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Antihyperlipidemic and antioxidant activities of "mimicking honey" extract from Basil (*Ocimum americanum* L.) stem waste

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ABSTRACT: Basil (*Ocimum americanum* L.) has broad pharmacological activity related to its phenolic content. This compound is a natural antioxidant, which also plays a role in reducing lipid profiles. Phenolics can be extracted with a "mimicking honey" (one type of the natural deep eutectic solvents, NADES) as an alternative solvent, that is greener than common volatile organic solvents, such as ethanol. This study aimed to evaluate the antihyperlipidemic and antioxidant activities of basil stem waste extract prepared using a mimicking honey solvent formulated from water, sucrose, glucose, and fructose. Extraction was conducted via ultrasonic-assisted methods. An *in vivo* test was performed on male Wistar rats induced with hyperlipidemia using quail egg yolk and propylthiouracil. The animals were divided into eight groups: normal control (no treatment), positive control (atorvastatin), mimicking honey solvent (MHS), honey solvent (HS), water solvent (WS), basil stem mimicking honey extract (MHE), basil stem honey extract (HE), and basil stem water extract (WE). Measured parameters included total cholesterol (TC), triglycerides (TG), and malondialdehyde (MDA) levels. Results indicated that the MHE group showed significantly different levels of TC (123.00 ± 12.85 mg/dL; P <0.01), TG (85.25 ± 22.29 mg/dL; P <0.001), and MDA (8.10 ± 0.20 nmol/mL; P <0.0001) compared to the WS group. These findings suggest that synthetic honey solvent is an effective "green solvent" for extracting bioactive compounds from basil stem, offering potential antihyperlipidemic and antioxidant benefits.

KEYWORDS: Antihyperlipidemic; antioxidant; green solvents; *Ocimum americanum* L.; phenolic.

INTRODUCTION

Hyperlipidemia is a chronic metabolic disorder characterized by elevated levels of cholesterol and triglycerides in the blood, exceeding normal physiological limits. This condition significantly increases the risk of cardiovascular complications, including atherosclerosis and hypertension [1]. According to data from the World Health Organization (WHO), hypercholesterolemia is responsible for approximately 2.6 million deaths and causes disability in around 29.7 million individuals annually. In Indonesia, the National Health Survey reports that 39.5% of the population suffers from hypercholesterolemia, while 18.9% experience hypertriglyceridemia [2]. The conventional approach to treating hyperlipidemia typically involves pharmacological interventions using synthetic drugs. However, the prevalence of adverse effects such as myopathy, liver damage, gout, and increased bleeding risk has prompted a growing interest in alternative therapies based on natural products. Among the plants widely utilized in traditional medicine across various regions of the world are species from the *Ocimum* genus [3]–[5].

Members of the *Ocimum* genus belong to the *Lamiaceae* family and are well-known for their strong aromatic properties [6]. One widely distributed species, *Ocimum americanum* L., commonly referred to as kemangi or white basil in Indonesia, can also be found in the United Kingdom, India, Africa, and several other countries [7]. This erect, branching herbaceous plant, which grows to a height of 15–60 cm, has

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numerous parts (leaves, flowers, seeds, and entire plant) that have traditionally been used for a range of medicinal purposes, including antioxidant, antihypertensive, antidiabetic, antipyretic, antimalarial, antibacterial treatments, and for managing eye and ear disorders, hemorrhoids, and more [7].

To date, there has been no specific research reporting the hypolipidemic activity of *O. americanum* [7]. Nevertheless, aqueous extracts of *O. americanum* at a dose of 200 mg/kg BW have been shown to significantly (P<0.05) reduce lipid peroxidation (as an antioxidant agent) compared to negative controls [8]. Moreover, previous studies have reported that aqueous extracts from the stems of *O. americanum* L. were capable of reducing malondialdehyde (MDA) levels by as much as 77.5% in rats with hepatotoxicity-induced conditions [9]. The *O. americanum* L. plant contains a diverse array of phytochemical compounds, including phenolics, flavonoids, tannins, steroids, and terpenoids [7]. Among these, polyphenolic compounds exert antihyperlipidemic effects by inhibiting the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [10], [11]. Tannins contribute to cholesterol reduction by enhancing the metabolic conversion of cholesterol into bile acids and promoting their excretion through feces [10]. In addition, steroid and terpenoid compounds have been reported to lower LDL and VLDL levels, increase the expression of LDL receptors, and regulate the activity of lipoprotein lipase, thereby facilitating triglyceride breakdown and maintaining a healthy balance between HDL and LDL cholesterol [12]. Furthermore, phenolic and tannin compounds demonstrate antioxidant activity by chelating metal ions and thereby inhibiting oxidative enzymatic activity [13], [14]. Saponins and triterpenoids can also generate hydroperoxides and superoxide anions, both of which are reactive oxygen species (ROS), contributing to their antioxidant roles [15].

Traditionally, the extraction of phytochemicals from *O. americanum* has relied on the use of organic solvents. However, these solvents are typically volatile and toxic, posing both health and environmental hazards [16]. As an environmentally friendly alternative, natural deep eutectic solvents (NADES) have emerged as a green solvent option. NADES are non-volatile, non-toxic, and advantageous in pharmaceutical formulation development due to their safety and practicality [17].

The efficacy of NADES in extracting various phytochemicals has been demonstrated in numerous studies. These include the extraction of polyphenols from *Pluchea indica* (L.) Less. leaves [18], saponins from *Polygonatum sibiricum* [19], triterpenoid saponins from *Aralia elata var. mandshurica (Rupr. & Maxim.) J. Wen* [20], and both alkaloids and phenolics from *Peumus boldus* [21]. The compound daidzin (a type of polyphenol) has been successfully extracted from the roots of *Pueraria candollei var. mirifica* using a sugar-based NADES solvent composed of water:sucrose:glucose:fructose (in a weight ratio of 18:3:18:22) [22]. This method yielded a daidzin concentration of 75.8±3.67 µg/mL, which was significantly higher than that obtained using honey-based NADES (27.4±1.88 µg/mL), conventional organic solvents such as absolute ethanol (9.31±0.663 µg/mL), water (3.44±0.409 µg/mL), and hydroethanolic solution (66.1 ± 8.08 µg/mL) [22].

Despite the promising extraction efficiency of sugar-based NADES, the antihyperlipidemic and antioxidant activity of basil (*O. americanum* L.) stem extract obtained using a mimicking honey NADES has not yet been reported. Therefore, the objective of this study is to evaluate the in vivo antihyperlipidemic and antioxidant potential of basil stem extract prepared using a MHS (comprising water, sucrose, glucose, and fructose), and to compare its efficacy against extracts prepared using honey and aqueous solvents in hyperlipidemic rat models.

▪ MATERIALS AND METHODS

Plant material preparation

Samples of *O. americanum* L. were collected from Pasar Bayam, Babelan District, Bekasi Regency, Indonesia. Botanical identification was conducted at the Laboratory of Plant Taxonomy, Structure, and Development, Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia (No. 00776/UN10.F0916/B/TA.00.02.3/2024). The basil stems were subjected to wet sorting, which involved the removal of foreign matter and plant parts other than the stems. The selected stems were then thoroughly washed and air-dried at room temperature (27±1 °C) for approximately seven days. Once dried, the stems were ground into a fine powder and sieved using a No. 40 mesh. The dried

powder was stored in an airtight container at room temperature until further extraction procedures were carried out.

Preparation of MHS and honey as solvent extraction

The MHS was formulated using a composition of water:sucrose:glucose:fructose (in a weight ratio of 18:3:18:22) [22]. Each component was weighed precisely and mixed in an Erlenmeyer flask. The mixture was then stirred and heated using a magnetic stirrer (Cimarec+™, Thermo Scientific) at 80 °C with a stirring speed of 1000 rpm until a clear and homogenous solution was achieved. The MHS was prepared at a concentration of 50% (v/v) in water, while the honey solvent (HS) used as a comparison, was prepared at a concentration of 20% (v/v) in water [18], [22]. Each solvent was divided into three portions designated for extraction, characterization, and in vivo animal testing. The portion intended for animal testing was further divided into seven separate containers for daily dosing and stored in a -20 °C freezer protected from light exposure [23].

Preparation of extracts

Extraction of *O. americanum* L. stem powder was carried out using three different solvents: MHS, HS, and water solvent (WS). The ratio of plant powder to solvent was 1:20 (b/v), and extraction was performed using an ultrasonic bath at 42 kHz (Branson 5510, Shanghai, China) at a temperature of 40 °C for 60 minutes. Following sonication, the mixture was centrifuged using a Universal Centrifuge (Gemmy PLC-025, Taipei, Taiwan) at 4000 rpm for 20 minutes. The supernatant was then filtered through a glass syringe connected to a filter holder containing Whatman filter paper [18]. The resulting extracts were classified as the basil stem mimicking honey extract (MHE), basil stem honey extract (HE), and basil stem water extract (WE). Each extract was divided into two portions for characterization and animal testing. The portion designated for animal testing was distributed into seven individual containers corresponding to daily doses and stored at -20°C, protected from light exposure [23].

Solvent and extract characterization

Characterization tests were conducted on a portion of the MHS, HS, and the corresponding extracts, basil stem mimicking honey extract (MHE), basil stem honey extract (HE), and basil stem water extract (WE). The tests included organoleptic evaluation (color, appearance, odor, and taste), pH measurement using a benchtop pH meter (OHAUS AQUASEARCHER™ AB33PH-F, China), viscosity measurement using a viscometer (Visco QC300, Anton Paar, Graz, Austria), and density determination using a pycnometer and analytical balance (OHAUS Instruments, China), all performed at room temperature.

Identification of secondary metabolites

Qualitative identification of secondary metabolites, such as phenolics, flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids, was conducted on pure honey (PH), MHS, HS, MHE, HE, and WE. The presence of phenolic compounds was detected using 5% $FeCl_3$ reagent, flavonoids using concentrated HCl and magnesium powder, and alkaloids with Dragendorff, Bouchardat, and Mayer reagents. Tannins were tested using 10% NaCl and 1% gelatin solutions, saponins with hot water and 2N HCl, steroids with a mixture of acetic anhydride and concentrated H_2SO_4, and terpenoids with chloroform and concentrated H_2SO_4 reagents [24], [25].

Evaluation of antihyperlipidemic activity

The experimental protocol in this study was approved by the Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, under ethical approval number: KET-1195/UN2.F1/ETIK/PPM/00.02/2024. The test subjects were 32 male Wistar rats (*Rattus norvegicus*), aged 10 weeks, weighing between 175–200 g, sourced from Kemuning (https://kemuning.co.id). All animals underwent a 7-day acclimatization period with standard food and water before being randomly assigned into eight groups, each consisting of four rats. The groups were as follows: (1) normal control, (2) positive control (atorvastatin 1.2 mg/kg BW, orally), (3) MHS control (0.8 mL/200 g BW, orally), (4) HS control (0.8 mL/200 g BW, orally), (5) WS control (0.8 mL/200 g BW, orally), (6) MHE treatment (0.8 mL/200 g BW, orally), (7) HE treatment (0.8 mL/200 g BW, orally), and (8) WE treatment (0.8 mL/200 g BW, orally). Except for the normal control group, all rats were induced with hyperlipidemia using a mixture of quail egg yolk and propylthiouracil (PTU) at a dose of 1.8 mg/200 g BW and quail egg yolk at 2 mL/200 g BW administered twice daily [26], [27]. The induction was carried out for 28 consecutive days, from day 8 to day

35 of the experiment. On day 36, following an 18-hour fasting period after the final treatment, blood samples were collected via the lateral tail vein for total cholesterol (TC) and triglyceride (TG) analysis using the Lipid Pro test strip method (South Korea) [28]. Additionally, lymph organs were collected for the assessment of lipid peroxidase activity.

Evaluation of antioxidant activity

Lymphatic organs were homogenized in phosphate-buffered saline (PBS) at a 1:9 ratio and centrifuged at 4000 rpm for 10 minutes [29]. Antioxidant activity was assessed using the thiobarbituric acid reactive substances (TBARS) method, which measures malondialdehyde (MDA) levels as an indicator of lipid peroxidation in the lymph of hyperlipidemic rats. A total of 250 µL of the supernatant was reacted with 250 µL of 20% trichloroacetic acid (TCA) and 0.5 mL of 0.67% thiobarbituric acid (TBA), followed by heating at 100°C for 10 minutes. The reaction mixture was then rapidly cooled to approximately 0 °C and centrifuged at 3000 rpm for 10 minutes at 4 °C. Absorbance was measured at a wavelength of 532 nm using a UV-Vis Spectrophotometer (UV-1601 Series, Shimadzu, Kyoto, Japan) [30].

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Prior to analysis, the data were tested for normality and homogeneity. Statistical analysis and graphical presentations were performed using GraphPad Prism 10 software (San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to compare differences among the groups. The different was statistically significant if $P < 0,05$ [31].

▪ RESULTS

Characteristics of solvents and extracts

The organoleptic of solvents and extracts are summarized in **Table 1**. In terms of consistency and odor, all extracts showed similar characteristics; however, differences were observed in taste and color. MHE and HE had a sweet and slightly astringent taste, while WE was distinctly bitter and astringent. In terms of consistency, MHS was slightly more viscous than HS, which was more fluid. Visually, MHS appeared pale yellow, whereas HS had a deeper yellow hue. It suggests that the solvent type influenced extract characteristics.

Table 1. Organoleptic characteristics of solvents and extracts.

Samples	Parameters			
	Color	Consistency	Odor	Taste
MHS	Slightly yellowish clear	Slightly viscous liquid	Odorless	Sweet
HS	Clear yellow	Liquid	Odorless	Sweet
MHE	Clear light brown	Liquid	Distinctive	Slightly astringent sweet
HE	Clear light brown	Liquid	Distinctive	Slightly astringent sweet
WE	Clear light brown	Liquid	Distinctive	Bitter astringent

Notes: MHS (mimicking honey solvent with 50% (v/v) in water concentration), HS (honey solvent with 20% (v/v) in water concentration), MHE (basil stem mimicking honey extract), HE (basil stem honey extract), WE (basil stem water extract).

The pH analysis was conducted to determine the acid-base characteristics of both solvents and extracts, as this property influences the efficiency of compound extraction, particularly polyphenols [32]. The complete results for pH, density, and viscosity are presented in **Table 2**. As shown in **Table 2**, all solvents and extracts exhibited acidic pH values (pH < 7). The highest viscosity was observed in MHE at 5.004 mPa's, followed closely by the solvent MHS at 4.492 mPa's. The elevated viscosity in MHE is likely influenced by the viscous nature of its solvent (MHS), which has a thicker consistency. In contrast, the viscosities of HE and HS were lower, possibly due to a higher proportion of water in their formulation. The lowest viscosity was recorded for WE. A similar pattern was observed for density measurements. MHE exhibited the highest density, while WE had the lowest, closely approximating that of pure water.

Table 2. pH, density, and viscosity of solvents and extracts.

Samples	Parameters		
	pH	Density (g/mL)	Viscosity (mPa's)
MHS	3.64	1.1740	4.492
HS	3.66	1.0762	2.701
MHE	4.80	1.1777	5.004
HE	5.37	1.0842	2.701
WE	6.72	1.0030	1.848

Notes: MHS (mimicking honey solvent with 50% (v/v) in water concentration), HS (honey solvent with 20% (v/v) in water concentration), MHE (basil stem mimicking honey extract), HE (basil stem honey extract), WE (basil stem water extract).

Identification of secondary metabolites

The qualitative identification of secondary metabolites is aimed to identify the presence of secondary metabolite compounds within a sample. This technique is favored due to its simplicity, minimal sample requirements, and the limited use of reagents. The results of the phytochemical screening for various solvents and basil stem extracts are presented in **Table 3**. Based on **Table 3**, phenolic and tannin compounds were detected in PH, HS, HE, MHE, and WE, whereas MHS did not exhibit these constituents. No detection was observed for flavonoids, alkaloids, saponins, steroids, or terpenoids in any of the solvents or extracts.

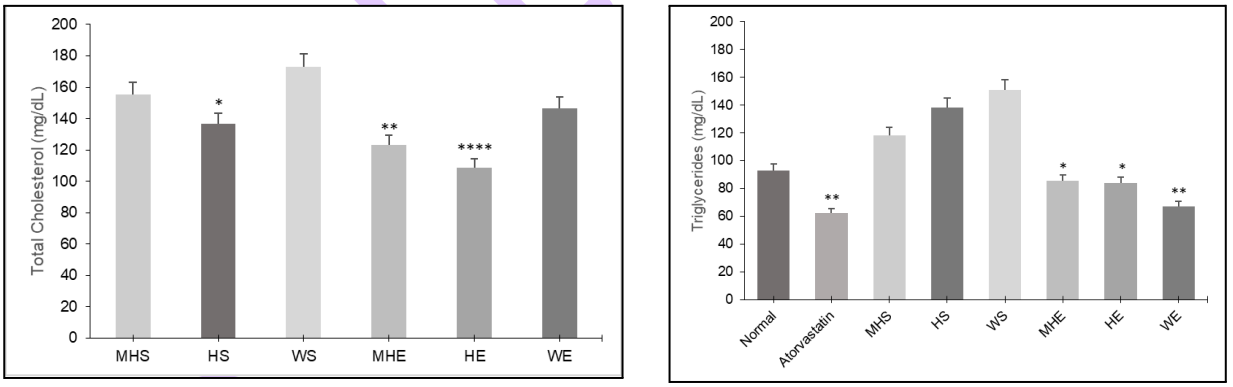
Table 3. Qualitative identification of secondary metabolite content in solvents and extracts.

Class of compounds	Samples					
	PH	HS	MHS	HE	MHE	WE
Phenolics	+	+	-	+	+	+
Flavonoids	-	-	-	-	-	-
Tannins	+	+	-	+	+	+
Alkaloids	-	-	-	-	-	-
Saponins	-	-	-	-	-	-
Steroids	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-

Notes: PH (pure honey), HS (honey solvent with 20% (v/v) in water concentration), MHS (mimicking honey solvent with 50% (v/v) in water concentration), HE (basil stem honey extract), MHE (basil stem mimicking honey extract), WE (basil stem water extract).

Antihyperlipidemic activity

Effect of the extracts on Total Cholesterol (TC) level



a b

Figure 1. Effects of *O. americanum* extracts on total cholesterol (a) and triglycerides levels (b) in hyperlipidemic rats. MHS: mimicking honey solvent with 50% (v/v) in water concentration; HS: honey solvent with 20% (v/v) in water concentration; WS: water solvent; MHE: basil stem mimicking honey extract; HE: basil stem honey extract; WE: basil stem water extract. *significantly different to WS at $P \leq 0.05$; **significantly different to WS at $P \leq 0.01$; ****significantly different to WS at $P \leq 0.0001$.

Measurement of TC levels revealed that both the normal and positive control groups had concentrations below the detection limit of the Lipid Pro test strip (<100 mg/dL), thus making quantification impossible for these groups. The use of the Lipid Pro test strip in this study was based on its efficiency and validity as a lipid monitoring device [33]. Although this instrument has a lower detection limit, it does not diminish the validity of the overall study findings, as the focus of the analysis was on evaluating the efficacy of treatment in groups with high cholesterol levels (above the detection limit). As illustrated in **Figure 1a**, the HE groups

exhibited the most significant reduction in total cholesterol with a mean concentration of 108.75 ± 8.20 mg/dL, statistically different from the WS group at $P \leq 0.0001$. The WS group, which received induction and water only, recorded the highest mean TC level at 172.75±6.94 mg/dL. Because all groups received water, the effect of water as a variable is considered uniform across treatments. The MHE group also demonstrated a significant reduction in TC ($P < 0.01$), with a mean value of 123.0 ± 12.85 mg/dL. Meanwhile, the WE group showed a reduction as well (146.50 ± 5.02 mg/dL), though the difference compared to the WS group was not statistically significant. These findings suggest that the MHS can serve as an effective alternative to real honey in extracting bioactive compounds from basil stems and appears to be more efficient than water alone.

Effect of the extracts on Triglyceride (TG) level

As illustrated in **Figure 1b**, the WE group demonstrated the most pronounced TG lowering effect, with a mean TG level of 67.25±13.24 mg/dL. This was significantly lower than that of the negative control group (WS), which recorded an average TG level of 150.75 ± 28.57 mg/dL ($P \leq 0.01$). The WS group served as the negative control, receiving induction and water only. Since all groups received water, its influence on TG levels is assumed to be consistent across treatments. Statistical analysis showed that the TG-lowering effect of the WE group was comparable to that of atorvastatin, which recorded a mean TG level of 62.5±8.62 mg/dL. Additionally, significant reductions in TG levels compared to the WS group ($P < 0.001$) were also observed in the MHE and HE groups, with mean values of 85.25 ± 22.29 mg/dL and 84.0±21.55 mg/dL, respectively. These findings suggest that MHS is a viable alternative to both pure honey and water as a solvent for extracting bioactive compounds from basil stems with triglyceride-lowering potential in hyperlipidemic rats.

Antioxidant activity

As illustrated in **Figure 2**, the MHE and HE groups exhibited comparable MDA levels, both averaging 9.32±0.11 nmol/mL and 8.49±0.14 nmol/mL, respectively. These values were significantly lower than that of the WS group (14.61±1.44 nmol/mL, $P \leq 0.0001$), indicating a robust antioxidant effect. The WE group also showed a marked reduction in MDA levels, with a mean concentration of 10.54±0.53 nmol/mL. These results suggest that MHS is equally effective as natural honey and more efficient than water in extracting bioactive compounds from basil stems.

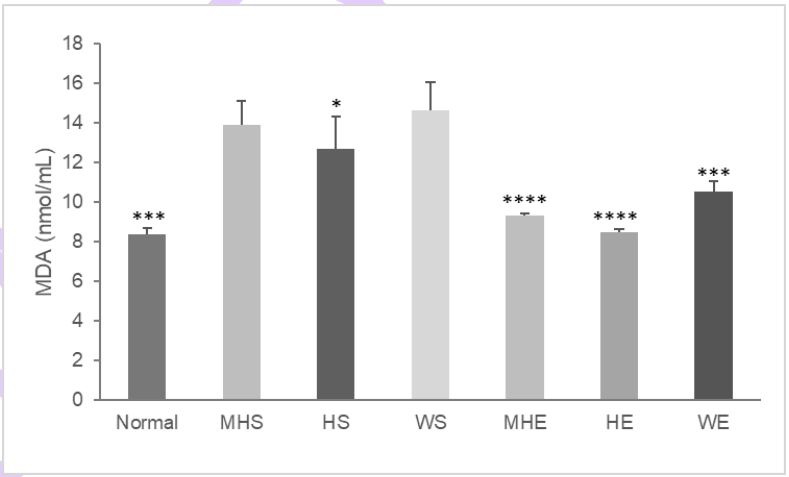


Figure 2. The antioxidant effects of *O. americanum* in hyperlipidemic rats. MHS: mimicking honey solvent with 50% (v/v) in water concentration; HS: honey solvent with 20% (v/v) in water concentration; WS: water solvent; MHE: basil stem mimicking honey extract; HE: basil stem honey extract; WE: basil stem water extract. ****significantly different to WS at P ≤0.001.

DISCUSSION

NADES is a green solvent that is non-volatile, non-toxic, and tends to be more viscous than conventional organic solvents [34]. The physicochemical properties of NADES, such as pH, viscosity, density, etc., influence its capacity to extract plant metabolites [18]. According to previous literature, both natural honey and NADES synthesized from sugar-based components generally have pH values ranging from 3.5 to 5.5. Earlier research reported pH values of 4.31 for MHS and 3.75 for HS [35]. However, in the current study, MHS was found to be more acidic than HS. This deviation is likely attributed to the different volumes of deionized water added, 50% in MHS compared to 20% in HS, since deionized water typically has a neutral pH of 6.0–6.4, which can influence the final acidity. The basil (*O. americanum* L.) stem extracts demonstrated pH levels ranging from acidic to near neutral, consistent with their polyphenolic content [7]. The type of solvent also had a notable influence on the pH, with the water extract (WE) showing a pH closer to neutral compared to MHE and HE, likely due to the absence of added acidic or sugar-based components. On the other hand, viscosity is significantly affected by solvent composition; interactions between sucrose, glucose, and fructose with water can restrict molecular mobility, thereby increasing thickness [36]. Conversely, increasing water content dilutes the solution, reducing viscosity [37]. These findings suggest a direct correlation between viscosity and density. This is consistent with previous studies stating that the density of deep eutectic solvents (DES) is generally higher than that of water [38]. The addition of water reduces density by lowering the concentration of dissolved sugars in the solution [39].

Next, phytochemical screening aims to identify the classes of secondary metabolites present in the samples. Phenolic and tannin components in the extracts were among the compounds successfully investigated in this test. The presence of phenolics was indicated by a color change to green upon the addition of $FeCl_3$, while the formation of a white precipitate upon the addition of 10% NaCl and 1% gelatin signified the presence of tannins. This precipitation is attributed to the formation of insoluble protein–tannin complexes in water [40]. The detection of phenolic and tannin compounds in PH and HS is consistent with previous studies reporting that Indonesian honey often contains various bioactive compounds, including flavonoids, phenolics, tannins, alkaloids, and steroids [41]. The presence of phenolic compounds in MHE and HE indicates that both MHS and HS were effective in extracting these compounds from the basil stem matrix, which aligns with earlier findings [22]. Similarly, the detection of tannins in MHE, HE, and WE corroborates previous research that has demonstrated the presence of tannins in *O. americanum* L. extracts [42]. Although sugar-based NADES is often reported to optimally extract total phenolics from plants, some researchers have also reported low yields of less polar flavonoids using this solvent. While the polarity of NADES can be adjusted to increase total phenolic yields, it does not necessarily guarantee optimal extraction of certain flavonoids [43]. Furthermore, the secondary metabolite detection system in this study employed only a preliminary qualitative test using simple drop or precipitant reagents. The low flavonoid content in the extract (which does not necessarily mean no extraction) and the varying visual observations of each individual explain the limitations of this testing system. These results are often referred to as false negatives. Further confirmation of the presence of compounds, that should be present in the extract (such as flavonoids and alkaloids in this study), is crucial using more sensitive chemical assays, such as liquid chromatography-mass spectrometry (LC-MS) [44].

Phytochemical screening results confirmed the presence of phenolic and tannin compounds in the HS as well as in all basil stem extracts. These compounds are likely responsible for the observed cholesterol-lowering effects. Caffeic acid, a type of phenolic, inhibits hepatic HMG-CoA reductase, thereby reducing cholesterol biosynthesis [11]. Rosmarinic acid also contributes to cholesterol reduction by enhancing the expression of SR-BI and LDL-R receptors, the transporters ABCCG5/ABCG8, and the enzyme CYP7A1, which together facilitate cholesterol transport and excretion via bile [45]. Tannins are known to lower cholesterol levels by accelerating its conversion to bile acids and increasing its excretion through feces [10]. The presence of phenolic and tannin compounds in the HS as well as in all basil stem extracts. These bioactive compounds are believed to contribute to the reduction in TG levels. Rosmarinic acid, one of the phenolic compounds identified, plays a role in lowering triglycerides by upregulating ABCG5/ABCG8 transporters and the CYP7A1 enzyme, which enhances cholesterol excretion. It also activates AMPK, thereby stimulating CPT1A, which supports fatty acid oxidation and reduces triglyceride accumulation in the liver [45]. Additionally, gallotannin has been reported to lower triglyceride levels by inhibiting the proliferation of preadipocyte cells [46].

In the antioxidant activity assay, the spleen was selected as the test organ for measuring malondialdehyde (MDA) levels. The spleen is critical in the immune system, producing antibodies, filtering aged erythrocytes, storing blood, and initiating immune responses to blood-borne antigens. Due to its immunological activity, the spleen is highly susceptible to oxidative damage from free radical accumulation. Additionally, it contains a high density of phagocytic cells that generate reactive oxygen species (ROS), making it a reliable organ for assessing lipid peroxidation under systemic oxidative stress [47]. This finding aligns with previous research demonstrating that *O. americanum* L., at a dose of 200 mg/kg BW, can suppress lipid peroxidation and reduce MDA levels [8]. Phytochemical analysis identified tannins and phenolics as key antioxidant constituents. Both classes of compounds act as radical scavengers. Tannins, by binding to body proteins, form a protective layer on the intestinal wall, which may reduce lipid absorption [48]. Phenolic compounds exhibit antioxidant effects by chelating metal ions and inhibiting enzyme activity [13]. These compounds also participate in hydrogen atom transfer, donating hydrogen from phenolic hydroxyl groups to stabilize radicals via the formation of H–O transition bonds with unpaired electrons [49].

Based on the findings of this study, the antioxidant effect of *O. americanum* L. appears to support its antihyperlipidemic activity. The presence of active phytochemicals provides protection against oxidative stress and enhances the therapeutic reduction of total cholesterol and triglycerides. This is further supported by studies indicating that antioxidant mechanisms, through inhibition of oxidative stress, modulation of lipid metabolism via pathways such as AMPK and PI3K/AKT, and reduction of lipid peroxidation, can significantly potentiate the antihyperlipidemic efficacy of medicinal plants [50].

CONCLUSION

This study demonstrates that basil (*O. americanum* L.) stem extract extracted using a MHS possesses significant antihyperlipidemic activity. MHE was more effective in reducing total blood cholesterol levels compared to WE. Furthermore, MHE exhibited a triglyceride-lowering effect comparable to that of atorvastatin. In addition to its lipid-lowering capabilities, MHE also showed strong antioxidant activity, as evidenced by a significant reduction in MDA levels in the spleen. These findings suggest that the MHS could serve as a promising, safe, and efficient natural alternative for extracting bioactive compounds from basil stems with potential antihyperlipidemic and antioxidant benefits.

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