

Microwave-Assisted Extraction, *In vitro* Antioxidant Activity, and Hepatoprotective Potential of Flavonoid-Rich Watermelon (*Citrullus lanatus*) Seed Extract in Acetaminophen-Induced Liver Damage in Wistar Rats

^{*1}Ruth Onyeje Akor, ¹Abdullahi Abdulkadir, ²Amodu Ameh Danladi, ¹Maimuna Bello Umar, ¹Abdulkadir Abubakar, ³Daganna Muhammad Chatta and ⁴Ndamitso Muhammad Muhammad

¹Department of Biochemistry, Federal University of Technology, Minna, Nigeria

²Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria

³Department of Plant Biology, Federal University of Technology, Minna, Nigeria

⁴Department of Chemistry, Federal University of Technology, Minna, Nigeria

*Corresponding authors' email: akorruthonyeje@gmail.com Phone: +2349035996944

ABSTRACT

Watermelon (*Citrullus lanatus*) seed is an underutilised agro-industrial by-product in Nigeria containing bioactive flavonoids with antioxidant potential. This study evaluated microwave-assisted extraction (MAE) of a flavonoid-enriched extract from watermelon seed, evaluated its *in vitro* antioxidant activity, and investigated its hepatoprotective potential against acetaminophen (APAP)-induced liver injury in Wistar rats. Eight MAE conditions varying in solid-to-solvent ratio, microwave power, and extraction time were screened. The highest total flavonoid content (TFC) of 8.03 mg QE/g DW and extraction yield of 5.96% were obtained at a 1:10 solid-to-solvent ratio, 60% microwave power, and 30 min extraction time. The extract showed concentration-dependent antioxidant activity, with IC₅₀ values of 41.88, 31.67, and 55.30 µg/mL for DPPH, FRAP, and lipid peroxidation inhibition assays, respectively, compared with 15.91, 12.00, and 19.35 µg/mL for ascorbic acid. Acute oral toxicity testing (OECD 425) established an LD₅₀ >2000 mg/kg. APAP significantly elevated ALT, AST, and ALP and reduced haemoglobin. The highest extract dose (LD₅₀ × 1/10) reduced ALT and AST to 61.11 and 41.76 U/L, restored haemoglobin to 20.68 g/dL, improved lipid profile, restored hepatic antioxidant enzymes, and reduced malondialdehyde from 33.82 to 20.22 nmol/mg protein. Histopathology confirmed dose-dependent preservation of hepatic architecture. These findings demonstrate promising antioxidant and hepatoprotective properties of the flavonoid-enriched extract and its potential as a low-cost functional ingredient.

Received: 23 July 2026

Accepted: 02 Aug. 2026

Published: 15 Sept. 2026

Keywords:

Watermelon Seed, Flavonoid Extraction; Microwave-Assisted Extraction; Antioxidant Activity, Hepatoprotection

INTRODUCTION

Watermelon (*Citrullus lanatus*) seeds, which constitute 15–20% of total fruit weight, is widely cultivated and consumed across Nigeria, with annual production exceeding 4 million tonnes (FAO, 2022). They are routinely discarded as waste, resulting to environmental pollution and leading to loss of valuable bioactive compounds (Espinosa-Leal *et al.*, 2018). Watermelon seeds are a recognized source of phytochemicals including flavonoids, alkaloids, tannins, glycosides, and saponins (Manivannan *et al.*, 2020). Naringenin, catechin and other flavonoid compounds present in watermelon seeds have potential hepatoprotective properties (Tzortzakis *et al.*, 2018). Oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defence system of biological systems, is a crucial pathogenetic mechanism of different chronic diseases, such as cardiovascular diseases, neurodegenerative diseases, diabetes, and liver injury. Approximately 70% of global deaths per year are due to oxidative stress associated non-communicable diseases (WHO, 2021). Synthetic antioxidants (such as BHT and BHA) have been used widely to protect against oxidative damage, but because of safety issues, there has been a growing interest in naturally occurring antioxidant compounds, especially flavonoids (Shahidi & Zhong, 2015). Flavonoids exert their antioxidant effects through multiple mechanisms, including free radical scavenging, metal ion

chelation, inhibition of lipid peroxidation, and modulation of endogenous antioxidant defence pathways (Panche *et al.*, 2016).

Flavonoids are polyphenolic secondary metabolites that have the ability to neutralize free radicals and stabilize reactive oxygen species (Panche *et al.*, 2016). Flavonoids also have antioxidant, hepatoprotective, anti-inflammatory, and cardioprotective properties (Hasnat *et al.*, 2024; Khan *et al.*, 2021). The liver is especially vulnerable to oxidative damage because of its pivotal role in xenobiotic metabolism. The acetaminophen (APAP) overdose has been commonly employed as an experimental model of oxidative hepatotoxicity, and causes glutathione depletion, excessive formation of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI), mitochondrial oxidative stress, lipid peroxidation and increased serum liver enzymes, closely mimicking clinical drug-induced liver injury (Jaeschke & Ramachandran, 2024).

Solvent extraction methods commonly used for obtaining flavonoids from plants are time consuming, labor intensive and consume significant amounts of organic solvents. Rapid volumetric heating (Sharma *et al.*, 2022) is one of the advantages of microwave-assisted extraction (MAE), which is an environmentally friendly alternative technique that has substantial advantages, including faster extraction times, less solvent consumption, and higher extraction efficiency.

However, extraction efficiency is highly dependent on extraction parameters including solvent-to-solid ratio, microwave power and extraction time, all of which influence flavonoid recovery and stability. Despite these advantages however, the use of MAE for flavonoid extraction from watermelon seeds and the subsequent assessment of the extract's *in vivo* hepatoprotective activity remain largely unexplored, particularly for Nigerian watermelon varieties. Furthermore, although previous studies have reported the antioxidant potential of watermelon seed extracts, limited information is available regarding the chromatographic characterization of their flavonoid constituents and the possible molecular interactions of these compounds with antioxidant-related protein targets. Integrating phytochemical characterization with molecular docking analysis may provide mechanistic insight into the biological activities of the identified flavonoids.

The aim of this study was therefore to evaluate microwave assisted extraction conditions for the maximum extraction of flavonoids from watermelon seeds, evaluate the *in vitro* antioxidant activity of the extract using the DPPH radical scavenging, FRAP (ferric reducing antioxidant power) and lipid peroxidation inhibition assays; and investigate the hepatoprotective activity of the extract against liver damage caused by acetaminophen treatment in Wistar rats and provide scientific evidence for the potential conversion of this underutilised agricultural by-product into functional health ingredients. In addition, High-Performance Liquid Chromatography (HPLC) was employed to characterize the major flavonoids present in the extract, while molecular docking was performed to investigate the interactions of the identified compounds with the Keap1 protein in order to provide mechanistic support for their antioxidant and hepatoprotective activities.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh watermelon seeds were purchased from Kure Ultra-Modern Market, Minna, Niger State, Nigeria, and authenticated by the Department of Plant Biology, Federal University of Technology, Minna. The seeds were washed and air-dried for 3–5 days to avoid thermal degradation of bioactive compounds (Bagade & Patil, 2021). Subsequently, they were milled into a fine powder and sieved through a 40–60 mesh screen to obtain a uniform particle size distribution (Bagade *et al.*, 2021). The resulting powder was stored in airtight amber glass containers at 4°C until extraction to minimise oxidative degradation and preserve phytochemical integrity (Lourenço *et al.*, 2019).

Microwave-Assisted Extraction of Flavonoids

Distilled water was used as the extraction solvent for the microwave-assisted extraction process. Eight extraction conditions (W1–W8) were evaluated by varying the solid-to-solvent ratio (1:10 or 1:20, w/v), microwave power level (40 or 60), and extraction duration (5–30 min) as presented in Table 1. For each experimental run, 5g of the powdered watermelon seed sample was mixed with the appropriate volume of distilled water in an Erlenmeyer flask and exposed to microwave irradiation at the specified power setting and extraction time. A microwave oven equipped with an integrated temperature monitoring system was employed to minimise overheating during extraction (Jokić *et al.*, 2012). Upon completion of irradiation, the extracts were rapidly cooled in an ice bath to limit thermal degradation of heat-sensitive constituents (Peixoto *et al.*, 2022). The liquid extract was then separated from the solid residue by filtration using

Whatman No. 1 filter paper. To enhance extraction efficiency and maximise flavonoid recovery, the residue was subjected to a second extraction under identical conditions, and the resulting filtrates were combined (Peixoto *et al.*, 2022). The pooled filtrates were concentrated in a water bath maintained at 40°C to remove excess solvent, producing a concentrated aqueous gel-like extract, which was subsequently stored in amber bottles at –20°C until further analyses were conducted (Peixoto *et al.*, 2022).

Extraction yield was calculated as:

$$\text{Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dry seed powder}} \times 100$$

UV-Vis Spectroscopic Characterisation

The UV-Vis absorption spectrum of the plant extract was determined using a Shimadzu UV-1800 spectrophotometer. The extract was scanned over a wavelength range of 200–700 nm at a scanning speed of 1 nm interval. The characteristic absorption peaks were recorded and used to confirm the presence of flavonoid compounds in the extract based on their intrinsic UV absorption profile.

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminium chloride (AlCl₃) colorimetric method (Kumar & Pandey, 2021). A 0.5 mL aliquot of diluted extract was mixed with 1.5 mL of 95% ethanol, 2.8 mL of distilled water, 0.1 mL of 10% AlCl₃·6H₂O, and 0.1 mL of 1 M potassium acetate (Lourenço *et al.*, 2019). The mixture was incubated for 30 min at room temperature in the dark, with distilled water substituted for AlCl₃ in the blank. Absorbance was read at 415 nm on a UV-Vis spectrophotometer (Wang *et al.*, 2023). A calibration curve was prepared using quercetin dihydrate standard solutions (10–100 µg/mL) (Tungmunthum *et al.*, 2018), and TFC was expressed as mg quercetin equivalent per gram dry weight (mg QE/g DW) (Panche and Diwan 2016).

High-Performance Liquid Chromatography (HPLC) Analysis

Prior to injection, the extract was passed through a 0.45 µm membrane filter. A C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size) kept at 25–30°C will be used to accomplish separation. Using a gradient elution program designed for flavonoid separation, the mobile phase was composed of solvent A (water acidified with 0.1% formic acid or phosphoric acid) and solvent B (acetonitrile or methanol) (Siger *et al.*, 2021). A photodiode array detector (PDA) was used for detection at wavelengths of 280, 320, and 360 nm, which are typical of several flavonoid classes. Retention durations, UV-Vis spectra, and comparison with genuine standards (quercetin, kaempferol, rutin, catechin, apigenin, and luteolin) was used for identification (Jokić *et al.*, 2019).

In vitro Antioxidant Assays

The antioxidant activity of the extracts was assessed against ascorbic acid, as reference standard at the concentrations of 6.25, 12.5, 25, 50 and 100 µg/mL *in vitro*. Each experiment was repeated three times.

The radical scavenging activity was tested on the basis of 2,2-diphenyl-1-picrylhydrazyl (DPPH) method as described by Oyaizu (1986). The solutions to be tested were prepared by dissolving the extract or ascorbic acid into methanol. After that, 2 mL of 0.004% DPPH solution in methanol was added to 1 mL of each concentration of the test sample and standard. The reaction mixtures were incubated in the dark at 25°C for 30 min; absorbance was read at 517 nm against a blank (blank is made by replacing the product with distilled water) with a

Shimadzu UV-1800 series double-beam spectrophotometer. The experiment was carried out in triplicates and the percentage inhibition was calculated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100$$

The ferric reducing antioxidant power (FRAP) was determined according to the method developed by Oyaizu (1986). Briefly, 1 mL of each concentration of the extract or ascorbic acid was mixed with 1 mL of 0.2 M sodium phosphate buffer and 1 mL of 1% potassium hexacyanoferrate (III) solution. After 20 min incubation at 50°C the mixture was added with 1 mL of 10% trichloroacetic acid (TCA) and then centrifuged. After centrifugation, 1 mL of the supernatant was mixed with 1 mL of distilled water and 0.2 mL of 0.1% Ferric chloride solution and absorbance was measured at 700nm. The experiment was carried out in triplicates and the percentage antioxidant activity was calculated using the formula below:

$$\% \text{ activity} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100$$

The inhibitory effects of the extracts on lipid peroxidation was determined via thiobarbituric acid reactive substances (TBARS) assay (Halliwell et al., 1995). 0.5 mL of 10% egg homogenate was added to 0.1 mL of extracts at various concentrations and made up to 1 mL with distilled water. 0.05 mL of FeSO₄ was added and incubated for 30 minutes. Then, 1.5 mL of acetic acid and thiobarbituric acid in sodium dodecyl sulphate were added. The reaction mixtures were incubated at 95°C for 1 hour, cooled, and 5 mL of butanol was added. 5.

Absorbance of the upper organic layer was read at 532 nm. The percentage inhibition of lipid peroxidation was calculated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100$$

Ethical Approval

The study was approved by the Directorate of Research and Innovative Studies (DRID), Federal University of Technology, Minna, with assigned number 000117. The study was conducted in accordance with the relevant ethical guidelines and regulations.

Acute Oral Toxicity Study

The acute oral toxicity of the watermelon seed extract was determined in healthy albino rats (n = 7) using the up-and-down procedure outlined in OECD Guideline 425. A single animal was administered a starting dose of 5 mg/kg, with doses subsequently adjusted based on the survival outcome of the previous animal (5, 17.5, 55, 175, 550, and 2000 mg/kg). Each animal was continuously monitored for signs of toxicity for 4 hours post-administration, followed by daily monitoring for 7 days. No mortality or signs of toxicity were observed at any dose up to and including the limit dose of 2000 mg/kg; the median lethal dose (LD₅₀) was therefore estimated to be greater than 2000 mg/kg (LD₅₀ > 2000 mg/kg), which is considered non-toxic.

Experimental Animals and Induction of Hepatotoxicity

A total of 30 Wistar albino rats (3 months old) were purchased from Top Murine Farms and acclimatised to the standard laboratory conditions for two weeks with free access to feed and water. The thirty (30) rats were assigned to six groups (n = 5) analyzed per group following balancing) as follows:

Group 1 (Normal control): received no treatment.

Group 2 (Negative control): Hepatotoxicity was induced by daily acetaminophen (APAP, 500 mg/kg, p.o.) administration during days 1-3 without further treatment.

Group 3 (Positive control): APAP induced as above and treated with 10mg/kg silymarin which is used as the standard hepatoprotective agent.

Group 4: LD₅₀ × 1/20 of watermelon seed extract was applied in APAP-induced.

Group 5: APAP induced and treated with watermelon seed extract at LD₅₀ × 1/15.

Group 6: APAP induced with a treatment of watermelon seed extract of LD₅₀ × 1/10.

Body weight measurements were taken at intervals during the study. At the completion of treatment animals were humanely sacrificed under anaesthetic, and blood samples taken for haematology and biochemistry. The antioxidant activities of the plants were evaluated *in vivo* using the method developed by Reddy et al., 2017.

Biochemical and Haematological Analyses

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin and total bilirubin were used to assess liver function. The lipid profile parameters (total cholesterol, triglycerides, LDL and HDL) were measured by standard enzymatic kits. In order to assess the antioxidant status of the liver, the activities of catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione (GSH) were measured. Haematological parameters were determined using an automated haematology analyser and included haemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLC) and total white blood cell count (TWBC).

Molecular Docking Analysis

Molecular docking was performed on selected HPLC-identified compounds, namely catechin, quercetin and naringenin, together with gallic acid as the reference compound, and silibinin, the primary active flavonolignan found in silymarin. The docking experiment was carried out to investigate their interactions with Kelch-like ECH-associated protein 1 (Keap1), a key regulator of the Nrf2 antioxidant pathway. The three-dimensional crystal structure of Keap1 was obtained from the Protein Data Bank (PDB ID 4L7B). Protein preparation involved removal of water molecules, addition of hydrogen atoms, and energy minimization where appropriate. Ligand structures were obtained from the PubChem database and prepared using Open Babel before docking. Molecular docking was performed using PyRx employing the AutoDock Vina algorithm. Binding affinity was expressed as binding energy (kcal/mol), and ligand-protein interactions were visualized using Discovery Studio Visualizer.

Statistical Analysis

Data were presented as mean ± standard deviation (SD) as indicated. The comparisons between groups were carried out by Two-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test and statistical significance was set at p < 0.05. *In vitro* antioxidant assays, two-way ANOVA was used to compare the effects of concentration, sample type, and their interactions.

RESULTS AND DISCUSSION

Effect of Extraction Parameters on Flavonoid Yield

The total concentration of flavonoids varied considerably across the eight extraction conditions studied (Table 1). The highest concentration (8.03 mg/mL) was achieved with a

solid-to-solvent ratio of 1:10, 60 microwave power and extraction time of 30 min (W1) and also with a more concentrated sample-to-solvent ratio when compared to all other conditions tested. With the more dilute of the two ratios (1:20), the yields of flavonoids were generally lower and not consistently found to rise monotonically with the microwave power or time. For example, two runs at the same conditions (1:20, 60 microwave power, 20 min; W4 and W5) resulted in concentrations of 1.12 and 1.39 mg/mL, respectively, suggesting some degree of batch-to-batch variability in the manual extraction and filtration method. The lowest yield (0.16 mg/mL) was obtained at the shortest extraction time (5 min; W8) indicating that very short microwave time was not

enough to provide adequate cell wall disruption and release of flavonoids.

Based on these preliminary screening results, it is concluded that higher solid-to-solvent ratio has a more significant effect on the extraction efficiency of flavonoids than the microwave power or time within the ranges tested with the most concentrated solid to solvent ratio of 1:10 showing the best extraction efficiency of flavonoids over all the 1:20 conditions. This is in line with the report that with a lower solvent-to-solid ratio, a higher concentration gradient of compound release to the solvent, up to a certain limit, exists (Hammi *et al.*, 2021).

Table 1: Total Flavonoid Concentration of Watermelon Seed Extracts Under Varying Microwave-Assisted Extraction Conditions

Sample	Ratio (solid:solvent)	Microwave Power (%)	Time (min)	Absorbance (415 nm)	Concentration (mg/mL)
W1	1:10	60	30	0.760	8.03
W2	1:20	40	30	0.201	0.57
W3	1:20	40	20	0.423	3.56
W4	1:20	60	20	0.242	1.12
W5	1:20	40	20	0.262	1.39
W6	1:20	60	10	0.608	6.03
W7	1:20	40	10	0.665	6.76
W8	1:20	60	5	0.170	0.16

The Highest Extraction Yield at the Selected Extraction Condition (W1) was 5.96%

UV-Vis Spectroscopic Characterisation of Flavonoid Extract

The UV-Vis absorption spectrum of the flavonoid-rich *Citrullus lanatus* extract is shown in Figure 1. The spectrum revealed two characteristic absorption bands at 220 nm and 325 nm, corresponding to Band II (benzoyl system, A-ring)

and Band I (cinnamoyl system, B-ring) of the flavonoid skeleton respectively. These absorption bands are consistent with the typical UV spectral profile of flavonoid compounds reported in literature (Panche *et al.*, 2016). The presence of these characteristic bands suggests the presence of flavonoid-like phenolic constituents.

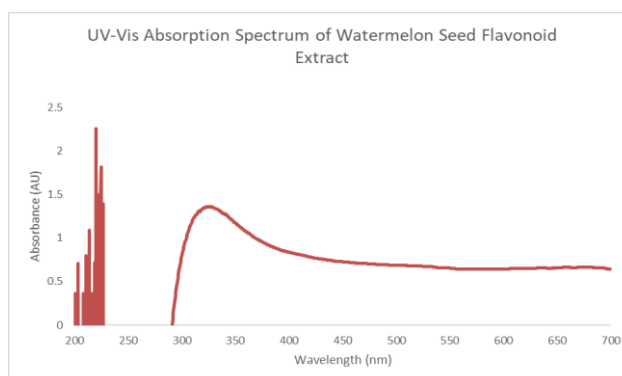


Figure 1: UV-Vis Absorption Spectrum of Watermelon Seed Flavonoid Extract

The figure above shows the characteristic absorption bands at 220 nm and 325 nm, corresponding to the benzoyl (Band II) and cinnamoyl (Band I) systems of flavonoids respectively.

HPLC Characterization of Flavonoids

High-performance liquid chromatography (HPLC) analysis of the watermelon seed extract identified four major flavonoid compounds: catechin, quercetin, and naringenin and a

phenolic compound (gallic acid, which was used as a reference compound in docking studies). Catechin was detected as the predominant compound, while quercetin, and naringenin were present in lower concentrations. The chromatographic profile confirms that the extract is rich in bioactive phenolic and flavonoid constituents known to possess antioxidant and hepatoprotective activities.

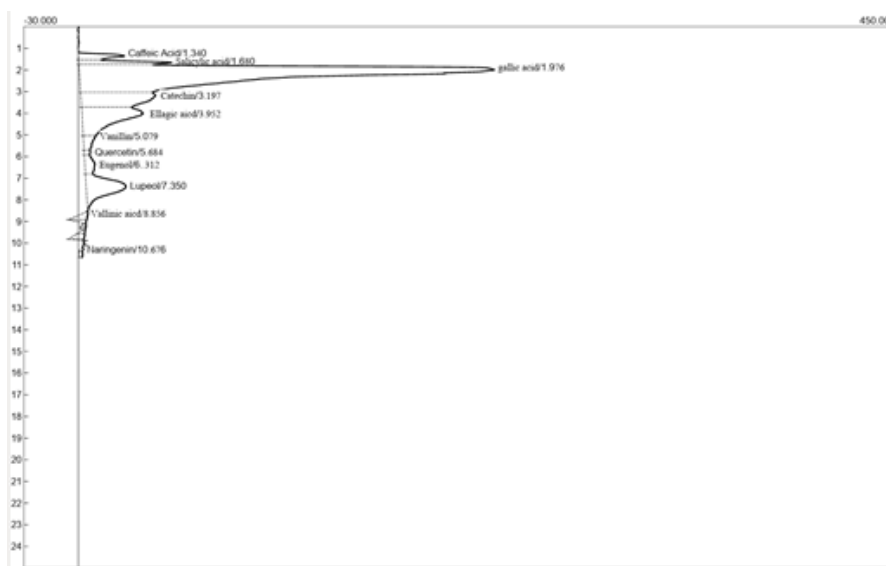


Figure 2: High Performance Liquid Chromatography (HPLC) Chromatogram of *Citrullus lanatus* Seed Extract

Table 2: Bioactive Compounds of *Citrullus lanatus* Seed Extracts Through High Performance Liquid Chromatography Analysis

Flavonoid	Retention Time (min)	Peak Area	Peak Height
Gallic acid	1.976	9168.3120	9168.312
Catechin	3.197	1563.0130	43.143
Quercetin	5.684	45.8340	3.478

The figure above shows the major flavonoid constituents of *Citrullus lanatus* seed extract.

***In vitro* Antioxidant Activity**

In this study, the flavonoid-rich *Citrullus lanatus* seed extract showed a concentration dependent free radical scavenging activity in DPPH assay with % inhibition of 6.14% at 6.25

µg/mL and 65.28% at 100 µg/mL (Figure 3). The scavenging activity of the extract was significantly lower than that of ascorbic acid at all concentrations tested ($p < 0.05$); however, the dose dependent trend was similar to the reference compound, and is typical of scavenging activity of extracts of plants rich in flavonoids (Seidu & Otutu, 2016).

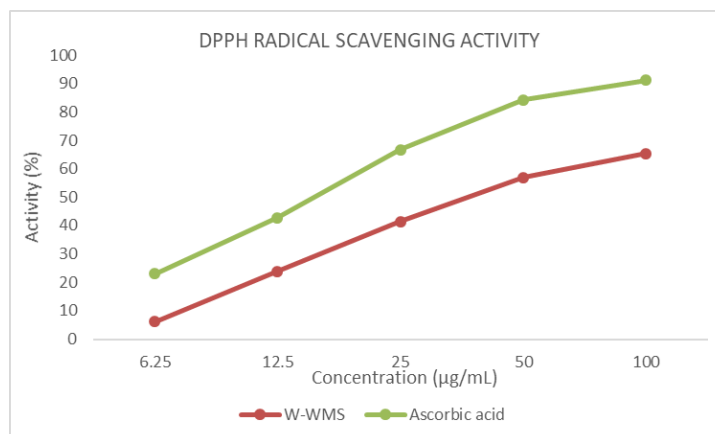


Figure 3: DPPH Radical Scavenging Activity (% Inhibition) of Watermelon Seed Flavonoid Extract and Ascorbic Acid. The IC_{50} of the Flavonoid Extract for DPPH was 41.88 µg/mL Compared to 15.91µg/mL for Ascorbic Acid, Indicating Concentration-Dependent Antioxidant Activity.

Similarly, the FRAP activity increased from 10.00% to 69.89% between doses of 6.25 and 100 µg/mL (figure 4) and the lipid peroxidation inhibition was enhanced from 2.65% to 59.67% in the same dose range (figure 5). In both assays, the extract once more exhibited significantly less activity

compared to ascorbic acid ($p < 0.05$), but a distinct concentration-dependent response indicating that genuine but moderate reducing and lipid protecting activity are present in the extract.

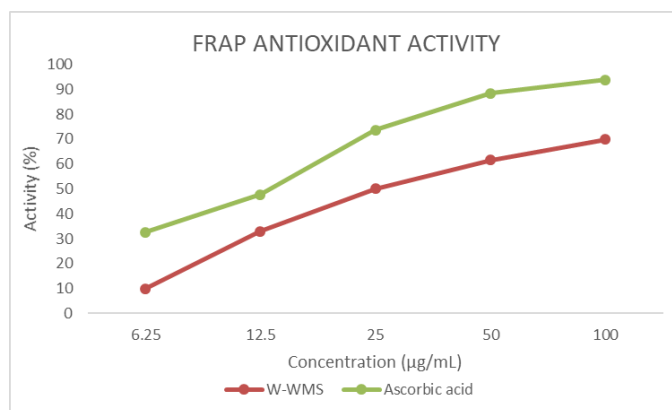


Figure 4: FRAP Activity (%) of Watermelon Seed Flavonoid Extract and Ascorbic Acid. The IC_{50} of the Flavonoid Extract for FRAP was 31.67 µg/mL Compared to 12.00 µg/mL for Ascorbic Acid, Indicating Concentration-Dependent Antioxidant Activity

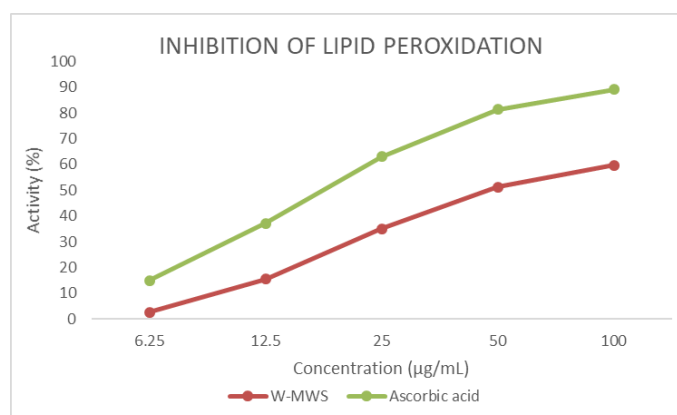


Figure 5: Inhibition of Lipid Peroxidation (%) of Watermelon Seed Flavonoid Extract and Ascorbic Acid

The IC_{50} of the flavonoid extract for ILP was 55.30 µg/mL compared to 19.35 µg/mL for ascorbic acid, indicating concentration-dependent antioxidant activity.

Effect on Body Weight

The body mass of all the treatment groups increased progressively with time during the study (Figure 6), which

shows that there was general body tolerance for both APAP and extract treatments. The body weight gain of the extract treated groups (Groups 4-6) was not different from that of the normal control group which indicated that the watermelon seed extract has no noticeable adverse effects on growth at the doses applied.

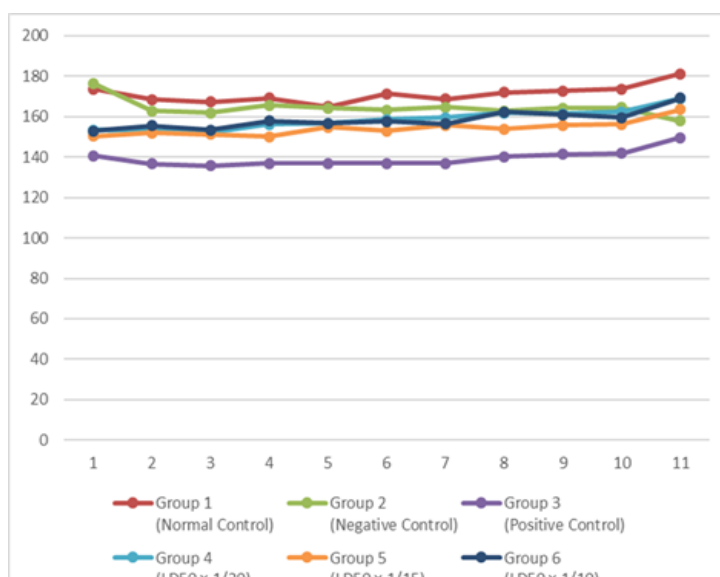


Figure 6: Body Weights of Rats Across All Treatment Groups

Hepatoprotective Effect Against APAP-Induced Liver Damage

The negative control group showed significant elevation in ALT (52.49 U/L), AST (81.68 U/L), ALP (114.55 U/L) ($p < 0.05$) and significant decrease in total protein and albumin, when compared with normal control (Table 3, $p < 0.05$). The highest dose ($LD_{50} \times 1/10$, Group 6) significantly decreased

AST and ALT levels to 41.76 U/L and 61.11 U/L, respectively, which was in the same range as the levels in the silymarin-treated positive control (Group 3), and significantly lower than those of the untreated negative control. The hepatoprotective effect was found to be dose-responsive, as the treatment dose ($LD_{50} \times 1/20$) in Group 4 had intermediate effect.

Table 3: Liver Function Test Parameters of Wistar Rats Across Treatment Groups

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Protein (g/dL)	Albumin (g/dL)	Total Bilirubin (mg/dL)
Group 1 (Normal Control)	31.35 $\pm$ 3.45 ^a	55.13 $\pm$ 3.61 ^a	83.18 $\pm$ 3.74 ^a	36.57 $\pm$ 5.66 ^b	24.91 $\pm$ 4.43 ^b	0.75 $\pm$ 0.10 ^a
Group 2 (Negative Control)	52.49 $\pm$ 3.34 ^d	81.68 $\pm$ 4.80 ^d	114.55 $\pm$ 5.85 ^c	21.36 $\pm$ 2.37 ^a	14.15 $\pm$ 1.21 ^a	1.21 $\pm$ 0.10 ^c
Group 3 (Positive Control)	37.10 $\pm$ 4.31 ^{ab}	63.74 $\pm$ 4.62 ^b	85.48 $\pm$ 5.72 ^a	30.78 $\pm$ 4.23 ^b	22.76 $\pm$ 3.43 ^b	0.82 $\pm$ 0.07 ^a
Group 4 ($LD_{50} \times 1/20$)	45.64 $\pm$ 4.35 ^c	72.60 $\pm$ 5.36 ^c	93.96 $\pm$ 4.79 ^b	24.44 $\pm$ 3.24 ^a	14.39 $\pm$ 1.96 ^a	0.94 $\pm$ 0.04 ^b
Group 5 ($LD_{50} \times 1/15$)	37.54 $\pm$ 5.03 ^{ab}	63.88 $\pm$ 3.81 ^b	88.17 $\pm$ 4.67 ^{ab}	30.84 $\pm$ 2.55 ^b	22.11 $\pm$ 2.78 ^b	0.82 $\pm$ 0.06 ^a
Group 6 ($LD_{50} \times 1/10$)	41.76 $\pm$ 3.57 ^{bc}	61.11 $\pm$ 3.86 ^b	83.26 $\pm$ 3.98 ^a	33.28 $\pm$ 3.31 ^b	24.59 $\pm$ 2.79 ^b	0.78 $\pm$ 0.07 ^a

Values are Mean $\pm$ SD (n = 5). Different Superscripts within a Column Differ Significantly ($p < 0.05$, Duncan's test). ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; ALP = Alkaline Phosphatase

The negative control showed an alteration in lipid profile with increased total cholesterol (469.40 mg/dL), triglycerides (146.40 mg/dL) and LDL (126.40 mg/dL) while HDL (73.02 mg/dL) decreased compared to the normal control (Table 4).

Lipid parameters showed a dose dependent improvement with the highest dose ($LD_{50} \times 1/10$) bringing HDL to a level statistically similar to normal control and reducing the cholesterol level in comparison to the negative control.

Table 4: Lipid Profile Parameters of Wistar Rats Across Treatment Groups

Group	Cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Group 1 (Normal Control)	384.51 $\pm$ 5.74 ^a	126.86 $\pm$ 3.37 ^a	114.20 $\pm$ 4.19 ^a	92.35 $\pm$ 2.42 ^b
Group 2 (Negative Control)	469.40 $\pm$ 13.87 ^d	146.40 $\pm$ 5.70 ^c	126.40 $\pm$ 4.39 ^c	73.02 $\pm$ 5.45 ^a
Group 3 (Positive Control)	392.52 $\pm$ 4.34 ^{ab}	128.82 $\pm$ 3.15 ^a	115.79 $\pm$ 6.29 ^a	100.98 $\pm$ 3.45 ^c
Group 4 ($LD_{50} \times 1/20$)	423.87 $\pm$ 7.19 ^c	138.07 $\pm$ 2.51 ^b	123.07 $\pm$ 3.44 ^{bc}	78.36 $\pm$ 6.59 ^a
Group 5 ($LD_{50} \times 1/15$)	424.56 $\pm$ 6.10 ^c	129.77 $\pm$ 3.90 ^a	117.94 $\pm$ 3.58 ^{ab}	87.82 $\pm$ 4.86 ^b
Group 6 ($LD_{50} \times 1/10$)	400.25 $\pm$ 8.58 ^b	129.01 $\pm$ 4.02 ^a	116.77 $\pm$ 3.66 ^{ab}	92.77 $\pm$ 5.21 ^b

Values are Mean $\pm$ SD (n = 5). Different Superscripts within a Column Differ Significantly ($p < 0.05$, Duncan's Test). LDL = Low-Density Lipoprotein; HDL = High-Density Lipoprotein

APAP exposure significantly reduced the activity of antioxidant enzymes in the liver of the negative control, the level of CAT was reduced to 19.99 U/mg protein, the level of SOD was reduced to 27.95 U/mg protein, the level of GSH was reduced to 81.22 μ mol/g tissue, and the level of MDA, a marker of lipid peroxidation, elevated to 33.82 nmol/mg protein (Table 5). In the same way, antioxidant enzyme activity was restored dose-dependently by extract, the highest

dose ($LD_{50} \times 1/10$) getting the enzyme activities of CAT, SOD and GSH to be statistically similar to normal and positive controls; while the level of MDA was decreased to 20.22 nmol/mg protein, which showed that the extract suppressed the oxidative lipid damage, supporting a true hepatoprotective mechanism rather than just a symptomatic effect on the antioxidant enzyme activity.

Table 5: *In vivo* Antioxidant Enzyme Activities of Wistar Rats Across Treatment Groups

Group	CAT (U/mg protein)	SOD (U/mg protein)	MDA (nmol/mg protein)	GSH (μ mol/g tissue)
Group 1 (Normal Control)	32.51 $\pm$ 3.30 ^b	51.65 $\pm$ 5.17 ^c	20.60 $\pm$ 2.96 ^a	107.48 $\pm$ 3.81 ^c
Group 2 (Negative Control)	19.99 $\pm$ 3.91 ^a	27.95 $\pm$ 2.97 ^a	33.82 $\pm$ 3.65 ^d	81.22 $\pm$ 6.21 ^a
Group 3 (Positive Control)	32.79 $\pm$ 3.73 ^b	48.41 $\pm$ 6.22 ^c	23.85 $\pm$ 3.79 ^{ab}	103.15 $\pm$ 4.28 ^c
Group 4 (LD ₅₀ $\times$ 1/20)	22.96 $\pm$ 2.32 ^a	33.13 $\pm$ 2.82 ^{ab}	31.18 $\pm$ 3.12 ^{cd}	83.66 $\pm$ 4.79 ^a
Group 5 (LD ₅₀ $\times$ 1/15)	28.96 $\pm$ 3.24 ^b	38.22 $\pm$ 3.43 ^b	27.05 $\pm$ 3.24 ^{bc}	93.58 $\pm$ 6.38 ^b
Group 6 (LD ₅₀ $\times$ 1/10)	32.82 $\pm$ 3.19 ^b	46.81 $\pm$ 2.99 ^c	20.22 $\pm$ 3.16 ^a	101.66 $\pm$ 5.38 ^c

Values are Mean $\pm$ SD (n = 5). Different Superscripts within a Column Differ Significantly (p < 0.05, Duncan's Test). CAT = Catalase; SOD = Superoxide Dismutase; MDA = Malondialdehyde; GSH = Glutathione.

The haematological assessment showed marked APAP-induced anaemia in the negative control group with haemoglobin (Hb) reduced to 11.76 g/dL and RBC count reduced to $4.65 \times 10^6/\mu$ L relative to the normal control (20.68 g/dL and $7.80 \times 10^6/\mu$ L respectively); there was an increased total white blood cell count (TWBC) in the negative control group, which indicated an inflammatory response (Table 6).

The lowest dose (LD₅₀ $\times$ 1/20) showed a lesser recovery of haematological parameters, though statistically the same as the normal control, while the two higher doses (LD₅₀ $\times$ 1/15 and 1/10) resulted in a statistically similar recovery of all haematological parameters to the normal control, indicating a dose-dependent protective effect.

Table 6: Haematological Parameters of Wistar Rats Across Treatment Groups

Group	HB (g/dL)	MCV (fL)	PCV (%)	RBC ($\times 10^6/\mu$ L)	MCHC (g/dL)	PLC ($\times 10^3/\mu$ L)	MCH (pg)	TWBC ($\times 10^3/\mu$ L)
Group 1 (Normal Control)	20.68 $\pm$ 1.40 ^b	43.00 $\pm$ 2.16 ^a	39.50 $\pm$ 1.00 ^a	7.80 $\pm$ 0.62 ^b	40.25 $\pm$ 1.26 ^b	128.00 $\pm$ 3.74 ^a	29.00 $\pm$ 0.82 ^b	7.71 $\pm$ 1.52 ^a
Group 2 (Negative Control)	11.76 $\pm$ 2.68 ^a	42.50 $\pm$ 1.29 ^a	40.25 $\pm$ 1.89 ^a	4.65 $\pm$ 0.96 ^a	28.75 $\pm$ 2.99 ^a	126.25 $\pm$ 3.30 ^a	21.25 $\pm$ 2.22 ^a	10.00 $\pm$ 2.03 ^b
Group 3 (Positive Control)	21.04 $\pm$ 2.60 ^b	40.75 $\pm$ 1.71 ^a	40.25 $\pm$ 1.71 ^a	8.68 $\pm$ 0.32 ^b	43.50 $\pm$ 2.38 ^b	127.75 $\pm$ 2.63 ^a	29.50 $\pm$ 1.29 ^b	8.34 $\pm$ 1.06 ^{ab}
Group 4 (LD ₅₀ $\times$ 1/20)	20.66 $\pm$ 0.87 ^b	41.00 $\pm$ 2.71 ^a	40.00 $\pm$ 1.41 ^a	5.67 $\pm$ 1.22 ^a	41.25 $\pm$ 2.22 ^b	128.50 $\pm$ 4.44 ^a	30.50 $\pm$ 1.29 ^b	7.83 $\pm$ 0.45 ^a
Group 5 (LD ₅₀ $\times$ 1/15)	22.22 $\pm$ 2.19 ^b	42.25 $\pm$ 1.71 ^a	40.00 $\pm$ 1.41 ^a	7.43 $\pm$ 1.68 ^b	40.25 $\pm$ 1.71 ^b	131.00 $\pm$ 4.97 ^a	28.00 $\pm$ 0.82 ^b	7.66 $\pm$ 0.34 ^a
Group 6 (LD ₅₀ $\times$ 1/10)	18.20 $\pm$ 2.62 ^b	42.75 $\pm$ 2.22 ^a	40.75 $\pm$ 0.96 ^a	8.14 $\pm$ 1.11 ^b	43.75 $\pm$ 2.99 ^b	126.00 $\pm$ 4.97 ^a	32.75 $\pm$ 0.96 ^c	7.41 $\pm$ 0.42 ^a

Values are Mean $\pm$ SD (n = 5). Different Superscripts within a Column Differ Significantly (p < 0.05, Duncan's Test). Hb = Haemoglobin; PCV = Packed Cell Volume; RBC = Red Blood Cell Count; MCHC = Mean Corpuscular Haemoglobin Concentration; TWBC = Total White Blood Cell Count

Collectively, these findings suggest that the hepatoprotective effect of the watermelon seed flavonoid extract against the APAP-induced liver injury is dose-dependent and consistently lowers the enzyme leakage, dyslipidaemia, oxidative stress and anaemia among the liver injury profile, and a dose of LD₅₀ $\times$ 1/10 is comparable in effects to the standard hepatoprotective agent, silymarin. This protection is in line with the *in vitro* free radical scavenging, reducing and lipid peroxidation-inhibiting activity of the extract, which suggests that the antioxidant activity of flavonoids is the

probable mechanism of the protective effect (Roy *et al.*, 2022).

Histopathological Findings

Liver sections from all experimental groups were stained with H&E and photographed at $\times 400$ and histological interpretation is provided below on each of the plates 1–6, each of which shows a photomicrograph of a liver section, with the corresponding histological interpretation.

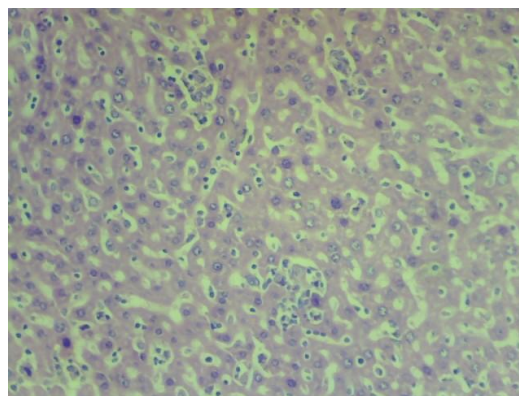
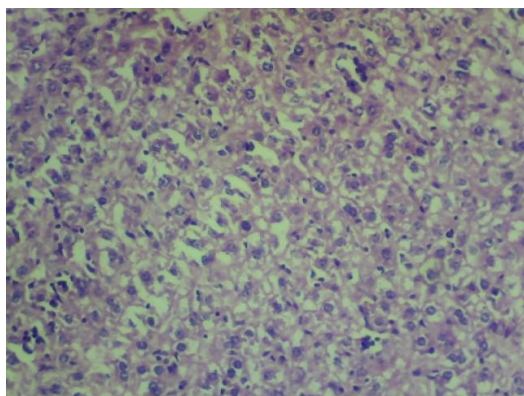


Figure 7: Normal Control

Figure 7(a) and (b): Photomicrographs of liver sections, Normal Control group (H&E stain, ×400). In the sections, the liver architecture appears normal, featuring cords of normal hepatocytes, normal portal tracts,

sinusoids and central veins. There are no features of acute or chronic damage.

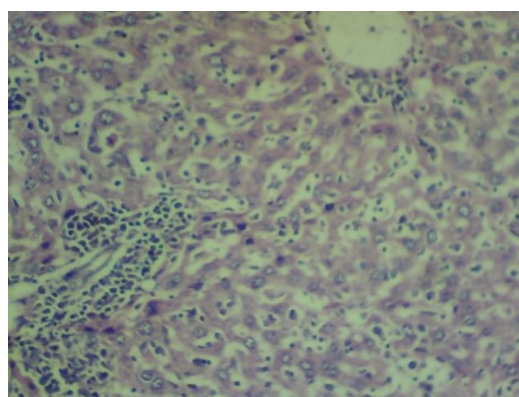
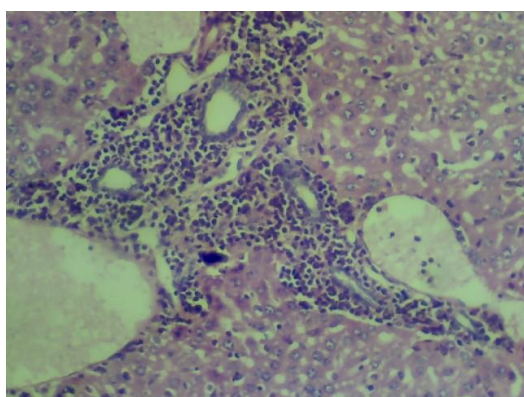


Figure 8: Negative Control (APAP-induced, untreated)

Figure 8(a) and (b): Photomicrographs of liver sections, Negative Control (APAP-induced, untreated) group (H&E stain, ×400).

The hepatic tissue in the sections is severely necrotic with hepatocytes and is distorted, with the central vein. The sinusoids were also enlarged, which was also associated with hepatotoxicity, induced by acetaminophen (APAP).

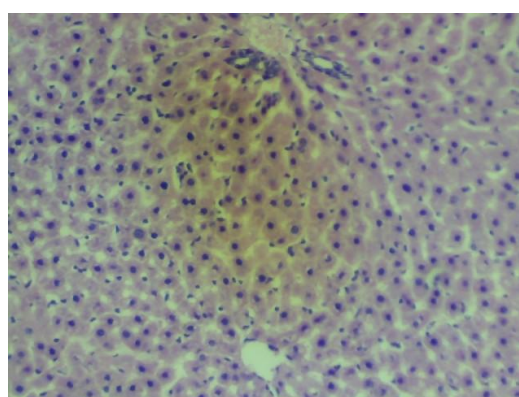
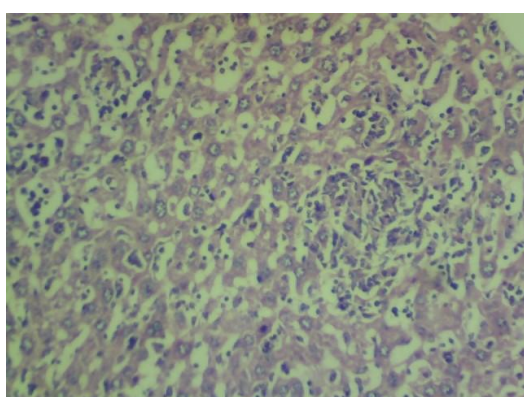


Figure 9: Positive Control (APAP-induced + Silymarin)

Figure 9(a) and (b): Photomicrographs of liver sections, Positive Control (APAP-induced + Silymarin) group (H&E stain, ×400).

In the sections, the hepatic tissue has a well-preserved architecture in which the hepatic portal tracts and sinusoids

are preserved as well as the central vein. Appeared mild necrotic liver cells and mild enlargement of sinusoids, suggesting the protective effects of silymarin against APAP-induced injury.

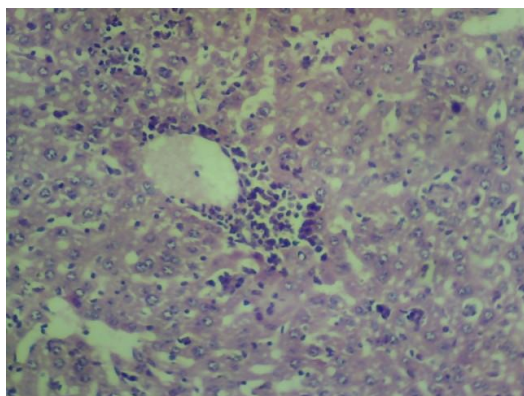
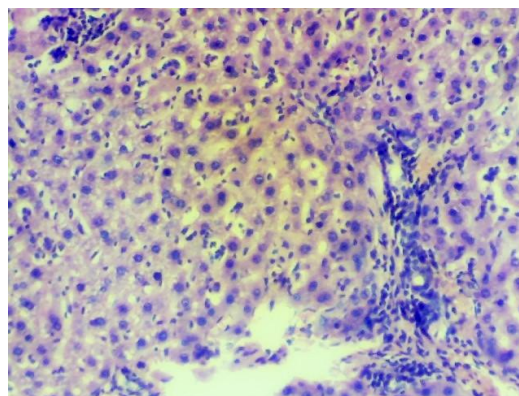


Figure 10: Low Dose (LD) Extract

Figure 10(a) and (b): Photomicrographs of liver sections, Low Dose (LD) Extract group (H&E stain, ×400). Sections demonstrate marked necrosis of the hepatocytes and distortion of the portal tracts with central vein. The sinusoids



were also seen to be enlarged, suggesting that the low dose was not protective against hepatotoxicity induced by APAP.

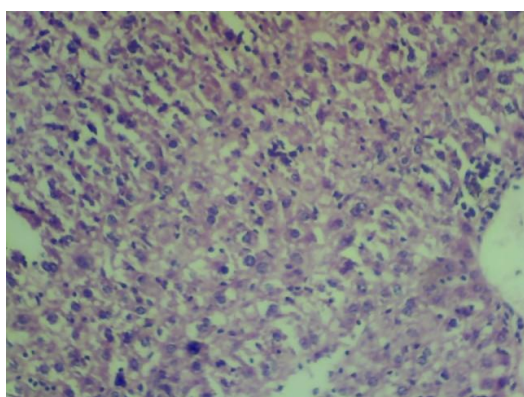
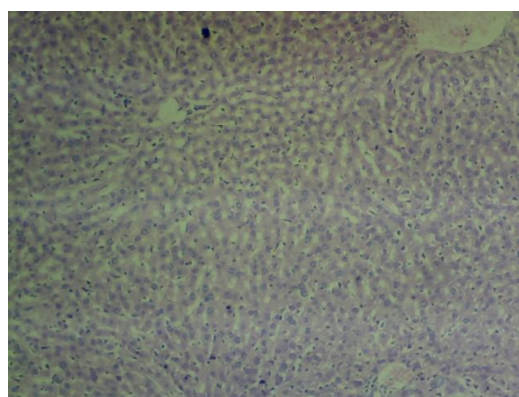


Figure 11: Medium Dose (MD) Extract

Figure 11(a) and (b): Photomicrographs of liver sections, Medium Dose (MD) Extract group (H&E stain, ×400). The hepatic tissue of the sections has a normal architecture consisting of cords of normal hepatocytes and normal portal



tracts and central vein. Some mild necrosis of hepatocytes and mild sinusoidal enlargement were noted which represent partial hepatoprotection at this dose.

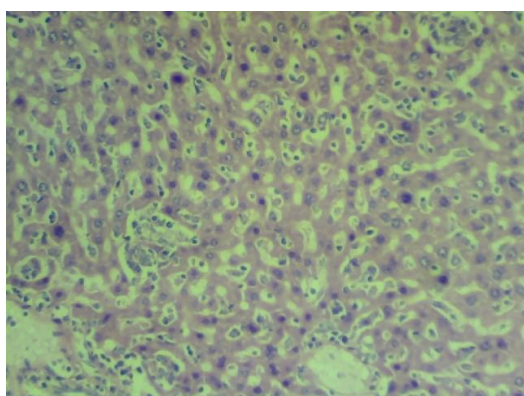
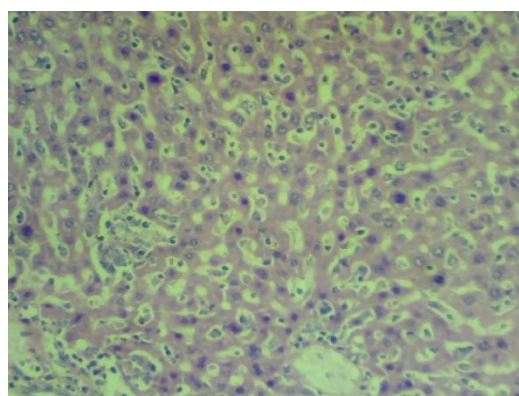


Figure 12: High Dose (HD) Extract

Figure 12(a) and (b): Photomicrographs of liver sections, High Dose (HD) Extract group (H&E stain, ×400). In this sections, normal hepatic tissue with preserved architecture of hepatocyte cords, portal tracts and central veins are seen. The hepatoprotective effect was seen to be significant as the sinusoids were only slightly enlarged,



similar to the effect observed in the silymarin-treated positive control.

Histopathological examination showed a liver protective effect in a dose dependent manner. Liver architecture was preserved in the normal control group, whereas in the negative control (APAP-induced and untreated) group, the liver architecture was severely disturbed with numerous

hepatocellular necrosis, portal distortion and sinusoidal enlargement, confirming successful hepatotoxicity induction. The liver histology in the low dose (LD) extract group was similar to the negative control group and not different from that of other treatment groups. The medium dose (MD) and high dose (HD) extracts showed a progressive preservation of hepatic architecture with a mild degree of necrosis and sinusoidal changes, similar to the positive control treated with silymarin. Based on these results, it can be concluded that the

hepatoprotective activity of the extract is dose-dependant, and that the medium and high doses provide a significant hepatoprotective effect against APAP-induced hepatotoxicity.

Molecular Docking Analysis

All identified compounds demonstrated favourable binding affinities toward the Keap1 binding pocket, although the interaction strengths differed among the compounds.

Table 7: Energy Scores for Each Ligand PyRx

Compound	Binding energy (kcal/mol)
Silibinin	-9.6
Quercetin	-8.4
Naringenin	-8.4
Catechin	-8.0
Gallic acid	-6.0

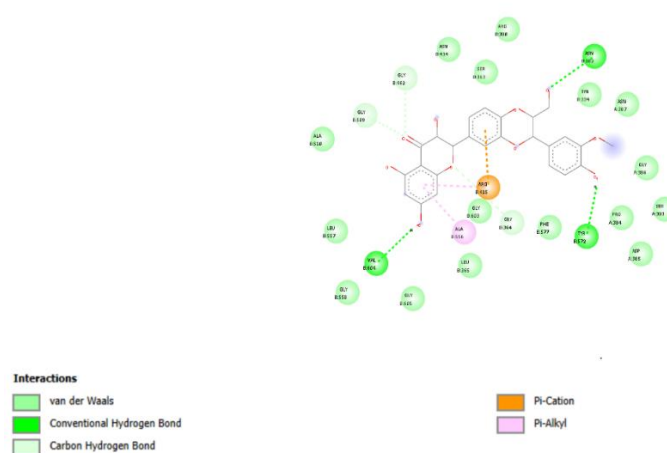


Figure 13: Visualization of 2D Structure of gallic Acid and KEAP1 Protein. The Cartoon view Showing the Amino Acid Residues Involved in the Interaction

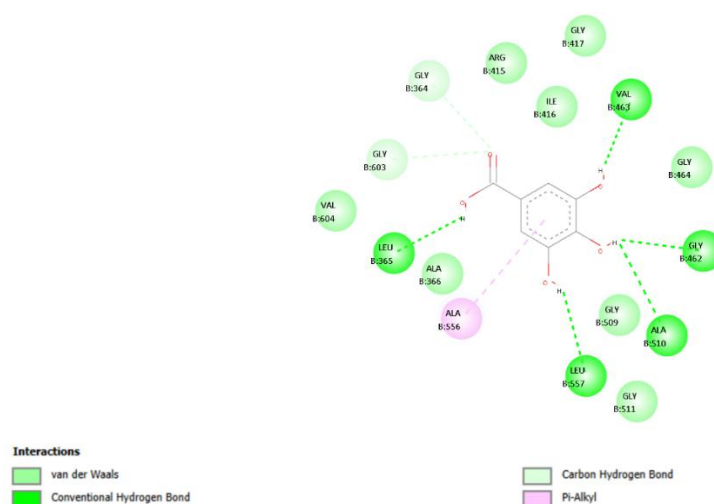


Figure 14: Visualization of 2D Structure of Gallic Acid and KEAP1 Protein. The Cartoon View Showing the Amino Acid Residues Involved in the Interaction.

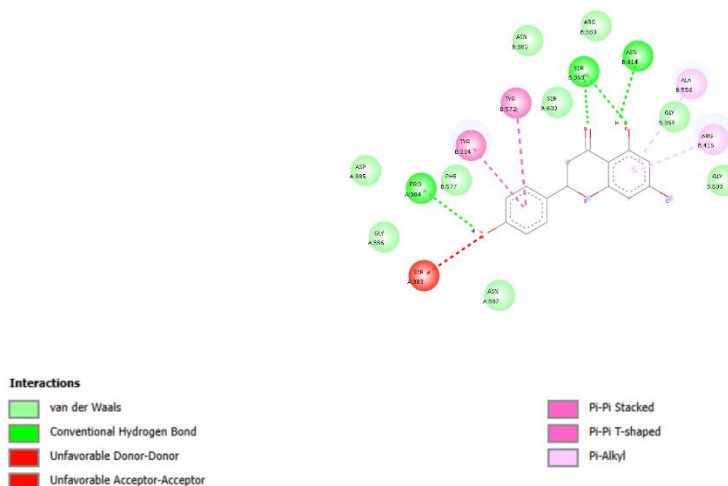


Figure 15: Visualization of 2D Structure of Naringenin and KEAP1 Protein. The Cartoon View Showing the Amino Acid Residues Involved in the Interaction

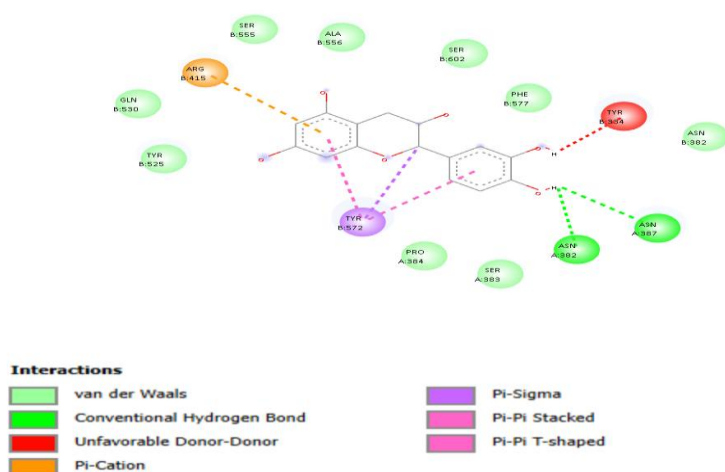


Figure 16: Visualization of 2D Structure of Catechin and KEAP1 Protein. The Cartoon View Showing the Amino Acid Residues Involved in the Interaction

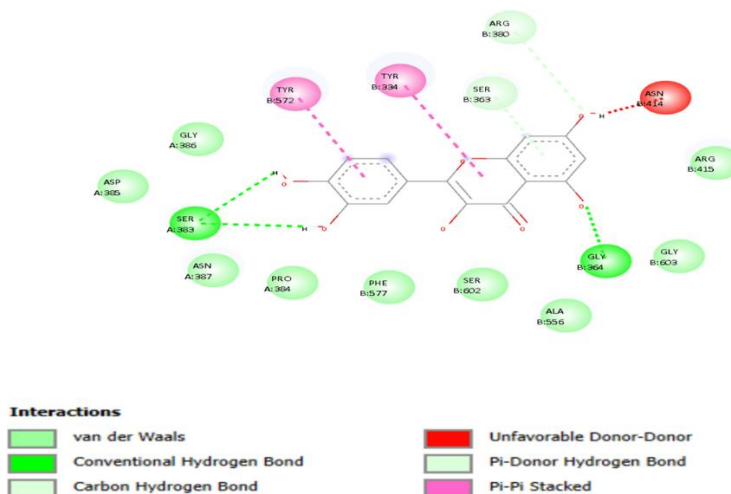


Figure 17: Visualization of 2D Structure of Quercetin and KEAP1 Protein. The Cartoon View Showing the Amino Acid Residues Involved in the Interaction

Discussion

In this study, the solid-to-solvent ratio had the highest effect on the extraction of flavonoids from *Citrullus lanatus* seeds by using microwave-assisted extraction (MAE). Of the extraction conditions tested, 1:10 (w/v) solid to solvent ratio gave the highest yield of flavonoids, increasing the solvent amount to 1:20 did not increase the extraction efficiency although there was an increase in microwave power and extraction time. This result suggests that the availability of solvents and mass transfer were more significant than microwave power in the successful recovery of flavonoids in the conditions studied. The observed effect of the ratio of solid to solvent is in accordance with other reports which show that the solvent-to-solid ratio is one of the key factors that determines the efficiency of microwave-assisted extraction. Bagade and Patil (2021) found that the extraction efficiency depends on the combination of the volume of solvent, extraction time, and microwave power, and the properties of the solvent, which affect the process of dielectric heating and mass transfer during MAE.

The aqueous extract of *Citrullus lanatus* seeds, containing flavonoids, showed antioxidant activity, which was concentration dependent in the DPPH, FRAP and lipid peroxidation assays. The antioxidant activity in the extract was found to be significantly lower than ascorbic acid in all the tests but it increased gradually as the concentration of the extract was increased, suggesting that the antioxidant constituents present in the extract still possess significant antioxidant activity and reducing power. The lower antioxidant activity compared to ascorbic acid was anticipated because ascorbic acid is a pure form of an antioxidant compound while the watermelon seed extract is a mixture of compounds containing flavonoids and other phytochemicals, which may be present at different concentrations. The antioxidant activity was found to increase with concentration in the present study as observed in other studies with watermelon seed extracts. Seidu and Otutu (2016) reported that watermelon seed flour showed appreciable DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) which increased as the concentration of the extract increased.

The aqueous extract of *Citrullus lanatus* seeds, when administered to rats with acetaminophen (APAP) liver toxicity, significantly reduced the level of serum liver enzymes (ALT, AST, ALP and bilirubin) and restored total protein and albumin concentration. The results suggest hepatocyte protective effect of the extract against APAP-induced membrane damage and maintenance of hepatosynthetic function. Raised ALT, AST and ALP seen in negative control group is a typical biochemical parameter of hepatocellular damage. In the case of acetaminophen overdose, excessive amounts of the reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) are formed, consume glutathione (GSH) and contribute to oxidative stress, mitochondrial dysfunction and hepatocyte necrosis. The elevated serum transaminases seen in APAP intoxication are due to leakage of intracellular enzymes into the blood due to disruption of the cell membranes. This mechanism has been well described by Jaeschke *et al.* (2012) and was corroborated by Vargas-Mendoza *et al.* (2019) who identified oxidative stress coupled with mitochondrial injury as key events in APAP hepatotoxicity. The hepatoprotective activity seen in the study is in agreement with the other experimental studies testing natural antioxidants to protect the liver from injury caused by APAP. Toklu *et al.*, (2019) showed that the administration of watercress extract for 14 days significantly decreased ALT and AST activities in the APAP-treated rats,

and improved antioxidant parameters, suggesting antioxidant role on hepatic tissues against oxidative damage in the APAP-treated rats. In a similar manner, Papackova *et al.* (2018) demonstrated that silymarin pretreatment significantly inhibited the increase in serum ALT and AST levels induced by APAP, which was attributed to the antioxidant and anti-nitrosative properties as well as to the ability to limit APAP-induced hepatocellular necrosis. The findings of the study are also dose dependent further supporting the biological importance of its hepatoprotective effect. Liver enzyme activities were statistically similar in the group treated with the highest dose ($LD_{50} \times 1/10$), while intermediate and lower doses were effective in a proportionately less extent. Several other plant extracts containing flavonoids have also been shown to be hepatoprotective in a dose-dependent manner, with higher doses leading to greater decreases in oxidative stress and liver damage. This pattern confirms the pharmacological significance of the hepatoprotective effect of watermelon seed extract, as opposed to an incidental effect.

The aqueous extract of *Citrullus lanatus* seeds significantly restored the activities of the endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) and significantly reduced malondialdehyde (MDA) in acetaminophen-treated rats. The results show that the extract was able to significantly reduce the oxidative stress caused by intoxication with acetaminophen and increase the antioxidant defense system. The drug overdose of acetaminophen has been reported to generate the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI), which quickly exhausts the intracellular glutathione pool and triggers the onset of mitochondrial oxidative stress. This overproduction of ROS and RNS then leads to a dysfunction of the endogenous antioxidant enzymes, lipid peroxidation, and finally hepatocellular necrosis. Jaeschke and Ramachandran (2024) extensively summarized this mechanism, concluding that mitochondrial oxidant stress is the underlying cause of liver injury caused by acetaminophen.

The marked increase in GSH that was seen in the current study indicates that the watermelon seed extract may have been counteracting the oxidative consumption of GSH, or it may have been promoting the regeneration of GSH during the process of detoxification of acetaminophen. The result is in line with Du *et al.* (2016), which showed the ability of the extracts from plant rich in flavonoids to preserve the intracellular glutathione, this by reducing the ROS and enhancing the cellular redox homeostasis. Likewise, Jaeschke and Ramachandran (2020) showed that hepatic glutathione is one of the most dependable markers of hepatoprotection against oxidative damage caused by acetaminophen, as glutathione is the major intracellular defense against the toxicity of NAPQI. The increases in SOD and CAT activities further demonstrate restoration of endogenous antioxidant defense mechanisms. The superoxide radical is converted to hydrogen peroxide by the action of superoxide dismutase and hydrogen peroxide is then turned to water and molecular oxygen by catalase; this helps to avoid the accumulation of highly reactive oxygen species. The restoration of these enzymes shows that the extract treatment has helped to lower the oxidative burden and maintain the normal function of these antioxidant enzymes. This finding is similar to that of other research that acid-washed flavonoids were found to boost the activity of endogenous antioxidant enzymes through a reduction of oxidative damage and an increase of antioxidant capacity. In addition, induction of antioxidant defense mechanisms has often been correlated with increased resistance to acetaminophen-induced hepatotoxicity. This is

one of the significant results of the present study, that malondialdehyde (MDA) concentration was significantly decreased after treatment. Malondialdehyde is a commonly accepted marker for lipid peroxidation and is a measure of the oxidation of polyunsaturated fatty acids in membrane. The high MDA levels seen in untreated acetaminophen-treated group and significant decrease after the administration of the extract suggest that it has inhibited lipid peroxidation and caused extensive membrane damage due to oxidative stress. The hepatoprotective activity observed in this study agrees with the report of Ogeyemhe and Odigie (2026), who demonstrated that *Alstonia boonei* extract exerted significant protective effects against aspirin-induced hepatotoxicity through improvement of liver function and attenuation of hepatic tissue damage. Although the hepatotoxic agent and plant source differed from those used in the present study, both findings support the potential of plant-derived bioactive compounds as effective hepatoprotective agents against drug-induced liver injury.

HPLC analysis of the optimized *Citrullus lanatus* seed extract revealed the presence of catechin, quercetin, and naringenin, with catechin showing the highest peak area (1563.0130), followed by quercetin (45.8340) and naringenin (29.3200). Gallic acid was also detected in the chromatographic profile and was included as an analytical reference in the present study. The occurrence of these phenolic and flavonoid constituents provides a plausible chemical basis for the antioxidant activity demonstrated by the extract. Flavonoids such as catechin, quercetin, and naringenin possess structural features that enable them to participate in free-radical scavenging and other antioxidant processes (Panche et al., 2016; Tungmunthum et al., 2018). The identification of quercetin in the present study is consistent with previous chromatographic investigation of *Citrullus lanatus* seeds, in which quercetin was reported among the phytochemical constituents of the seed extract (Dhanalakshmi & K., 2013). The detection of the other compounds further contributes to the characterization of the phytochemical composition of the watermelon seed extract investigated in the present study.

The molecular docking analysis provided further insight into the possible molecular basis of the antioxidant activity of the extract. The selected compounds showed favourable interactions with Keap1, with silibinin exhibiting the strongest binding affinity (−9.6 kcal/mol), followed by quercetin and naringenin (−8.4 kcal/mol), catechin (−8.0 kcal/mol), while the gallic acid artifact recorded a comparatively weaker binding energy of −6.0 kcal/mol.

The two-dimensional interaction structures further showed that the docked compounds were accommodated within the Keap1 binding pocket through different non-covalent interactions, including hydrogen bonding, van der Waals interactions and π -related interactions. For silibinin, the interaction profile revealed a conventional hydrogen bond with Asn382, along with additional hydrogen bonding involving Val604 and Tyr572, which likely anchor the ligand within the binding cleft. A π -cation interaction with Arg415 and a π -alkyl interaction with Ala556 further stabilised the complex, while extensive van der Waals contacts with surrounding residues (including Gly509, Gly603, Gly605, Leu365, Phe577, and Tyr334, among others) contributed to the overall fit of the compound within the pocket. This combination of hydrogen bonding, π -mediated, and van der Waals interactions likely accounts for the notably stronger binding energy observed for silibinin relative to the other compounds evaluated.

These interactions can contribute to stabilization of ligand–protein complexes and may account, in part, for the

favourable binding energies observed. The strong predicted affinity of silibinin is consistent with its established recognition as a potent Nrf2/Keap1 pathway modulator in the literature, while the favourable interaction of quercetin with Keap1 is consistent with previous reports linking quercetin to modulation of the Keap1–Nrf2 antioxidant pathway (Yang et al., 2015); broader docking studies have similarly reported favourable interactions between antioxidant phytochemicals and Keap1 (Li et al., 2020). The markedly stronger predicted affinity of silibinin, together with the comparatively favourable affinities of quercetin and naringenin, therefore provide additional *in silico* support for their possible contribution to the antioxidant activity observed experimentally. However, these docking interactions represent computational predictions and do not by themselves establish activation of the Nrf2 pathway. Overall, the docking findings, together with the 2D interaction profiles, complement the HPLC and antioxidant results and suggest that the identified phytochemicals may contribute to the biological activity of the *Citrullus lanatus* seed extract.

CONCLUSION

The findings demonstrate that *Citrullus lanatus* seed extract possesses significant antioxidant and hepatoprotective potential. The HPLC findings establish the presence of several bioactive flavonoids, while the docking results provide supportive *in silico* evidence for possible interactions with Keap1. The combined findings suggest that flavonoids present in watermelon seeds may contribute to protection against oxidative and acetaminophen-induced hepatic injury.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the Tertiary Education Trust Fund (TETFund) and the Federal University of Technology, Minna (FUTMinna) for funding this research through the Institution-Based Research (IBR) grant [TETFund Grant No.: TETFUND/FUTMINNA/2025/022/VOL.1].

REFERENCES

- Bagade, S. B., & Patil, M. (2021). Recent advances in microwave-assisted extraction of bioactive compounds from complex herbal samples: A review. *Critical Reviews in Analytical Chemistry*, 51(2), 138–149. <https://doi.org/10.1080/10408347.2019.1686966>
- Espinosa-Leal, C. A., Puente-Garza, C. A., & García-Lara, S. (2018). *In vitro* plant tissue culture: means for production of biological active compounds. *Planta*, 248(1), 1–18.
- Food and Agriculture Organization of the United Nations. (2022). FAOSTAT statistical database. FAO.
- Hasnat, H., Shompa, S. A., Islam, M. M., Alam, S., Richi, F. T., Emon, N. U., ... & Ahmed, F. (2024). Flavonoids: A treasure house of prospective pharmacological potentials. *Heliyon*, 10(6). <https://doi.org/10.1016/j.heliyon.2024.e27533>
- Jaeschke, H., & Ramachandran, A. (2020). The role of oxidant stress in acetaminophen-induced liver injury. *Current Opinion in Toxicology*, 20–21, 9–14. <https://doi.org/10.1016/j.cotox.2020.03.003>

- Jaeschke, H., & Ramachandran, A. (2024). Acetaminophen hepatotoxicity: Paradigm for understanding mechanisms of drug-induced liver injury. *Annual Review of Pathology: Mechanisms of Disease*, 19, 453–478. <https://doi.org/10.1146/annurev-pathmechdis-051122-094016>
- Jaeschke, H., McGill, M. R., & Ramachandran, A. (2012). Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: Lessons learned from acetaminophen hepatotoxicity. *Drug Metabolism Reviews*, 44(1), 88–106. <https://doi.org/10.3109/03602532.2011.602688>
- Jokić, S., Cvjetko, M., Božić, Đ., Fabek, S., Toth, N., & Vorkapić-Furač, J. (2012). Optimisation of microwave-assisted extraction of phenolic compounds from broccoli and its antioxidant activity. *International Journal of Food Science & Technology*, 47(12), 2613–2619. <https://doi.org/10.1111/j.1365-2621.2012.03143.x>
- Khan, J., Deb, P. K., Priya, S., Medina, K. D., Devi, R., Walode, S. G., & Rudrapal, M. (2021). Dietary flavonoids: Cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. *Molecules*, 26(13), 4021. <https://doi.org/10.3390/molecules26134021>
- Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, 24(22), 4132. <https://doi.org/10.3390/molecules24224132>
- Manivannan, A., Lee, E. S., Han, K., Lee, H. E., & Kim, D. S. (2020). Versatile nutraceutical potentials of watermelon—A modest fruit loaded with pharmaceutically valuable phytochemicals. *Molecules*, 25(22), 5258. <https://doi.org/10.3390/molecules25225258>
- Ogeyemhe, B. E., & Odigie, E. B. (2026). Hepatoprotective effects of *Alstonia boonei* against aspirin-induced liver injury in albino rats. *FUDMA Journal of Sciences*, 10(2), 108–116. <https://doi.org/10.33003/fjs-2026-1002-4604>
- Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5, e47. <https://doi.org/10.1017/jns.2016.41>
- Papackova, Z., Heczkova, M., Dankova, H., Sticova, E., Lodererova, A., Bartonova, L., Poruba, M., & Cahova, M. (2018). Silymarin prevents acetaminophen-induced hepatotoxicity in mice. *PLoS ONE*, 13(1), e0191353. <https://doi.org/10.1371/journal.pone.0191353>
- Peixoto, F. B., Raimundini Aranha, A. C., Nardino, D. A., Defendi, R. O., & Suzuki, R. M. (2022). Extraction and encapsulation of bioactive compounds: A review. *Journal of Food Process Engineering*, 45(12), e14167. <https://doi.org/10.1111/jfpe.14167>
- Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab., babalghith, A.O., & Islam, M.R. (2022). Flavonoids: A bioactive compound from medicinal plants and its therapeutic applications. *BioMed Research International*, 2022 (1), 5445291. <https://doi.org/10.1155/2022/5445291>
- Seidu, K. T., & Otutu, O. L. (2016). Phytochemical composition and radical scavenging activities of watermelon (*Citrullus lanatus*) seed constituents. *Croatian Journal of Food Science and Technology*, 8(2), 83–89.
- Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods*, 18, 757–781. <https://doi.org/10.1016/j.jff.2015.01.047>
- Sharma, M., Kohli, D., Soni, N., & Mitra, J. (2021). Microwave assisted extraction of bioactive compounds: A Review. *Octa Journal of Biosciences*, 9(2).
- Tungmunthum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, 5(3), 93.
- Tzortzakis, N., Chrysargyris, A., & Petropoulos, S. A. (2018). Phytochemicals content and health effects of cultivated and underutilized species of the cucurbitaceae Family. In *Phytochemicals in vegetables: A valuable source of bioactive compounds* (pp. 99-165). Bentham Science Publishers.
- Wang, L., Xu, H., Li, X., Chen, H., Zhang, H., Zhu, X., ... & Zhang, H. (2023). Cucurbitacin E reduces IL-1 β -induced inflammation and cartilage degeneration by inhibiting the PI3K/Akt pathway in osteoarthritic chondrocytes. *Journal of Translational Medicine*, 21(1), 880.
- WHO. (2021). Noncommunicable diseases progress monitor 2021. World Health Organization.

