidative stress biomarkers. While oxidative stress biomarkers are vital indices for assessing disease progression and the result of therapeutic interventions, the effects of the usage of *Opa eyin* on these biomarkers remain essentially unidentified. This study addresses the substantial gap in the current literature by investigating the *in vitro* antioxidant activity of *Opa eyin* as well as its *in vivo* effect on oxidative stress biomarkers in Wistar rats.

2.0 Materials And Methods

The sample of *Opa eyin* was obtained from a traditional medicine hawker in the Mushin market in the Mushin Local Government Area of Lagos State, Nigeria. There was no evidence of official registration of this remedy. The hawkers interviewed were not willing to give out their recipes due to their oath of secrecy.

2.1 Phytochemical screening

Phytochemical analysis of *Opa eyin* sample was carried out using the methods of Sofowora (1999).

2.2 In vitro antioxidant activity

2.2.1 Quantitative DPPH radical scavenging assay

The method of Gyamfi *et al*., (1999) was adapted for the determination of the scavenging activity of the concoction on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. To 2 ml of the sample at four different concentrations (25, 50, 75 and 100 µg/ml) by diluting 10-fold in 80% methanol was added 2 ml of 0.1 mM solution of DPPH in methanol. The mixtures were allowed to stand at room temperature in the dark for 30 minutes. Thereafter, absorbance was taken 517 nm. Ascorbic acid was taken as the standard while methanol was used as the blank (control). The percentage of DPPH scavenging capacity was calculated at each concentration using equation 1

$$\% \text{ DPPH scavenging activity} = \frac{A_{control} - A_{sample}}{\text{Absorbance of Control}} \times 100 \quad (1)$$

where $A_{control}$ and A_{sample} are the absorbances of the control and of the sample respectively.

2.2.2 Reducing power method (RP)

The method described by Oyaizu (1986) was used with slight modifications. An aliquot of 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of Potassium ferricyanide (1% w/v) was added to 1.0 ml of sample (at four different concentrations 25, 50, 75 and 100 µg/ml) in methanol.

The resulting mixture was incubated at 50 ºC for 20 min, followed by the addition of 2.5 ml of Trichloroacetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of Ferric chloride (0.1%, w/v). 1 ml of methanol was used as a blank. The absorbance is then measured at 700 nm against blank sample. An increase in absorbance of the reaction mixture indicates the reducing power of the samples.

2.2.3 Nitric oxide inhibition

An aliquot of 2 ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of the sample at various concentrations 25, 50, 75 and 100 µg/ml). The mixture was then incubated at 25 ºC for 150 min. Three milliliters (3 ml) of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)] was added to the mixture. The same mixture without the sample was used as the control. The mixture was incubated at room temperature for 30 min and its absorbance measured at 546 nm (Alam *et al*., 2012). The amount of nitric oxide radical inhibition is calculated following the equation

$$\% \text{ inhibition of nitric oxide} = \frac{A_{control} - A_{sample}}{\text{Absorbance of Control}} \times 100 \quad (2)$$

where $A_{control}$ and A_{sample} are the absorbances of the control and of the sample respectively.

2.3 Experimental Animals

A total of 20 healthy male Wistar rats were obtained from the Department of Pharmacognosy, University of Lagos. The animals weighing between 200-220g were housed in plastic cages within the animal house of the department. The experimental animals were allowed to acclimatize for 7 days before experimentation during which they were fed with pelletized animal feed and clean water. The NIH Guide for the Care and Use of Laboratory Animals (National Institute of Health, 2011) was strictly followed.

The twenty (20) male albino Wistar rats were assigned to 4 groups (n=5). The first group received 0.4 ml/kg of normal saline and served as control, while the 2nd, 3rd, and 4th groups received graded doses of *Opa eyin* (0.20, 0.40, and 0.80 ml/kg bodyweight respectively). These doses were chosen based on the acute toxicity study carried out earlier on the concoction (Aletan, *et al*.,2022). At the end of the 28-day treatment period, the animals were deprived of feed but had free access to drinking water for 24 hours before being anesthetized under inhaled chloroform. The abdomen and the thorax were opened and the organs (liver, kidney, testis) were quickly removed and placed in sterile containers. A portion of 0.5g of tissue of each organ was homogenized in 4.5 ml of buffer solution (ice-cold phosphate buffer, pH 7.4) using homogenizer. The resulting homogenates were centrifuged in sterile bottles at 15,000 rms for 10 mins in a centrifuge. The resulting supernatant was collected and stored at 4º C until required for the biochemical investigations.

2.4 In vivo antioxidant activity

2.4.1 Determination of Superoxide Dismutase Activity

Superoxide Dismutase activity was determined by the ability of the enzyme to inhibit the auto-oxidation of epinephrine. This was determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of tissue homogenate, and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of the substrate (epinephrine), and

0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

2.4.3 Determination of Catalase activity

Catalase activity was determined according to Sinha *et al.* (1972). It was assayed colorimetrically at 620 nm and expressed as μmoles of H_2O_2 consumed/min/mg protein at 25ºC. The reaction mixture (1.5 ml) contained 1.0 ml of 0.01M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate, and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

2.4.3 Determination of Reduced Glutathione

The reduced glutathione (GSH) content of the tissues as non-protein sulphydryls was estimated according to the method described by Sedlak

and Lindsay (1968). To the homogenate, 10% Trichloroacetic acid (TCA) was added and centrifuged. 1.0 ml of supernatant was treated with 0.5ml of Ellman's reagent (19.8 mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

2.5 Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24 N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100ºC for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDA-TBA a complex of 1.56×10^5 M^{-1} cm^{-1}.

2.6 Statistical analysis

The results from the investigations were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to determine the differences in the means between groups. Tukey's multiple comparison test was used to separate the means where significant differences existed. Differences in means were considered significant at $p < 0.05$. All analyses were performed in R statistical program version 4.3.0

3.0 Results and Discussion

3.1 Phytochemical Analysis

A preliminary qualitative screening of *Opa eyin* revealed the presence of phenol, alkaloid, flavonoid, tannin, saponin, terpenoid, steroid, phlobatanin and reducing sugar

The result of the quantitative phytochemical analysis of *Opa eyin* is presented in Table 1. The result reveals a total anthocyanin content of 109.91 mg/100g and a reducing sugar of 31.30 mg/100g. An appreciable amount (98.82 mg/100g) of flavonoids was also recorded in comparison to the other phytochemicals present. Also present are phenol, tannin, terpenoid, steroid, saponin, and alkaloids.

Table 1. Phytochemical constituents of *Opa eyin*

Phytochemical	Quantity (mg/100 g)
Flavonoids	98.82 ± 0.38
Steroid	58.39 ± 1.28
Alkaloid	56.55 ± 2.38
Reducing sugar	31.30 ± 0.12
Tannin	55.77 ± 0.32
Terpenoids	51.20 ± 0.36
Saponin	56.11 ± 1.12
Total Anthocyanin	109.91 ± 5.13

****Values are expressed as mean ± SD**

3.2 Antioxidant activity

The *in vitro* antioxidant activity of *Opa eyin* was evaluated using three methods: DPPH scavenging activity, reducing power, and nitric oxide scavenging activity. The results are presented in Table 2. In these three methods, the concoction showed appreciable antioxidant activity. The antioxidant ability of *Opa eyin* showed an increase with

increasing concentration. The values were comparable to those observed in ascorbic acid (the reference antioxidant). Using the reducing power method, *Opa eyin* was shown to exhibit a higher antioxidant capacity from the concentration of 50 ug/ml and above than the reference (ascorbic acid). Lower but still appreciable values were observed using the DPPH and nitric acid scavenging methods when compared to the reference.

Table 2. *In vitro* anti-oxidant activity of *Opa-eyin*

	DPPH Scavenging activity (% Inhibition)			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Agbo	35.8 ± 0.24	51.47 ± 0.81	63.86 ± 0.40	72.57 ± 0.88
Ascorbic acis	45.05 ± 0.68	56.55 ± 1.36	76.92 ± 0.76	89.82 ± 0.50
	Reducing power			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Agbo	0.215 ± 0.005	0.440 ± 0.011	0.495 ± 0.005	0.693 ± 0.002
Ascorbic acid	0.238 ± 0.006	0.381 ± 001	0.483 ± 003	0.625 ± 0.001
	Nitric oxide Scavenging activity (% Inhibition)			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Agbo	32.50 ± 0.99	55.30 ± 0.36	66.48 ± 0.63	79.76 ± 0.26
Ascorbic acid	47.89 ± 0.34	63.09 ± 0.59	76.07 ± 1.16	84.91 ± 0.75

*****Values represent mean ± standard deviation***

3.3 Oxidative stress studies

The results of the one-way ANOVA for the oxidative stress markers of the liver demonstrate significant dose-dependent effects of *Opa eyin* on various parameters. In Figure 1A, glutathione levels increased significantly across treatment groups, peaking at 0.8 ml, with the control and 0.2 ml group showing lower levels. Also, superoxide dismutase (SOD) activity also showed a significant dose-dependent increase. The control and 0.2 ml groups displayed similar activity, but higher concentrations (0.4 ml and 0.8 ml) led to marked increases, with the highest activity observed at 0.8 ml (Fig. 1B). Additionally, Figure 1C illustrates the effects on catalase activity, where the 0.2 ml group exhibited the highest activity compared to all other groups, including the control. However, catalase activity decreased significantly at 0.4 ml and moderately recovered at 0.8 ml. Finally, Figure 1D reveals significant reductions in malondialdehyde (MDA) levels, a marker of lipid peroxidation, at higher extract doses. The control and 0.2 ml groups had elevated MDA levels, suggesting oxidative damage, whereas the 0.4 ml and 0.8 ml groups showed a substantial reduction, indicating the mitigation of lipid peroxidation and oxidative stress.

In this study, there was a significant variation in glutathione levels across the groups in the kidney assay. The control group exhibited moderately high glutathione levels (~40 µmol/ml). However, treatment with 0.2 ml of the concoction significantly reduced

glutathione levels to ~25 µmol/ml, while a partial recovery was observed with the 0.4 ml dose (~35 µmol/ml). The 0.8 ml dose resulted in the highest glutathione levels (~45 µmol/ml), (Fig2A), suggesting that higher doses of the concoction enhance antioxidant capacity, whereas t. he lowest dose appears to suppress glutathione synthesis or regeneration.

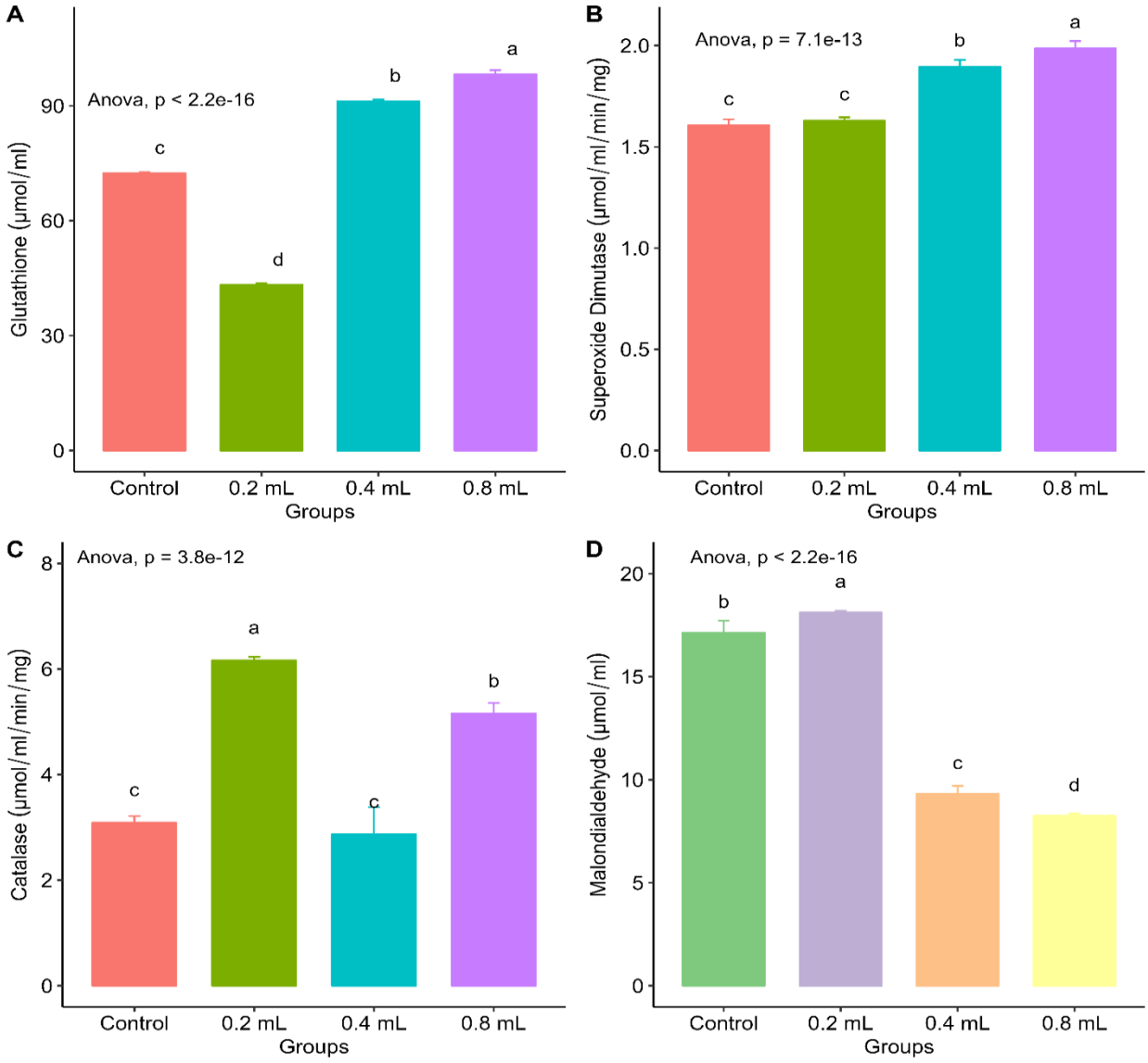

Fig. 1: Hepatic oxidative stress response to *Opaeyin*

Superoxide dismutase (SOD) activity (Figure 2B) also showed significant changes across the group. The control group recorded the lowest activity (~1.2 µmol/ml/min/mg), whereas treatment with 0.2 ml of the extract markedly increased activity to ~1.6 µmol/ml/min/mg, a level that was maintained at both 0.4 ml and 0.8 ml doses. These findings demonstrate that the concoction stimulates SOD activity, with the lowest dose (0.2 ml) being sufficient to elicit a strong antioxidant response.

Catalase activity (Figure 2C) exhibited a more complex trend. The control group showed relatively low activity (~3 µmol/ml/min/mg), while treatment with 0.2 ml of the extract resulted in the highest catalase activity (~8 µmol/ml/min/mg). In contrast, the 0.4 ml dose caused a significant reduction in activity (~2.5 µmol/ml/min/mg), and the 0.8 ml dose led to a partial recovery (~5 µmol/ml/min/mg). These results suggest a biphasic response, where lower doses

maximally stimulate catalase activity, but higher doses moderate its effect.

Finally, malondialdehyde (MDA) levels (Figure 2D), an indicator of lipid peroxidation, also varied significantly across the groups. The control group displayed moderate MDA levels (~15 µmol/ml). Treatment with 0.2 ml increased MDA levels significantly (~20 µmol/ml), indicating enhanced oxidative damage at this dose. Conversely, the 0.4 ml and 0.8 ml doses reduced MDA levels to ~18 µmol/ml and ~12 µmol/ml, respectively, indicating a protective effect of the concoction against lipid peroxidation at higher doses.

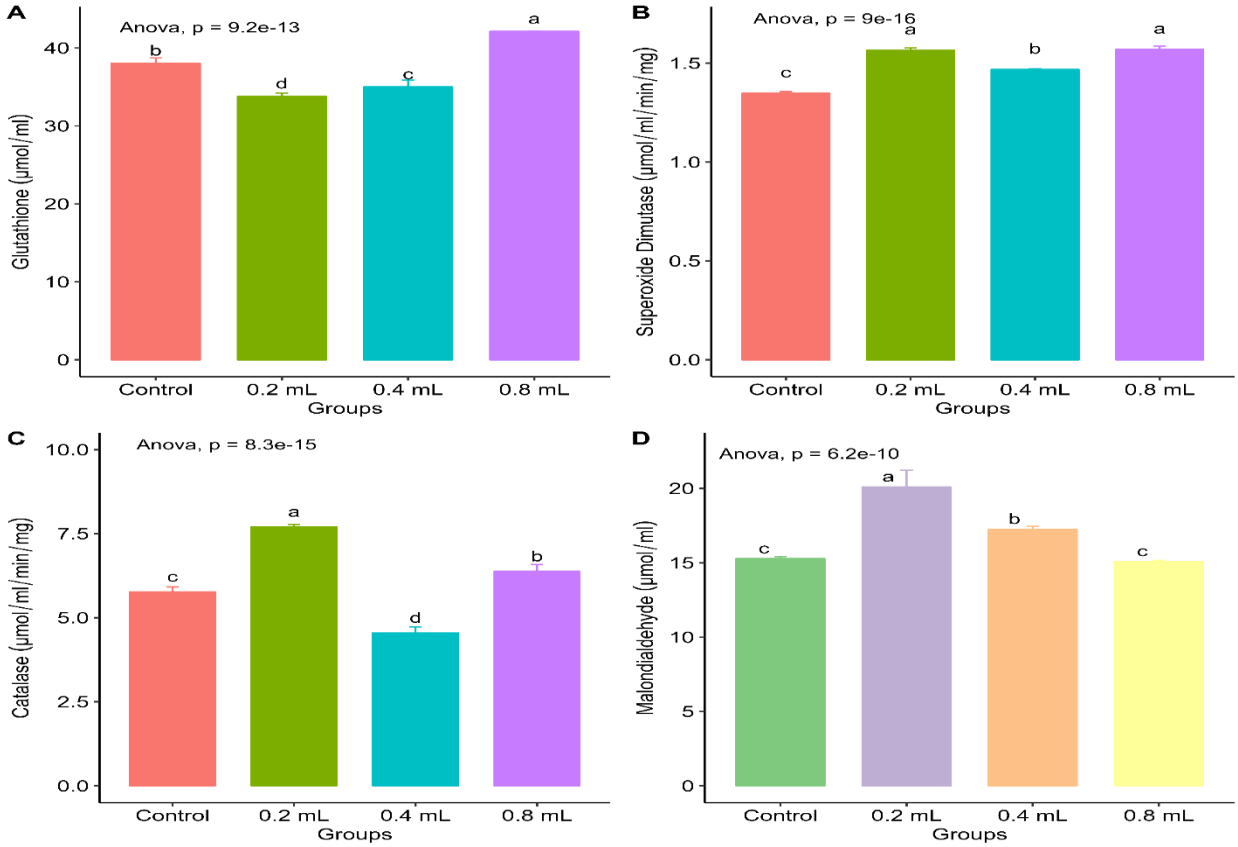

Fig. 2: Oxidative stress response for Kidney

The oxidative response in the testes of are hereby presented. A significant variation was observed in glutathione levels among the groups. The control group exhibited moderate glutathione levels (~60 µmol/ml). Treatment with 0.2 ml of the concoction caused a slight but significant decrease to ~55 µmol/ml. However, a clear dose-dependent increase was observed with higher doses. Glutathione levels rose to ~65 µmol/ml with 0.4 ml of the extract and peaked at ~75 µmol/ml in the 0.8 ml group (Figure 3A). These results suggest that higher doses of the concoction enhance glutathione production or recycling, contributing to an improved antioxidant defense system. Similarly, SOD activity (Figure 3B) showed significant differences across the groups. The control group recorded the highest SOD activity (~1.5 µmol/ml/min/mg), while the 0.2 ml and 0.4 ml treatments caused a marked reduction to ~1.0 µmol/ml/min/mg. Interestingly, activity partially recovered at the highest dose of 0.8 ml, reaching ~1.3 µmol/ml/min/mg. These results indicate that while lower doses of the concoction may suppress SOD activity, higher doses partially restore it, suggesting a potential regulatory effect of the concoction on enzymatic antioxidant mechanisms.

For catalase activity (Figure 3C), there was also a significant dose-dependent trend. The control group exhibited the lowest catalase activity (~2.5 µmol/ml/min/mg). Treatment with 0.2 ml increased activity significantly to ~5 µmol/ml/min/mg, while the 0.4 ml dose elicited the highest activity (~7.5

µmol/ml/min/mg). However, catalase activity declined at the 0.8 ml dose, dropping to ~4 µmol/ml/min/mg. These results suggest a biphasic response, where intermediate doses (0.4 ml) maximize catalase activity, while higher doses (0.8 ml) lead to a decline, possibly due to saturation effects or feedback regulation.

Finally, MDA levels (Figure 3D), an indicator of lipid peroxidation and oxidative damage, were significantly influenced by the treatments. The control group exhibited the highest MDA levels (~8 µmol/ml), indicating high oxidative stress. Treatment with 0.2 ml of the extract slightly reduced MDA levels to ~6 µmol/ml. A further reduction was observed with the 0.4 ml dose (~4 µmol/ml), and the lowest levels (~3 µmol/ml) were achieved at 0.8 ml. These findings suggest that higher doses of the concoction effectively mitigate lipid peroxidation, reducing oxidative damage.

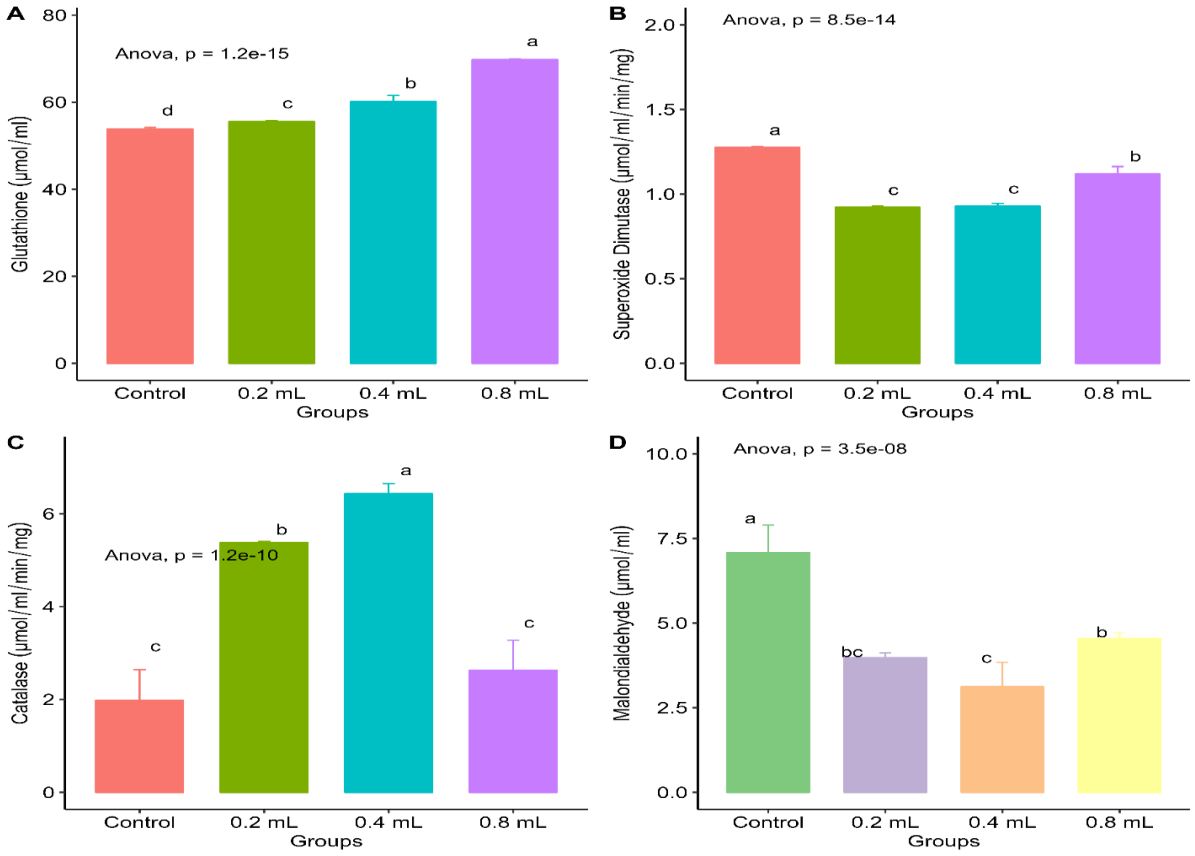

Fig. 3: Oxidative stress response across in the testes

The current research highlights the potential of *Opa eyin* regarding its phytochemical composition and antioxidant properties. High levels of flavonoids (98.82 mg/100 g) and anthocyanins (109.91 mg/100 g) suggest strong antioxidant properties that are commonly associated with cardiovascular and neuroprotective health (Dahanayake *et al.*,2019; Roy *et al.*, 2022). Steroids (58.39 mg/100 g) and terpenoids (51.20 mg/100 g) offer anti-inflammatory and antimicrobial effects, while alkaloids (56.55 mg/100 g) and saponins (56.11 mg/100 g) contribute to analgesic, cholesterol-lowering, and immune-boosting benefits (Kumar *et al.*, 2023). Tannins (55.77 mg/100 g) provide antimicrobial properties but may affect iron absorption. Reducing sugars (31.30 mg/100 g) are important in suppllying energy but may be influence by blood sugar levels. These findings underscore *Opa eyin* therapeutic and nutritional potential (Dahanayake *et al.*,2019; Roy *et al.*, 2022). The phytochemical constituents of some plants have been reputed to have high antioxidant abilities in both *in vitro* and *in vivo* studies (Rodríguez-Negrete *et al.*,2024).

Antioxidant molecules prevent excessive damage of cells by free radicals. Our study showed that *Opa eyin* exhibits promising antioxidant activity, albeit lower than ascorbic acid in most assays. Its performance in reducing power, particularly at higher concentrations, warrants further exploration. The present finding indicate that this herbal mixture could serve as a complementary natural antioxidant, potentially valuable in mitigating oxidative stress-related conditions. We found that upon exposure to *Opa eyin* high activity of DPPH and nitric oxide scavenging activity comparable to the reference ascorbic acid was observed. Radical scavenging molecules interact indirectly with peroxide radicals, scavenging them rapidly through a mechanism involving direct inhibition of lipid peroxidation (Gulcin & Alwasel, 2023). This is of importance considering the uncertainty around the physiological roles of nitric oxide as a bioactive molecule (Andrabi *et al.*,2023) and its indirect effect leading to the generation of free radicals (Belenichev *et al.*, 2023). The *in vitro* antioxidant ability of this herbal mixture to scavenge nitric oxide implies potential cytoprotective properties.

In the liver, the results showed that *Opa eyin* has potent antioxidant effects, particularly at higher doses (0.4 ml and 0.8 ml) thereby enhancing antioxidant defense and reducing oxidative damage to the hepatic cells. The 0.2 ml dose showed inconsistent effects, sometimes appearing to induce oxidative stress or have limited protective capacity. The observed higher levels of glutathione (GSH) and superoxide dismutase (SOD) in the kidney is an indication of an enhanced antioxidant defense system throughout the experiment. These two molecules play critical roles in mitigating oxidative stress by neutralizing reactive oxygen species (ROS) and preventing cellular damage. The present investigation revealed that the herbal mixture can stimulate improved kidney health.

Overall, *Opa eyin* has potent *in vivo* antioxidant effects, particularly at higher doses (0.4 ml and 0.8 ml), thereby enhancing antioxidant defense and reducing oxidative damage. The 0.2 ml dose showed inconsistent effects, sometimes appearing to induce oxidative stress or have limited protective capacity. For instance, high MDA levels triggered by the administration of 0.2ml of *Opa eyin* corresponded with high catalase activity in this study. This implies high oxidative stress caused by exposure to 0.2 ml of *Opa eyin* likely induced excessive production of reactive oxygen species (ROS). In response, catalase activity increased as an adaptive mechanism to detoxify the system of hydrogen peroxide (H_2O_2), a major by-product of ROS. Several studies have shown that elevated catalase or peroxidases helps protect cells from oxidative damage by converting H_2O_2 into water and oxygen, hence restoring redox balance (Yisa et al., 2023; Osés *et al*., 2024). The *in vivo* antioxidant activity of *Opa eyin* may have a positive effect on the reproductive health of the exposed animals. This is because compared to the unexposed group, reduced MDA levels in the testes of the animals were recorded in the animals that received the 0.2ml, 0.4ml, and 0.8ml of the herbal blend. Reproduction is crucial to the continuity of life. Herbs that boost reproductive fitness may be a ready therapy for people with sexual dysfunction as demonstrated in this study. This may account for the wide circulation and consumption of *Opa eyin* in the area. In a similar study, *Chlorophytum borivilianum* reduced testicular oxidative stress in mice (Mararajah *et al*., 2024).

4.0 Conclusion

The study revealed that *Opa eyin* contains various phytochemicals, including flavonoids, steroids, alkaloids, tannins, terpenoids, saponins, phenols, and reducing sugars, with total anthocyanin and flavonoid content being notably high. The antioxidant activity evaluation demonstrated that *Opa eyin* exhibited significant free radical scavenging abilities, with its reducing power surpassing that of ascorbic acid at higher concentrations. The oxidative stress studies showed that treatment with *Opa eyin* influenced antioxidant markers in the liver,

kidney, and testes in a dose-dependent manner. Glutathione levels increased at higher doses, while SOD and catalase activities exhibited variations depending on the organ and extract concentration. The extract also reduced malondialdehyde levels, indicating its protective effect against lipid peroxidation and oxidative stress.

The findings suggest that *Opa eyin* possesses substantial antioxidant potential, which may contribute to mitigating oxidative stress-related damage in biological systems. The observed variations in enzymatic activity and oxidative stress markers highlight its potential as a natural antioxidant source, though the effects appear dose-dependent. The study confirms the traditional use of *Opa eyin* in herbal medicine and supports further investigation into its bioactive properties.

Based on the results, it is recommended that further studies be conducted to elucidate the specific bioactive compounds responsible for the observed antioxidant effects. More detailed toxicity and pharmacokinetic studies are necessary to establish safe and effective dosage levels. Additionally, exploring the potential therapeutic applications of *Opa eyin* in oxidative stress-related diseases could provide valuable insights into its medicinal value.

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Compliance with Ethical Standards Declaration

Ethical Approval

Not Applicable

Competing interests

The authors declare no known competing financial interests

Data Availability

Data shall be made available on request

Conflict of Interest

The authors declare no conflict of interest

Ethical Considerations

This research adhered to ethical guidelines, ensuring that all data collection, experimental procedures, and analyses complied with scientific, environmental, and biomedical research standards. The study was conducted following institutional and national ethical regulations for research involving herbal medicine, animal models, and in vitro assays. Ethical approval was obtained from the appropriate institutional review board, and all efforts were made to minimize harm and ensure the humane treatment of experimental animals in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement). Informed consent was obtained from participants involved in ethnobotanical surveys, and confidentiality was maintained throughout the study

Funding

The authors declared no external source of funding

Authors' Contributions:

UIA conceived the work and wrote the initial manuscript. **SA** designed the laboratory studies. **AGY** carried out the data analysis and result presentation, **AEA** carried out the literature search.

UIA and **AGY** wrote the manuscript. All the authors read through and approved the final draft.