Antioxidant Potential of *Curculigo pilosa* Rhizome Extracts in Hydrogen Peroxide Induced Oxidation *In Vivo*

Damilola A. Omoboyowa[1*], Elijah A. Agboola [1], Damilola S. Bodun [1], Simbo T. Akinsulure [1], Samia Belahcene [2], Dhurgham Al-Fahad [3]

Abstract

Background: The scavenging of oxidants and the inhibition of their conversion into harmful metabolites are essential for maintaining the health of living organisms. As a result, there has been significant interest in identifying natural antioxidants. This study investigates the antioxidant potential of Curculigo pilosa rhizome extracts in mitigating oxidative stress in Drosophila melanogaster (Harwich strain) induced with hydrogen peroxide (H_2O_2). Methods:The radical scavenging activity of butanol (BECP), methanol (MECP), and ethyl acetate (EECP) extracts of C. pilosa was evaluated using standard assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), lipid peroxidation (LPO), nitric oxide (NO) inhibition, and ferric reducing antioxidant power (FRAP). The most potent extracts (MECP and BECP) were further assessed *in vivo* using H_2O_2-induced Drosophila. Molecular docking studies were conducted using the Schrödinger suite to evaluate the interaction between BECP-derived compounds, identified via HPLC, and Drosophila glutathione-S-transferase (GST). Results: MECP and BECP demonstrated significant antioxidant activity, comparable to butylated hydroxytoluene (BHT), in DPPH, LPO, NO inhibition, and FRAP assays. In vivo, these extracts enhanced the survival rate of Drosophila at concentrations of 2 mg and 4 mg/10 g diet. Flies treated with ascorbic acid, MECP, and BECP exhibited significantly increased ($P < 0.05$) negative geotaxis performance and reduced NO levels compared to H_2O_2-induced flies on a normal diet. Additionally, MECP and BECP significantly ($P < 0.05$) upregulated mRNA expression of GST and glutathione peroxidase (GPx) in H_2O_2-induced flies. Molecular docking revealed that phlorizin and curculigoside had high binding affinities to *Drosophila* GST (-6.65 kcal/mol and -6.54 kcal/mol, respectively), comparable to glutathione (-5.97 kcal/mol). **Conclusion:** The butanol and methanol extracts of *Curculigo pilosa* rhizome enhanced the free radical scavenging capacity of *Drosophila melanogaster* and mitigated H_2O_2-induced oxidative stress, likely due to their bioactive antioxidant compounds. These findings suggest the potential of *C. pilosa* as a natural source of antioxidants for managing oxidative stress-related conditions.

Keywords: Antioxidant; Free radicals; Natural compounds; Hydrogen peroxide; Drosophila

Significance | *Curculigo Pilosa* extracts exhibit strong antioxidant properties, mitigating oxidative stress in Drosophila melanogaster, highlighting potential therapeutic applications.

*Correspondence. Damilola A. Omoboyowa, Phyto-medicine and Computational Biology Laboratory, Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria
E-mail: damilola.omoboyowa@aaua.edu.ng

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Introduction

Free radicals, also known as reactive oxygen species (ROS), are highly reactive molecular entities characterized by the presence of unpaired electrons in their atomic orbitals. These unstable molecules readily donate or accept electrons, allowing them to act

Author Affiliation.
[1] Phyto-medicine and Computational Biology Laboratory, Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria
[2] Laboratory of Biotechnology, Environment and Health, Faculty of Nature and Life Sciences, University of Jijel, Jijel 18000, Algeria
[3] Deparment of Pharmaceutical Science, College of Pharmacy, University of Thi-Qar, Iraq

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as both oxidants and reductants in biochemical reactions (Chaudhary et al., 2023). While ROS play essential roles in physiological processes at low concentrations, their excessive accumulation leads to oxidative stress, which can result in cellular damage and various pathological conditions (Phaniendra et al., 2015). Oxidative stress arises from an imbalance between ROS production and the antioxidant defense system, which neutralizes free radicals through enzymatic and non-enzymatic mechanisms (Omoboyowa et al., 2022a). The body's antioxidant defense mechanisms involve metal chelation, free radical scavenging, and enzymatic detoxification of ROS to mitigate oxidative damage (Chaudhary et al., 2023).

Hydrogen peroxide (H_2O_2) is a non-radical ROS with the ability to cross biological membranes, making it a significant inducer of oxidative stress. Cells can also generate H_2O_2 as a signaling molecule or second messenger (Omoboyowa et al., 2022a; Hachiya & Akashi, 2005). In erythrocytes, superoxide dismutase (SOD) converts superoxide anions into H_2O_2, which, if not efficiently neutralized, can lead to irreversible cellular injury (Omoboyowa et al., 2022a). Catalase and peroxidase enzymes play crucial roles in detoxifying H_2O_2 by converting it into water and molecular oxygen, thereby preventing oxidative damage (Subedi et al., 2017). Given the harmful effects of oxidative stress, there is increasing interest in the development of potent natural antioxidants to counteract ROS-induced cellular injury. In particular, plant-derived antioxidants have been widely reported for their significant free radical scavenging potential and therapeutic applications (Shabir et al., 2022).

Curculigo pilosa, a member of the Hypoxidaceae family commonly known as African crocus, is among the medicinal plants recognized for its antioxidant properties (Karigidi et al., 2020). In African traditional medicine, its rhizome is utilized as a supplement for sorghum beer and infant nutrition. Phytochemical analyses have revealed that C. pilosa rhizome contains bioactive compounds such as phenols, terpenoids, coumarins, steroids, and phlobatannins, which contribute to its pharmacological activities (Gbadamosi & Egunyomi, 2010). Additionally, studies have identified the presence of essential nutrients, including proteins, dietary fiber, and minerals, further highlighting its nutritional significance (Adefegha et al., 2018). The therapeutic potential of C. pilosa has been demonstrated in experimental models; for instance, Karigidi and Olaiya (2021) reported that C. pilosa-supplemented diets improved glucose and lipid metabolism in streptozotocin-induced diabetic rats. Similarly, the plant exhibited antioxidant and antidiabetic effects when incorporated into yam flour formulations (Karigidi & Olaiya, 2021).

Drosophila melanogaster has emerged as a valuable *in vivo* model for evaluating the medicinal properties of natural compounds due to its genetic similarities with humans and its suitability for disease modeling (Men et al., 2022). The free radical scavenging potential of various plant extracts, including Amaranth leaf, has been demonstrated in H_2O_2-induced *D. melanogaster* models (Johnmark & Kinyi, 2021). Furthermore, natural antioxidants such as quercetin, gallic acid, ascorbic acid, and caffeine have been shown to mitigate oxidative stress markers in H_2O_2-induced Drosophila (Omoboyowa et al., 2022a).

Given the need for effective natural antioxidants, this study aimed to investigate the free radical scavenging potential of solvent extracts of C. pilosa rhizome. The study employs an H_2O_2-induced oxidative stress *D. melanogaster* model alongside *in vitro* antioxidant assays and an in silico approach to elucidate the plant's antioxidative properties.

Materials and Methods

Procurement of C. pilosa rhizomes

The C. pilosa rhizomes used for this study were purchased from Okitipupa main market, Southwest Nigeria. The plant was identified and authenticated by a botanist from Adekunle Ajasin University, Akungba-Akoko, Nigeria with the voucher number (PSBH-2308) deposited.

Chemical and Reagents

Hydrogen peroxide (H2O2) was procured from Merck (Darmstadt, Germany), thiobarbibituric acid, 2,4,6-tripyridyl-s-troazine (TPTZ), 5, 5'-dithiobis(2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were procured from Sigma Aldrich (USA). All other chemicals were of analytical grade from the laboratory shelf.

Culturing of Drosophila stock

Dr. Abolaji of Drosophila Research Laboratory, University of Ibadan, Nigeria donated the Harwich strain of Drosophila melanogaster. The flies were transported and cultured on cornmeal diet including brewer yeast (2.5 g), agar-agar (3.79 g), blended corn (29 g) and nipagin (0.3 g) in 500 mL of water at the Laboratory of phyto-medicine and computational Biology, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

Extraction of C. pilosa Rhizome

Fresh C. pilosa rhizomes were rinsed with water, cut into pieces, air-dried at room temperature for three weeks and milled using mechanical blender. Exactly 176 g of the powdered plant was macerated with one liter (1 L) of ethylacetate for 72 hours. Thereafter, the solvent-plant mixture was filtered, the filtrate was allowed to pass through whatman No 1 filter paper, then concentrated with rotary evaporator and allowed to dry as ethylacetate extract (EECP). The residue was soaked in 1000 mL of n-butanol, separated after 72 hours; the filtrate was concentrated and dried as n-butanol extract (BECP). The same procedure was repeated for methanol to obtain the methanol extract. The three

extracts (EECP, BECP, and MECP) were subjected to *in vitro* antioxidant assays.

***In vitro* free radical scavenging Assays**

Estimation of DPPH Scavenging potential

In order to estimate the free radical scavenging ability of the plant extracts, DPPH radical optical density was monitored based on the method of Ruch et al. (1989) as reported by Omoboyowa et al. (2020). Briefly, 0.2 mL of the extracts dissolved in water was added to 2 mL of 0.5 mM DPPH dissolve in methanol, water was used as control and butrylated hydroxyl toluene (BHT) as standard. The mixture was incubation for 30 min, and then absorbance was read at 517 nm and the DPPH percentage inhibition was calculated thus;

$$\%\ DPPH\ inhibition = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

Nitric Oxide Scavenging activity

The estimation of stable products (nitrate and nitrite) produced by nitric oxide radical (NO*) in aerobic condition with or without C. pilosa extracts was estimated using Griess reagent as described previously by Sreejayan and Rao, (1997). Briefly, 2 mL of 10 mM sodium nitroprusside prepared in phosphate buffer with pH 7.4 was added with 0.5 mL of the extracts, incubated for 150 mins at 25°C. Exactly 0.5 mL of the mixture was reacted with Griess reagent (5 % glacial acetic acid, 2% sulfanilic acid and 0.2% naphthylethylenediamine dichloride). The solution was allowed to stand for 30 mins at 27°C and the absorbance read at 546 nm, with nitric oxide radical inhibition was calculated using the equation:

$$\%\ NO\ radical\ inhibition = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{Control}} \times 100$$

Lipid Peroxidation Inhibitory Assay

The thiobarbituric acid reactive species method was used to estimate the formation of lipid peroxide in lipid-rich substrate (egg yolk homogenates) according to modified method of Ruberts et al. (2000). Briefly, 300 µL of plant extracts and standard was mixed with 500 µL of 10 % egg yolk homogenate in phosphate buffer saline (pH-7.4) and make up to 1.0 mL with distilled water, then sequential addition of 50 µL of 10 µM FeSO4 and 0.5 mM ascorbic acid (20 µL). After incubation at 37°C for 1 hr, 10 µM of EDTA (0.2 mL) and 1.5 mL of TBA reagent (15 % trichloroacetic acid, 0.375 % TBA and 2.5 M HCl) were added. The resultant solution was incubated for 15 mins at 100°C, centrifuged for 10 mins at 3000 ×g, absorbance of the supernatant was read at 532 nm against blank.

Ferric Reducing Antioxidant Potential of C. pilosa Extracts

Ferric-tripyridyl-s-triazine complex reduction to its violet-coloured ferrous form was determined using the method of Pulido et al. (2000). Exactly 300 µL of the extracts and standard were mixed with freshly prepared FRAP reagent (900 µL) consisting of 0.3 M of acetate buffer, 10 mM of 2,4,6-tripyridyl-s-troazine solution prepared in 40 mM of HCl and 20 nM of ferric chloride in ratio 10:1:1. The mixture was allowed to stand at 37°C for 30 min, thereafter, absorbance of the chromophore developed was read at 593 nm. FRAP value was extrapolated from FeSO4.7H2O standard curve

High Performance Liquid Chromatography (HPLC) Analysis of C. pilosa

Based on the *in vitro* antioxidant result, the n-butanol extract (BECP) was observed to be the most potent; it was therefore subjected to HPLC analysis according to method reported by Omoboyowa et al. (2023). Briefly, 5 g of the extract was dissolved in 20 mL of acetonitrile/methanol and mixed vigorously for 30 mins. The organic solvent was separated into 25 mL flask and makes up to the mark. The standard analytes were previously inserted into the chromatograph and the chromatogram peaks were used to generate a window on the HPLC machine (Shi-Madzu Co., Kyoto, Japan) for comparison with the extract. The extract was filtered using 0.45 µm pore size syringe filter and 50 µL of the filtrate was inserted into the HPLC to generate corresponding peak profile of the extract in the chromatograph. The retention time and peak area of the compounds from the extract was compared with the reference compounds and compounds' concentration of the extract was estimated by the equation:

$$Compound\ concentration = \frac{Compound\ peak \times Standard\ concentration}{Standard\ peak\ area}$$

Effect of the extracts on the survival of D. melanogaster

To determine the concentration of the methanol and n-butanol extracts of C. pilosa (MECP and BECP) that extend life span of *D. melanogaster*, 1-4 days old flies of both sexes with 30 flies/vial in triplicates were cultured with diet incorporated with 2.0, 4.0 and 6.0 mg/10 g diet of MECP and BECP for 30 days (Omoboyowa et al. 2023). The flies' diet was changed every five days with similar treatment. The daily mortality of the flies were recorded and used to evaluate the survival rate of the flies. From the outcome of this study, the concentration of the extracts for the *in vivo* study using *D. melanogaster* was determined.

In vivo* study: Effects of MECP and BECP on H2O2-induced toxicity in *D. melanogaster

Oxidative stress induction and Experimental design

Based on the previous study by Omoboyowa et al. (2022a), the concentration of H2O2 (0.2 mL of 6% H2O2) for induction of oxidative stress in *D. melanogaster* was adopted. The flies were exposed to H2O2 via diet for five (5) days. The rescue action of MECP and BECP on H2O2¬-induced toxicity was carried out by

orally exposing 1-4 days old D. melanogatser grouped into seven (35 flies/vial, n = 6) to normal diet, ascorbic acid, MECP and BECP incorporated diet at 2 mg and 4 mg/10 g of diet. Groups 2-7 were exposed to H2O2 prior to the ascorbic acid (standard control) and extracts exposure for 5 days.

At the end of 5 days treatment, flies were anaesthetized with ice, blot-dried and homogenized in 0.1 M phosphate buffer (pH-7.4) at 1 mg : 10 µL. The homogenates were centrifuged at 4000×g for 10 mins at 4°C in 17R centrifuge (Thermo Scientific). The supernatants were transferred into Eppendorf tubes, labeled for the biochemical assays. Five flies from each group were homogenized in 100 µL TRIzol for DNA extraction used for glutathione-s-transferase and glutathione peroxidase mRNA expression.

Geotaxis Assay

The locomotor ability of the flies was performed following the method reported by Adedara et al. (2022). Briefly, exactly 10 experimental flies were transferred into glass column (15 × 15 cm). Gently tapped to move the flies down the column, total number of flies that climbed up above the 6 cm mark was recorded and the percentage locomotor's ability was evaluated using the equation below. The experiment was repeated four times.

$$Locomotor\ ability\ (\%) = \frac{Total\ flies - Climbing\ flies}{Total\ flies} \times 100$$

Biochemical Assays

The formation of yellow-coloured complex of reduced glutathione reaction with DTNB was determined based on the method of Moron et al. (Moron et al. 1979). Nitric oxide level of the flies' homogenate was measured using Griess reaction method based on the method reported by Adedara et al. (2022). The malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids in the flies' homogenate was determined as lipid peroxidation inhibition by the method of Hogberg et al. (Hogberg et al. 1974). Total thiol content of the flies' homogenate was determined according to the method of Ellman, (1959).

mRNA expression of glutathione-s-transferase and glutathione peroxidase

The mRNA expression of selected genes was carried out using blotting system as reported by Omoboyowa et al. (2023). Briefly, isolation of total mRNA from the flies homogenized in 100 µL of TRIzol was carried out using Kit (Quick-RNA MiniPrep™). DNA contaminants were removed by treatment with DNAse I (NEB, Cat: M0303S). The quantification and purification of RNA was carried out at 260 nm and 280 nm respectively using Spectrophotometer. DNA-free RNA (1 µg) was converted to cDNA with cDNA protoscript II synthesis through the method of reverse transcriptase reaction (Omoboyowa et al. 2023).

Genes of interest were amplified by the method of Polymerase chain reaction (PCR) with OneTaqR2X Master Mix (NEB) using the appropriate primers for *D. melanogaster* (table 1) obtained from Inqaba Biotec, (Hatfield, South Africa).

Molecular Docking Study

The structure of the bioactive compounds identified through HPLC analysis of the butanol extract of C. pilosa rhizome were retrieved from pubchem.ncbi.nlm.nih.gov and prepared using Ligprep tool of Schrodinger suite (2021 v2). The crystallographic structure of *D. melanogaster* glutathione-s-transferase (dGST PDB ID = 1MOU) was retrieved from www.rcsb.org, the protein was prepared using protein preparation wizard of Schrodinger suite (2021 v2). The receptor grid generation tool of the software was used to generate a grid coordinate (x = 20.5, y = 21.4 and z = 25.0) at the binding domain of the co-crystalized ligand (glutathione) (Omoboyowa et al. 2022a). The prepared compounds were screened against the target's binding site to estimate the binding affinity and the 2D-interaction of the protein-ligand complexes were visualized with discovery studio 2020. Prior to the virtual screening, the in-silico procedure was validated by extracting and re-docking the molecule that was co-crystallized with the crystallographic structure of the target. The root mean deviation of the superimposed molecules was evaluated in Chimera 1.14.

Statistical Analysis

Data obtained were analyzed using GraphPad Prism 9; Kaplan-Meier method was used to analyze the survival rate with Log-rank test used for the mean comparisons. The biochemical data were analyzed with one way analysis of variance (ANOVA) followed by Tukey post hoc test at $P < 0.05$ considered statistically significant. Results were presented in mean ± SEM.

Results

In vitro antioxidant potential of Curculigo pilosa extracts

The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical inhibition by methanol, butanol and ethylacetate extracts of C. pilosa rhizome was presented in figure 1. The results revealed that, the percentage DPPH inhibition of the extracts at high concentration (1000, 1500 and 2000 µg/mL) was significantly ($P < 0.05$) increased compared with the extracts at 500 µg/mL. The extracts across concentrations showed non-significant ($P > 0.05$) increase in percentage DPPH inhibition compared with the standard antioxidant (butylated hydroxyltoluene). From table 2, the butanol extract showed low IC50 of 30.18 followed by the methanol extract with IC50¬ of 60.9 while the ethylacetate extract has higher IC50 of 102.5. Therefore, both methanol and butanol extracts were the most potent extract for scavenging DPPH radical.

The ability of the extracts to inhibit nitric oxide (NO) generation was depicted in figure 2a, the result showed that the extracts showed non-significant ($P > 0.05$) increase in percentage NO inhibition compared with butylated hydroxytoluene (BHT). Figure 2b showed that, the extracts have significant ($P < 0.05$) higher percentage lipid

peroxidation inhibition compared with BHT. Methanol and butanol extracts were observed to show non-significant ($P > 0.05$) reduction in percentage lipid peroxidation inhibition compared with ethylacetate extract. Although there was significant ($P < 0.05$) increase in the ferric reducing antioxidant power (FRAP) of methanol and butanol extracts compared with BHT, the ethylacetate extract showed no significant difference in ferric reducing antioxidant power compared with BHT (figure 2c).

Identification of bioactive compounds in BECP using HPLC

The bioactive compounds present in butanol extract of C. pilosa (BECP) were identified using HPLC; ten (10) peaks depicting 10 active compounds were observed in the HPLC chromatogram. The compounds were observed to include sitosterol, curculigol, curculin, curculigenin A, curculigenin B, curculigoside, stigmasterol, phlorizin, pomiferin and scandenin. From figure 3 and table 3, curculigol showed the highest peak and composition of 24.41 ppm, while sitosterol showed the lowest peak among the bioactive compounds.

Effect of C. pilosa extracts on percentage survival of D. melanogaster

The percentage survival of the flies treated with varying concentration of butanol and methanol extracts of C. pilosa rhizome is represented in figure 4. The flies treated with 2 mg and 4 mg of BECP / 10 g of diet revealed significantly ($P < 0.05$) higher percentage of survival compared with flies exposed to normal diet. There was significant ($P < 0.05$) decrease in the percentage survival of flies treated with 6 mg of MECP / 10 g diet compared with flies exposed to normal diet. Flies treated with 2 mg and 4 mg of MECP / 10 g diet revealed significantly ($P < 0.05$) higher percentage survival compared with the flies treated with 6 mg MECP / 10 g diet.

Effect of C. pilosa extracts on geotaxis activity of H2O2-induced D. melanogaster

The locomotor activity of H2O2-induced flies treated with butanol and methanol extracts of C. pilosa is presented in figure 5. The result revealed that, H2O2-induced flies exposed to normal diet showed significant ($P < 0.05$) reduction in the percentage negative geotaxis compared with the control flies. Treatment of *D. melanogaster* with ascorbic acid, methanol extract and butanol extracts of C. pilosa at 2 mg and 4 mg / 10 g diet after oxidative stress induction showed significant ($P < 0.05$) increase in percentage negative geotaxis compared with H2O2-induced flies exposed to normal diet.

In vivo Antioxidant level of H2O2-induced D. melanogaster treated with C. pilosa

Figure 6 showed the *in vivo* antioxidant potential of C. pilosa rhizomes in H2O2-induced *D. melanogaster*. The flies induced with H2O2 exposed to normal diet showed significant ($P < 0.05$) decrease in reduced glutathione compared with control flies. Oxidative stress induced flies treated with ascorbic acid and C. pilosa extracts showed significant ($P < 0.05$) increase in reduced glutathione level compared with H2O2-induced flies exposed to normal diet. The H2O2-induced flies exposed to 2 mg and 4 mg of BECP and MECP/10 g diet showed significant ($P < 0.05$) decrease in reduced glutathione concentration compared with the control group (figure 6a).

H2O2-induced flies exposed to normal diet showed significant ($P < 0.05$) reduction in total thiol content compared with control flies. The flies treated with 4 mg ascorbic acid and methanol extract of C. pilosa showed significant ($P < 0.05$) increase in total thiol content compared with H2O2-induced exposed to normal diet. The 4 mg methanol extract of C. pilosa /10 g diet treated flies after H2O2-induction showed significant ($P < 0.05$) increase in total thiol content compared with H2O2-induced flies treated with ascorbic acid (figure 6b).

Oxidative stress markers in H2O2-induced D. melanogaster treated with C. pilosa

From figure 7a and b, the flies induced with H2O2 and exposed to normal diet showed significant ($P < 0.05$) increase in nitric oxide and malondialdehyde level compared with the normal control flies. Treatment of flies with C. pilosa extracts after H2O2 induction revealed significant ($P < 0.05$) reduction in the nitric oxide concentration compared with H2O2 induced flies exposed to normal diet. There was significant ($P < 0.05$) reduction in the nitric oxide concentration of H2O2-induced flies treated with methanol and butanol extracts of C. pilosa rhizome compared with H2O2-induced flies treated with 4 mg of ascorbic acid / 10 g of diet.

The H2O2-induced flies treated with ascorbic acid and 2 mg of methanol extract of C. pilosa showed significant ($P < 0.05$) reduction in malondialdehyde level compared with H2O2-induced flies exposed to normal diet (figure 7b).

GPx and GST mRNA expression of H2O2-induced flies treated with C. pilosa rhizome

The results presented in figures 8a-b revealed that the H2O2-induced flies exposed to normal diet showed significant ($P < 0.05$) down-regulation of glutathione peroxidase (GPx) and glutathione-s-transferase (GST) mRNA expression compared with control flies on standard diet. There was non-significant ($P > 0.05$) up-regulation in the GPx mRNA expression of oxidative stress induced flies treated with 4 mg of ascorbic acid and 2 mg of BECP/ 10 g diet compared with H2O2-induced flies on normal diet. H2O2-induced flies treated with 4 mg of BECP and MECP at 2 mg and 4 mg /10 g diet showed significant ($P < 0.05$) up-regulation of GPx mRNA expression compared with the oxidative stress induced flies exposed to normal diet as shown in figure 8a. H2O2-induced flies treated with ascorbic acid, BECP and MECP showed significant ($P < 0.05$) up-regulation in GST mRNA expression compared with flies exposed to normal diet after oxidative stress induction (figure 8b).

Table 1. Primers sequences

Gene	Forward primers	Reverse primers
GstD1	CGACTCCCTGTACCCTAAGT	GTAGTAGTTGGCGAACTCTG
PHGPX	GATACCCATGGCAACGATGT	CCTTTAGATCCGTCAGCCTTCTC
Gpdh	TCGGACTGCGTAGACACTAGA	AGCGCCATCTATGTAAGGATGT

GstD1: Glutathione-s-transferase; PHGPX: Glutathione peroxidase

Table 2. IC50 parameters of DPPH inhibition of solvent extracts of *Curculigo pilosa* rhizome

Parameters	Methanol Extract	Butanol Extract	Ethylacetate Extract
Log IC50	2.207	1.480	2.011
IC50	60.9	30.18	102.5
R^2	0.9765	0.9969	0.8922

Table 3. Bioactive compounds from n-butanol extract of *Curculigo Pilosa*

S/N	Compounds	Retention time	Area	Composition (ppm)
1	Sitosterol	1.16	33.16	3.32
2	Curculigol	1.30	244.10	24.41
3	Curculin	1.85	102.78	10.28
4	Curculigenin A	2.18	73.19	7.32
5	Curculigenin B	3.05	73.00	7.30
6	Curculigoside	4.00	84.10	8.41
7	Stigmasterol	5.32	74.50	0.00
8	Phlorizin	6.20	391.25	0.00
9	Pomiferin	8.47	34.99	0.00
10	Scandenin	9.48	37.43	0.00

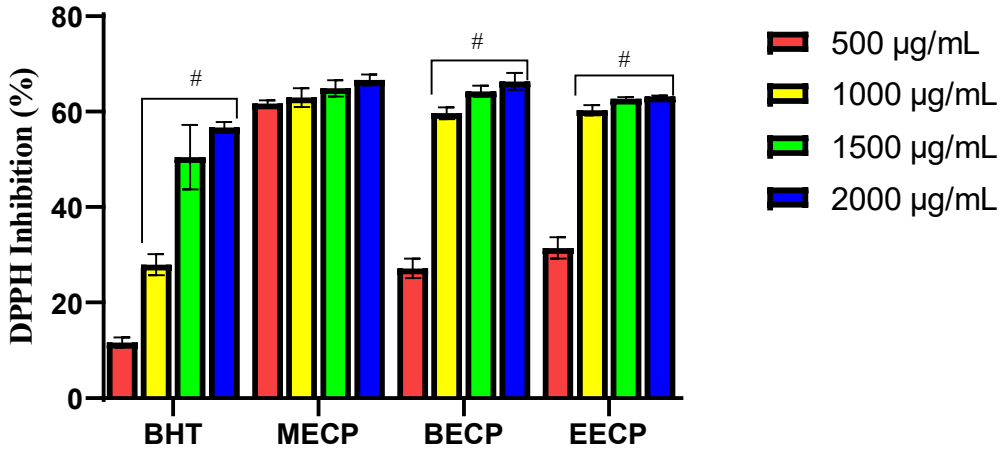


Figure 1. DPPH inhibitory potential of solvent extracts of *Curculigo pilosa* rhizome

#[P < 0.05]; significant compared with 500 µg/mL

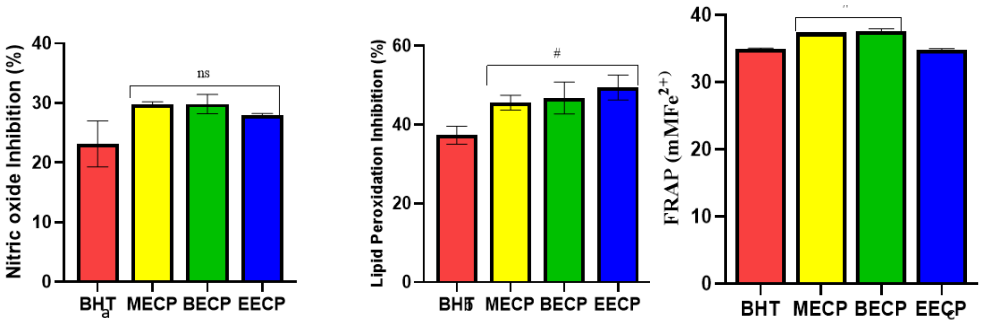


Figure 2. *In* vitro antioxidant potential of solvent extracts of *Curculigo pilosa rhizome*

#, ns[P < 0.05]; significant compared with BHT

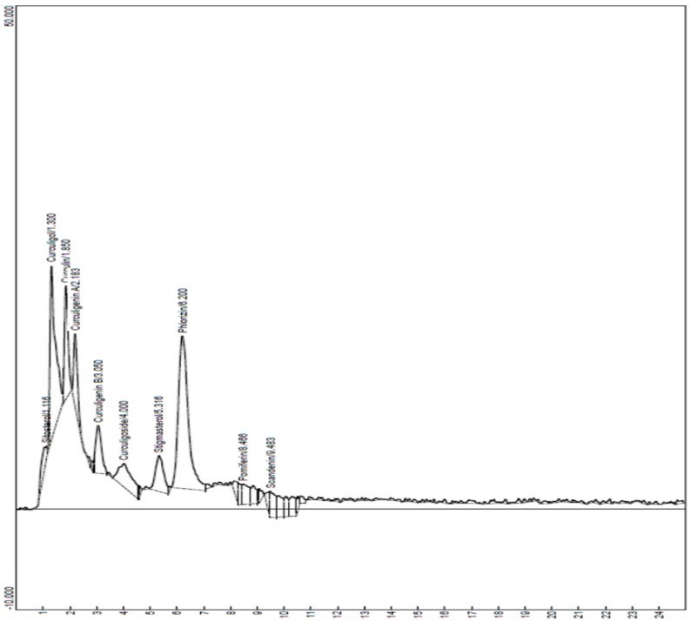


Figure 3. HPLC chromatogram of n-butanol extract of *Curculigo pilosa*

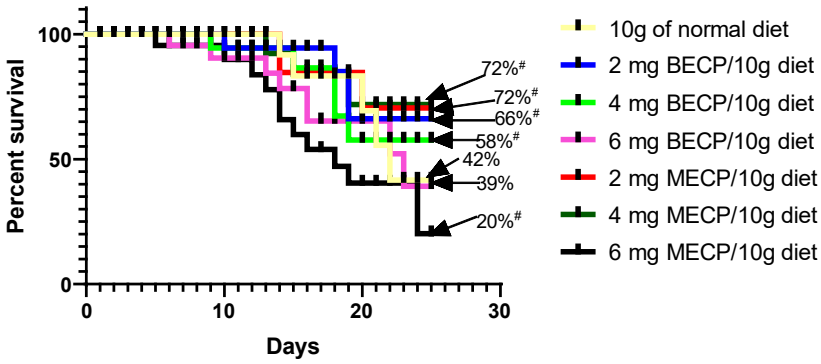


Figure 4. Percentage survival of D. melanogaster administered *Curculigo pilosa* extracts.

#[P < 0.05] significant compared to the CRTL flies; BECP: Butanol extract of *C. pilosa*; MECP: Methanol extract of *C. pilosa*

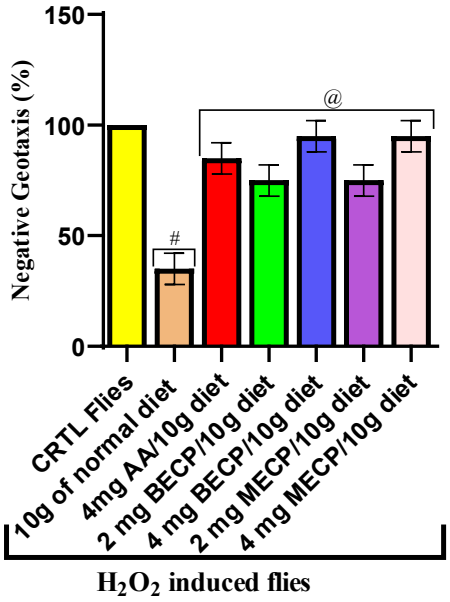


Figure 5. Climbing ability of H2O2-induced D. melanogaster treated with *Curculigo pilosa* rhizome.
#[P < 0.05] significant compared to the CRTL flies; @[P < 0.05] significant compared to H2O2 induced flies exposed to normal diet
CRTL flies: Flies without induction, BECP: Butanol extract of *C. pilosa*; MECP: Methanol extract of *C. pilosa*

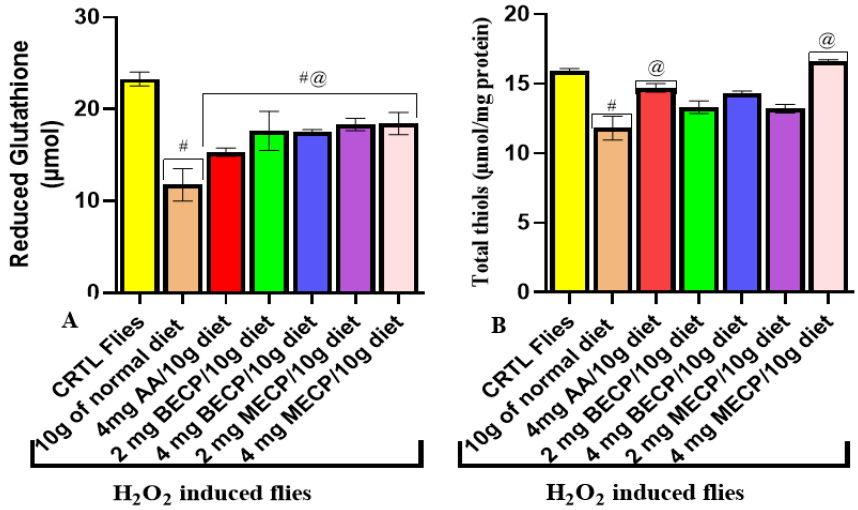


Figure 6. In vitro non-enzyme antioxidant level of H2O2-induced D. melanogaster treated with *Curculigo pilosa* rhizome.
#[P < 0.05] significant compared to the CRTL flies; @[P < 0.05] significant compared to H2O2-induced flies exposed to normal diet
CRTL flies: Flies without induction, BECP: Butanol extract of *C. pilosa*; MECP: Methanol extract of *C. pilosa*

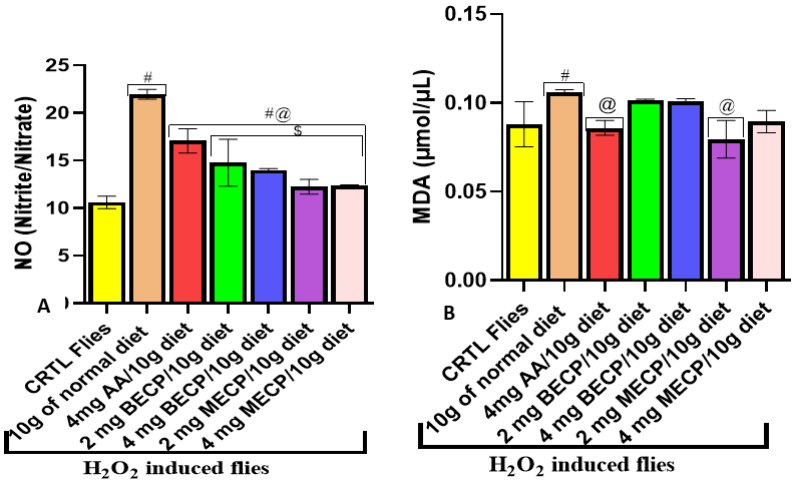


Figure 7. oxidative stress markers of H2O2-induced D. melanogaster treated with *Curculigo pilosa* rhizome

#[P < 0.05] significant compared to the CRTL flies; @[P < 0.05] significant compared to H2O2 induced flies exposed to normal diet; $[P < 0.05] significant compared to the 4 mg AA /10g diet flies

CRTL flies: Flies without induction, BECP: Butanol extract of *C. pilosa*; MECP: Methanol extract of *C. pilosa*

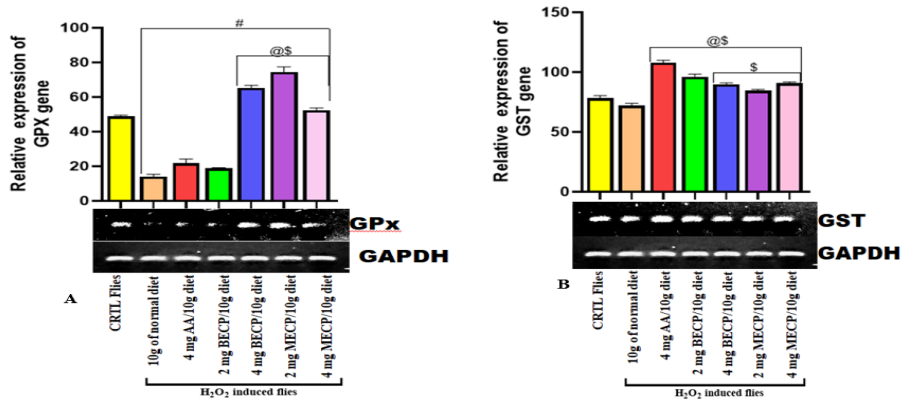


Figure 8. (a) GPx and (b) GST mRNA expression of H2O2-induced D. melanogaster treated with *Curculigo pilosa* rhizome

#[P < 0.05] significant compared to the CRTL flies; @[P < 0.05] significant compared to H2O2 induced flies exposed to normal diet; $[P < 0.05] significant compared to the 4 mg AA /10g diet flies; GPx: Glutathione peroxidase; GST: Glutathione-s-transferase; AA: ascorbic acid

CRTL flies: Flies without induction; BECP: Butanol extract of *C. pilosa*; MECP: Methanol extract of *C. pilosa*

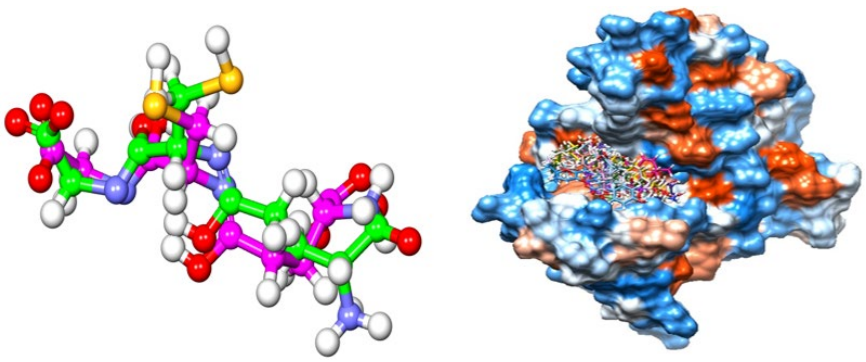


Figure 9: (a) Superimposition of re-docked ligand (green) and the co-crystallized (pink) ligand at the binding site of the target (b) Binding site of all the molecules.

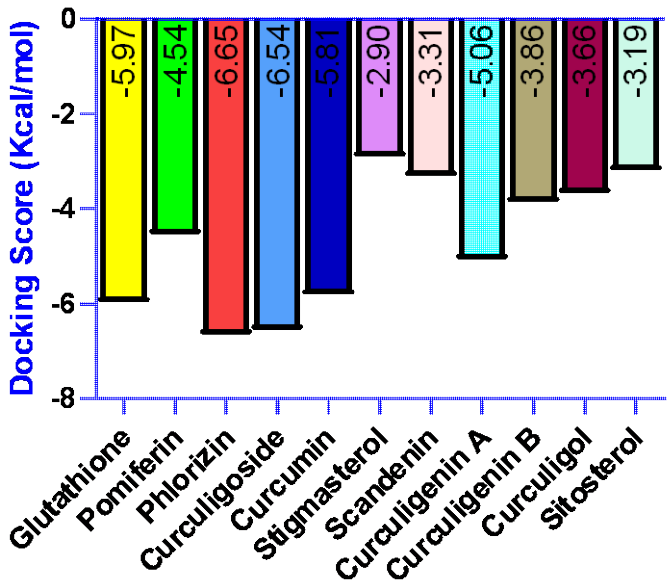
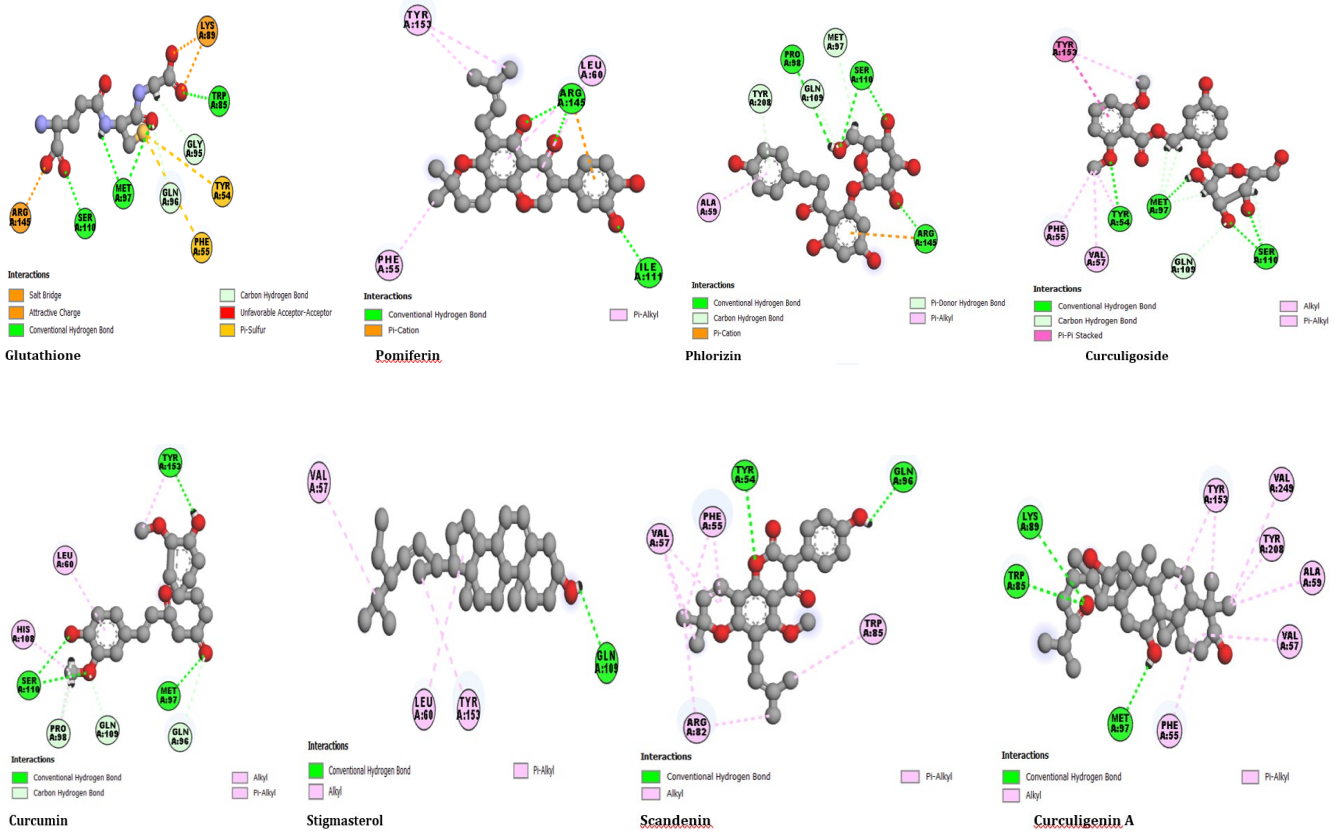
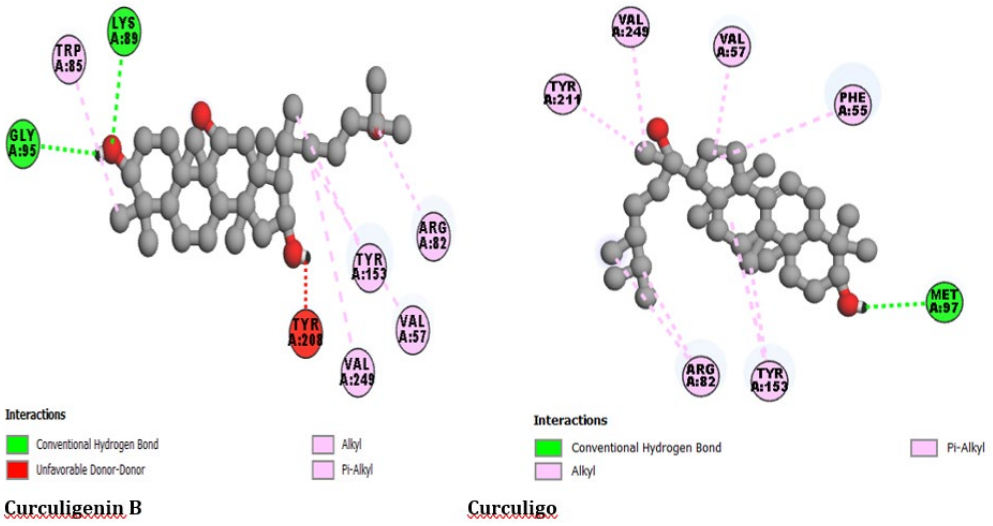
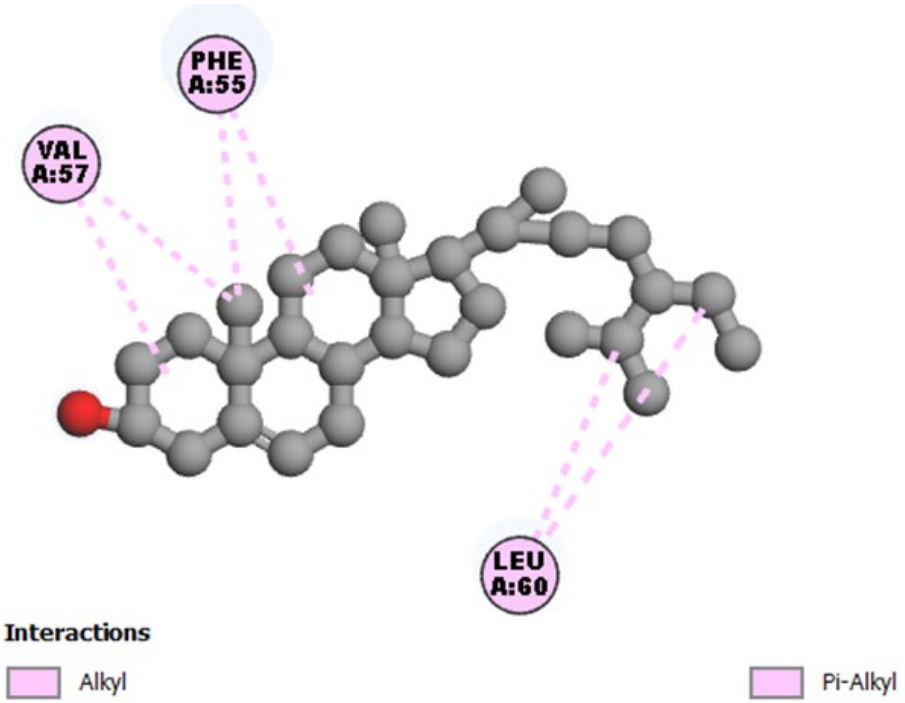


Figure 10. Graphical representation of binding affinity of compounds from *C. pilosa* against glutathione-s-transferase







Sitosterol

Figure 11. 2D interactions of compounds from *C. pilosa* with binding site of glutathione-s-transferase (1M0U).

Molecular docking of bioactive compounds against glutathione-s-transferase

The validation of the docking protocol is shown in figure 9a by re-docking and superimposition of the co-crystalized ligand. The root square deviation of the docking validation was 1.990 Å. The results of the molecular docking of the bioactive compounds identified from the HPLC chromatogram of n-butanol extract of C. pilosa rhizome against Drosophila glutathione-s-transferase (dGST) is presented in figure 10. The result revealed that phlorizin (-6.65 kcal/mol) and curculigoside (-6.54 kcal.mol) exhibit high binding affinity against dGST comparable to glutathione (-5.97 kcal/mol) the co-crystalized ligand. Other bioactive compounds showed varying degrees of binding affinity with sitosterol (-3.19 kcal/mol) having the lowest binding affinity. Figure 9b showed that all the bioactive compounds bind at the binding pocket on the target. The compounds interacted with the amino acid residues of the target binding site with various interactions, notably is the hydrogen bond interaction. As shown in figure 11, phlorizin, curculigoside and curcumin formed four (4) hydrogen bonds with PRO 38, SER 110, ARG 145, TRY 54, MET 97 and TRY 152 residues at the binding site of dGST which is comparable with glutathione that formed four H-bond with similar amino acid residues. Other compounds such as pomiferin and curculigenin A formed three (3) H-bond interaction, scandenin and curculigenin B formed two (2) H-bond while stigmasterol and curculigo formed one H-bond interaction with GLN 109 and MET 97 respectively.

Discussion

The redox status of the cell is estimated by the relationship between antioxidants and oxidants. An exaggerated production of reactive oxygen species (ROS) and the lack of sufficient antioxidants create a vicious circle connecting oxidative stress and its pathophysiological disorders such as apoptosis and necrosis (Shabir et al. 2022). Natural antioxidants derived from plants have significant inhibitory potential against free radicals (Belahcene et al. 2023). Free radicals scavenging ability of various extracts of C. pilosa rhizome were studied via DPPH, nitric oxide, lipid peroxidation inhibition and ferric reducing antioxidant potential.

1,1-diphenyl-2-picrylhydrazyl is used to estimate antioxidant potential of plant extracts. The ability of medicinal plants to convert1,1-diphenyl-2-picrylhydrazyl to diphenylpicrylhydrazine (its reduced form) is associated with the ability of the natural compounds in the plant to act as hydrogen donator via rupturing the O-H bond so as to transfer hydrogen atoms, then inactivating free radicals (Lang et al. 2024). The DPPH scavenging potential of methanol, butanol and ethylacetate extracts of C. pilosa rhizome is presented in figure 1. The percentage DPPH inhibition of the extracts were non-significantly ($P > 0.05$) higher compared with the standard antioxidant (butylated hydroxyl toluene). Butanol extract was the most potent inhibitor of DPPH among the extracts with IC50 of 30.18 followed by the methanol extract (IC50 = 60.9). Ethylacetate extract was less potent compared with other extracts with IC50 of 102.50 as shown in table 2. The result of the percentage DPPH inhibition obtained is consistent with the findings of Karigidi and Olaiya, (2021) who reported high percentage DPPH inhibition and low IC50 for yam flour supplemented with C. pilosa rhizome.

The ferrous (Fe3+) to ferric (Fe2+) reducing ability in the presence of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) to form blue Fe2+-TPT complex (Jiménez-Estrada et al. 2013) was used to estimate the antioxidant potential of C. pilosa extracts. Figure 2c showed significant ($P < 0.05$) increase in ferric reducing antioxidant power (FRAP) of methanol and butanol extracts of C. pilosa compared with BHT. Ethylacetate extract showed no significant difference in the FRAP compared with BHT. Karigidi and Olaiya, (2021) also reported high FRAP in C. pilosa supplemented yam flour. Nitric oxide (NO) is a mediator involved in physiological process regulation; excessive release of NO has been attributed with pathogenic disorders (Adebayo et al. 2019). The percentage NO inhibition by C. pilosa extracts was significantly ($P < 0.05$) higher compared with BHT. Methanol and butanol extracts of C. pilosa were observed to be better NO inhibitor than the ethylacetate extract as shown in figure 2a. One more attribute appraised in the present study was the Malondealdehyde (MDA) content which is known to be a reliable indicator of lipid peroxidation (LPO) and extent of oxidative injury to the cell machinery (Wided et al. 2015). LPO is the glycation and protein modification reaction in cellular component mediated by free radicals (Arika et al. 2019). It destroy cellular membrane lipids and initiates a pathway of the oxidation of poly-unsaturated fatty acids (PUFA), ultimately synthesizing MDA via a cascade of chain reactions (Mohideen et al. 2023). MDA is the most considerable end product of lipid peroxidation which can oxidize thiols in protein resulting into protein dysfunction (Poodineh and Nakhaee, 2019). The percentage lipid peroxidation inhibitor of solvent extracts of C. pilosa shown in figure 2b revealed that, methanol, butanol and ethylacetate extracts significantly ($P < 0.05$) inhibit lipid peroxidation compared with BHT. The antioxidant ability of the butanol extract of C. pilosa may be attributed to the presence of phenolic compounds observed in the HPLC analysis of the extract (figure 3, table 2). Since phenolic compounds are class of antioxidants that act as reducing agents, singlet oxygen quenchers, hydrogen donors or metal chelators (Ebrahimzadeh et al. 2010). The HPLC result revealed the presence of eight (8) phenolics and two (2) sterols as shown in table 2.

The ability of methanol (MECP) and butanol (BECP) extracts to improve survival of Drosophila melanogaster was studied for 30 days using varying concentration to determine the concentration of the MECP and BECP to be use in this study. From figure 4, 4 mg of

BECP/10 g diet increase the survival rate of the flies compared with the 6 mg of BECP/10 g diet. 2 mg and 4 mg of MECP / 10 g diet significantly ($P < 0.05$) increase the survival rate of the flies compared with 6 mg of MECP / 10 g diet. Chattopadhyay et al. (2015) stated that longevity is one of the indicators of good health. From figure 5, the H2O2-induced flies exposed to normal diet showed significant ($P < 0.05$) reduction in percentage geotaxis compared with the control flies. The experimental flies induced with H2O2 and treated with ascorbic acid and varying concentrations of BECP and MECP showed significant ($P < 0.05$) increase in survival rate compared with oxidative stress induced control flies. The reduction in the negative geotaxis observed in this study might result from the effect of the hydrogen peroxide on peripheral neuropathy resulting into muscle weakness and loss of coordination. The ameliorative action of the C. pilosa extracts might be attributed to its antioxidant potential which agrees with the finding of Omoboyowa et al. (2022a) who reported the modulatory effects of phenolics compounds on oxidative stress induced *D. melanogaster*.

Reduced glutathione (GSH) is abundant in the cellular system as a non-enzymatic antioxidant which plays vital role in the detoxification of foreign substances through electrophilic molecules (Averill-Bates, 2023). Production of GSH at low concentration leads to cellular damage by oxidative stress (Anadozie et al. 2023). The significant ($P < 0.05$) reduction of GSH caused by H2O2 induction compared with control flies was notably restored by ascorbic acid, BECP and MECP treatment in H2O2-induced flies suggesting that MECP and BECP protected the *D. melanogaster* cellular system from oxidative damage by elevating cellular GSH generation even better that standard antioxidant (ascorbic acid) as shown in figure 6a. Nitric oxide (NO) is a vital chemical mediator generated by the endothelial cells and macrophages as an unstable gas which can be converted into stable metabolites as nitrite and nitrate (Anadozie et al. 2023). Although NO is involve in various physiological events and important in the defense mechanism of the cellular system, excessive production of NO can activate pro-inflammatory responses leading to tissue damage (Njoya et al. 2017). The result presented in figure 7a showed that MECP and BECP were able to ameliorate the toxicity caused by H2O2 on *D. melanogaster*, thus suggesting its therapeutic efficacy.

Lipid peroxidation is a chain reaction of oxidative lipid degradation with malondialdehyde (MDA) as by product and oxidative stress marker Fatoki et al., 2022). The increase of MDA level in H2O2-induced flies was significantly ($P < 0.05$) reduced after exposure to 4 mg of ascorbic acid per 10 g diet and 2 mg of MECP per 10 g diet while other treatments showed non-significant ($P > 0.05$) reduction as shown in figure 7b. The results of the non-enzymatic antioxidants obtained in this study were consistent with the findings of Anadozie et al. (2023) who reported similar trends in oxidative stress parameters of copper induced *D. melanogaster* after exposure to alkaloids fraction of Buchholzia coriacea seed. The decrease total thiol level observed in flies exposed to H2O2 incorporated diet was restored by ascorbic acid, MECP and BECP. Suggesting the ability of the plant extracts to increase the organic compounds containing sulfhydryl (-SH) group. This result is consistent with the findings of Omoboyowa et al. (2022a) who reported increase in total thiol concentration of H2O2-induced flies after exposure to antioxidant compounds.

The activities of cellular antioxidant enzymes such as glutathione peroxidase (GPx) and glutathione-s-transferase (GST) are modulated in disease conditions due to the presence of reactive oxygen species (ROS) (Lee et al. 2001). The free radicals scavenging activity of superoxide dismutase is effective by the detoxifying activity of GPx on hydroperoxides generated from superoxide radicals (Ugbaja et al. 2021). mRNA expression of GPx and GST revealed significant ($P < 0.05$) down-regulation by H2O2 induction in *D. melanogaster*. Ascorbic acid, MECP and BECP significantly ($P < 0.05$) up-regulate GST mRNA expression while 4 mg of BECP and both concentration of MECP significantly ($P < 0.05$) up-regulate GPx compared with the H2O2-induced flies (figure 8). Down-regulation of GPx and GST in H2O2- induced flies suggests a compromised oxidant-antioxidant balance, thereby increasing reactive oxygen species, ultimately inducing oxidative stress. MECP and BECP were able to mitigate the oxidative stress caused by H2O2 by up-regulation of GPx and GST genes, suggesting the free radical scavenging potential of C. pilosa extracts.

Molecular docking studies of the bioactive compounds from butanol extract C. pilosa rhizome identified by HPLC analysis was performed against the binding site of glutathione-s-transferase. This enzyme was targeted to further complement the modulatory efficacy of the extract observed in the *in vivo* study. The root means square deviation (RMSD) of 1.990 Å observed during the validation of the docking procedure showed that the docking protocol is reliable and reproducible since the RMSD is less than 2.00 Å (Oyibo et al., 2023; Balogun et al., 2021). The phytocompounds such as phlorizin and curculigoside were observed to have binding affinity of -6.65 kcal/mol and -6.54 kcal/mol respectively than the standard (glutathione = -5.97 kcal/mol) as shown in figure 10 suggesting the ability of the phytochemicals in the extract to modulate GST in order to scavenge free radicals. The molecular interaction of antioxidant compounds against GST binding site has been reported by Omoboyowa et al. 2022a). The interaction of molecules with the amino acid residues of target binding site plays vital role in the modulatory activity of such molecules (Omoboyowa et al. 2022b). From figure 11, Phlorizin formed four hydrogen bonds (H-bonds) with PRO 98. SER 110 and ARG 145, curculigoside and curcumin

formed four H-bonds with TYR 54, MET 97 and SER 110 which were comparable with the standard (glutathione).

Conclusion

In conclusion, the outcomes presented in this study revealed that the investigated plant is rich in a plethora of crucial chemical compounds of a high biological value. MECP and BECP exhibit antioxidant activity and might prevent the flies from oxidative stress caused by hydrogen peroxide toxicity. These C. pilosa extracts were observed to be safe to the flies at the concentration used for this study, restored locomotive activity, antioxidant homeostasis and up-regulation of antioxidant genes of H2O2-exposed flies. Methanol and butanol extracts of C. pilosa rhizomes could be potential sources of therapeutic agent in the management of oxidative stress associated disorders. Therefore, isolation of these bioactive compounds and further experimental study in vertebrate animals are proposed to validate the findings from this study.

Author contributions

DAO conceptualized the study, supervised the research process, and took the lead in writing the original draft. DAO also validated the data, curated the dataset, developed the methodology, conducted the investigation, and managed the overall project administration. EAA contributed to the conceptualization and methodology of the study and was involved in reviewing and editing the manuscript. DSB and STA both contributed to the methodological framework and participated in the review and editing of the manuscript. SB and DA provided critical feedback through the review and editing of the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

References

Adebayo SA, Ondua M, Shai LJ, Lebelo SL (2019) Inhibition of nitric oxide production and free radical scavenging activities of four South African medicinal plants. J. Inflamm. Res. 12: 195-204

Adedara AO, Babalola AD, Stephano F, Awogbindin IO, Olopade JO, Rocha JBT, Whitworth AJ, Abolaji AO (2022) An assessment of the rescue action of resveratrol in parkin loss of function induced oxidative stress in Drosophila melanogaster. Sci. Rep. 12: 3922–3934

Adefegha SA, Oyeleye SI, Oboh G (2018) African crocus (Curculigo pilosa) and wonderful kola (Buchholzia coriacea) seeds modulate critical enzymes relevant to erectile dysfunction and oxidative stress. J. Complemen. Integrat. Med. 15: 1-12

Anadozie SO, Asueiza UA, Olusola BA (2023) Alkaloid-rich extract of Buchholzia coriacea seed mitigate the effect of copper-induced toxicity in Drosophila melanogaster. Vegetos, (https://doi.org/10.1007/s42535-023-00760-9

Arika W, Kibiti CM, Njagi JM, Ngugi MP (2019) *In Vitro* Antioxidant Properties of Dichloromethanolic Leaf Extract of Gnidia glauca (Fresen) as a Promising Antiobesity Drug. Journal of Evidence-Based Integrative Medicine, 24: 1-17

Averill-Bates DA (2023) Chapter 5: The antioxidant glutathione. Vitamins and hormones. G Litwack Acad Press 121: 109–141

Belahcene S, Widad K, Omoboyowa DA, Alshihri AA, Alelyani M, Bakkour Y, Leghouchi EJ (2023) Unveiling the Chemical Profiling Antioxidant and Anti-Inflammatory Activities of Algerian Myrtus communis L. Essential Oils, and Exploring Molecular Docking to Predict the Inhibitory Compounds against Cyclooxygenase-2. Pharmaceuticals, 16: 1343.

Balogun, T.A., Iqbal, M.N., Saibu, O.A., Akintubosun, M.O., Lateef, O.M., Nneka, U.C., Abdullateef, O.T., Omoboyowa, D.A., 2021. Discovery of potential HER2 inhibitors from Mangifera indica for the treatment of HER2-positive breast cancer: an integrated computational approach. J. Biomol. Struct. Dyn. 39, 1–12.

Chattopadhyay D, James J, Roy D, Sen S, Chatterjee R, Thirumurugan K (2015) Effect of semolina-jaggery diet on survival and development of Drosophila melanogaster, Fly 9: 16–21

Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull-Razis AF, Modu B, Calina D, Sharifi-Rad J (2023) Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front. Chem. 11: 1158198.

Ebrahimzadeh MA, Nabavi1 SF, Nabavi1 SM, Pourmorad F (2010) Nitric oxide radical scavenging potential of some Elburz medicinal plants. Afri. J. Biotechnol. 9: 5212-5217

Ellman GL (1959) Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82: 70–77

Fatoki JO, Kehinde SA, Adegoke AA, Faokunla O, Atere TG, Olajide AT (2022) Inhibition of lipid peroxidation and free radical scavenging activities of methanolic leaf extract of Psidium guajava. Res. J. of Health Sci. 10: 139-150

Gbadamosi IT, Egunyomi A (2010) Phytochemical screening and invitro anti-candidal activity of extracts and essential oil of Curculigo pilosa (Schum and Thonn) Engl. Hypoxidaceae, Afr. J. Biotechnol. 9: 1236–1240

Hachiya M, Akashi M (2005) Catalase regulates cell growth in HL60 human promye-locytic cells: evidence for growth regulation by H2O2, Radiat. Res. 163: 271–282

Hogberg J, Larsen ER, Kristogerson A, Ovrhenius S (1974) NADPH-dependent reductase solubilised from microsomes of peroxidation and its activity. Biochem. and Biophys. Res. Commu. 56: 836.

Jiménez-Estrada M, Velázquez-Contreras C, Garibay-Escobar A, Sierras-Canchola D, Lapizco-Vázquez R, Ortiz-Sandoval C, Burgos-Hernández A, Robles-Zepeda RE (2013) *In vitro* antioxidant and antiproliferative activities of plants of the ethnopharmacopeia from northwest of Mexico. BMC Complementary and Alternative Medicine 13: 12-20

Johnmark N, Kinyi HW (2021) Amaranth leaf extract protects against hydrogen peroxide induced oxidative stress in Drosophila melanogaster , BMC Res. Notes, 14: 188–193 .

Karigidi KO, Akintimehin ES, Omoboyowa DA, Adetuyi FO, Olaiya CO (2020) Effect of Curculigo pilosa supplemented diet on blood sugar, lipid metabolism, hepatic oxidative stress and carbohydrate metabolism enzymes in streptozotocin-induced diabetic rats. J. Diabetes Metab. Disord. 19: 1173-1184

Karigidi KO, Olaiya CO (2021) Effects of Curculigo pilosa supplementation on antioxidant and antidiabetic activities of yam flour. J. Food Sci. Technol. 58: 4110–4117

Lang Y, Ningxuan G, Zhihuan Z, Xianjun M, Yang L, Shufang Y, Yiyun Y, Zhufeng J, Bin L (2024) Classification and antioxidant assays of polyphenols: a review, Journal of Future Foods 4: 193-204

Lee SE, Ju EM, Kim JH (2001) Free radical scavenging and antioxidant enzyme fortifying activities of extracts from Smilax china root. Experimental and Molecular Medicine, 33: 263-268

Men TT, Khang DT, Tuan NT, Trang DTX (2022) Anti-aging effects of Lasia spinosa L. stem extract on Drosophila melanogaster. Food Sci. Technol, Campinas 42: e38721

Mohideen K, Krithika C, Saranya R, Chandini R, Snehashish G, Safal D (2023) Assessment of Oxidative Stress by the Estimation of Lipid Peroxidation Marker Malondialdehyde (MDA) in Patients with Chronic Periodontitis: A Systematic Review and Meta-Analysis, International Journal of Dentistry. 23: 6014706.

Moron MS, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim. Biophys. Acta. 582: 67–78

Njoya EM, Munvera AM, Mkounga P, Nkengfack AE, McGaw LJ (2017) Phytochemical analysis with free radical scavenging, nitric oxide inhibition and antiproliferative activity of Sarcocephalus pobeguinii extracts, BMC Complementary and Alternative Medicine 17: 199-208

Omoboyowa DA, Agoi MD, Shodehinde SA, Saibu OA, Saliu JA (2023) Antidiabetes study of Spondias mombin (Linn) stem bark fractions in high-sucrose diet-induced diabetes in Drosophila melanogaster. Journal of Taibah University Medical Sciences 18: 663e675

Omoboyowa DA, Karigidi KO, Aribigbola TC (2020) Bridelia ferruginea Benth leaves attenuates diabetes nephropathy in STZ-induced rats via targeting NGAL/KIM-1/cystatin c gene. Clin. Phytosci. 6: 63-73

Omoboyowa DA, Olugbenga ST, Adetuyi FD, Akinsulure ST, Akinwande KM, Iwuji CB, Ayekolu SF (2022a) Modulatory effects of selected compounds on oxidative stress in hydrogen peroxide-induced Drosophila melanogaster. Pharmacol. Res.-Modern Chin. Med. 5: 100169

Omoboyowa DA, Singh G, Fatoki JO, Oyeneyin OE (2022b) Computational investigation of phytochemicals from Abrus precatorius seeds as modulators of peroxisome proliferator-activated receptor gamma (PPAR γ), J. Biomol. Struct. and Dyn. 35: 1–5

Oyibo, A., Abolaji, A. O., Omoboyowa, D. A., Odunola, O.A. (2023). Vitellaria paradoxa inhibits arsenic-induced toxicity in Drosophila melanogaster via the augmentation of antioxidant system. Toxicology 494 (2023) 153590

Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J. Clin. Biochem. 30: 11–26.

Poodineh J, Nakhaee A (2019) *In Vitro* Antioxidant and Anti-Lipid Peroxidation Activities of Hydroalcoholic Extracts of Caralluma tuberculata, Root and Aerial Parts. Jundishapur J Nat Pharm Prod. 14: e69685.

Pulido R, Bravo L, Sauro-Calixto F (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry, 48: 3396–3402

Ruberto G, Baratta MT (2000) Antioxidant activity of selected essential oil components in two lipid model systems. Food Chemistry, 69: 167–174.

Ruch RJ, Cheng SJ, Klaunig JE (1989) Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen 10: 1003–1008.

Shabir S, Yousuf S, Singh SK, Vamanu E, Singh MP (2022) Ethnopharmacological Effects of Urtica dioica, Matricaria chamomilla, and Murraya koenigii on Rotenone-Exposed *D. melanogaster*: An Attenuation of Cellular, Biochemical, and Organismal Markers. Antioxidants. 11: 1623

Sreejayan N, Rao MNA (1997) Nitric oxide scavenging by curcuminoids. J. of Pharm. and Pharm. 49: 105–107.

Subedi RP, Vartak RR, Kale PG, (2017) Management of stress exerted by hydrogen per-oxide in Drosophila melanogaster using Abhrak bhasma, J. Appl. Pharm. Sci. 7: 65–71

Ugbaja RN, Akinhanmi TF, James AS, Ugwor EI, Babalola AA, Ezenandu EO, Ugbaja VC, Emmanuel EA (2021) Flavonoid-rich fractions from Clerodendrum volubile and Vernonia amygdalina extenuates arsenic-invoked hepatorenal toxicity via augmentation of the antioxidant system in rats. Clinical Nutrition Open Science 35: 12e25

Wided K, Rouibah H, Lahouel M (2015) Quercetin Protects Liver Cells and Mitochondria Against Doxorubicin Induced Oxidative Stress in Albinos' Rats, Journal of Biologically Active Products from Nature 5: 331-38.