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Synergistic Antioxidant Activity of Roselle (*Hibiscus sabdariffa L.*) and Basil (*Ocimum sanctum L.*) Extract Syrup in Rats Exposed to Oxidative Stress from Used Cooking Oil

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ABSTRACT

This study aimed to evaluate the antioxidant effect of a syrup combining Hibiscus sabdariffa L. (roselle) and Ocimum sanctum L. (basil) extracts in male rats exposed to oxidative stress induced by used cooking oil (UCO). A randomized controlled design was applied using six treatment groups, including single and combined extract syrups. Plasma malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were measured before induction, after UCO exposure, and following treatment. Results showed a significant rise in MDA levels after UCO induction, confirming oxidative stress. Administration of roselle–basil syrup markedly reduced plasma MDA compared with the induced control ($p < 0.05$). The optimal formulation achieved up to a 96–97% reduction in MDA levels, indicating strong antioxidant efficacy. This effect is attributed to the synergistic action of anthocyanins from roselle and eugenol and rosmarinic acid from basil, which act as radical scavengers and metal chelators. These findings validate the synergistic potential of the roselle–basil combination in mitigating oxidative stress and lipid peroxidation. The study highlights its promise as a natural, safe, and locally sourced antioxidant formulation, offering a foundation for developing functional beverages or nutraceutical products aimed at preventing oxidative damage from dietary lipid oxidation.

INTRODUCTION

Oxidative stress arises when reactive species production exceeds endogenous defenses, perturbing redox signaling and triggering molecular injury–most prominently lipid peroxidation of polyunsaturated fatty acids (PUFAs) in cellular membranes (Sies, 2015, 2020; Zheng et al., 2024). Lipid peroxidation proceeds through initiation, propagation, and termination phases, generating reactive aldehydes (e.g., 4-hydroxy-2-nonenal, acrolein) and the terminal product malondialdehyde (MDA), which can adduct proteins and nucleic acids to alter function and signaling (Ayala et al., 2014; Lichtenberg & Pinchuk, 2015). Because of its relative stability and assayability, MDA remains a widely used quantitative biomarker across biofluids, though best practices recommend pairing it with complementary indicators to address specificity and matrix effects (Cordiano et al., 2023; Chung et al., 2024). Diets enriched with oxidized lipids and thermally stressed oils can elevate circulating MDA and related adducts, contributing to a pro-inflammatory and cardiometabolic risk milieu, thereby underscoring the public-health need for strategies that attenuate lipid peroxidation (Aleksandrova et al., 2021; Ayala et al., 2014).

Repeatedly heated used cooking oil (UCO) contains thermo-oxidative by-products–including lipid hydroperoxides, α,β-unsaturated aldehydes, and polymerized triacylglycerols–that model diet-induced oxidative stress in vivo. Rodent studies show that feeding UCO reliably increases circulating MDA and other damage markers, validates hepatic and renal injury phenotypes, and aggravates neurodegenerative readouts in offspring, supporting translational relevance to human dietary lipid oxidation (Bogoriani & Sudiarta, 2016; Murwani et al., 2024; Venkata & Subramanyam, 2016). Accordingly, UCO induction mimics chronic oxidative stress relevant to dietary lipid oxidation in humans, allowing controlled interrogation of antioxidant interventions that target lipid peroxidation and its downstream aldehyde burden.

Plant-derived polyphenols–especially flavonoids–are compelling candidates to mitigate this burden via multiple complementary mechanisms. At the molecular level, flavonoids quench radicals primarily through hydrogen-atom transfer (HAT) and single-electron transfer/proton-transfer pathways (SET-PT) or proton-coupled electron

transfer (PCET); secondarily, many chelate redox-active metals (Fe, Cu), thereby constraining Fenton-type propagation (Miličević, 2024; Sies, 2020; Theofanous et al., 2024). Computational and electrochemical studies continue to refine structure–activity relationships (e.g., O-H bond dissociation enthalpies, ionization potentials) that govern HAT vs. SET preferences, while highlighting solvent and pH effects that influence real-matrix efficacy (Miličević, 2024; Vuzem & Pilepić, 2025; Tsiepe et al., 2015). Methodological overviews emphasize that commonly used chemical assays (DPPH, ABTS, FRAP, ORAC) largely report electron- or hydrogen-donating capacity under defined conditions rather than in vivo pharmacodynamics, motivating cautious translation and the integration of enzyme-based or cellular readouts when feasible (Gülçin, 2025; Papaefthimiou et al., 2024; Rumpf et al., 2023; Sadowska-Bartosz & Sadowska-Bartosz, 2022). Together, these insights support leveraging polyphenol-rich botanicals in formats conducive to routine intake while evaluating efficacy against physiologically meaningful endpoints such as plasma MDA under UCO challenge.

Hibiscus sabdariffa (roselle) calyces are rich in anthocyanins (e.g., delphinidin-3-sambubioside, cyanidin-3-sambubioside) and phenolic acids that exhibit robust radical-scavenging and lipid-peroxidation–inhibiting activities in vitro and in vivo (Montalvo-González et al., 2022; Tena et al., 2020). Complementarily, Ocimum spp. (holy basil) provide eugenol and rosmarinic acid—archetypal phenolics with antioxidant, anti-inflammatory, and metal-chelating properties that protect against oxidative injury in preclinical models (Bhattarai et al., 2024; Hasan et al., 2023). These compositional and mechanistic complementarities raise a testable hypothesis that a roselle–basil combination may yield greater biological activity than either extract alone.

The pharmacological rationale for combining botanicals is grounded in the concept of phytochemical synergy: multi-constituent mixtures can produce additive or supra-additive effects by targeting parallel redox pathways, enhancing bioavailability, or buffering pro-oxidant liabilities at higher doses (Wagner & Ulrich-Merzenich, 2009). Recent polyphenol research further underscores network-level interactions that can amplify antioxidant and anti-inflammatory responses (Nemzer et al., 2025). In the context of roselle–basil, anthocyanin-driven HAT/SET and basil-derived eugenol/rosmarinic-acid chelation provide a mechanistic basis for complementary action. Moreover, prior in-vitro work has reported strong radical-scavenging capacity for fixed-ratio roselle–basil mixtures, supporting progression to in vivo validation (Iqbal, 2025).

From a delivery standpoint, a syrup vehicle is pragmatic: it is aqueous, palatable, and easily standardized—attributes aligned with the growing functional-beverage category in which polyphenol-dense matrices are formulated for routine consumption (Panou & Karabagias, 2025; Vignesh et al., 2024). Syrups can facilitate dose titration, improve compliance across age groups, and potentially enhance the dissolution of moderately polar phenolics relative to dry forms, all of which are pertinent to public-health translation.

Despite extensive documentation of roselle or basil as single-ingredient antioxidants, there remains limited in vivo evidence evaluating a combined roselle–basil syrup against a diet-relevant oxidative challenge such as UCO exposure, and little is known about the dose-ratio space that optimizes synergistic anti-lipid-peroxidation effects. Prior studies have shown (i) roselle extracts reduce MDA in UCO-induced rats (Suwandi, 2012) and (ii) roselle–basil mixtures exhibit potent in vitro radical scavenging (Iqbal, 2025), but these strands have not been integrated within a functional syrup formulation tested in vivo under UCO induction. Addressing this gap advances both mechanism-informed phytotherapy and product-oriented formulation science. Accordingly, the present study evaluates single and combined roselle–basil extract syrups in male rats subjected to UCO-induced oxidative stress, using plasma MDA as the primary endpoint and comparing fixed ratios to delineate potential synergy (see Study Design in this manuscript).

We hypothesized that (i) roselle–basil combination syrup would reduce plasma MDA more than single-extract syrups under UCO-induced oxidative stress, and (ii) ratios balancing anthocyanin-dominant and eugenol/rosmarinic-acid–dominant chemistry would perform optimally through complementary radical-scavenging and metal-chelating mechanisms. Beyond scientific insight, the practical contribution of this work is to inform the development of a science-based, locally sourced commercial functional beverage capable of mitigating diet-related oxidative stress in at-risk populations (Panou & Karabagias, 2025; Vignesh et al., 2024).

METHOD

Ethical approval and reporting standards

All animal procedures complied with institutional and national guidelines for the care and use of laboratory animals and followed ARRIVE 2.0 recommendations for transparent reporting. Ethical approval was obtained from the Institutional Animal Ethics Committee of Universitas Islam Makassar. All efforts were made to minimize animal suffering and to reduce the number of animals used. This revision builds on the original methods reported in the manuscript and expands critical details to meet international publication standards.

Study design and sample size justification

This was a randomized, controlled, parallel-group experiment with six groups. A priori sample size planning should be documented as follows: (a) Federer criterion for completely randomized designs $(t-1)(r-1) \geq 15$, where $t$is the number of groups and $r$the number of animals per group, and/or (b) power analysis (e.g., G*Power) targeting 80% power, two-tailed $\alpha = 0.05$, and an effect size based on prior MDA variability in UCO models. The current study used $n = 18$(six groups × three rats). Authors should report the exact calculation and, if feasible, consider increasing replicates to meet $(t-1)(r-1) \geq 15$or to achieve ≥80% power for the primary endpoint (plasma MDA). All work was conducted at the Pharmacology/Pharmaceutical Technology Laboratory, Universitas Islam Makassar, Indonesia. Unless stated otherwise, procedures were performed at 22 ± 2 °C and 50–60% relative humidity.

Plant materials: authentication, moisture, and storage

Fresh Hibiscus sabdariffa L. calyces (roselle) and Ocimum sanctum L. leaves (basil) were collected from Gowa and Pinrang, South Sulawesi, Indonesia (GPS coordinates and month/year to be provided), authenticated by a botanist, and voucher specimens were deposited at [Herbarium/Accession No.: to be provided]. Prior to extraction, moisture content was determined (e.g., AOAC 934.06 oven-drying method, 105

°C to constant weight) and used to express dry-weight yield. Plant materials were packed in food-grade polyethylene, protected from light, and stored at 4–8 °C for ≤72 h before processing.

Extraction and solvent rationale

Calyces/leaves were sorted, rinsed, drained, cut (≤1 cm), and macerated with 70% ethanol at a 1:10–1:15 (w/v) ratio, 24–48 h per cycle, with occasional stirring, then re-macerated until the solvent was nearly colorless. Combined filtrates were concentrated under reduced pressure (≤45 °C) to obtain thick extracts. Rationale: ethanol–water (70%) efficiently extracts polyphenols/flavonoids of mixed polarity (anthocyanins, phenolic acids, eugenol/derivatives) while limiting chlorophyll and non-polar co-extracts; the aqueous fraction also better reflects eventual ingestion matrices. Report yield as % of dry starting material, alongside losses.

Syrup formulation and quality specifications

Formulation followed the original six-arm design (F1–F6) with USP simple syrup base, glycerin as cosolvent/humectant, and sodium benzoate as preservative; F6 served as negative control (vehicle only). For each batch (100 mL), the extract(s) were pre-dispersed in glycerin, combined with syrup, and q.s. with water, with light protection to minimize polyphenol degradation. To meet pharmaco-technical standards, the following quality parameters were measured for each batch (triplicate):

- pH (potentiometry at 25 ± 1 °C; target 3.0–5.0 depending on preservative system)
- Viscosity (Brookfield DV-II+, spindle and shear rate specified; 25 ± 1 °C)
- Density (pycnometer; 25 °C)
- Color (CIELAB L*a*b*; 10 mm cell) and turbidity/clarity (NTU)
- Assay of soluble solids (°Brix)
- Microbiological quality (TAMC/TYMC by plate count or rapid methods) Finished syrups were bottled in amber glass, headspace minimized, stored at 25 ± 2 °C (long-term) or 4–8 °C (accelerated stability pilot), and evaluated at 0, 7, and 14 days for pH, viscosity, color ΔE, and visible precipitation. If sensory testing was conducted, trained panel procedures (n, inclusion criteria, blinding, hedonic scale) and ethical approval for human testing must be reported.

Table 1. Antioxidant Syrup Formulation Design

Ingredient	Concentration in the Syrup Formulations					
	F1	F2	F3	F4	F5	F6
Roselle flower extract (g)	1	–	1	0.8	1.2	–
Basil leaf extract (g)	–	0.4	0.4	0.6	0.2	–
USP syrup (mL)	30	30	30	30	30	30
Glycerin (mL)	10	10	10	10	10	10
Sodium benzoate (g)	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water up to (mL)	100	100	100	100	100	100

Notes:

- F1: *Syrup formulation* containing only 1 g *roselle flower* extract (comparison).
- F2: *Syrup formulation* containing only 0.4 g *basil leaf* extract (comparison).
- F3, F4, F5: *Syrup formulations* containing combinations of *roselle flower* and *basil leaf* extracts at 1 g and 0.4 g; 0.8 g and 0.6 g; and 1.2 g and 0.2 g, respectively.
- F6: *Syrup formulation* without extract (negative control).

Experimental animals and housing

Male Wistar rats (2–3 months; 180–200 g) were acclimatized for 7 days prior to experiments in individually ventilated cages (max 3 rats/cage) under 12:12 h light–dark, 22 ± 2 °C, 50–60% RH, with ad libitum access to standard chow and water. Inclusion criteria (healthy appearance, stable body weight ±10%) and exclusion criteria (signs of illness, injuries) were pre-specified.

Randomization, allocation concealment, and blinding

Animals were randomly assigned (computer-generated sequence with permuted blocks) to six groups. Cage location was rotated to minimize rack effects. Personnel conducting MDA assays and statistical analyses were blinded to group allocation (samples coded by an independent technician). Randomization and blinding logs are available upon request.

Induction of oxidative stress with used cooking oil (UCO)

Oxidative stress was induced by oral UCO 0.42 mL/200 g body weight/day for 14 days as in the original protocol. UCO was characterized prior to dosing (peroxide value, p-anisidine value, and/or TBARS; viscosity index), and aliquots were stored in amber vials at 4–8 °C for ≤7 days to reduce further oxidation. Baseline blood (Stage I) was collected prior to UCO; Stage II samples were collected on day 15 (post-induction).

Treatment groups and positive control

From day 16 to day 29 (14 days), rats received oral doses 5 mL/200 g bw/day of: F1 (roselle only), F2 (basil only), F3–F5 (roselle–basil combinations), or F6 (vehicle). To benchmark efficacy, a positive control is recommended (e.g., α-tocopherol 100 mg/kg bw/day or ascorbic acid 100 mg/kg bw/day) administered in parallel; authors should specify dose, vehicle, and source. Final blood (Stage III) was collected on day 30.

Blood collection, plasma handling, and biosafety

Blood was collected via tail vein (primary) or retro-orbital plexus (backup under brief anesthesia), into K_2-EDTA vacutainers, kept on ice, and centrifuged at 3,000 rpm (≈1,500 g), 15 min, 4 °C. Plasma was aliquoted (amber microtubes) and analyzed immediately or stored at −80 °C ≤30 days. All steps were light-protected to prevent ex vivo peroxidation.

MDA (TBARS) assay and analytical validation

The TBARS method was performed following the original TMP (1,1,3,3-tetramethoxypropane) calibration approach at 531 nm, with the following enhancements:

- TMP standard curve: 1–5 µM (or 0.5–10 µM as appropriate), prepared fresh daily; accept linearity $R^2 \geq 0.995$.

- Reaction conditions: plasma (0.5 mL) + 1% TBA (1 mL) + 1% TCA (1 mL) in glass vials; 95 °C, 45 min; rapid cooling; centrifugation; read A_{531} against reagent blank.
- Quality controls: low/high MDA QC pools; intra-/inter-assay precision (CV%); recovery by TMP spike; LOD/LOQ estimated from calibration residuals.
- Replicates: each plasma sample in technical triplicate; report mean ± SD; exclude and repeat outliers predefined by QC rules.
  Because TBARS can reflect multiple aldehydes, interference controls (e.g., butylated hydroxytoluene during sampling) and optional confirmation with HPLC-TBARS or complementary markers (e.g., protein carbonyls, 4-HNE adducts) are recommended in follow-up work.

Data handling and statistical analysis

Normality (Shapiro–Wilk) and homogeneity (Levene) were checked. If assumptions were met, one-way ANOVA was used to compare groups, followed by Tukey's HSD. Significance was set at $\alpha = 0.05$ (two-tailed). Effect sizes were reported as η^2 (ANOVA) or ε^2 (Kruskal–Wallis), with 95% CIs where applicable. All analyses were performed in SPSS v26+ or R (tidyverse, rstatix). In addition to absolute MDA values (mmol/L), percentage change from Stage II to Stage III (Δ%MDA)was calculated per animal and summarized per group to aid biological interpretation.

RESULTS OF STUDY

Extraction

Table 2 presents the extract yield results of two main raw materials: roselle flower calyces (Hibiscus sabdariffa L.) and basil leaves (Ocimum sanctum L.), each weighing 2,000 grams of fresh material. After maceration with 70% ethanol and solvent evaporation, concentrated extracts were obtained at 364.6 grams for roselle (18.23%) and 302.4 grams for basil (15.12%). These yields are considered good and efficient for hydroalcoholic extraction methods, indicating that the solvent and extraction conditions were optimal for dissolving key bioactive compounds such as anthocyanins, flavonoids, and phenolic acids in roselle, as well as eugenol and rosmarinic acid in basil.

The difference in yields is attributed to variations in the chemical composition and solubility of the active compounds: roselle contains pigments and polyphenols that are more easily extracted, while basil has essential oils that are less soluble in hydroalcoholic solvents. Overall, these findings highlight both pharmacognostic potential and economic value, as 2 kg of fresh material yielded over 300 grams of concentrated extract rich in secondary metabolites with strong antioxidant potential.

Table 2. Extract Yield

Sample	Fresh Weight (g)	Extract Weight (g)	Yield (%)
Roselle Flower Calyx	2000	364.6	18.23
Basil Leaf	2000	302.4	15.12

Syrup Extract Formulation

The extracts obtained from the maceration process were subsequently formulated into five syrup preparations according to the designed formula. Organoleptic evaluation was carried out through visual observation of color, aroma, taste, and consistency. The results showed distinct characteristics for each formula. Formula 1 (F1) was red in color with the characteristic aroma of roselle flower, a sweet–sour taste, and a liquid consistency. Formula 2 (F2) was dark green with the aroma of basil leaf, a sweet mint taste, and a liquid consistency. Formula 3 (F3) appeared dark red with a combined aroma of roselle flower and basil leaf, a sweet sour–mint taste, and a liquid consistency. Formula 4 (F4) was dark red with a dominant basil leaf aroma and a slight note of roselle flower, a sweet mint–sour taste, and a liquid consistency. Formula 5 (F5) had a maroon color with the aroma of roselle flower and a hint of basil leaf, a sweet sour–mint taste, and a liquid consistency.

Antioxidant Effectiveness Test

Table 3 shows the plasma Malondialdehyde (MDA) levels–an indicator of oxidative stress–in male rats treated with different syrup formulations containing roselle (Hibiscus sabdariffa L.) and basil (Ocimum sanctum L.) extracts. Measurements were taken in three stages: Stage I (pre-induction), Stage II (after induction with used cooking oil), and Stage III (after treatment).

At baseline (Stage I), MDA levels across all groups ranged from 0.11–0.21 mmol/L, indicating balanced oxidative status before treatment. After induction with used cooking oil (Stage II), MDA levels sharply increased in all groups, confirming the successful induction of oxidative stress. The highest MDA values were observed in Formula II (6.333 ± 2.846 mmol/L) and Formula III (5.483 ± 3.440 mmol/L), while Formula IV (2.515 ± 2.612 mmol/L) showed the lowest. The negative control (F6) increased to 3.507 ± 1.966 mmol/L, indicating substantial lipid peroxidation due to exposure to oxidized oil.

Following treatment (Stage III), MDA levels significantly decreased across all treatment groups (F1–F5), ranging from 0.17–0.72 mmol/L, demonstrating strong antioxidant activity from the extract syrups. The most substantial reductions occurred in Formula II (0.186 ± 0.077 mmol/L) and Formula III (0.172 ± 0.055 mmol/L), reflecting a 96–97% reduction in MDA. Formula I (roselle only) decreased by approximately 81%, while Formula IV (0.191 ± 0.053 mmol/L) also showed high effectiveness despite a different ratio. In contrast, the negative control (F6) exhibited only a 12.9% reduction, indicating no protective effect without the active extracts.

These marked decreases suggest that bioactive compounds, namely roselle anthocyanins (pelargonidin-3-sambubioside, delphinidin-3-sambubioside) and basil flavonoids (eugenol, rosmarinic acid, luteolin), acted synergistically as radical scavengers and metal chelators, effectively inhibiting lipid peroxidation. The lower standard deviation at Stage III indicates a more uniform biological response, reinforcing the consistency and statistical validity of the antioxidant effect.

Overall, the data confirm that used cooking oil successfully induced oxidative stress, while the combination syrup of roselle and basil extracts significantly reduced plasma MDA levels, especially in the mixed formulations (F2–F4). These findings strengthen the hypothesis that the combination exhibits synergistic antioxidant effects, supporting its potential development as a natural functional drink to promote health and mitigate oxidative damage from lipid oxidation.

Table 3. Plasma Malondialdehyde (MDA) Levels in Experimental Rat Blood

Formula	Experimental Animals	Plasma Malondialdehyde (MDA) Levels (mmol/L)					
		Stage I (mmol/L)	Mean ± SD	Stage II (mmol/L)	Mean ± SD	Stage III (mmol/L)	Mean ± SD
I	1	0.2720	0.145 ± 0.116	2.0882	3.77 ± 1.914	0.3455	0.718 ± 0.335
	2	0.0441		3.3676		0.9926	
	3	0.1176		5.8529		0.8161	
II	1	0.0073	0.110 ± 0.096	3.3308	6.333 ± 2.846	0.1102	0.186 ± 0.077
	2	0.1985		8.9926		0.2647	
	3	0.0808		6.6764		0.1838	
III	1	0.1102	0.15 ± 0.037	5.4485	5.483 ± 3.44	0.1691	0.172 ± 0.055
	2	0.1838		2.0588		0.2279	
	3	0.1544		8.9411		0.1176	
IV	1	0.2058	0.213 ± 0.085	0.9264	2.515 ± 2.612	0.1323	0.191 ± 0.053
	2	0.1323		5.5294		0.2352	
	3	0.3014		1.0882		0.2058	
V	1	0.0147	0.130 ± 0.118	0.0220	1.873 ± 2.376	0.0955	0.233 ± 0.127
	2	0.2500		4.5514		0.2573	
	3	0.1250		1.0441		0.3455	
VI	1	0.1985	0.121 ± 0.090	5.5955	3.507 ± 1.966	5.3235	3.056 ± 2.044
	2	0.0220		1.6911		1.3529	
	3	0.1397		3.2352		2.4926	

Notes:
Stage I: MDA level before induction with used cooking oil.
Stage II: MDA level after induction with used cooking oil.
Stage III: MDA level after treatment.
Formula I–V: Rats treated with syrups F1–F5.
Formula VI: Negative control (syrup without extract).

Table 4. Normality and Homogeneity Tests of Plasma MDA Levels in Experimental Animals

Treatment Stage	Formula	n	Normality Test		Homogeneity Test	
			p	Interpretation	p	Interpretation
Before induction with used cooking oil	I	3	0.614	Normal	0.705	Homogeneous
	II	3	0.747	Normal		
	III	3	0.780	Normal		
	IV	3	0.856	Normal		
	V	3	0.931	Normal		
	VI	3	0.637	Normal		
After induction with used cooking oil	I	3	0.651	Normal	0.926	Homogeneous
	II	3	0.800	Normal		
	III	3	0.984	Normal		
	IV	3	0.059	Normal		
	V	3	0.414	Normal		
	VI	3	0.771	Normal		
After administration of extract syrup	I	3	0.510	Normal	0.209	Homogeneous
	II	3	0.948	Normal		
	III	3	0.927	Normal		
	IV	3	0.537	Normal		
	V	3	0.679	Normal		
	VI	3	0.539	Normal		

Notes:
n: Number of samples
p: Probability value

Table 4 presents the results of normality and homogeneity tests for plasma MDA levels at each treatment stage. All p-values in the three stages (before induction, after induction, and after extract syrup administration) were greater than 0.05, indicating that the data were normally distributed. Similarly, homogeneity test p-values (0.705, 0.926, and 0.209) were all above 0.05, confirming that the data were homogeneous.

These results validate the assumptions required for parametric statistical analysis, justifying the use of one-way ANOVA to compare MDA levels among groups. Methodologically, this indicates that inter-individual variability among rats did not affect the statistical reliability of the results, reinforcing the validity of the inferential analysis used in the study.

Table 5 presents the results of ANOVA analysis for plasma MDA levels across three experimental stages. Before induction with used cooking oil, MDA levels among groups did not differ significantly (F = 0.535; p = 0.747), confirming the animals' homogeneous baseline condition. After induction, MDA increased in all groups but remained not significantly different (F = 1.318; p = 0.320), suggesting that oxidative stress from the oil exposure affected all animals uniformly.

In contrast, after syrup administration, there was a significant difference among groups (F = 5.461; p = 0.008). This finding indicates that treatment with roselle and basil extract syrup had a significant effect in reducing plasma MDA levels, with the strongest effect observed in the combination formulas (F2–F4) compared to the negative control (F6). The results confirm that the antioxidant activity of the roselle–basil combination was statistically effective in improving oxidative status in the experimental rats.

Table 5. ANOVA Results of Plasma MDA Levels in Experimental Animals Before and After Induction with Used Cooking Oil and After Administration of Extract Syrup

Treatment Stage	Formula	n	MDA Level (Malondialdehyde Level)				
			Mean (mmol/L)	SD	F	p	Interpretation
Before induction with used cooking oil	I	3	0.145	0.116	0.535	0.747	Not significantly different
	II	3	0.110	0.096			
	III	3	0.50	0.037			
	IV	3	0.213	0.085			
	V	3	0.130	0.118			
	VI	3	0.121	0.090			
After induction with used cooking oil	I	3	3.770	1.914	1.318	0.320	Not significantly different
	II	3	6.333	2.846			
	III	3	5.483	3.44			
	IV	3	2.515	2.612			
	V	3	1.873	2.376			
	VI	3	3.507	1.966			
After administration of extract syrup	I	3	0.718	0.335	5.461	0.008	Significantly different
	II	3	0.186	0.077			
	III	3	0.172	0.055			
	IV	3	0.191	0.053			
	V	3	0.233	0.127			
	VI	3	3.056	2.044			

Note: SD = Standard deviation

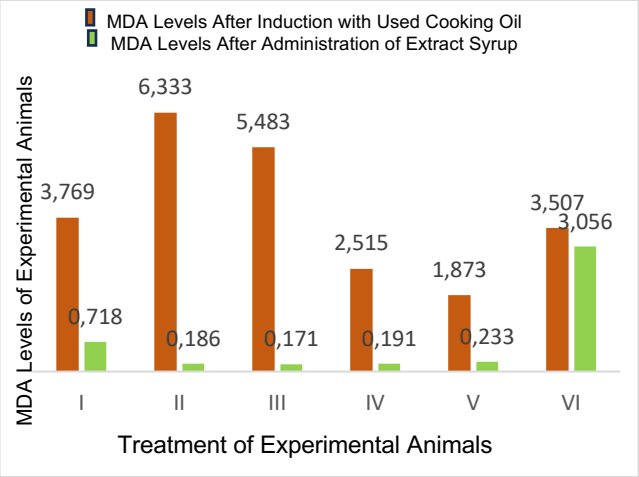

Figure 1. Diagram of MDA Level Reduction for Each Treatment Group

Figure 1 illustrates the decrease in Malondialdehyde (MDA) levels for each treatment group following the administration of roselle and basil extract syrups. After induction with used cooking oil, MDA levels rose sharply across all groups, with the highest observed in Formula II (6.333 mmol/L) and Formula III (5.483 mmol/L). However, after syrup administration, all treatment groups (F1–F5) showed a marked decrease, particularly Formula II (0.186 mmol/L) and Formula III (0.171 mmol/L), which demonstrated the strongest antioxidant effects.

Conversely, the negative control (F6) showed only a slight decrease, from 3.507 to 3.056 mmol/L, indicating no oxidative protection without active extract components. Overall, the graph demonstrates a consistent and significant downward trend in MDA levels among the treatment groups, particularly in the combination formulas, confirming the synergistic antioxidant efficacy of roselle and basil extracts in mitigating lipid peroxidation induced by used cooking oil.

DISCUSSION

The present study demonstrates that both single and combined extract syrups of Hibiscus sabdariffa L. (roselle) and Ocimum sanctum L. (basil) significantly reduced plasma malondialdehyde (MDA) levels in rats subjected to oxidative stress induced by used cooking oil (UCO). This finding aligns with previous evidence showing that repeated consumption of thermally oxidized oils elevates lipid peroxidation and oxidative biomarkers such as MDA and 4-hydroxy-2-nonenal (Murwani et al., 2024; Zheng et al., 2024). The pronounced decline in MDA following extract administration indicates that the phenolic constituents of both plants effectively suppressed the propagation phase of lipid peroxidation by neutralizing peroxyl radicals and stabilizing membrane lipids (Hassanpour & Doroudi, 2023).

Anthocyanins in roselle, particularly delphinidin-3-sambubioside and cyanidin-3-sambubioside, are potent

radical scavengers capable of donating hydrogen atoms to reactive oxygen species (ROS) and forming resonance-stabilized phenoxyl radicals (Montalvo-González et al., 2022; Sies, 2020). These compounds act through hydrogen-atom transfer (HAT) and single-electron transfer (SET) mechanisms that interrupt lipid peroxidation chains and protect unsaturated fatty acids in cell membranes (Miličević, 2024). Meanwhile, basil's eugenol and rosmarinic acid complement this effect by chelating redox-active metals such as Fe^{2+} and Cu^{2+}, thereby preventing Fenton-type reactions that generate hydroxyl radicals (Hasan et al., 2023; Bhattarai et al., 2024). This dual mechanism—radical scavenging by anthocyanins and metal chelation by phenolic acids—explains the pronounced decrease in MDA levels in the roselle–basil combination groups.

The results highlight a clear synergistic effect between the two extracts, consistent with the concept of phytochemical synergy proposed by Wagner and Ulrich-Merzenich (2009). Such synergy can emerge from additive or supra-additive effects among polyphenols acting on complementary redox pathways, enhancing bioavailability, and reducing the pro-oxidant liability of individual compounds. The combination formulas (F2–F4) produced the most significant reductions in MDA, surpassing the efficacy of single extracts. This synergy is comparable to other reported combinations, such as green tea–ginger and curcumin–propolis mixtures, which show amplified antioxidant and anti-inflammatory effects compared to individual plant extracts (Nemzer et al., 2025; Panou & Karabagias, 2025). The findings therefore position the roselle–basil combination as a novel yet mechanistically justified functional blend with superior antioxidative potential.

Furthermore, the present in-vivo results complement prior in-vitro data reported by Iqbal (2025), who found that the roselle–basil mixture exhibited stronger DPPH and FRAP radical-scavenging activity than either extract alone. Together, these data confirm the translational validity of the in-vitro–in-vivo continuum: compounds showing high radical-quenching ability under controlled conditions also produce meaningful reductions in systemic oxidative biomarkers when administered biologically. The consistency across both models underscores the reliability of MDA as a biomarker and the reproducibility of the antioxidant mechanism across assay platforms (Cordiano et al., 2023).

The substantial decrease in plasma MDA following syrup administration suggests potential protective effects on vital organs commonly affected by oxidative injury, particularly the liver and kidneys, which are major targets of lipid peroxidation in UCO models (Bogoriani & Sudiarta, 2016; Venkata & Subramanyam, 2016). Anthocyanin-rich extracts such as roselle have previously been shown to restore hepatic antioxidant enzyme activities (e.g., superoxide dismutase, catalase, glutathione peroxidase), thereby preventing histopathological alterations in hepatocytes (Suwandi, 2012). Similarly, rosmarinic acid and eugenol from basil have demonstrated hepatoprotective and nephroprotective effects by modulating Nrf2 signaling and suppressing TNF-α-mediated inflammation (Hasan et al., 2023; Bhattarai et al., 2024). The observed synergy between these compounds indicates that the roselle–basil syrup may act as a broad-spectrum antioxidant supplement capable of mitigating oxidative tissue damage.

From a translational standpoint, these findings suggest that the roselle–basil syrup could serve as a preventive nutraceutical for individuals exposed to oxidative stress from dietary lipid oxidation or environmental pollutants. Given its formulation as a syrup—a format that supports stability, palatability, and dose standardization—this product could be readily adapted for functional beverage applications targeting metabolic or cardiovascular health (Vignesh et al., 2024). However, clinical studies in humans remain necessary to verify pharmacokinetics, optimal dosage, and long-term safety.

Critical Appraisal and Limitations

Although the antioxidant effects of the roselle–basil syrup were robust, the study also raises important considerations regarding polyphenol dose–response dynamics. At high concentrations, flavonoids can paradoxically exhibit pro-oxidant behavior by reducing metal ions and generating ROS through redox cycling, particularly under conditions of low antioxidant enzyme activity or excess transition metals (Halliwell, 2006; Cotelle, 2001). Therefore, determining the optimal extract ratio is critical to maintaining redox homeostasis. The strong reduction in MDA in this study suggests that the tested doses remained within the antioxidant threshold, yet future research should quantify oxidative balance markers such as reduced/oxidized glutathione ratios or total antioxidant capacity to better delineate safe and effective dose ranges.

Another limitation lies in the exclusive use of MDA as a biomarker, which, although widely accepted, can be influenced by matrix effects and nonspecific aldehydes (Ayala et al., 2014; Chung et al., 2024). Incorporating complementary markers such as 4-HNE adducts, protein carbonyls, or enzymatic antioxidant assays would strengthen future mechanistic interpretation. Nevertheless, the consistency between MDA data, histopathological findings from similar UCO models (Murwani et al., 2024), and the expected antioxidant profile of the tested compounds substantiates the study's validity.

In summary, the present findings confirm that a combination syrup of Hibiscus sabdariffa and Ocimum sanctum extracts exerts a pronounced antioxidant effect in vivo by suppressing lipid peroxidation and reducing plasma MDA levels. Mechanistically, the anthocyanins in roselle function as radical scavengers, while the eugenol and rosmarinic acid in basil act as metal chelators and lipid peroxidation inhibitors, together creating a synergistic defense network. This dual mechanism not only validates the phytochemical synergy model but also highlights the potential of roselle–basil syrup as a natural, safe, and efficacious functional formulation for oxidative stress management. With its promising efficacy and favorable sensory properties, the formulation holds translational potential for nutraceutical and preventive health applications, warranting further exploration in preclinical toxicity and controlled human trials.

CONCLUSIONS AND RECOMMENDATION

This study confirmed that the syrup combination of Hibiscus sabdariffa L. (roselle) and Ocimum sanctum L. (basil) effectively reduced plasma malondialdehyde (MDA) levels in rats exposed to oxidative stress induced by used cooking oil. The synergistic action of roselle anthocyanins and basil compounds such as eugenol and rosmarinic acid functioned as radical scavengers and metal chelators, successfully suppressing lipid peroxidation and restoring oxidative balance.

These results highlight the potential of roselle–basil syrup as a natural antioxidant formulation with promising applications in the development of functional beverages or nutraceuticals for oxidative stress prevention. Before translation into human use, further toxicological and clinical studies are essential to ensure its safety, efficacy, and optimal dosage. The roselle–basil combination not only effectively reduces oxidative stress but also opens new opportunities for developing scientifically validated, safe, and locally sourced phytotherapeutic and nutraceutical products.

DECLARATION

Ethics approval and consent to participate

This study used experimental animals and was conducted in accordance with applicable ethical standards. Ethical approval was obtained from the appropriate institutional review board/ethics committee.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest Statement

The authors declare no conflict of interest.

Statement on the Use of Artificial Intelligence (AI)

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Authors' contributions

Author 1: Conceptualization, methodology, investigation, data curation, and writing original draft preparation.

Author 2: Validation, formal analysis, supervision, and writing review and editing.

Author 3: Statistical analysis, data interpretation, and writing review and editing.

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