

Potential Combination of *Annona squamosa* Leaves and *Moringa oleifera* Leaves as Pancreatic Lipase Inhibitor

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Abstract: Obesity is a metabolic disorder characterized by a body mass index (BMI) >30 and is associated with an increased risk of degenerative diseases such as diabetes mellitus type 2, stroke, and cardiovascular disease. Anti-obesity drugs such as orlistat are effective; however, they may cause side effects, highlighting the need for natural alternatives. This study aimed to evaluate the effect of a combination of 70% ethanol extracts of srikaya leaves (*Annona squamosa* L.) and kelor leaves (*Moringa oleifera* Lam) based on phytochemical content, antioxidant capacity, and pancreatic lipase inhibitory activity. Extraction was carried out using a sonication method, followed by formulation of extract combinations and evaluation of total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity using DPPH and FRAP methods, and lipase inhibition. The results showed that the single extract of *A. squamosa* leaves exhibited the highest activity, with TPC of 95.15 mg GAE/g, TFC of 47.57 mg QE/g, DPPH of 395.10 mg TE/g, FRAP of 476.30 mg TE/g, and lipase inhibition of 64.15%. The extract combination showed synergistic effects on DPPH activity and pancreatic lipase inhibition, but tended to be antagonistic for TPC, TFC, and FRAP.

Keywords: Anti-obesity, *Annona squamosa*, *Moringa oleifera*, Pancreatic Lipase

Abstrak: Obesitas merupakan kelainan metabolik dengan indeks massa tubuh (IMT) >30 yang meningkatkan risiko penyakit degeneratif seperti diabetes melitus tipe 2, stroke dan penyakit jantung. Obat antiobesitas seperti orlistat efektif, namun berpotensi menimbulkan efek samping sehingga diperlukan alternatif dari bahan alami. Penelitian ini bertujuan menentukan efek kombinasi ekstrak etanol 70% daun srikaya (*Annona squamosa* L) dan daun kelor (*Moringa oleifera* Lam) berdasarkan kadar fitokimia, kapasitas antioksidan, serta aktivitas inhibisi lipase pankreas. Ekstraksi dilakukan menggunakan metode sonikasi, diikuti formulasi kombinasi dan pengujian kadar fenolik total (TPC), flavonoid total (TFC), kapasitas antioksidan metode DPPH dan FRAP, serta inhibisi lipase pankreas. Hasil menunjukkan ekstrak tunggal daun srikaya memiliki aktivitas tertinggi, dengan TPC 95,15 mg GAE/g, TFC 47,57 mg QE/g, DPPH 395,10 mg TE/g, FRAP 476,30 mg TE/g, serta inhibisi lipase 64,15%. Kombinasi ekstrak menunjukkan efek sinergis pada aktivitas DPPH dan inhibisi lipase pankreas, namun cenderung antagonis pada TPC, TFC, dan FRAP.

Kata kunci: Anti-obesitas, *Annona squamosa*, *Moringa oleifera*, Lipase Pankreas

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1. Introduction

Obesity is a condition characterized by excessive fat accumulation that increases health risks and reduces life expectancy. According to the World Health Organization, more than 890 million adults worldwide were classified as obese in 2022, while the prevalence of obesity in Indonesia reached 23.4% in 2023 (World Health Organization, 2025; Kemenkes, 2023). Obesity is strongly associated with metabolic disorders, including diabetes mellitus, hypertension, and cardiovascular diseases (Hossain *et al.*, 2016). Excessive fat accumulation also increases the production of reactive oxygen species (ROS) (Hidayati and Hardani, 2019), leading to oxidative stress and decreased antioxidant enzyme activity, which further contributes to metabolic complications (Zulfahmidah *et al.*, 2021).

One of the main therapeutic targets in obesity management is pancreatic lipase, an enzyme responsible for lipid hydrolysis and fat absorption in the body (Olivecrona, 2016).

Inhibition of pancreatic lipase can reduce dietary fat absorption, making it an important strategy for anti-obesity treatment. Orlistat is a widely used pancreatic lipase inhibitor approved by the Food and Drug Administration (Khushboo *et al.*, 2020). However, its long-term use is associated with several side effects, including steatorrhea, diarrhea, abdominal pain, kidney injury, and pancreatitis (Bansal and Khalili, 2022). Therefore, the exploration of plant-based anti-obesity agents has gained increasing attention.

Annona squamosa L. and *Moringa oleifera* Lam. are medicinal plants reported to possess anti-obesity potential. Previous studies demonstrated that *A. squamosa* exhibits anti-obesity activity through in silico, in vitro, and in vivo approaches (Singh and Pattnaik, 2023). Meanwhile, *M. oleifera* contains bioactive compounds such as flavonoids, phenolics, tannins, alkaloids, and saponins, which may inhibit pancreatic lipase activity and improve metabolic disorders related to obesity (Rahmayanti *et al.*, 2021; Haningtyas *et al.*, 2022). Furthermore, combining plant extracts may produce synergistic effects, resulting in greater biological activity compared to individual extracts alone (Uduwana *et al.*, 2023). Therefore, this study aims to evaluate the potential synergistic anti-obesity activity of combined *A. squamosa* and *M. oleifera* extracts through pancreatic lipase inhibition and antioxidant-related mechanisms.

2. Materials and Methods

The instruments used in this study included standard laboratory glassware, spatulas, micropipettes (NICHIRYO Le, Japan), vial bottles, filter paper, 60-mesh sieve, funnels, volumetric flasks, stirring rods, nanospectrophotometer (SPECTROstarNano, BMG-LABTECH, Germany), 96-well microplates, sonicator (Decon F5 Major, Decon Laboratories, United States), oven, desiccator, blender, rotary vacuum evaporator (LabTech Ltd., United States), analytical balance, micropipette tips of various sizes, and statistical software including IBM SPSS Statistics 25 and GraphPad Prism 8.

The plant materials used in this study were leaves of *Annona squamosa* and *Moringa oleifera* obtained from Tropical Biopharmaca Research Center, IPB University, Bogor, West Java, Indonesia. The chemicals and reagents used included distilled water (distilled water), 70% ethanol, Folin–Ciocalteu reagent (10%), Na₂CO₃ (10%), gallic acid, AlCl₃ (10%), glacial acetic acid, analytical-grade ethanol, quercetin, TPTZ (2,4,6-tripyridyl-s-triazine), HCl, FeCl₃, acetate buffer (pH 3.6), Trolox, DPPH (1,1-diphenyl-2-picrylhydrazyl), pancreatic lipase enzyme (Sigma-Aldrich, United States), orlistat, phosphate-buffered saline (PBS; NaCl and Triton X-100), and p-NPB (para-nitrophenyl butyrate).

The methods used in this study included the determination of moisture content, extraction yield, phytochemical content consisting of total phenolic content (TPC) and total flavonoid content (TFC), antioxidant capacity assays including 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), as well as pancreatic lipase inhibition activity assay.

2.1. Sample Preparation

The preparation of *Annona squamosa* and *Moringa oleifera* leaves dried leaf powder was conducted according to Alkhalidy *et al.* (2023) and Ogundipe *et al.* (2022), respectively. Fresh leaves were washed, air-dried at room temperature without direct sunlight exposure, ground into powder, and sieved through a 60-mesh sieve to obtain dried leaf powder with uniform particle size.

2.2. Moisture Content Determination of the Dried leaf powder

Moisture content was determined by the gravimetric oven-drying method following Fikriyah and Nasution (2021). Approximately 2 g of sample was dried at 105 °C to a constant weight, cooled in a desiccator, and weighed. Moisture content was calculated based on the weight loss after drying. All analyses were performed in triplicate using the following equation:

$$\text{Moisture content (\%)} : \frac{\text{Sample Fresh Weight (g)} - \text{Sample Dry Weight (g)}}{\text{Sample Fresh Weight (g)}} \times 100\%$$

2.3. Extraction of Dried leaf powder

Dried leaf powder (20 g) was extracted twice with 70% ethanol (200 mL) by ultrasound-assisted extraction for 30 min. The combined extracts were concentrated using a rotary evaporator (45–50 °C) and stored at 4 °C until analysis.

$$\text{Yield (\%)} : \frac{\text{Extract Weight (g)}}{\text{Sample Weight (g)} - (1 - \text{Moisture content})} \times 100\%$$

2.4. Combined Extract Formulation

The crude extracts obtained from both samples were formulated according to the method described by Haqiqi (2025). The formulated extracts were dissolved in 70% ethanol to obtain a final concentration of 1000 ppm (1 mg/mL) and prepared at ratios of 1:0, 0:1, 3:1, 2:1, 1:1, 1:2, and 1:3.

2.5 Total Phenolic Content (TPC) Analysis

TPC was determined using the Folin–Ciocalteu method according to Yatma *et al.*, (2023). Absorbance was measured at 765 nm using a nanospectrophotometer, with gallic acid (0, 20, 40, 60, 80, 100, 120, and 140 µg/mL) as the standard. Analyses were performed in triplicate, and results were expressed as mg gallic acid equivalent per gram of extract (mg GAE g⁻¹).

2.6 Total Flavonoid Content (TFC) Analysis

TFC was determined using the aluminum chloride colorimetric method according to Arista *et al.* (2023). Absorbance was measured at 415 nm, with quercetin (0, 50, 100, 150, 200, 250, 300, 350, 400, and 450 µg/mL) as the standard. Analyses were performed in triplicate and results were expressed as mg quercetin equivalent per gram of extract (mg QE g⁻¹).

2.7 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Capacity Assay

DPPH radical scavenging capacity was determined according to Hasti and Makbul (2022). Trolox (0, 3.125, 5, 12.5, 20, 40, and 50 µg/mL) was used as the standard, and absorbance was measured at 517 nm after incubation. Analyses were performed in triplicate and results were expressed as mg trolox equivalent (TE) g⁻¹ extract.

2.8 Ferric Reducing Antioxidant Power (FRAP) Radical Scavenging Capacity Assay

FRAP antioxidant capacity was determined according to Yatma *et al.* (2023). Trolox (0, 25, 50, 100, 200, 300, and 400 µg/mL) was used as the standard, and absorbance was measured at 595 nm. Analyses were performed in triplicate and results were expressed as mg trolox equivalent (TE) g⁻¹ extract.

2.9 Pancreatic Lipase Inhibition Activity Assay

Pancreatic lipase inhibitory activity was determined following Chedda *et al.* (2016) and Pliego *et al.* (2015). Samples were prepared at 1000 µg/mL and incubated with pancreatic lipase and p-NPB substrate under optimized assay conditions. Absorbance was measured at 400 nm, and inhibitory activity was calculated using a p-NP standard curve (25, 50, 75, 100, 125, 150, 175, 200, 225, and 250 µM). All analyses were performed in triplicate, with orlistat as the positive control.

2.10 Data Analysis

Data were analyzed using One-Way ANOVA ($\alpha = 0.05$), followed by Duncan's post hoc test at 95% confidence level ($\alpha = 0.05$). Pearson's correlation analysis was performed to assess the relationship between variables.

3. Results

3.1. Moisture Content and Crude Extract Yield of *Annona squamosa* Leaves and *Moringa oleifera* Leaves

The analysis results showed that the moisture content of *A. squamosa* leaves dried leaf powder had an average value of 8.10%, while *M. oleifera* leaves dried leaf powder showed a moisture content of 5.54% (Table 1). Based on these data, the moisture content of both samples can be considered low, as they were below 10%. The extract yield was calculated based on the ratio between the crude extract weight and the dried leaf powder weight by considering the moisture content of the samples. Based on the calculations presented in Table 1, the crude extract yield of *A. squamosa* leaves was 15.28%, whereas the crude extract yield of moringa leaves was 17.55%

Table 1. Moisture Content and Yield of *Annona squamosa* and *Moringa oleifera* Leaves

Test	Average Result (%)
Moisture Content of <i>A. squamosa</i> leaves dried leaf powder	8.10 ± 0.16
Moisture Content of <i>M. oleifera</i> leaves dried leaf powder	5.54 ± 0.07
Yield crude extract of <i>A. squamosa</i> leaves	15.28 ± 0.60
Yield crude extract of <i>M. oleifera</i> leaves	17.55 ± 4.44

3.2. Total Phytochemical Content

The total phytochemical content in this study was evaluated through Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) analyses. The total phenolic content of each sample extract combination was expressed as mg GAE/g extract, calculated based on the gallic acid standard curve equation using the same solvent as the sample extracts. Based on the data presented in Figure 1, the highest total phenolic content was observed in the single extract of *A. squamosa* leaves, with a value of 95.15 ± 0.32 mg GAE/g extract.

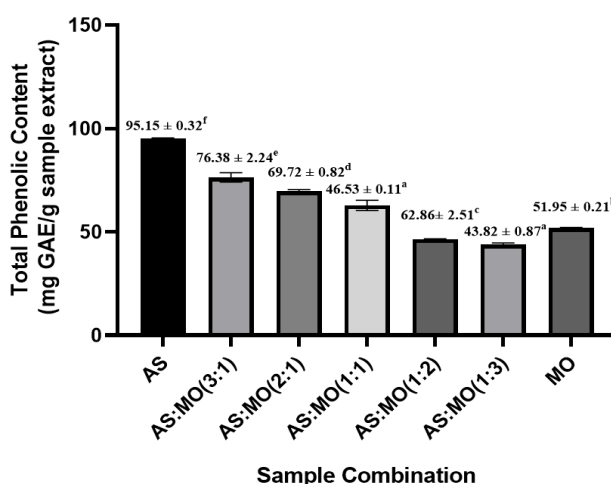


Figure 1. Total phenolic content of sample combination. Values with different letters are significantly different ($p < 0.05$) based on Duncan's post hoc test.

The total flavonoid content of each sample extract combination was expressed as mg QE/g extract, calculated based on the quercetin standard curve equation using the same solvent as the sample extracts. Based on the results presented in Figure 2, the single extract of *A. squamosa* leaves and the combined extract at a 1:1 ratio exhibited the highest total flavonoid content and were not significantly different, with values of 47.57 ± 0.00 and 46.40 ± 1.64 mg QE/g extract, respectively.

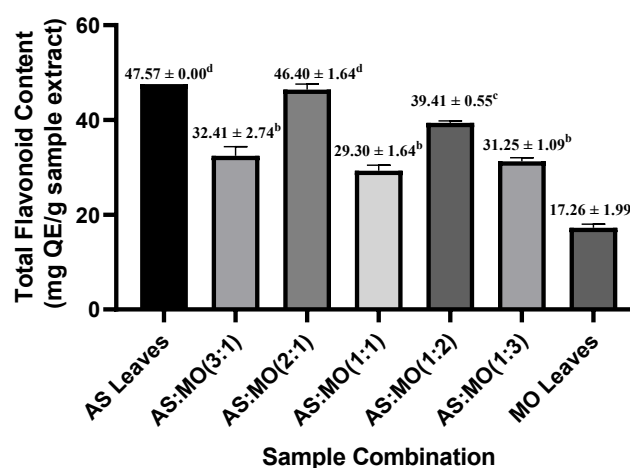


Figure 2. Total flavonoid content of sample combination. Values with different letters are significantly different ($p < 0.05$) based on Duncan's post hoc test.

3.3. Antioxidant Capacity

The antioxidant capacity in this study was evaluated using the DPPH and FRAP methods. The DPPH antioxidant capacity of each sample extract combination was expressed as mg TE/g extract, calculated based on the Trolox standard curve equation using the same solvent as the sample extracts. Based on the data presented in Figure 3, the single extract of *A. squamosa* leaves exhibited the highest DPPH antioxidant capacity, with a value of 395.10 ± 3.31 mg TE/g extract. In contrast, the lowest antioxidant capacity was observed in the single extract of *M. oleifera* leaves, with a value of 18.24 ± 1.42 mg TE/g extract.

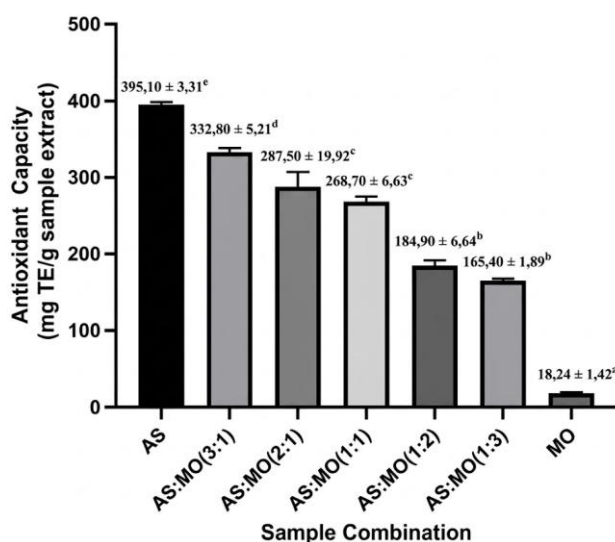


Figure 3. Antioxidant capacity DPPH of sample combination. Values with different letters are significantly different ($p < 0.05$) based on Duncan's post hoc test.

The antioxidant capacity using the FRAP method for each sample extract combination was expressed as mg TE/g extract, calculated based on the Trolox standard curve equation using the same solvent as the sample extracts. Based on the data presented in Figure 4, the highest antioxidant capacity was observed in the single extract of *A. squamosa* leaves, with a value of 476.30 ± 1.34 mg TE/g extract. The lowest antioxidant capacity was found in the single extract of *M. oleifera* leaves, with a value of 46.31 ± 1.34 mg TE/g extract.

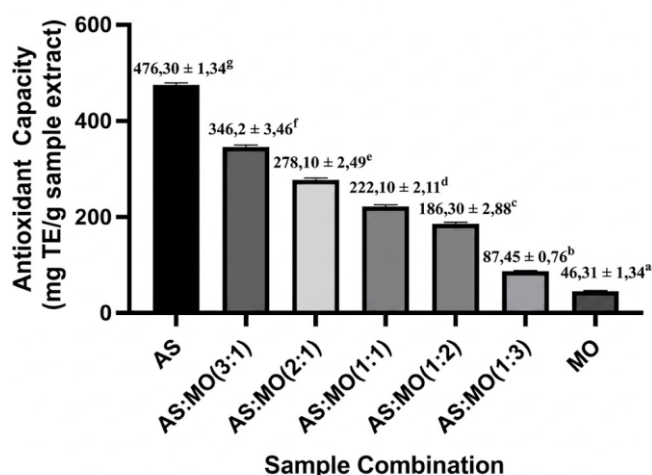


Figure 4. Antioxidant capacity FRAP of sample combination. Values with different letters are significantly different ($p < 0.05$) based on Duncan's post hoc test.

3.6. Pancreatic Lipase Inhibition Activity

Pancreatic lipase inhibitory activity was calculated from enzyme activity based on p-nitrophenol production and expressed as the percentage inhibition relative to the control. As shown in Figure 5, the inhibition activity of the extract combinations ranged from 42–64%. The single extract of *A. squamosa* leaves exhibited the highest inhibitory activity ($64.15 \pm 2.70\%$), whereas the single extract of *M. oleifera* leaves showed the lowest activity ($42.36 \pm 1.35\%$).

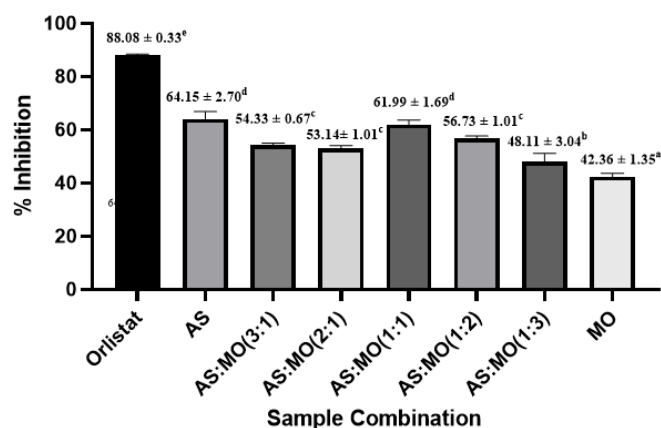


Figure 5. Inhibition pancreatic lipase of sample combination. Values with different letters are significantly different ($p < 0.05$) based on Duncan's post hoc test.

3.7. Correlation Between Phytochemical and Antioxidant Capacity

Pearson correlation analysis revealed a positive but non-significant correlation between TPC and DPPH antioxidant capacity ($r = 0.509$, $p = 0.2433$) (Figure 6A). In contrast, TPC showed a strong positive and significant correlation with FRAP antioxidant capacity ($r = 0.934$, $p = 0.0021$) (Figure 6B). TFC was strongly and positively correlated with DPPH antioxidant capacity ($r = 0.866$, $p = 0.0117$) (Figure 6C), whereas its correlation with FRAP antioxidant capacity was positive but not significant ($r = 0.521$, $p = 0.2710$) (Figure 6D). These results indicate that phenolic compounds were more closely associated with FRAP values, while flavonoids contributed more strongly to DPPH scavenging activity.

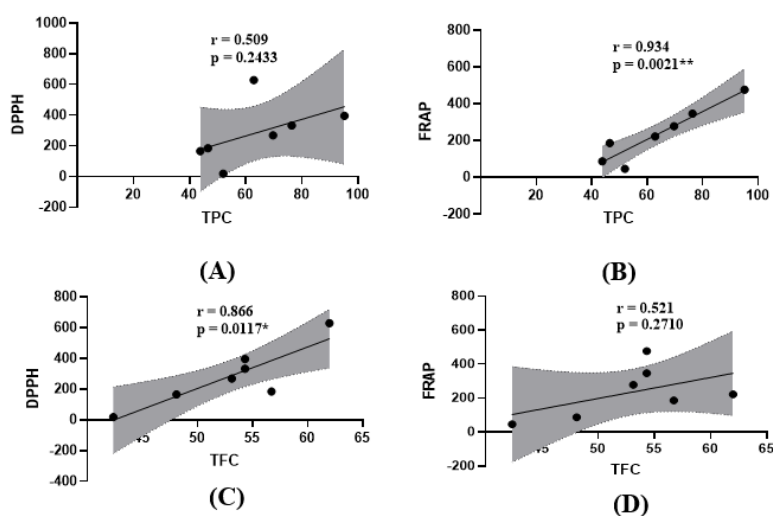


Figure 6. Correlation graphs between total phenolic content and DPPH (A), total phenolic content and FRAP (B), total flavonoid content and DPPH (C), total flavonoid content and FRAP (D) of the combined extracts of *A. squamosa* and *M. oleifera* leaves.

3.8. Correlation Among Phytochemical, Antioxidant, and Pancreatic Lipase Inhibition Activity

Pearson correlation analysis showed positive but non-significant correlations between lipase inhibition activity and both TPC ($r = 0.622$, $p = 0.1355$) and TFC ($r = 0.648$, $p = 0.1151$) (Figures 7A and 7B). In contrast, DPPH antioxidant capacity was strongly and significantly correlated with lipase inhibition activity ($r = 0.838$, $p = 0.0185$) (Figure 7C). Similarly, FRAP antioxidant capacity showed a significant positive correlation with lipase inhibition activity ($r = 0.782$, $p = 0.0377$) (Figure 7D). These findings suggest that antioxidant capacity was more closely associated with pancreatic lipase inhibition than phytochemical content alone.

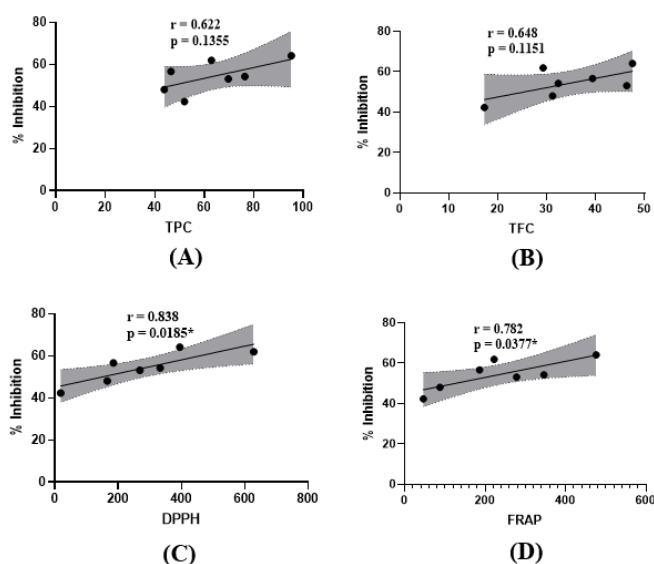


Figure 7. Correlation graphs between total phenolic content (A), total flavonoid content (B), antioxidant capacity measured by the DPPH method (C), and the FRAP method (D) of the combined extracts of *A. squamosa* and *M. oleifera* leaves with pancreatic lipase inhibition percentage.

4. Discussion

4.1. Moisture Content and Crude Extract Yield of *Annona squamosa* Leaves and *Moringa oleifera* Leaves

The determination of dried leaf powder moisture content was carried out using gravimetry method. The results obtained (Table 1) showed moisture content values below 10% for each sample. This is consistent with the statement of Wandira *et al.* (2023), that the acceptable moisture content requirement for dried leaf powder is below 10%. Therefore, the *A. squamosa* and *M. oleifera* leaves dried leaf powder samples were suitable for the subsequent extraction process.

The extraction yield of *M. oleifera* leaves in this study was 17.55%, which met the standard extraction yield requirement for *M. oleifera* leaves extract of not less than 9.2%, as stated by the Ministry of Health of the Republic of Indonesia (2017). Meanwhile, the extraction yield of *A. squamosa* leaves was 15.28%, which is comparable to several previous studies reporting extraction yields in the range of 10–25%, depending on the extraction method and conditions used. A relatively high extraction yield may indicate the efficiency of the extraction process in dissolving compounds from the raw material. In general, a high yield may reflect the extraction of both active and non-active compounds, so differences in extraction yield values may influence the concentration of bioactive compounds contained in the extract (Senduk *et al.* 2020).

4.2. Phytochemical Content and Antioxidant Capacity of Combination Extract

Phenolic and flavonoid compounds are important plant secondary metabolites and are synthesized via biosynthetic such as shikimic acid and phenylpropanoid pathways that contribute to antioxidant activity (Diniyah & Lee, 2020; Liunokas & Karwur, 2020). The total phenolic content (TPC) and total flavonoid content (TFC) showed similar trends across all treatments (Figures 1 and 2). The single extract of *A. squamosa* leaves exhibited significantly higher TPC (95.15 ± 0.32 mg GAE g⁻¹) and TFC (47.57 ± 0.00 mg QE g⁻¹) than *M. oleifera* leaves (51.95 ± 0.21 mg GAE g⁻¹ and 17.26 ± 1.99 mg QE g⁻¹, respectively). Consequently, increasing the proportion of *A. squamosa* in the mixtures increased both phytochemical parameters.

Conversely, equal or *M. oleifera* dominated mixtures exhibited lower phytochemical contents than expected, suggesting antagonistic interactions among secondary metabolites that may reduce the release or availability of phenolic and flavonoid compounds (Wardhani *et al.*, 2024). Differences in phytochemical values compared with previous studies may be attributed to variations in extraction techniques, solvent systems, and environmental factors affecting secondary metabolite biosynthesis.

A similar pattern was observed for antioxidant capacity. The single extract of *A. squamosa* leaves showed the highest DPPH and FRAP values (395.10 and 476.30 mg TE g⁻¹, respectively), whereas *M. oleifera* leaves exhibited the lowest antioxidant capacity (18.24 and 46.31 mg TE g⁻¹, respectively) (Figures 3 and 4). Antioxidant capacity increased significantly ($p < 0.05$) with increasing proportions of *A. squamosa* extract. Among the combinations, the 3:1 ratio exhibited the highest antioxidant activity, reaching 332.80 mg TE g⁻¹ for DPPH and 346.20 mg TE g⁻¹ for FRAP. The enhanced antioxidant performance is likely associated with the higher phenolic and flavonoid contents of *A. squamosa* leaves and potential additive interactions between bioactive compounds. Variations from previously reported antioxidant values may reflect differences in extraction procedures and environmental conditions affecting phytochemical composition.

TPC showed a positive but non-significant correlation with DPPH antioxidant capacity ($r = 0.509$, $p = 0.2433$) (Figure 6A), indicating that phenolic compounds contributed to radical scavenging activity but were not the sole determinants of DPPH antioxidant capacity. In contrast, TPC exhibited a strong positive and significant correlation with FRAP antioxidant capacity ($r = 0.934$, $p = 0.0021$) (Figure 6B), suggesting that phenolic compounds played a major role in ferric ion reduction. This finding is consistent with the ability of phenolic compounds to donate electrons or hydrogen atoms through hydroxyl groups, thereby neutralizing free radicals and reducing oxidizing agents (Maryam *et al.*, 2015; Zuraida *et al.*, 2017). The resulting phenoxyl radicals are relatively stable due to resonance stabilization, preventing further radical chain reactions (Dareda *et al.*, 2020).

Similarly, TFC showed a strong positive and significant correlation with DPPH antioxidant capacity ($r = 0.866$, $p = 0.0117$) (Figure 6C), indicating that flavonoids contributed substantially to free radical scavenging activity. However, the correlation between TFC and FRAP antioxidant capacity was positive but not significant ($r = 0.521$, $p = 0.2700$) (Figure 6D), suggesting a less dominant role of flavonoids in ferric ion reduction. As a major subclass of polyphenolic compounds, flavonoids exert antioxidant activity through hydrogen atom donation and electron transfer mechanisms, which are strongly influenced by the number and position of hydroxyl groups within their structure (Nur *et al.*, 2019; Taufiq *et al.*, 2017). The resulting flavonoid radicals are stabilized through electron delocalization and resonance, enabling them to terminate free radical chain reactions and reduce oxidative stress (Pietta, 2000; Cetinkaya *et al.*, 2017; Sadeer *et al.*, 2020).

4.3. Pancreatic Lipase Inhibition Activity of Combination Extract

Pancreatic lipase is a key enzyme involved in dietary fat digestion and a major target for obesity management. The single extract of *A. squamosa* leaves exhibited the highest lipase inhibitory activity ($64.15 \pm 2.70\%$), whereas *M. oleifera* leaves showed the lowest inhibition ($42.36 \pm 1.35\%$) (Figure 5). Among the combinations, the 1:1 ratio showed the highest inhibition ($61.99 \pm 1.69\%$), suggesting favorable interactions between bioactive compounds from both extracts. The inhibitory activity may be attributed to phenolic and flavonoid compounds, which have been reported to interfere with pancreatic lipase activity (Chedda *et al.*, 2016; Meila & Noraini, 2017). Although all extract treatments were less effective than orlistat ($88.08 \pm 0.33\%$), the relatively high inhibition observed for *A. squamosa* and the 1:1 combination highlights their potential as natural anti-obesity agents.

Pearson correlation analysis revealed positive but non-significant correlations between lipase inhibition activity and both TPC ($r = 0.622$, $p = 0.1355$) and TFC ($r = 0.648$, $p = 0.1151$) (Figures 7A and 7B). This suggests that lipase inhibition was influenced more by the composition and structural characteristics of individual compounds than by total phenolic or flavonoid content alone. Other bioactive constituents, such as terpenoids, may also contribute to the observed activity (Moreno-Córdova *et al.*, 2020; Singh & Pattnaik, 2024).

In contrast, DPPH and FRAP antioxidant capacities showed strong and significant positive correlations with lipase inhibition activity ($r = 0.838$, $p = 0.0185$ and $r = 0.782$, $p = 0.0377$, respectively) (Figures 7C and 7D). These findings indicate that extracts with greater antioxidant capacity tended to exhibit stronger lipase inhibitory activity. Since antioxidant assays reflect the combined effects of multiple bioactive compounds, antioxidant capacity may better represent the overall biological activity of the extracts than TPC or TFC alone. Overall, antioxidant capacity showed a stronger association with pancreatic lipase inhibition than total phytochemical content, suggesting that multiple bioactive constituents contribute to the anti-obesity potential of the extracts.

5. Conclusion and Recommendation

The combination of 70% ethanol extracts of *Annona squamosa* and *Moringa oleifera* leaves exhibited different interaction patterns across the tested parameters. Antagonistic effects were observed in total phenolic content, total flavonoid content, and FRAP antioxidant capacity, whereas synergistic effects were found in DPPH antioxidant capacity and pancreatic lipase inhibition activity. Overall, the single extract of *A. squamosa* leaves showed the highest activity in all parameters tested, and increasing the proportion of *A. squamosa* leaves in the combinations generally enhanced the activity values, although not always synergistically. Correlation analysis indicated that antioxidant capacity was positively associated with pancreatic lipase inhibition, while phytochemical content showed no significant direct correlation, suggesting that lipase inhibitory activity may involve multiple bioactive compounds and complex interaction mechanisms.

Further studies are recommended to evaluate other phytochemical groups, particularly total alkaloid and terpenoid contents, as potential contributors to pancreatic lipase inhibition. Comprehensive phytochemical profiling using mass spectrometry instrument and molecular docking analyses of *A. squamosa* leaves extract is also required to identify the compounds responsible for the observed bioactivity. In addition, determination of concentrated extract moisture content and in vivo studies are necessary to validate the anti-obesity potential of *A. squamosa*, *M. oleifera*, and their combinations.

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