

The effect of green tea and *Moringa oleifera* tea brewing on lipid profiles in overweight and obese subject: a clinical trial

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Abstract

Obesity and overweight significantly increase the risk of dyslipidemia and chronic diseases, such as coronary heart disease, diabetes mellitus, and hypertension. Previous studies have highlighted the benefits of green tea, made from tender shoots and leaves of *Camellia sinensis*, and *Moringa oleifera* tea brewing in improving fat metabolism, fasting blood glucose, and HbA1c levels. Although various studies have investigated the individual effects of green tea (GT) and *M. oleifera* tea (MT) on lipid profiles, evidence of their combined effects remains limited to animal studies. This study aimed to analyze the levels of phytochemicals in GT, MT and combination green tea–*M. oleifera* tea brewing (CTB); evaluate the organoleptic properties of the combination; analyze the efficacy of the products in improving the lipid profile in overweight/obese individuals. In a four-week intervention trial, 40 overweight/obese individuals were divided into four groups based on the intervention given: (a) GT, (b) MT, (c) CTB, and (d) a control group. Bioactive analysis showed that CTB had significantly higher antioxidant capacity compared to GT and MT, while total phenolic content was higher in GT and CTB compared to MT. The best organoleptic results were observed in formulation F5 (50% green tea and 50% *M. oleifera* tea), which was used for the intervention, along with the other groups. The 4-week intervention indicated that GT, MT, and CTB may influence lipid profile improvement. However, the differences between the groups were not statistically significant. In conclusion, CTB enhanced antioxidant activity, but no significant improvement in lipid profile was observed across the groups.

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Introduction

Overweight and obesity have become serious global health issues that increase the risk of chronic illnesses, including dyslipidemia^[1]. Dyslipidemia is characterized by decreased levels of high-density lipoprotein (HDL) and increased levels of triglyceride, total cholesterol, and low-density lipoprotein (LDL)^[2]. Obesity, high blood pressure, and dyslipidemia are the three primary risk factors for cardiovascular disease. Dyslipidemia, a major cause of morbidity and death and an epidemic that is expanding globally, is caused by its role in atherosclerosis, which is the constriction of blood vessels caused by cholesterol plaques^[3]. According to the 2018 Basic Health Research (Riset Kesehatan Dasar)^[4], 27.9% of Indonesians aged 15 years and older had triglyceride levels ≥ 150 mg/dL, and 28.8% had cholesterol levels over the normal threshold of ≥ 200 mg/dL. Furthermore, data from the Institute for Health Metrics and Evaluation^[5] indicate that the disease burden associated with elevated low-density lipoprotein cholesterol has increased by 185.7 per 100,000 population in Disability-Adjusted Life Years (DALYs) from 2011 to 2021. These results show that the prevalence of dyslipidemia in Indonesia has increased significantly. A high-fat diet is an example of a bad lifestyle choice that is usually overlooked until it causes cardiovascular problems^[6]. One alternative that offers potential for managing dyslipidemia and other cardiovascular risk factors is *M. oleifera* and green tea. Their rich phytochemical profiles contribute to their medicinal properties, making them attractive options for those seeking natural health management approaches^[7,8].

Green tea (GT), made from tender shoots and leaves of *Camellia sinensis*, and *M. oleifera* tea (MT) are believed to have functional

capabilities through their antioxidant activity. Catechins in tea, especially epigallocatechin gallate (EGCG), have been shown to lower total cholesterol, triglycerides, and LDL-C, and reduce oxidative stress in moderate smokers^[9,10]. Previous studies have shown the potential of *M. oleifera* leaf plants to improve lipid profiles, lower blood glucose levels, and stabilize the blood pressure. The ethanol extract of *M. oleifera* leaves reduced the expression of adipogenesis-related genes and decreased triglyceride accumulation^[11], and clinical trials in animals and humans have shown that *M. oleifera* consumption can reduce blood pressure^[12].

In this study, the combination of functional food ingredients, which are GT and MT, is expected to increase their role as antioxidants. EGCG in green tea is relatively stable in heat, whereas the antioxidant properties of *M. oleifera* have the potential to protect against oxidative damage caused by free radicals, sunlight, oxygen, and heat^[13,14]. Previous animal studies have shown that combination green tea–*M. oleifera* brewing (CTB) can reduce plasma triglyceride and total cholesterol levels compared to GT or MT^[15–17]. Despite these promising findings, human clinical studies exploring the combined effects of CTB are limited, and the lack of research emphasizes the importance of scientific inquiry and the necessity for further studies. Therefore, this study aimed to analyze the bioactive compounds of GT and MT and evaluate the potential synergistic effects of their combination on lipid profiles.

Materials and methods

The materials used in this study were GT and MT, purchased commercially from the Tea and Kina Research Center in Bandung, Indonesia, and the Center for Medicinal and Aromatic Research

(Balitro) in Bogor, Indonesia. The samples were divided into tea bags with several formulations: F1 (4 g GT), F2 (4 g MT), F3 (2.8 g GT and 1.2 g MT), F4 (2.8 g MT and 1.2 g GT), and F5 (2 g GT and 2 g MT). The tea bags were then packed into sealed plastic clips and stored dry at room temperature of less than 30 °C until the samples were needed for consumption or analysis.

Sample preparation

Antioxidant and total phenolic analysis: Tea from various formulations was brewed with distilled water at a ratio (1:10) or 4 g of tea in 40 mL of distilled water for 6 min, as described by Sudaryat et al.^[18]. Samples F1, F3, F4, and F5 were brewed using distilled water at 85 °C, and sample F2 was brewed using distilled water at 70 °C. The brewing temperatures of the GT and CTB were obtained from Saklar et al.^[19]. MT temperature refers to the research of Prasetya et al.^[20].

The examination of EGCG and catechin required drying teas of diverse formulations in a vacuum oven at 40 °C until a minimum moisture content of 10% was reached. The sample was subsequently processed to a uniform 60 mesh size. 5 g of the material was brewed during the optimization phase using 200 mL of boiling water as the solvent for 60 min. The brewed solution was filtered using a 0.45 µm Whatman micro membrane filter before being injected into the HPLC devices.

Antioxidant activity

DPPH radical-scavenging activity

The antioxidant capacities of various GT and MT samples were measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) method, as described by Molyneux^[21]. Tea samples brewed in a ratio of 1:10 (4 g in 40 mL of hot water) were diluted to various concentrations (6.25–200 ppm), and vitamin C standard curves with the same concentration series were prepared. The sample and vitamin C solutions were then placed in a test tube with a volume of 1 mL. Each sample received 1 mL of 0.2 mM DPPH solution. The samples were vortexed and incubated at room temperature in the dark for 30 min. The absorbance of the solution was then read on a spectrophotometer at a wavelength of 516 nm. Antioxidant activity was calculated and expressed as equivalents of vitamin C, referred to as Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) (mg AEAC/100 g material).

Total Phenolic Folin-Ciocalteu method

The total phenolic approach employed pertains to the study conducted by Musci and Yao^[22]. One hundred microliters of tea brew or gallic acid standard (ranging from 100 to 500 µL) were pipetted into a test tube, followed by the addition of 750 µL of Folin reagent, which had been diluted with distilled water in a 1:1 ratio. After 5 min at room temperature, 3.75 mL of 10% Na₂CO₃ was added, and the mixture was incubated for 30 min at room temperature. The absorbance of the solution was measured at 765 nm using a spectrophotometer in conjunction with a blank sample. Gallic acid was used as the standard, with values expressed in milligrams of gallic acid per gram.

EGCG and catechin analysis

EGCG and catechin levels in the tea samples were determined by injecting the brewed solution into the HPLC instrument Krauner GMBH Germany and Chromagel software version 3.3.2. as a data integrator. The injection volume used was 20 µL at 30 °C under the following conditions: mobile phase H₃PO₄ 0.1%; water : Acetonitrile : Methanol (14:7:3:1 v/v/v/v) pH 4 isocratic with a flow rate of 1.2 mL/min using UV detection at 280 nm with a column of 250 mm × 4.6 mm, 5µm. This analytical method was based on Fernando & Soysa^[23], with modifications.

Hedonic test

The organoleptic test in this study used human senses as an instrument, referring to the research by Atmadja & Yuniarto^[24] and ISO 6658. The organoleptic test was performed by 30 semi-trained panelists. The organoleptic test used was the hedonic test (preference), which included color, aroma, and taste, with a 10 cm linear rating scale^[25]. The scoring was on a scale of 1 to 9, with the provisions of 1 = very strongly dislike, 2 = very dislike, 3 = dislike, 4 = slightly dislike, 5 = neutral, 6 = slightly like, 7 = like, 8 = strongly like, and 9 = very strongly like. The product was recognized by a good level of acceptance if the overall value was more than 4.50 (moderately acceptable).

Study design, sample size, and participants

This study used a clinical trial method with stratified groups, which were divided into several groups: A (GT group), B (MT group), C (CTB group), and D (control group). This study was approved by the Ethical Commission of Dental Medicine Health Research Universitas Airlangga with number 1360/HRECC.FODM/XII/2023. The calculation of the number of subjects in this study refers to the research of Zheng et al.^[26], with a minimum number of subjects of nine and an anticipated dropout rate of 10%, leaving a total of ten subjects needed for each group. 40 subjects were included in the four experimental groups of 40 people.

This study was conducted on IPB University students with the following inclusion criteria: 18–45 years old, total cholesterol value ≥ 160 mg/dL to ≤ 240 mg/dL, body mass index ≥ 23 kg/m², and blood pressure < 139/89 mmHg. The exclusion criteria for this study were those who consumed anti-cholesterol medications or medicinal products that influence metabolism, patients with diabetes mellitus, hypertension, and other metabolic syndrome conditions. Informed consent was obtained from each subject before starting the research procedure.

The intervention stage began with screening by distributing flyers on social media (Twitter, Instagram, and WhatsApp). A total of 151 prospective subjects of green tea and *M. oleifera* intervention filled out the Google form provided as registration media, and 96 prospective subjects met the criteria of respondents who had a body mass index ≥ 23 kg/m², had an age range of 18–45 years, and were undergraduate and postgraduate students of IPB Dramaga. The total cholesterol levels of 71 respondents were examined using a finger-prick test. As a result, 58 subjects met the inclusion criteria for total cholesterol levels in the range of 160–240 mg/dL. After determining the research schedule, 40 participants (31 women and nine men) agreed to participate. These 40 subjects subsequently underwent venous blood sampling for a complete lipid profile analysis and were categorized into 33 subjects with dyslipidemia or meeting at least one or more lipid profile values with indicators of total cholesterol ≥ 200 mg/dL, LDL ≥ 130 mg/dL, HDL < 40 mg/dL (men) < 50 mg/dL (women), and triglycerides ≥ 150 mg/dL, and the other seven subjects were categorized as subjects at risk of dyslipidemia, with total cholesterol values ≥ 160 mg/dL and LDL ≥ 100 mg/dL^[27,28]. Subjects were then stratified based on sex, age, body mass index, and total cholesterol level into four intervention groups: GT, MT, CTB, and control.

The intervention in this study was carried out for four weeks. In the first week, all groups (GT, MT, CTB, and control) received education on the Balanced Nutrition Guidelines. This education included messages to consume a diverse diet consisting of adequate amounts of carbohydrates, proteins, fats, vitamins, and minerals, engage in regular physical activity for at least 30 min per day, maintain an ideal body weight, and practice clean and healthy behavior. In addition, subjects were recommended to consume a

minimum of eight glasses of mineral water per day to maintain body hydration and limit their intake of sugar, salt, and fat. The recommended daily consumption limits were a maximum of 50 g of sugar, a maximum of 5 g of salt, and a maximum of 67 g of fat. Food consumption guidelines were also adjusted to the principle of 'My Plate Guideline', which consists of 1/3 of the plate filled with protein sources, 1/3 of the plate filled with fruit, 2/3 of the plate filled with staple foods, and 2/3 of the plate filled with vegetables.

Besides nutrition education, subjects in the GT, MT, and CTB groups were required to consume one tea bag brewed in 200 mL of hot water for 3 min, once a day, 2 h after meals. The GT group consumed 4 g of green tea, the MT group consumed 4 g of *M. oleifera* leaves, and the CTB group consumed a combination of 2 g of green tea and 2 g of *M. oleifera* leaves brewed in 200 mL of hot water. The CTB formulation (F5: 2 g green tea and 2 g *M. oleifera* leaves) was selected based on the preferred composition determined from the hedonic test. Meanwhile, the control group only received nutrition education in the first week without any additional intervention.

Blood samples were collected twice, before the intervention started and after four weeks of intervention. Subjects were also asked to conduct food intake interviews (2 × 24 h food recall) and physical activity interviews four times, two times at week zero (1 × weekday and 1 × weekends) and two times at week three (1 × weekday and 1 × weekends). Samples were distributed daily for four

weeks. Respondents were advised to avoid consumption of health supplements and were encouraged to maintain a balanced nutritional diet to prevent confounding factors during the intervention process. Figure 1 illustrates the stages of this study.

Lipid profile measurement

Lipid profile analysis began with a finger-prick test (Easy Touch) during the recruitment process to measure total cholesterol. Participants with total cholesterol between 160–240 mg/dL were eligible for the study and proceeded to venous blood sampling. Venous blood samples (6 mL) were collected at the beginning and end of the study. Plasma lipid profiles, such as triglycerides, total cholesterol, and HDL, were analyzed using an Elitech reagent kit, and absorbance was measured at $\lambda = 500$ nm using a Selectra Pro M Spectrophotometer (ELITechGroup, Puteaux, France). LDL levels were calculated using the Friedewald formula: $LDL = TC - (HDL + 0.2 \text{ TG})$. The lipid profile was analyzed at Bogor Health District Clinical Laboratory.

Statistical analysis

Data analysis was performed using Microsoft Excel and Statistical Package for the Social Sciences (SPSS). Bioactive compounds, including antioxidant capacity and total phenolic, were replicated three times. Data normality and homogeneity analyses were performed at the beginning of the study. Subject characteristics, including sex, age, and anthropometry, were analyzed using the chi-square test at

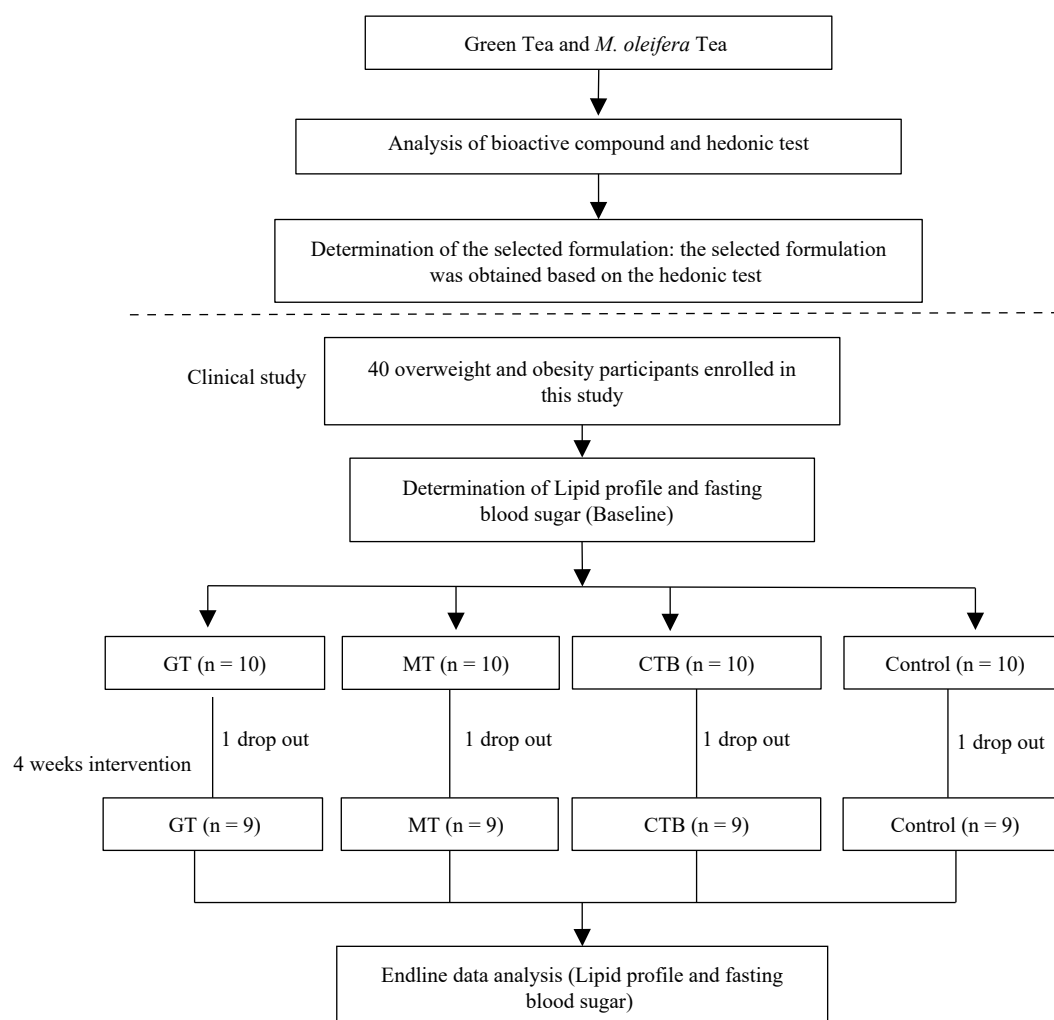


Fig. 1 Study phases of the GT (Green tea), MT (*M. oleifera* tea), CTB (combination green tea–*M. oleifera* brewing), and control groups.

a significance level of $p \leq 0.05$. One-way ANOVA was conducted to analyze the data on lipid profiles, organoleptic tests, and antioxidant capacity at a significance level of $p < 0.05$. Wilcoxon analysis was used to evaluate the consumption patterns and physical activity levels (PAL).

Results

The results of catechin analysis in the table below (Table 1) show a range of catechin concentrations from 0.081% to 0.282%, characterized by the highest catechin content in sample F5 (0.282% w/w), followed by samples F3 (0.245% w/w) and F1 (0.242% w/w). The lowest catechin content was observed in F2 (0.081% w/w). The highest concentration of EGCG was found in sample F1 (2.387% w/w) and the lowest in sample F5 (0.078% w/w). Consistent with the EGCG levels, F1 had the highest total phenolic content, followed by the highest proportion of green tea, which was $F3 > F5 > F4 > F2$.

Antioxidant capacity

The antioxidant properties of GT, MT, and CTB were assessed due to the high antioxidant capacity and total phenolic content of green tea and, related to their significant levels of ascorbic acid, quercetin, and catechin[29,30].

The results of antioxidant capacity analysis above were replicated three times where F1 (4 g green tea), F2 (4 g *M. oleifera*), F3 (2.8 g green tea, 1.2 g *M. oleifera*), F4 (1.2 g green tea, 2.8 g *M. oleifera*), F5 (2 g green tea, 2 g *M. oleifera*) (Table 2).

Hedonic test

Hedonic value or level of preference for CTB was intended to measure the score of respondents' favorite combination tea-*M. oleifera* drinks for new products that have not been found in the market. Hedonic values are shown in Table 3.

Hedonic test scores from the organoleptic evaluation of CTB in this study showed a significant difference in the variables of color and aroma, while no significant differences were observed in taste and overall acceptance.

Effects of consumption of green tea and *M. oleifera* tea brewing on lipid in overweight and obese subject

There were no significant differences among the groups in terms of age, sex, anthropometric measurements, or baseline lipid profiles. Lipid profile changes including total cholesterol, triglycerides, HDL-C, LDL-C in all treatments showed no significant difference ($p > 0.05$). Details of the subjects characteristics were presented in Table 4.

These non-significant results do not necessarily indicate that there is no improvement in lipid profiles in this study. Several factors may have influenced the outcomes, including large standard deviation, dietary intake and physical activity levels among subjects. The changes observed in the GT, MT, CTB, and control groups are presented in Table 5.

The analysis indicated no significant differences between the groups ($p > 0.05$). Although there was some improvement in lipid profile values in some groups, the results also showed that CTB may not be more effective in reducing lipid profiles when compared to the GT intervention group. The consumption patterns in this study may have influenced the responses of the various tea interventions and control groups to the lipid profile levels. Table 6 presents data on subjects' nutrient adequacy compared to the 2019 Recommended Dietary Allowance (RDA) during weeks zero and three of the intervention.

Table 1. Catechin, EGCG and total phenolic of difference tea brewing.

Tea	Catechin % (w/w)*	EGCG % (w/w)*	Total phenolic content (mg GAE/g)**
F1 (4 g green tea)	0.242	2.387	1.66
F2 (4 g <i>M. oleifera</i> tea)	0.081	0.424	0.35
F3 (2.8 g green tea, 1.2 g <i>M. oleifera</i>)	0.245	0.160	1.10
F4 (1.2 g green tea, 2.8 g <i>M. oleifera</i>)	0.227	0.088	0.67
F5 (2g green tea, 2g <i>M. oleifera</i>)	0.282	0.078	0.94

*% catechin and EGCG were expressed as % (w/w) of dry weight. **mg of Gallic Acid Equivalent (GAE) per g (dry weight).

Table 2. Antioxidant capacity and total phenolic tea (mg AEAC/g) of GT, MT and CTB.

Tea	Antioxidant capacity (mg AEAC/g)**	DPPH radical scavenging activity (% inhibition)
F1 (4 g green tea)	15.75 ± 0.89 ^a	58.54 ± 1.16 ^a
F2 (4 g <i>M. oleifera</i> tea)	15.50 ± 0.89 ^a	58.90 ± 1.10 ^a
F3 (2.8 g green tea, 1.2 g <i>M. oleifera</i>)	18.70 ± 0.45 ^b	63.27 ± 0.43 ^b
F4 (1.2 g green tea, 2.8 g <i>M. oleifera</i>)	22.98 ± 2.58 ^c	68.00 ± 3.39 ^c
F5 (2g green tea, 2 g <i>M. oleifera</i>)	18.07 ± 1.53 ^{ab}	62.28 ± 1.09 ^b
p-value	0.001	0.000

**mg of Antioxidant Equivalent Activity Concentration (AEAC) per g dry weight. Values with different letters in the same column were significantly different ($p < 0.05$).

Table 3. Hedonic score values of CTB with various concentrations.

Hedonic test	Formula			p-value
	F3	F4	F5	
Color	7.35 ± 1.60 ^b	4.44 ± 2.53 ^a	7.36 ± 1.29 ^b	0.000*
Aroma	6.49 ± 1.68 ^b	5.47 ± 1.92 ^a	6.53 ± 1.48 ^b	0.028*
Taste	4.11 ± 2.66 ^a	4.99 ± 2.36 ^a	5.14 ± 2.14 ^a	0.207
Overall	5.71 ± 2.07 ^a	5.43 ± 2.08 ^a	5.96 ± 1.48 ^a	0.569

Different superscript letters in the same row indicate statistically significant differences $p < 0.05$. * significant at $p < 0.05$

Discussion

Different formulations of green tea, *M. oleifera* tea brewing, and a combination of tea-*M. oleifera* affect antioxidant capacity and total phenolic compounds

Numerous studies have indicated that the antioxidant concentrations in vegetables, fruits, and grains are comparable. *M. oleifera* is acclaimed for its antioxidants, total phenolic compounds, and flavonoids[30–33]. In addition to *M. oleifera*, green tea possesses a distinct nutritional profile of polyphenols, including a mixture of polyhydroxy phenolic compounds, which include flavonols, flavones, catechins (epicatechin-gallate, epigallocatechin, and epigallocatechin gallate), and anthocyanidins[34].

The total phenolic analysis of various tea formulations showed the highest result in the composition of F1 (4 g green tea), followed by the composition of CTB with a higher proportion of green tea (Table 1). This was because green tea has catechins and other gallic compounds as the main phenolic compounds, which contributed 30% of the dry weight of green tea[35]. Overall, the total phenolic values of GT, MT and CTB were in the range of 0.35 to 1.66 mg/GAE/g or equivalent to 0.35–1.66 g/GAE/kg. Consistent with the results of Kopjar et al.[36], the total phenolic of green tea, yellow tea, and black tea ranged from 0.575 to 6.629 g/kg. When

Table 4. Baseline anthropometric and lipid profile characteristics of overweight and obese subjects in the four groups.

	Variable/parameter	Control (n = 9)	GT (n = 9)	MT (n = 9)	CTB (n = 9)	p-value
Sex	Males	2	2	2	3	0.931
	Females	7	7	7	6	
Age (years)	19–29	7	8	9	7	0.623
	30–45	2	1	0	2	
Anthropometric (kg/m ²)	Overweight (23–22.9)	2	3	0	0	0.370
	Obese I (25–29.9)	5	2	7	6	
	Obese II (≥ 30)	2	4	2	3	
TC (mg/dL)	Mean ± SD	184.7 ± 25.5 ^a	200.4 ± 28.6 ^a	203.1 ± 19.6 ^a	188.0 ± 27.0 ^a	0.350
TG (mg/dL)	Mean ± SD	110.8 ± 19.8 ^a	90.3 ± 31.8 ^a	106.5 ± 31.9 ^a	87.6 ± 13.2 ^a	0.158
LDL-C (mg/dL)	Mean ± SD	117.6 ± 22.3 ^a	134.6 ± 22.3 ^a	123.3 ± 36.3 ^a	116.7 ± 38.8 ^a	0.596
HDL-C (mg/dL)	Mean ± SD	44.7 ± 7.5 ^a	47.6 ± 14.6 ^a	56.7 ± 18.3 ^a	46.0 ± 5.6 ^a	0.194

TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol. Different superscript letters in the same row indicate statistically significant differences $p < 0.05$.

Table 5. Changes in lipid profiles of overweight and obese subjects after four weeks of green tea and *M. oleifera* tea brewing intervention.

Lipid Profile	Control (n = 9)	GT (n = 9)	MT (n = 9)	CTB (n = 9)	p-value
TC (mg/dL)	4.3 ± 17.5 ^a	−11.6 ± 25.9 ^a	−11.8 ± 22.1 ^a	5.8 ± 22.4 ^a	0.178
TG (mg/dL)	−8.4 ± 24.3 ^a	2.4 ± 11.7 ^a	4.3 ± 35.5 ^a	−3.3 ± 27.8 ^a	0.728
LDL-C (mg/dL)	3.0 ± 15.5 ^a	−16.4 ± 18.62 ^a	−3.5 ± 33.8 ^a	14.5 ± 36.83 ^a	0.141
HDL-C (mg/dL)	3.2 ± 9.9 ^a	4.2 ± 13.2 ^a	−7.7 ± 20.7 ^a	−0.2 ± 6.59 ^a	0.255

The differences in lipid profile levels before and after the intervention were analyzed using a one-way ANOVA with a significance level of $p < 0.05$. TC: Total cholesterol; TG: Triglycerides; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol.

Table 6. Subjects' nutrient adequacy percentages based on the Recommended Dietary Allowance (RDA).

Mean ± SD (min-max)	Week 0	Week 3	p-value
Energy (%)	69.63 ± 23.23 (39.64–132.28)	69.42 ± 25.61 (24.91–135.91)	0.561
Protein (%)	83.03 ± 31.16 (37.58–155.06)	78.67 ± 29.02 (38.10–159.15)	0.753
Fat (%)	80.76 ± 33.70 (28.56–182.43)	116.01 ± 43.05 (50.81–244.85)	0.000*
Carbohydrate (%)	68.75 ± 34.25 (19.82–178.75)	60.87 ± 24.90 (18.19–121.57)	0.069
PAL**	1.54 ± 0.16 (1.34–2.00)	1.55 ± 0.19 (1.24–2.42)	0.749

The interventions were analyzed using the WILCOXON test with a significance level of $p < 0.05$. * Significant at $p < 0.05$. ** PAL: Physical Activity Level.

compared with the total phenolics of green tea from Malang, East Java, Indonesia, which ranges from 0.21 to 0.25 mg GAE/mg^[37], the total phenolic compounds in this study are considered as superior.

The EGCG profile in green tea is higher when compared to non-esterified catechins (C). These results are in line with the research of Kim et al.^[38], and Paiva et al.^[39], which showed that the levels of non-esterified catechins GC (gallocatechin), EGC (epigallocatechin), C (catechin) are lower when compared to EGCG. However, the overall profile of EGCG and catechins is still quite low when compared to other conventional green tea sources, which amounted to 7.64 mg/100 mg and 0.77 mg/100 g^[38]. EGCG and catechin values are influenced by several factors, such as different environmental conditions, temperature, weather, and humidity during the planting period, which affect the quality of tea, especially catechin compounds^[40].

The combination of two types of bioactive compounds can be synergistic, additive or antagonistic^[41]. The increased antioxidant capacity of CTB (Table 2) should be attributed to the synergistic effect of the two groups of bioactive compounds present in the combination. Consistent with the study by Shokery et al.^[42], the addition of green tea to yogurt products increased antioxidant activity due to the synergistic effect between bioactive compounds. In general, the antioxidant capacity of CTB is dominated by polyphenolic compounds. *M. oleifera* leaves also have quercetin, kaempferol, ascorbic acid, carotene, isothiocyanate, and cryptochlorogenic acid compounds that can affect the increase in antioxidant capacity^[43,44]. However, it remains important to highlight that antioxidant activity

in this study was assessed using *in vitro* methods, which may not fully reflect the complex bioavailability, metabolism, and physiological interactions that occur in the human body. The functional health advantages of the tested formulations need to be confirmed by additional studies using *in vivo* analysis, as *in vivo* antioxidant testing is thought to be more indicative of actual biological effects.

Organoleptic test - hedonic response of combination green tea–*M. oleifera* brewing (CTB)

The organoleptic results of the CTB formulations showed significant differences in the hedonic attributes of color and aroma, with formulations F3 (2.8 g GT and 1.2 g MT) and F5 (2 g GT and 2 g MT) being preferred (Table 3). This preference may be attributed to the presence of terpenoids and amino acid-derived volatile compounds that enhance the aroma of green tea^[45]. However, no significant differences were found in flavor attributes and overall evaluation. Formulation F5 was selected as the most favorable overall in comparison with the other formulations; however, additional work is required to enhance the flavor quality to align with overall consumer preferences.

Effect of green tea brewing (GT), *M. oleifera* tea brewing (MT), and their combination-tea *M. oleifera* brewing (CTB) on lipid profile

Lipid profile values, including total cholesterol, triglycerides, HDL-C, and LDL-C levels, showed no significant effects in any of the experimental groups ($p > 0.05$). Several factors may influence the effects of GT and MT. Based on previous research, many

confounding factors that affect the results of clinical trials in tea research, such as lifestyle, dietary habits, ethnicity, genetic effects, and caffeine intake habits, contribute to the inconsistent results^[46]. Increasing the daily dose of tea consumption, randomizing the group in the study, and implementing a longer duration of tea intervention are considered necessary to obtain better and more significant lipid profile values^[47]. A meta-analysis by Momose et al.^[48] reported that improvements in lipid profiles can be achieved within an intervention period of three weeks to three months, with greater reductions observed in individuals with higher baseline LDL levels and BMI. These findings indicate that although the intervention duration in the present study was relatively short, extending the intervention period, particularly in individuals with a higher lipid profile, may enhance the lipid-lowering effects.

The non-significant results in this study may also be influenced by high standard deviation values, showing diversity with a wide range of standard deviations in line with the study of Lee et al.^[49], who showed high standard deviation values in the effect of nut intervention, which was not significant in improving the blood lipid profile. In addition to the high standard deviation value factor, based on 2×24 h food recall observation data, there was an increase in fat consumption in week three, which was significantly different ($p < 0.05$) (Table 6) when compared to week zero. Increased fat consumption may contribute to higher cholesterol levels and decreased HDL levels. Consistent with Wali et al.^[50], consumption of fat, especially saturated fatty acids such as those found in many dairy products, butter, cheese, and others, may contribute to increased LDL cholesterol levels and decreased HDL cholesterol levels. Increasing the daily dose of tea consumption, randomizing the groups in the study, and a longer duration of tea intervention are considered necessary to obtain better and more significant lipid profile values^[47]. Linear regression results between Body Mass Index (BMI) and lipid profile variables showed a positive correlation, especially cholesterol with BMI ($p = 0.018$), triglycerides with BMI ($p = 0.037$), and HDL with BMI ($p = 0.005$) (data not shown) in subjects with higher BMI in the GT group. Meanwhile, the results of the linear regression between the CTB and MT groups on BMI were not significantly different. This may prove that the consumption of CTB was not more beneficial than the consumption of GT in improving the lipid profile.

In this study, total cholesterol and LDL in both GT (green tea) and MT (*M. oleifera* tea) groups decreased although they did not reach significant levels. However, combined tea brewing (CTB) reduced triglyceride levels. Green tea epicatechin reduces total cholesterol by inhibiting the SCAP/SRAB-1 (Sterol Regulatory Element-Binding Protein-1) pathway, which is a critical regulator of cholesterol and fatty acid synthesis, thereby lowering cholesterol production and hepatic cholesterol synthesis^[51]. Meanwhile, *M. oleifera* inhibits HMG-CoA reductase activity, contributing to lower total cholesterol and LDL levels^[52]. The CTB group had lower triglyceride levels without a reduction in total cholesterol or LDL levels. This could be due to the synergistic actions of EGCG from green tea and isothiocyanate from *M. oleifera*, which both activate the lipolytic enzyme ATGL. According to Gigih et al.^[53], isothiocyanate inhibits lipogenic proteins (FAS, SREBP1, and FSP27), reducing fat synthesis and storage while enhancing ATGL activity, which aids in the breakdown of triglycerides into fatty acids and glycerol for energy use. EGCG has a lipolytic effect by enhancing lipophagy and lipolysis while inhibiting lipogenesis^[54]. Martini et al.^[17], who revealed that a combination of white tea and *M. oleifera* significantly lowered triglycerides in animal models compared to single doses of white tea, green tea, or *M. oleifera*, provide additional support for CTB's triglyceride-lowering mechanisms. The study's limitations include weak

randomization methods, a relatively short intervention period, non-specific dietary control, and a small sample size, all of which limit the generalizability of the findings.

Conclusions

The results showed that combination green tea–*M. oleifera* brewing (CTB) had higher antioxidant capacity than green tea (GT) and *M. oleifera* tea (MT). Formulations with a larger amount of green tea had a higher overall phenolic content. CTB was also shown to have beneficial synergistic characteristics in terms of antioxidant capacity. Organoleptic test results showed overall positive acceptance, as evidenced by an overall acceptance score of more than 4.5, suggesting good acceptability. Formulation F5 (2 g GT and 2 g MT) was the most popular among the subjects. The intervention results revealed no significant differences in lipid profile improvement across the GT, MT, CTB, and control groups. CTB did not exhibit any specific characteristics compared to GT or MT in terms of lipid profile improvement. Other metabolite profiles, including oxidative stress, should be examined in the future with higher dosages and extended intervention periods, particularly in populations with elevated LDL and BMI. Additionally, investigating potential ethnic variations may provide further insights into lipid profile modulation.

Author contributions

The authors confirm their contributions to the paper as follows: study conception and design: Rimbawan R, Dewi M; data collection: Alawiyah YS; analysis and interpretation of results: Rimbawan R, Dewi M, Alawiyah YS; draft manuscript preparation: Rimbawan R, Alawiyah YS. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data that support the findings of this study are available on request from the corresponding author.

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Conflict of interest

The authors declare that they have no conflict of interest.

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