



## Determination of Antioxidant Activity of Methanolic Leaf Extract of *Emilia sonchifolia* and its Effect on the Lipid Profile of Wistar Rats

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### Abstract

This study investigated the methanolic extract of *Emilia sonchifolia* leaves to provide a scientific basis for its ethnobotanical applications and to evaluate its impact on lipid profile parameters in Wistar rats. Pulverized leaves were macerated in 99.8% methanol for 72 hours, filtered, and concentrated via rotary evaporation at 40°C. The crude extract was partitioned exhaustively using a 1:3 extract-to-solvent ratio with hexane, dichloromethane (DCM), ethyl acetate, and n-butanol. Antioxidant capacity was assessed using Ferric Reducing Antioxidant Power (FRAP) and DPPH assays, alongside spectrophotometric quantification of total phenolic and flavonoid content. For the *in vivo* study, 24 Wistar rats were divided into four groups (n = 6). Group 1 (control) received normal saline, while groups 2 – 4 received incremental daily oral doses (500, 1000, and 1500 mg/kg) of the extract for 14 days. On day 15, serum was collected via cardiac puncture. Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) were measured calorimetrically, while LDL and VLDL were calculated using the Friedewald formula. Phytochemical screening confirmed the presence of flavonoids, tannins, saponins, alkaloids, and cardiac glycosides. The extraction process yielded 82.02g of crude extract (17.45% w/w). The Ethyl acetate and DCM fractions exhibited the highest antioxidant activities, with results comparable to standard Ascorbic Acid. Lipid profile analysis indicated no significant differences ( $p > 0.05$ ) in TC, TG, HDL, or VLDL levels. However, a highly significant increase in Low-Density Lipoprotein (LDL) was observed ( $p < 0.01$ ) across all treated groups compared to the control. While the potent antioxidant capacity of *Emilia sonchifolia* validates its traditional use in managing oxidative-related ailments, the significant elevation of LDL levels ( $p < 0.01$ ) highlights a critical metabolic safety concern. These findings suggest that the plant may adversely modulate lipid homeostasis, which could potentially increase cardiovascular risk. Therefore, despite its therapeutic benefits, caution is recommended in its medicinal and dietary consumption.

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## 1.0 Introduction

Oxidative stress is defined as a physiological state resulting from a chronic imbalance between the production of reactive oxygen and nitrogen species (ROS/RNS) and the body's endogenous antioxidant capacity. This state is central to the pathogenesis of various chronic conditions, including cancer, cardiovascular diseases, and neurodegenerative disorders (Udofia, 2024). When ROS levels exceed neutralizing thresholds, these unstable molecules induce oxidative damage to cellular DNA, proteins, and membrane lipids, often culminating in cell death (Sahnoun et al., 1997; Udo et al., 2009).

The rising incidence of oxidative abnormalities has intensified the search for reliable, plant-based antioxidants as alternative therapies (Okokon et al., 2012; Uffia et al., 2024). However, many phytochemical supplements significantly influence host lipid profiles, particularly cholesterol and triglyceride levels. Improper or excessive use of such extracts often driven by an unregulated "health-seeking" bias can lead to severe metabolic complications that may counteract their therapeutic benefits (Udofia et al., 2023). A critical case in point is *Emilia sonchifolia*, an important edible medicinal plant utilized in traditional salads and ethnomedicine across Africa, India, and Malaysia. While documentation exists regarding its use for ailments such as asthma, malaria, and inflammatory diseases (Devi et al., 2006), its systemic safety profile remains incomplete. Critically, previous research on *E. sonchifolia* has focused almost exclusively on isolated antioxidant screening, leaving a significant void in the literature regarding its physiological integration with lipid metabolism. Chemical radical-scavenging assays do not provide adequate insight into how the plant modulates cholesterol and triglycerides at graded doses. Furthermore, the absence of safety-linked lipid

data for a plant consumed as a food staple represents a substantial public health oversight. This study bridges this gap by evaluating the extract's toxicological markers alongside a comprehensive lipid profile analysis. These findings will provide the necessary evidence-based framework for healthcare workers to recommend safe consumption levels for both dietary and medicinal purposes.

## 2.0 Materials and Methods

### 2.1 Collection and Identification of Plant Material

Fresh leaves of *Emilia sonchifolia* were obtained from bushes in Ikot Akpaden, Akwa Ibom State, Nigeria. Authentication was performed by Prof. Margaret Bassey, a taxonomist at the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen (UUH/10(e)) was deposited at the Faculty of Pharmacy Herbarium.

### 2.2 Plant Extraction

Leaves were cleaned, air-dried, and pulverized. 470g of the material was macerated in 6.8L of 99.8% methanol at room temperature (25°C - 32°C) for 72 hours with periodic agitation. The mixture was filtered and concentrated via rotary evaporation at 40°C. **The process yielded 82.02g of crude methanol extract (17.45% w/w yield).** The extract was refrigerated at -4°C until use.

### 2.3 Phytochemical Screening

Qualitative screening for alkaloids, saponins, tannins, flavonoids, cardiac glycosides, and anthraquinones was performed according to the standard protocols of Sofowora (1993).

### 2.4 Fractionation (Partitioning of Extract)

30g of the crude extract was dissolved in 100mL of distilled water. This was partitioned exhaustively in a separating funnel using a 1:3 ratio of Hexane, Dichloromethane (DCM), Ethyl

acetate, and Butanol. Each fraction was dehydrated under reduced pressure at 35°C and stored in a desiccator.

## 2.5 Antioxidant Evaluation

### 2.5.1 DPPH Radical Scavenging Activity

The scavenging activity of the extract and fractions was evaluated against ascorbic acid at concentrations of 20, 40, 60, 80, and 100 µg/mL following the method of Shekhar and Anju (2014). 1mL of 0.1mM DPPH was added to 3mL of sample, incubated in the dark for 30 minutes, and measured at 700nm. The percentage inhibition was calculated as:

$$\text{DPPH Scavenging effect (\%)} = \frac{A_o - A_s}{A_o} \times 100 \quad (1)$$

Where  $A_o$  is the control absorbance and  $A_s$  is the sample absorbance.

### 2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP activity was determined by mixing 1mL of sample (20–100 µg/mL) with sodium phosphate buffer (pH 6.6) and 1% w/v potassium ferricyanide. After incubation at 50°C, 10% trichloroacetic acid was added, and the mixture was centrifuged. The supernatant was treated with 0.1% ferric chloride and absorbance was read at 700nm.

## 2.6 Quantitative Determination (TPC and TFC)

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were quantified via spectrophotometry using Folin-Ciocalteu and Aluminum Chloride methods, respectively, as described by Emmanuel et al. (2020). Results were expressed as mg Gallic Acid Equivalent (GAE/g) and mg Rutin Equivalent (RE/g).

## 2.7 Acute Toxicity Study

Acute toxicity was assessed following OECD (2001) guidelines. No morbidity was observed at 5000 mg/kg, leading to the selection of 500, 1000, and 1500 mg/kg for the study doses.

## 2.8 Animal Treatment

Twenty-four Wistar rats (140–180g) were acclimatized for two weeks. They were distributed into 4 groups (n=6):

- **Group 1:** Control (10mL normal saline).
- **Groups 2–4:** 500, 1000, and 1500 mg/kg of extract respectively.

Doses were administered daily via oral cannula for 14 days.

## 2.9 Analysis of Lipid Profile

On day 15, animals were euthanized via terminal inhalation anesthesia using chloroform. Deoxygenated blood was collected via cardiac puncture into EDTA bottles. Serum cholesterol (TC), triglycerides (TG), and HDL were measured calorimetrically, while LDL and VLDL were calculated using the Friedewald formula.

## 2.10 Statistical Analysis

Data are expressed as Mean ± SEM. Analysis was conducted using One-way Analysis of Variance (ANOVA) followed by the Tukey-Kramer post-test using SPSS. Statistical significance was set at ( $p < 0.05$ ), with ( $p < 0.01$ ) used for high-significance observations.

## 2.11 Ethical Approval

Approval for the use of the animals for the study was obtained from the Animal Ethics Committee of the faculty of Pharmacy, University of Uyo, Nigeria. All animal experiments were conducted in accordance with internationally accepted Laboratory Animal Use and Care of Laboratory Animals (1996) as adopted and promulgated by the National Institute of Health (NIH publication No. 85(23), revised (1996), based on Helsinki convention and guidelines and rules of faculty of Pharmacy, University of Uyo, Nigeria for animal experimentation.

## 3.0 Results

### 3.1 Phytochemical Screening and Yield

The extraction of 470g of dried *Emilia sonchifolia* leaves yielded 82.02g (17.45% w/w) of crude methanol extract. Qualitative screening confirmed the presence of alkaloids, saponins,

tannins, flavonoids, and cardiac glycosides. Anthraquinones were notably absent (Table 1).

### 3.2 Acute Toxicity Study

At the limit dose of 5000 mg/kg, the extract produced no signs of clinical toxicity, morbidity,

or mortality over a 14-day observation period. This indicates an oral LD<sub>50</sub> greater than 5000 mg/kg, categorizing the extract as relatively non-toxic according to OECD guidelines.

**Table 1:** Qualitative Phytochemical Composition of *Emilia sonchifolia* Leaf Extract

Phytochemical Constituent	Test/Method	Observation	Inference
Saponins	Frothing Test	Presence of persistent foam	+
Tannins	Ferric Chloride Test	Blue-black coloration	+
Alkaloids	Meyer's Test	Creamy precipitate	+
Flavonoids	Alkaline Reagent Test	Yellow coloration	+
Cardiac Glycosides	Salkowski Test	Reddish-brown ring	+
Anthraquinones	Borntrager's Test	No color change	—

**Key:** (+) = Presence; (—) = Absence

### 3.3 Lipid Profile Modulation

Lipid profile analysis of rats treated for 14 days revealed no significant changes ( $p > 0.05$ ) in Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), or Very Low-Density Lipoprotein (VLDL) compared to the control. However, a highly significant **increase** in Low-Density Lipoprotein (LDL) was observed at all treatment doses ( $p < 0.01$ ), as detailed in Table 2.

### 3.4 Antioxidant Capacity and Quantitative Determination

#### 3.4.1 DPPH Radical Scavenging Activity

The extract and its fractions demonstrated concentration-dependent scavenging of the DPPH radical. The Ethyl acetate fraction showed the highest potency, nearly matching the Ascorbic Acid standard at 100 µg/mL (Figure 1).

#### 3.4.2 Ferric Reducing Antioxidant Power (FRAP)

Reducing power increased linearly with concentration across all samples. The Ethyl acetate and Dichloromethane (DCM) fractions exhibited superior reducing capacities, exceeding the performance of the standard drug, Ascorbic Acid (Figure 2).

#### 3.4.3 Total Phenolic Content (TPC)

Quantitative determination via the Gallic Acid calibration curve ( $y = 0.0072x + 0.1346$ ) identified the Ethyl acetate fraction as having the

highest phenolic load (1.122 mg GAE/g) (Figure 3), followed by the DCM fraction (Table 3).

#### 3.4.4 Total Flavonoid Content (TFC)

Using the Rutin calibration curve ( $y = 0.0021x + 0.0526$ ), the DCM fraction was found to contain the highest flavonoid concentration (2.150 mg RE/g) (Figure 4). The negative values recorded for the Butanol and Aqueous fractions indicate that flavonoid levels in these solvents were below the analytical limit of detection (Table 4).

### 3.5 Statistical Analysis Summary

One-way ANOVA confirmed that while antioxidant markers varied significantly across fractions ( $p < 0.05$ ), the most significant physiological change was the elevation of LDL levels in the treated animal groups ( $p < 0.01$ ).

## 4.0 Discussion

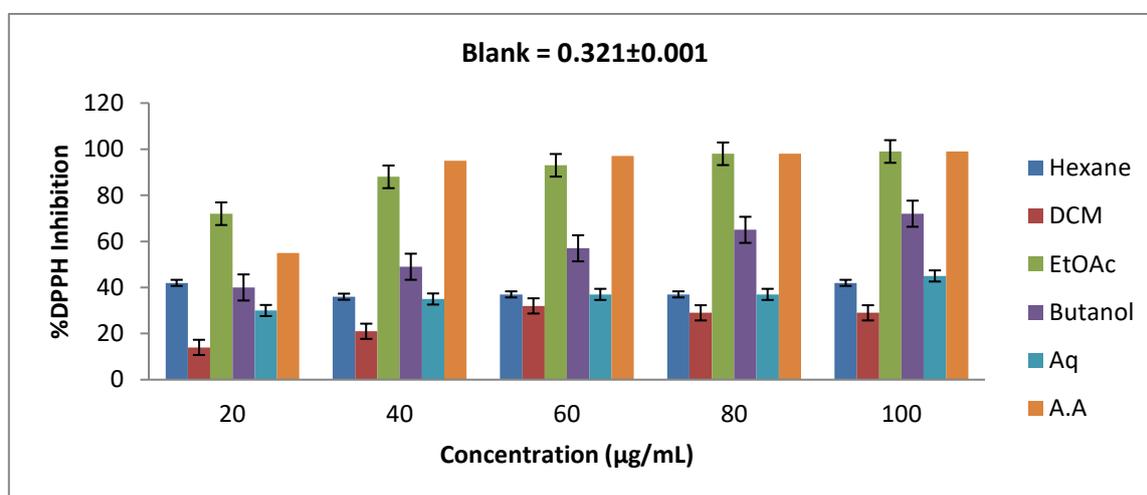
The phytochemical screening of the methanolic leaf extract of *Emilia sonchifolia* revealed a diverse array of bioactive secondary metabolites, including saponins, tannins, alkaloids, cardiac glycosides, and flavonoids. These results align with the findings of Neethu and Gangaprasad (2018), who identified similar compounds as the primary drivers of the plant's therapeutic efficacy. These metabolites are integral to the plant's bioactivity; for instance, flavonoids and tannins are well-recognized for their ability to inhibit oxidative stress and neuroinflammation (Cichon et al., 2020). Antioxidants function by neutralizing free radicals - highly reactive molecules capable

of damaging DNA, proteins, and cellular membranes by donating electrons to stabilize them (Anju et al., 2010). In this study, the antioxidant capacity of the extract and its fractions was evaluated using DPPH and FRAP assays.

**Table 2:** Lipid Profile Parameters of Wistar Rats Following 14-Day Administration of *Emilia sonchifolia* Methanol Extract

Dose (mg/kg)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
Control	1.66±0.15	1.95±0.29	1.91±0.00	0.94±0.62	0.93±0.00
500	1.71±0.00	1.14±0.51	2.26±0.00	1.06±0.25 <sup>b</sup>	0.50±0.19 <sup>ns</sup>
1000	1.76±0.01	1.21±0.16	2.14±0.08	0.89±0.07 <sup>b</sup>	0.55±0.07 <sup>ns</sup>
1500	1.79±0.06	1.24±0.30	2.17±0.07	0.94±0.16 <sup>b</sup>	0.56±0.13 <sup>ns</sup>

Note: Values are expressed as Mean ±SEM (n=6). Significance: <sup>b</sup>(p < 0.01) compared to control; <sup>ns</sup> = non-significant. Abbreviations: TC (Total Cholesterol), TG (Triglycerides), HDL (High-Density Lipoprotein), LDL (Low-Density Lipoprotein), VLDL (Very Low-Density Lipoprotein).



**Figure 1:** Bar Chart of DPPH Assay of Scavenging Activity of *Emilia sonchifolia* Leaf Extract and Fractions

The results indicated that the Ethyl acetate fraction possessed the highest potency, with a DPPH scavenging effect of 98% at 100 µg/mL, nearly identical to the Ascorbic Acid standard (99%). This high activity suggests that the semi-polar constituents of *E. sonchifolia* are particularly effective at hydrogen donation. Similarly, the FRAP assay showed that the Ethyl acetate and Dichloromethane (DCM) fractions exhibited superior reducing power (1.050 and 0.918 respectively) compared to Ascorbic Acid (0.626). This concentration-dependent increase in absorbance is consistent with the findings of Emmanuel et al. (2020), proving that the plant

fractions possess significant potential to reduce  $Fe^{3+}$  to  $Fe^{2+}$ . A point of technical clarification involves the negative TFC values recorded for the Butanol (-0.171) and Aqueous (-0.457) fractions. As these values are derived from the Rutin calibration curve ( $y = 0.0021x + 0.0526$ ), a negative result indicates that the sample absorbance was lower than the Y-intercept of the standard curve. In analytical chemistry and pharmacognosy, this does not represent a calculation error but rather confirms that the flavonoid concentration in these specific fractions is negligible or falls below the analytical limit of detection (LOD).

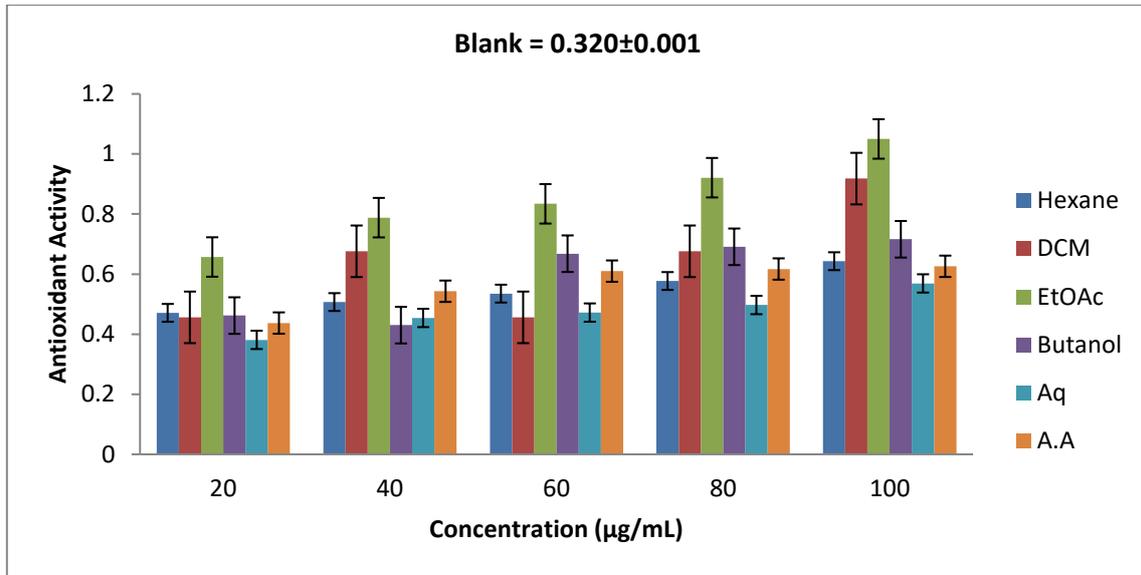


Figure 2: Bar Chart of FRAP ASSAY of *Emilia sonchifolia* Leaf Extracts and Fraction

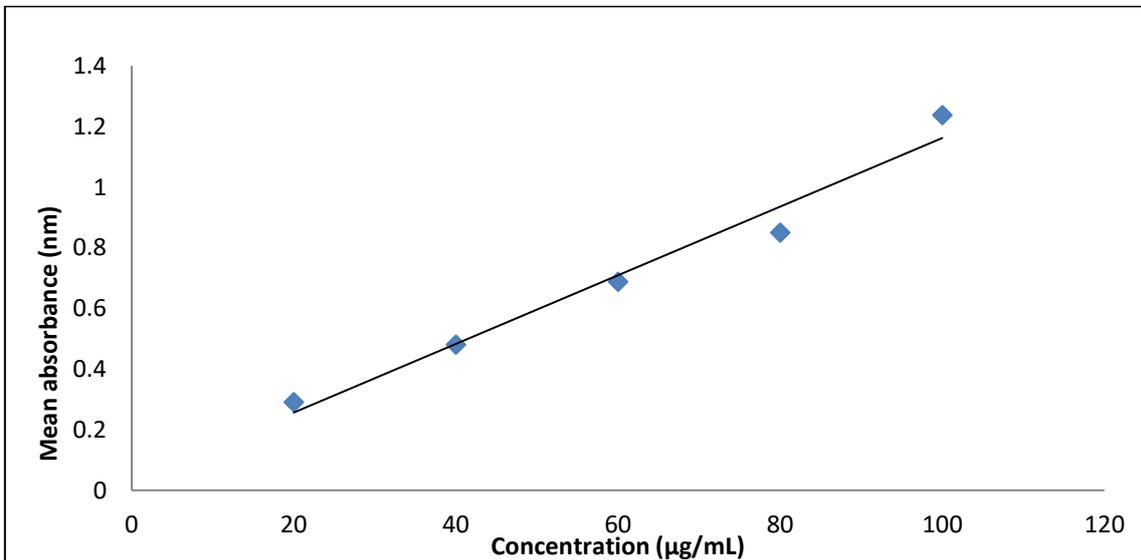


Figure 3: Gallic Acid Standard Calibration Curve for Total Phenolic content Assay.  $Y = 0.0072x + 0.1346$ ,  $X = (Y - 0.1346)/0.0072$ , where Y = Mean Absorbance of Samples and X = Total Phenolic Content

Table 3: The Absorbance of Gallic acid for Standard Calibration Curve for Total Phenolic Content of *Emilia sonchifolia* Leaves Extracts and Fractions

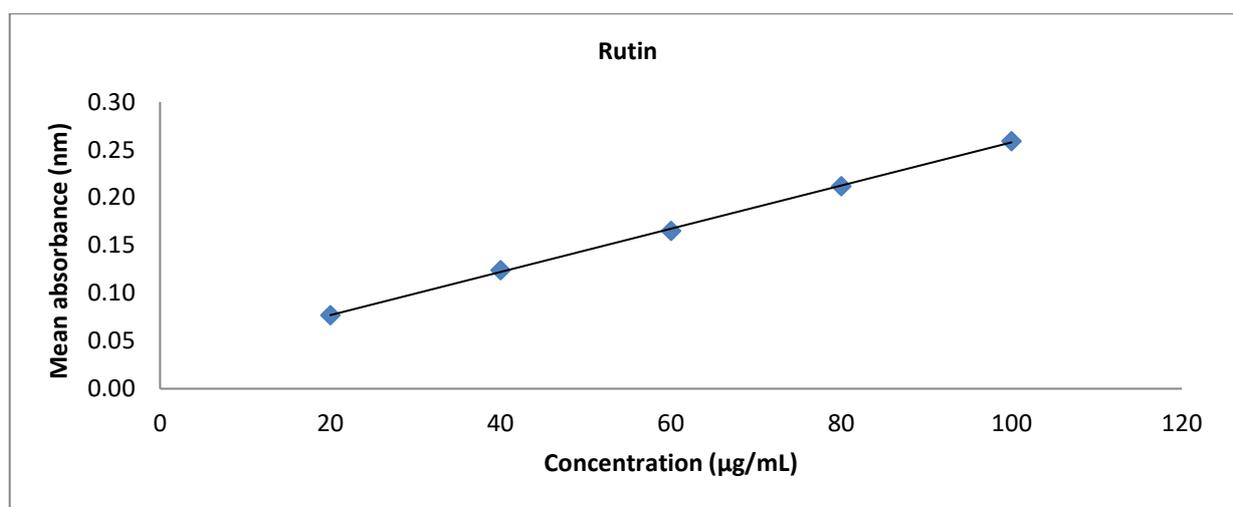
Conc. µg/mL	ABS1	ABS2	ABS3	Mean±SEM	Total phenolic content (x) MgRE/g
Extract	0.073	0.073	0.073	0.073±0.000	0.378
Hexane	0.067	0.067	0.067	0.067±0.001	0.325
Dichloromethane	0.118	0.118	0.118	0.118±0.001	0.776
Ethyl acetate	0.157	0.157	0.157	0.157±0.001	1.122
Butanol	0.075	0.075	0.075	0.075±0.001	0.396
Aqueous	0.041	0.041	0.041	0.041±0.000	0.095

Key: ABS = Absorbance

**Table 4:** Total Flavonoid Content from the Rutin Calibration Curve

Concentration ( $\mu\text{g/mL}$ )	Mean Absorbance $\pm$ SEM	Total flavonoid mg RE/g
Extract	0.032 $\pm$ 0.001	0.007
Hexane	0.080 $\pm$ 0.001	1.721
Dichloromethane	0.092 $\pm$ 0.000	2.150
Ethyl acetate	0.047 $\pm$ 0.000	0.542
Butanol	0.027 $\pm$ 0.001	-0.171
Aqueous	0.019 $\pm$ 0.001	-0.457

**KEY:** ABS = Absorbance SEM =Standard Error of Mean. Values were expressed as Mean  $\pm$  SEM



**Figure 4:** Rutin Standard Calibration Curve for Total Flavonoid.  $Y = 0.0021x + 0.0526$ ,  $X = (Y - 0.0526)/0.0021$ ; where Y = Mean Absorbance of Samples and X = Total Flavonoid Content

The physiological evaluation of the extract's impact on the lipid profile yielded significant results. While previous literature has often been inconclusive or suggested lipid-lowering potential (Dash et al., 2015; Ajen et al., 2022), this study observed a highly significant **increase** in Low-Density Lipoprotein (LDL) levels ( $p < 0.01$ ) across all treated groups. This elevation occurred despite the stability of Total Cholesterol (TC), Triglycerides (TG), and High-Density Lipoprotein (HDL).

This finding is of critical importance because LDL is the primary lipoprotein responsible for transporting cholesterol to peripheral tissues; its elevation is a major risk factor for cardiovascular complications. While Ajen et al. (2022) noted that *E. sonchifolia* could reduce LDL in models of cadmium-induced stress, our data suggests that in a standard physiological state, the extract

may adversely modulate lipid homeostasis. This could be due to an interference with the hepatic LDL-receptor activity or a modulation of the HMG-CoA reductase pathway that favors the accumulation of low-density lipoproteins. Consequently, while the potent antioxidant capacity validates the plant's ethnobotanical use for oxidative-related conditions, the significant elevation of LDL levels highlights a metabolic safety concern that necessitates caution in long-term or high-dose consumption.

## 5.0 Conclusion

The findings of this study provide empirical validation for the antioxidant potential of *Emilia sonchifolia*, supporting its traditional ethnobotanical applications. The leaf extract is rich in bioactive secondary metabolites specifically flavonoids, tannins, and saponins which correlate with the potent free-radical

scavenging and ferric-reducing activities observed in the Ethyl acetate and Dichloromethane fractions. These properties suggest that the plant may serve as a valuable natural source of antioxidants for mitigating oxidative stress.

However, the *in vivo* physiological evaluation reveals a critical metabolic oversight in traditional consumption. While the extract did not significantly alter Total Cholesterol, Triglycerides, or HDL levels, the highly significant increase in Low-Density Lipoprotein (LDL) levels ( $p < 0.01$ ) highlights a targeted adverse effect on lipid homeostasis. This finding suggests that *E. sonchifolia* may interfere with lipid metabolic pathways, posing a potential cardiovascular safety risk that has previously gone unrecorded.

This study was limited to a 14-day administration period in a healthy rat model; therefore, the long-term chronic effects of consumption remain unknown. Furthermore, while the physiological outcome is clear, the specific molecular mechanisms such as the extract's impact on HMG-CoA reductase activity or hepatic LDL-receptor expression were not evaluated in this initial study.

In conclusion, while *Emilia sonchifolia* is a potent antioxidant, the observed elevation of LDL necessitates caution in its medicinal and dietary use. Further research is urgently required to isolate the specific active components responsible for this lipid modulation and to conduct long-term cardiovascular safety trials to establish safe consumption thresholds for human health.

### Competing Interests

Authors have declared that no competing interests exist.

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