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Antioxidant Activity of Extract Combination from *Moringa oleifera* Leaves and *Garcinia mangostana* L. Pericarp

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Abstract
Radical-related diseases can be mitigated through natural antioxidant therapies that help regulate the formation and accumulation of free radicals. The pericarp of *Garcinia mangostana* L. and the leaves of *Moringa oleifera* are well-known for their antioxidant properties due to their high level of bioactive compounds, such as flavonoids, phenolics, and xanthones. This study aimed to explore the antioxidant capabilities of these two herbal extract combinations. Using the maceration method with 96% ethanol, the extraction yields were 11.4% w/w for *M. oleifera* and 24.11% w/w for *G. mangostana*. Qualitative phytochemical screening revealed differences in secondary metabolites, particularly steroids and triterpenes, between the two extracts. The antioxidant activity was categorized based on EC50 values, with crude *G. mangostana* showing very strong activity (<50 µg/mL) and M. oleifera classified as strong (50–100 µg/mL). Combinations of the extracts (2–10 ppm) produced very strong antioxidant activity but showed reduced efficacy compared to *G. mangostana* alone, suggesting antagonistic interactions based on combination index (IC) calculation. This study emphasizes the need for multiple antioxidant assays to evaluate diverse mechanisms and recommends further research to optimize synergistic effects in herbal extract combinations.

Keywords: Antioxidant, DPPH assay, *Garcinica mangostana*, *Moringa oleifera* combination

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Aktivitas Antioksidan Kombinasi Ekstrak Daun *Moringa oleifera* dan Kulit Buah *Garcinia mangostana* L.

Abstrak
Penyakit yang berkaitan dengan radikal bebas, dapat dikurangi dengan terapi antioksidan alami yang mengatur pembentukan dan akumulasi radikal bebas. Penyakit yang berhubungan dengan radikal bebas dapat dikurangi melalui terapi antioksidan alami yang membantu mengatur pembentukan dan akumulasi radikal bebas. Kulit buah *Garcinia mangostana* L. dan daun *Moringa oleifera* terkenal akan sifat antioksidannya karena tingginya kadar senyawa bioaktif, seperti flavonoid, fenolik, dan xanthone. Penelitian ini bertujuan untuk mengeksplorasi kemampuan antioksidan dari kedua kombinasi ekstrak herbal ini. Dengan menggunakan metode maserasi dengan etanol 96%, hasil ekstraksi adalah 11,4% b/b untuk *M. oleifera* dan 24,11% b/b untuk *G. mangostana*. Skrining fitokimia kualitatif mengungkapkan perbedaan metabolit sekunder, khususnya steroid dan triterpen, antara kedua ekstrak. Aktivitas antioksidan dikategorikan berdasarkan nilai EC50, dengan *G. mangostana* menunjukkan aktivitas yang sangat kuat (<50 µg/mL) dan *M. oleifera* diklasifikasikan sebagai kuat (50–100 µg/mL). Kombinasi ekstrak (2–10 ppm) menghasilkan aktivitas antioksidan yang sangat kuat tetapi menunjukkan kemanjuran yang berkurang dibandingkan dengan *G. mangostana* saja, yang menunjukkan interaksi antagonis berdasarkan perhitungan Indeks Kombinasi (IC). Studi ini menekankan perlunya beberapa uji antioksidan untuk mengevaluasi berbagai mekanisme dan merekomendasikan penelitian lebih lanjut untuk mengoptimalkan efek sinergis dalam kombinasi ekstrak herbal.

Kata Kunci: Antioksidan, uji DPPH, *Garcinica mangostana*, kombinasi *Moringa oleifera*.

1. Introduction

Indonesia's rich biodiversity offers various herbal plants with pharmacological benefits, particularly as antioxidants. The use of natural compounds is deeply rooted in its culture, as seen in the traditional practice of *jamu*. However, more than 90% of these products are still based on empirical evidence, with limited preclinical and clinical data available to support their safety and efficacy scientifically.[1] According to the BPOM Indonesia website, *jamu* products made from the pericarp of *Garcinia mangostana* (mangosteen) are available on the market and promote their potential to prevent various diseases.[2]

The pericarp of *G. mangostana*, often considered waste, contains a high concentration of xanthones. Studies on α-mangosteen have confirmed that it acts as a chemopreventive agent, providing antioxidant protection against DNA damage caused by reactive oxygen species (ROS). Neutralizing free radicals plays a crucial role in preventing mutagenesis and the initiation of carcinogenesis.[3] Despite its potent antioxidant properties, α-mangosteen has low absorption in the body, limiting its therapeutic potential. Studies show it is orally bioavailable, especially with an oily vehicle, but only about 2% of the ingested dose is absorbed in humans.[4] To overcome this limitation, the combination of α-mangosteen with herbal compounds could enhance its antioxidant effect.

Moringa oleifera leaves are rich in polyphenols, which exhibit strong free radical scavenging activity.[5] Besides its notable antioxidant properties, *M. oleifera* contains mineral nutrients such as iron (Fe) and calcium (Ca), which are highly bioaccessible, making it a valuable source of antioxidant supplementation. Furthermore, studies have shown that the antioxidant activity of *M. oleifera* is particularly effective in the intestinal environment, where it enhances gut health by improving antioxidant status, modulating the intestinal micro-ecosystem, and reducing inflammatory responses in mucosal tissues.[6] Additionally, active peptide compounds released from *M. oleifera* during gastrointestinal digestion have been found to exhibit significant antioxidant properties.[7]

Therefore, this paper will evaluate the antioxidant activity of the combination of these two herbs, aiming to explore their synergistic effects and potential as an effective strategy to combat oxidative stress and prevent degenerative diseases.

2. Materials and Methods

2.1. Materials

The plants materials used were *Garcinia mangostana* L. pericarp, from Manado, North Sulawesi, and *Moringa oleifera* L. leaves from KWT Mina Tani Lestari, Tangerang. The reagents used in this study included 96% ethanol (Merck), Dragendorff's reagent (Merck), magnesium powder (Merck), HCl, $FeCl_3$ (Merck), Liebermann-Burchard reagent, Aquadest (Pasifik Kimia Indonesia), ascorbic acid (Merck), and DPPH (Tokyo Chemical Industry, Japan.

2.2. Instruments

The tools for the extraction process included a set of rotary evaporator (Hei-VAP Value, Rotachill, Vacuum Pumping Unit), water bath (Merck), Oven (Memmert UF 55), UV-Vis spectrophotometer (Agilent), analytical balance (Ohaus), micropipette (DLab).

2.3. Methods

2.3.1. Determination

Garcinia mangostana L. pericarp and *Moringa oleifera* L. leaves were identified by the Plant Taxonomy Laboratory of the Department of Biology, FMIPA, Universitas Padjadjaran.

2.3.2. Sample Preparation

The fresh materials were cleaned, sliced, and dried at 50–70°C for 90 minutes before ground into a fine powder. One hundred grams of each extract were extracted with 96% ethanol (1:5 ratio). The mixture was stirred three times daily for 48 hours. After maceration, the solution was evaporated below 50°C to obtain a thick extract.[8]

2.3.3. Phytochemical screening

Alkaloids

A test solution (2 ml) is evaporated in a dish to obtain a residue. The residue is dissolved in 5 ml of 2N HCl and divided into three test tubes. In test tube 1, dilute acid is added as a blank. In test tube 2, three drops of Dragendorff's reagent are added, and in test tube 3, three drops of Mayer's reagent are added. A positive alkaloid result is indicated by the formation of an orange precipitate in test tube 2 and a yellow precipitate in test tube 3.[9]

Flavonoids

A total of 100 mg of each extract from mangosteen peel and moringa leaves is added to 2 ml of ethanol and shaken until homogeneous. Subsequently,

magnesium powder and 5 drops of concentrated HCl are added. The formation of red, yellow, or orange precipitates indicates a positive flavonoid result.[9]

Polyphenols and Tannins

A test solution (2 mL) is divided into three test tubes. In test tube 1, 1% $FeCl_3$ is added. In test tube 2, gelatin is added, and in test tube 3, Stiasny's reagent is added and then heated. The resulting solution from test tube 3 is filtered, and the filtrate is treated with sodium acetate and $FeCl_3$. A positive tannin result is indicated as follows:

- For test tube 1, a blue-black or green-black color indicates tannins.
- For test tube 3, a light red precipitate indicates catechin tannins, while a blue-black precipitate in the filtrate indicates gallic tannins.9

Saponin

A total of 100 mg of each extract from mangosteen peel and moringa leaves is placed into a test tube, followed by adding 5 ml of hot water. After cooling, the solution is vigorously shaken for 10 seconds. A positive result is observed if foam forms for approximately 10 minutes, with a height of 1–10 cm. Adding 1 drop of 2N HCl, with the foam persisting, confirms a positive result.[9]

Steroids and Triterpenes

A total of 1 gram of extract is macerated with 20 ml of ether for 2 hours and then concentrated. The residue is treated with 2 ml of Liebermann-Burchard reagent. A greenish-blue color indicates a positive result for steroids, while a reddish-brown color indicates a positive result for triterpenoids.[9]

2.3.4. Antioxidant assay using DPPH radical scavenging assay

Determination of operating time

Operating time was determined by reacting 50 µL of ascorbic acid standard with 4.0 mL of 0.1 mM DPPH solution. The mixture was homogenized with a stirrer for 1 minute, and absorbance was measured at the λmax every 5 minutes for 60 minutes.[10]

Antioxidant evaluation of individual and extract combination

Each extract (10 mg) was dissolved in 100 mL of pro-analysis ethanol to obtain a concentration of 100 ppm. From this stock solution, serial dilutions were prepared to concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. For each sample, 0.125 mL of the extract solution was pipetted into a tube, followed by 0.75 mL of 30 ppm DPPH solution. The tubes were vortexed and left to stand for 30 minutes. Absorbance was measured at a wavelength of 517 nm for antioxidant evaluation. All measurements were performed in triplicate.[10]

Free-radical scavenging activity determination

The scavenging activity is calculated using the following formula:

$$Scavenging\ activity\ (\%) = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100\%$$

Where:
A_blank = absorbance of the DPPH solution without the sample (blank)
A_sample = absorbance of the DPPH solution with the sample (extract or vitamin C)

IC_{50} calculation

To calculate the IC50 value using the linear regression equation, you use the formula:

$$y = bx + a$$

Where:
y = 50, (because IC50 corresponds to 50% scaveng ing activity)
x = the concentration of the test solution
a = the slope constant
b = the intercept constant

Determination of Combination Index of Extract Combination

The percentage of inhibition of the extract combination converted into fraction of effect (Fa) using this formula: Fa = 100% of activity/100. The treatment concentration (ppm) and Fa were automatically processed using CompuSyn software Version 1. Interaction of these two combinations was evaluated using the Combination Index (CI), whether a synergism (CI < 1), an additive effect (CI = 1), or an antagonism (CI >1).[11]

Table 1. Extract yield and characteristic

Sample	Simplicia (g)	Extract (g)	Yield (%)	Organoleptic
Garcinia mangostana L. pericarp	100	24.153	24.15	Brown, thick
Moringa oleifera L. leaves	100	11.146	11.14	Blackish Green, thick

Table 2. Qualitative analysis of secondary metabolites of extracts

Secondary metabolic	Test	*G. mangostana* L. pericarp	*M. oleifera* L. leaves
Alkaloids	Dragendorff	+	+
Flavonoids	Shinoda	+	+
Polyphenols	$FeCl_3$	+	+
Tannins	Stiasny	+	+
Saponin	Foam	-	+
Steroid	Liebermann- Burchard	-	+
Triterpenes	Liebermann- Burchard	+	-

(+) presence; (-) Absence

3. Results

3.1. Extraction result

The maceration process with 96% ethanol showed that the yield values in Table 1 met the requirements of the Indonesian Herbal Pharmacopoeia, which specifies a minimum yield of 7.2%.[12]

3.2. Secondary metabolic

Extraction using 96% ethanol produced secondary metabolites as listed in Table 2. The qualitative profile was consistent with the previous study on mangosteen pericarp[8] and moringa leaves.[13]

3.3. Antioxidant activity

The antioxidant activity was evaluated using the DPPH assay, which showed IC_{50} values for standard and individual extract (see Table 3). The synergistic effects of the constant ratio of the combination (1:1) were analyzed using CompuSyn software. The combination index plot (Figure 1) demonstrated antagonistic action detected in each combination (CI > 1) (Table 4).

4. Discussion

Antioxidant capacity indicates the ability of a substance to reduce the number of oxidant molecules, reflecting its stoichiometry.[14] The antioxidant properties of the extract combination are categorized based on its IC_{50} value: <50 µg/mL indicates very strong activity, 50–100 µg/mL indicates strong activity, 100–250 µg/mL indicates moderate, and 151-200 indicates weak activity.[15] In this study, a crude *G. mangostana* pericarp extract exhibits very strong antioxidant activity, while the *M. oleifera* leaf extract is classified as having strong antioxidant activity.

Complex combinations of phytochemicals have been shown to interact, either by enhancing the antioxidant impact or by interfering with one another's action.

Table 3. Calculation of inhibition percentage of standard and individual extract

Sample	Concentation (ppm)	Abs (λ= 518nm)	Inhibition (%)	IC50 (ppm)
Vitamin C	5	0.529 ± 0.006	45.45	
	10	0.500 ± 0.009	48.44	
	15	0.443 ± 0.004	54.25	10.66 ppm y = 0.8814x + 40.598 r^2 = 0.9925
	20	0.402 ± 0.009	58.54	
	25	0.364 ± 0.026	62.43	
	DPPH	0.969		
Garcinia mangostana L. pericarp extract	2	0.522 ± 0.011	40.82	
	4	0.434 ± 0.005	50.79	
	6	0.299 ± 0.045	66.06	3.46 ppm y = 0.4186x + 26.708 r^2 = 0.9848
	8	0.200 ± 0.042	77.29	
	10	0.133 ± 0.001	84.96	
	DPPH	0.882		
Moringa oleifera L. leaves extract	5	0.701 ± 0.003	27.81	
	10	0.685 ± 0.012	29.42	
	20	0.609 ± 0.001	37.21	55.64 ppm y = 0.8814x + 40.598 r^2 = 0.9925
	40	0.537 ± 0.089	44.70	
	80	0.395 ± 0.040	59.29	
	DPPH	0.971		

Table 4. Percentage of free radical inhibition of extract combination and calculation of combination index

Moringa (ppm) A	Mangostana (ppm) B	Abs (λ= 518nm)	Inhibition (%)	Combination Index (CI)
2	2	0.516	37.67	1.05360
	4	0.591	40.02	1.44485
	6	0.549	44.67	1.62742
	8	0.592	48.49	1.78161
	10	0.542	54.65	1.73596
4	2	0.591	34.26	1.81314
	4	0.530	41.04	1.85690
	6	0.509	43.41	2.12742
	8	0.473	46.74	2.27072
	10	0.407	54.78	2.01646
6	2	0.549	40.33	1.90430
	4	0.531	42.25	2.21820
	6	0.518	43.7	2.52659
	8	0.507	44.86	2.82897
	10	0.469	48.97	2.80419
8	2	0.592	34.66	2.97204
	4	0.532	41.24	2.76185
	6	0.454	49.85	2.38122
	8	0.362	59.97	1.93410
	10	0.336	62.84	1.97141
10	2	0.542	39.84	2.90927
	4	0.521	42.29	3.10097
	6	0.485	46.31	3.07315
	8	0.469	48.08	3.25229
	10	0.442	51.00	3.27168

Based on CI calculation, this crude extract combination performed CI combination at > 1. This value indicates antagonistic actions.[11] Antagonism in antioxidant activity occurs when certain compounds interfere with each other's effectiveness, reducing the overall efficacy of the mixture compared to a single extract.[16] For example, previous research has demonstrated that alkaloids and saponins may interact antagonistically, leading to a decrease in the antioxidant capacity of extracts containing both compounds.[17] The DPPH, as one of the popular methods, evaluates the capacity of antioxidants to convert the deep purple color of DPPH, as a radical, into the light yellow α, α-diphenyl-β-picrylhydrazine.[18] The antagonistic effect observed in this study could be attributed to variations in crude extract composition, which may hinder optimal hydrogen donation. This could occur due to steric hindrance, where specific molecules physically obstruct the active sites of antioxidants, or competitive interactions, where multiple compounds compete for the same target, ultimately reducing the binding efficiency to DPPH radicals.[10,19]

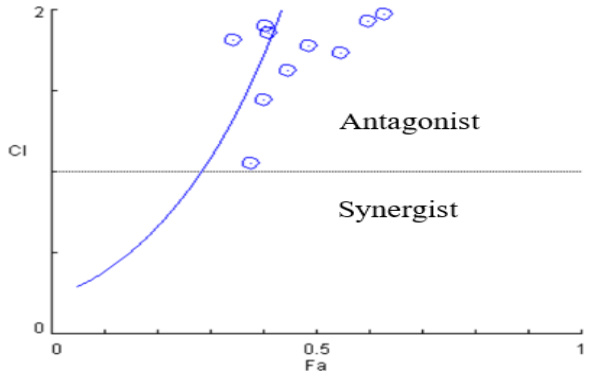

Figure 1. Combination Index Plot of combination extract (CompuSyn Report)

Future research should focus on utilizing multiple antioxidant evaluation methods to assess the diverse mechanisms of antioxidant activity comprehensively. Since different assays measure various aspects, such as radical scavenging, lipid peroxidation prevention, or metal ion reduction,[20] comparing results from these methods will enhance the accuracy and reliability of understanding the antioxidant potential of crude extracts.

5. Conclusion

This study showed that *Garcinia mangostana* pericarp and *Moringa oleifera* leaves extracts have strong antioxidant activity. However, their combination exhibited an antagonistic effect according to CI calculations. Further validation through different antioxidant assays is needed to confirm these results and investigate the mechanisms involved in reducing radicals.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Trifena BE, Analisis uji in vitro dan in vivo ekstrak kombinasi kulit manggis (*Garcinia mangostana* L.) dan pegagan (*Centella asiatica* L.) sebagai krim antioksidan (Thesis). Depok: Universitas Indonesia; 2012.
2. Badan Pengawas Obat dan Makanan (BPOM), Daftar Obat Tradisional [diakses pada: 3 Januari 2025]. Tersedia dari: https://cekbpom.pom.go.id/produk-obat-tradisional.
3. Naing S, Sandech N, Maiuthed A, Chongruchiroj S, Pratuangdejkul J, Lomarat P, *Garcinia mangostana* L. Pericarp Extract and Its Active Compound α-Mangostin as Potential Inhibitors of Immune Checkpoint Programmed Death Ligand-1. Molecules. 2023;28:6991.
4. Chitchumroonchokchai C, Riedl KM, Suksumrarn S, Clinton SK, Kinghorn AD, Failla ML, Xanthones in Mangosteen Juice Are Absorbed and Partially Conjugated by Healthy Adults. The Journal of Nutrition. 2012;142(4):675–680.
5. Yasoob TB, Yu D, Khalid AR, Zhang Z, Zhu X, Saad HM, et al. Oral administration of *Moringa oleifera* leaf powder relieves oxidative stress, modulates mucosal immune response and cecal microbiota after exposure to heat stress in New Zealand White rabbits. J Animal Sci Biotechnol. 2021;12(66).
6. Peñalver R, Martínez-Zamora L, Lorenzo JM, Ros G, Nieto G, Nutritional and Antioxidant Properties of *Moringa oleifera* Leaves in Functional Foods. Foods. 2022; 11:1107.
7. Avilés-Gaxiola S, León-Félix J, Jiménez-Nevárez YB, Angulo-Escalante MA, Ramos-Payán R, Colado-Velázquez J, Heredia JB. Antioxidant and anti-inflammatory properties of novel peptides from Moringa oleifera Lam. leaves. South African Journal of Botany. 2021;141:466–73.
8. Widyarman AS, Lay SH, Wendhita IP, Tjakra EE, Murdono FI, Binartha CTO. Indonesian Mangosteen Fruit (*Garcinia mangostana* L.) Peel Extract Inhibits *Streptococcus mutans* and *Porphyromonas gingivalis* in Biofilms In vitro. Contemp Clin Dent. 2019;10(1):123-128.
9. Ngibad K. Phytochemical Screening Of Sunflower Leaf (*Helianthus annuus*) and Anting-Anting (Acalypha Indica Linn) Plant Ethanol Extract. Borneo Journal of Pharmacy. 2019;2(1).
10. Gulcin İ, Alwasel SH. DPPH Radical Scavenging Assay. Processes. 2023;11(8):2248.
11. Smeriglio A, Iraci N, Denaro M, Mandalari G, Giofrè SV, Trombetta D. Synergistic Combination of Citrus Flavanones as Strong Antioxidant and COX-Inhibitor Agent. Antioxidants. 2023;12(4):972.
12. Departemen Kesehatan Republik Indonesia. Farmakope Herbal Indonesia. 2008;109-114.
13. Fatmawati A, Sucianingsih D, Kurniawati R, Abdurrahman M. Microscopic Identification and Determination of Total Flavonoid Content of Moringa Leaves Extract and Ethyl Acetate Fraction (*Moringa oleifera* L.). IJPST. 2021;1(1):66-74.
14. Shojaee MS, Moeenfard M, Farhoosh R. Kinetics, and stoichiometry of gallic acid and methyl gallate in scavenging DPPH radical as affected by the reaction solvent. Sci Rep. 2022;12(1):8765.
15. Maryam St, Razak R, Baits M, Salim A, Analysis of Vitamin C and Antioxidant Activity of *Capsicum frutescens* L. and *Capsicum annuum* L. (curly and large chili variety). IJPST. 2023;1(1): 57-64.
16. Rudiana T, Indriatmoko DD, Firginia K. Antioxidant Activity of The Combination of Ambarella Leaves (*Spondias dulcis* Parkinson) And Soursop Leaves (Annona Muricata Linn) Extract. JFSP. 2023;9(2):83-87.
17. Nakitto A, Byaruhanga Y, Wagner A, Muyonga J. Influence of ripeness stage on the bioactive compounds' contents and antioxidant activities of Solanum *anguivi* Lam. fruits accessions. Heliyon. 2023;9(11).
18. Munteanu IG, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci. 2021;22(7):3380.
19. Luís Â, Duarte AP, Pereira L, Domingues F. Interactions between the major bioactive polyphenols of berries: effects on antioxidant properties. European Food Research and Technology. 2017;244(1):175–185.
20. Nwachukwu ID, Sarteshnizi RA, Udenigwe CC, Aluko RE. A Concise Review of Current In Vitro Chemical and Cell-Based Antioxidant Assay Methods. Molecules. 2021; 26(16):4865.