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Potential Acetylcholinesterase, Lipase, α-Glucosidase, and α-Amylase Inhibitory Activity, as well as Antimicrobial Activities, of Essential Oil from Lettuce Leaf Basil (*Ocimum basilicum* L.) Elicited with Jasmonic Acid

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Abstract: The aim of this study was to investigate the influence of the elicitation with jasmonic acid on the biological activities of essential oils (EOs) from lettuce leaf basil (*Ocimum basilicum* L.). Specifically, 0.01 μM jasmonic acid (JA1), 1 μM jasmonic acid (JA2), and 100 μM jasmonic acid (JA3) were used as elicitors. The results indicated that the elicitation increased the acetylcholinesterase, lipase, and α-amylase inhibitory activity of essential oils. A significant difference in α-glucosidase inhibition was noted only for the JA3 extract (IC_{50} = 0.81 μL/mL), as this activity was lower than in the control sample without elicitation (IC_{50} = 0.68 μL/mL). The studied basil EOs exhibited similar activity against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). Based on the value of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentrations (MBC), the best antimicrobial activity was observed for JA2 and JA3.

Keywords: lettuce leaf basil (*Ocimum basilicum* L.); elicitation; essential oils; biological activities

1. Introduction

Herbs have had many applications as ornamental crops and raw material in the perfumery, food, and pharmaceutical industries for centuries. The use of herbs in the food and pharmaceutical industries is mainly connected with the beneficial health and preservative effects of their essential oils. The components of plant essential oils (EOs), which are a mixture of secondary metabolites in the form of volatile oily liquids, generally belong to two groups of compounds: Terpene compounds and aromatic compounds. EOs are multi-component mixtures of terpene compounds (mono-, sesqui-, and diterpenes) and their derivatives. These derivatives may be alcohols, ketones, aldehydes, esters, ethers, amines, and sulfides. A characteristic feature of the composition of essential oils is that it contains fairly high concentrations of two or three major components, while other components are present in trace amounts [1]. This enormous diversity of the chemical composition of essential oils determines the unusually wide range of their biological activity. The most frequently studied properties of essential oils are antimicrobial, antioxidant, and anti-inflammatory activities; however, in recent years, some information has been provided about the possibility of using plant essential oils to support the

treatment of certain diseases, e.g., diabetes mellitus or Alzheimer's disease (AD) [2]. These diseases are more frequent among ageing populations and their incidence is steadily on the increase. Both have long prodromal phases and are complex chronic disorders. Although these diseases seem very different, it is assumed that type 2 diabetes is a main risk factor for developing Alzheimer's disease, as there are many shared phenotypes, including mitochondrial dysfunction [3]. Moreover, as described in recent studies, disorders in insulin release or function may contribute to AD development, which may prompt a conclusion that it is actually a neuroendocrine disease. Insulin resistance, which is often the main risk factor of diabetes, influences the basic pathological process of AD, including the metabolism of the amyloid precursor protein (APP), forming a peptide (Ab) that generates the plaque, i.e., one of the most pathological features of the disease [4]. At the base of the development of abnormal glucose and lipid metabolism is obesity, and some studies have indicated that elevated cholesterol and triglyceride levels precede AD pathology [5]. On the other hand, recent reports have suggested that the composition and metabolic activity of the gut microbiota may affect the risk of developing obesity. Hence, one of the methods to support the pharmacotherapy of AD and diabetes is to inhibit enzymes involved in the pathogenesis of the diseases, e.g., acetylcholinesterase, lipase, or carbohydrate hydrolysis enzymes, and to inhibit pathogen growth. These compounds can be included in food ingredients and cause less serious side effects than medicines.

In fact, many herbal plants possess the ability to prevent some diseases, e.g., diabetes or Alzheimer's disease [6–10]. Basil is widely used in traditional medicine in different cultures and is also known for its culinary uses [11]. Among many herbal species, basil seems to be accepted by consumers not only as a medicinal plant but also as a culinary herb. It is well known that some diseases, for instance, diabetes, belong to the group of diet-related diseases and many food compounds may inhibit their development. Additionally, the phytochemical constituents contained in *O. basilicum*, especially linalool, methylchavikol, methyl cinnamate, linolen, rosmarinic acid, citral, eugenol, and geraniol, predispose this plant to the support of the treatment of such diseases as diabetes and Alzheimer's disease [8,11].

Some studies have indicated that compounds of essential oils of basil such as eugenol can reduce the blood glucose level in vivo [12]. In another in vivo study, oral administration of 1,8-cineole-rich essential oil resulted in a significant reduction in the fasting blood glucose level at 2 doses: 110 and 220 mg of essential oil per kg body weight [6]. The neuroprotective effectiveness of eugenol against the acrylamide-induced neuropathy model in rats has been observed as well [13]. The essential oils of *Ocimum basilicum* L were also found to have a positive effect in vivo in a β-amyloid rat model of Alzheimer's disease. Exposure of basil EO was carried out via an electronic vaporizer by 60 min inhalation [14]. The cited studies were conducted in vivo for about 96 h; certainly, further studies of the long-term use and clinical trials are needed, but the properties observed are promising.

It is known that the biological activity of essential oils depends to a large extent on the composition; therefore, factors responsible for the quantity and chemical composition of essential oils largely determine their bioactive properties [15]. The yield and chemical composition of essential oils are affected not only by methods of drying plant material or extraction processes, but also by some pre-harvest treatments and environmental conditions [7,8]. As indicated in some studies, elicitation may represent a group of determinants of the biosynthesis of many groups of plant secondary metabolites, including essential oil compounds [9,10]. Some studies have indicated that the exogenous application of some plant hormones (like salicylic acid, jasmonic acid, and arachidonic acid), which can be regarded as elicitors, may result in increased biosynthesis of plant bioactive compounds [15]. In our previous study, we found that jasmonic acid was the most effective in induction of the biosynthesis of plant secondary metabolites [16]. Additionally, there are many publications confirming the highly effective action of jasmonic acid in the improvement of some biological properties in lovage [17] and basil plants [18,19].

In many studies, basil essential oil was shown to act as an antidiabetic, anti-obesity, and anti-Alzheimer's agent [14,20]. There are many investigations confirming that such compounds of essential oils as eugenol, linalool, 1,8-cineole, and β-pinene (present in basil essential oil analyzed in

the present study) possess many biological activities connected with anti-diabetic and neuroprotective action [12,21].

The bioactivities of essential oils probably depend on their composition.

As differences in the composition of essential oils from control and jasmonic acid-elicited lettuce leaf basil leaves were observed in our previous study [22], it can be assumed that elicitation with jasmonic acid can also modulate the biological activities of essential oil obtained from these plants. Various components of essential oils are responsible for their diverse pro-health properties; hence, studies on other bioactivities may be very valuable. A novelty of this work is the determination of properties of basil essential oils that have not yet been tested in elicited herbs. Therefore, the aim of the present study was to evaluate the antimicrobial, potential anti-diabetic, and anti-Alzheimer's disease activity of essential oil obtained from control and jasmonic acid-elicited lettuce leaf basil.

2. Materials and Methods

2.1. Plant Material

Essential oils from control and jasmonic acid-elicited lettuce leaf basil leaves (*Ocimum basilicum L. cv. Crispum*) were the plant material used in this study. In addition, 0.01 µM jasmonic acid (JA1), 1 µM jasmonic acid (JA2), and 100 µM jasmonic acid (JA3) (Sigma) were used for elicitation of basil (1.5 mL per plant). The growth conditions and method of elicitation, as well as the isolation of essential oil, were described in our previous manuscript [22].

2.2. Acetylcholinesterase (AChE) Inhibitory Activity

To evaluate the inhibitory activity of essential oils on acetylcholinesterase, an assay described by Ellman, Courtney, Andres, and Featherstone [23] was used with some modification. Namely, 415 µL of Tris-HCl buffer (0.1 M, pH 8) was mixed with 20 µL of the enzyme (AChE, 0.5 U/mL) and 20 µL of oil samples with different concentrations (briefly dissolved in ethanol), and the mixture was incubated for 30 min at room temperature. Afterward, 75 µL of acethyl-thiocholine (15 mM) and 475 µL of 3 mM DTNB (5,5-dithio-bis-2-nitrobenzoic acid) were added and the mixture was incubated for 30 min. The absorbance of the final mixture after the incubation was measured at 412 nm.

The results were expressed as an EO concentration (µL/mL) that yielded 50% of AChE inhibition (IC_{50}).

2.3. Lipase Inhibitory Activity

Lipase activity was measured with the use of p-nitrophenyl acetate (pNPA) as a substrate according to the method described by Wang et al. [24] with modification.

2.4. α-Amylase Inhibitory Activity

The α-amylase inhibitory activity (αAI) of the protein hydrolyzates and peptide fractions was measured according to the method described by Świeca, Baraniak, and Gawlik-Dziki [25]. All assays were carried out in triplicate. For determination of the IC_{50} value, the inhibitory activity was investigated for four concentrations of samples.

2.5. α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was measured with the method described by Jakubczyk, Świeca, Gawlik-Dziki, and Dziki [26].

2.6. Antimicrobial Activity

The basil essential oils were tested against Gram-positive *Staphylococcus aureus* ATCC 29737 and Gram-negative *Escherichia coli* ATCC 25922. These strains were obtained from the American

Type Culture Collection (ATCC, distributors: LGC Standards, Łomianki, Poland) and stored at 4 °C. All strains were cultured at 37 °C on Nutrient Broth (NB) medium.

2.6.1. Disk Diffusion Method

The antimicrobial assays were performed using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute [27]. Sterile Petri dishes containing solid and sterile Mueller–Hinton Agar (MHA) were used. The samples were dissolved with sterile PBS and 1% Tween 80 and filtered through syringe filters (Ø = 0.22 µm). The sterile filter disk (Ø = 6 mm) was saturated with 10 µL of each sample at a concentration of 10 mg mL^{-1} and placed on the MHA with the tested bacteria. The bacterial suspension (100 µL) prepared from an overnight culture was adjusted to an inoculation of 10^8 CFU mL^{-1}. The plates were incubated at 37 °C for 18 h. Disks without samples were used as a negative control. Ampicillin (10 µg/disk), neomycin (30 µg/disk), oxytetracycline (30 µg/disk), and erythromycin (15 µg/disk) were used as positive controls for bacteria. Antimicrobial activity was evaluated by measuring the inhibition zone (mm) for the tested microorganisms and compared to the controls.

2.6.2. Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentrations (MBC)

Serial twofold dilution of each essential oil stock was made with Mueller Hinton Broth with 1% Tween 80, to yield final concentrations ranging from 2.5 to 0.156 mg mL^{-1}, and placed into tubes. Then, 500 µL of the bacterial (5×10^5 CFU mL^{-1}) culture was added. The tubes with the MHB or bacterial culture were the negative and positive controls, respectively. The minimum bactericidal (MBC) concentrations were determined after broth macrodilution by subculturing a sample from a tube showing no microbial growth onto the surface of the MHA medium. The plates were incubated at 37 °C for 18 h. The MBC is defined as the lowest concentration of an antimicrobial agent needed to kill 99.9% of the final inoculum after 18 h of incubation [27].

2.7. Statistical Analysis

All determinations were performed in triplicate. Statistical analysis was performed using STATISTICA 7.0 for comparison of means using Tukey's test at the significance level $p < 0.05$.

3. Results

3.1. Effect of JA Elicitation on Inhibition of Anticholinesterase and Pancreatic Lipase Activities by Basil Essential Oils

The effects of AChE and pancreatic lipase inhibition by the studied essential oils are presented in Table 1. The IC_{50} values for AChE of EOs from the control and JA-elicited basil were from 38.31 ± 3.30 µL mL^{-1} for the control to 16.89 ± 1.08 µL mL^{-1} in the case of the JA2 sample. It should be noted that the elicitation with all the concentrations of JA caused a statistically important increase in this activity exhibited by the essential oils from the basil plants. Elicitation with JA2 was the most effective, i.e., the IC_{50} value was lower by 56% than in the control.

The elicitation improved the inhibitory properties against pancreatic lipase. As shown by the data described in Table 1, the lowest IC_{50} value (0.09 µL mL^{-1}) was determined for the JA2 sample. The control sample was characterized by IC_{50} of 0.35 µL mL^{-1}.

Table 1. Effect of jasmonic acid elicitation on acetylcholinesterase and lipase inhibitory activity of essential oil from basil.

Sample	IC_{50} [μL mL^{-1}]	
	Acetylcholinesterase Inhibition	Lipase Inhibition
C	38.31 ± 3.30 ^{c}	0.35 ± 0.05 ^{c}
JA1	27.15 ± 1.33 ^{b}	0.24 ± 0.028 ^{b,c}
JA2	16.89 ± 1.08 ^{a}	0.09 ± 0.006 ^{a}
JA3	27.42 ± 0.24 ^{b}	0.13 ± 0.013 ^{a,b}
Synthetic positive control	Galantamine [6.62 μg/mL]	Orlistat [12.38 μg/mL]

Abbreviations: C, control; JA1, 0.01 μM jasmonic acid; JA2, 1 μM jasmonic acid; JA3, 100 μM jasmonic acid; Mean ± standard deviation. Statistically significant differences ($p < 0.05$) are indicated by various letters in columns (^{a},^{b},^{c}).

3.2. Effect of JA Elicitation on the Ability of Basil EOs to Inhibit Selected Enzymes Involved in Diabetes Pathogenesis

The potential inhibitory activity of essential oils from the control and JA-elicited basil against enzymes involved in the pathogenesis of diabetes was studied. It was evaluated based on α-amylase and α-glucosidase inhibitory activity and expressed as an IC_{50} value (μL mL^{-1})–Table 2. The highest potential ability to inhibit α-glucosidase activity was determined for the JA2 sample (IC_{50} = 0.66 ± 0.02 μL mL^{-1})–Table 2. However, it should be noted that, in the case of α-glucosidase inhibition activity, the elicitation with jasmonic acid did not cause a statistically significant difference in the inhibition ability of the EOs. In turn, all the concentrations of the elicitor resulted in an approx. 10-fold increase in the ability of α-amylase inhibition by the essential oils from the elicited basil. The lowest IC_{50} value for this enzyme was determined for the JA1 sample (IC_{50} = 1.32 ± 0.28 μL mL^{-1})–Table 2.

Table 2. Effect of jasmonic acid elicitation on α-amylase and α-glucosidase inhibitory activity of essential oil from basil.

Sample	IC_{50} (μL mL^{-1})	
	α-Amylase Inhibition	α-Glucosidase Inhibition
C	13.34 ± 0.05 ^{b}	0.68 ± 0.03 ^{a}
JA1	1.32 ± 0.28 ^{a}	0.73 ± 0.03 ^{a}
JA2	1.72 ± 0.19 ^{a}	0.66 ± 0.02 ^{a}
JA3	1.52 ± 0.08 ^{a}	0.81 ± 0.04 ^{b}
Synthetic positive control	Acarbose [0.258 mg/mL]	Acarbose [0.28 mg/mL]

Abbreviations: C, control; JA1, 0.01 μM jasmonic acid; JA2, 1 μM jasmonic acid; JA3, 100 μM jasmonic acid. Mean ± standard deviation. Statistically significant differences ($p < 0.05$) are indicated by various letters in columns (^{a},^{b}).

3.3. Effect of JA Elicitation on Antimicrobial Activities of Basil Essential Oils

The antimicrobial activity of the basil essential oils was evaluated against one Gram-positive (*Staphylococcus aureus*) and one Gram-negative (*Escherichia coli*) bacterial species–Table 3. These activities were expressed as inhibition of bacterial growth (inhibition zone). Additionally, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentrations (MBC) were determined. All the essential oils (from the control and JA-elicited basil) exhibited antibacterial properties against the tested microorganisms. As shown by the results of the agar disk-diffusion method, the JA1 and JA2 samples exhibited the highest antimicrobial activity against *Escherichia coli*, while the essential oil from the JA1-elicited basil had the strongest antimicrobial activity against *Staphylococcus aureus* as well. The MIC

and MBC values did not exactly confirm the results of the inhibition zone test, because the EO from the JA3-elicited plants had the lowest MBC for both tested microorganisms (0.625 and 0.312 μL mL^{-1} for *E. coli* and *S. aureus*, respectively)–Table 3. Additionally, the JA3 sample exhibited the highest effectiveness measured as MIC (0.156 μL mL^{-1}) against *S. aureus.* It should also be noted that the JA3 sample had a lower MIC value (0.312 μL mL^{-1}) against *E. coli*, compared to the control (0.625 μL mL^{-1}).

Table 3. Effect of jasmonic acid elicitation on antimicrobial activity of essential oil from basil.

Sample	*E. coli* ATCC 25922	*S. aureus* ATCC 29737
Inhibition zone (mm) *		
C	17.70 ^{a} ± 2.65	15.70 ^{a} ± 0.37
JA1	21.20 ^{a,b} ± 1.79	23.60 ^{b,e} ± 1.91
JA2	22.20 ^{b} ± 3.34	19.00 ^{a,c} ± 2.16
JA3	14.50 ^{c} ± 1.12	18.00 ^{a,c,d} ± 1.30
AMP	13.90 ^{c} ± 0.29	13.00 ^{a,d} ± 0.44
NE	22.90 ^{b} ± 0.45	26.40 ^{e} ± 0.98
OT	15.70 ^{b} ± 0.43	16.50 ^{a} ± 0.22
E	10.00 ^{c,d} ± 0.63	17.00 ^{a} ± 0.31
	MIC (μL mL^{-1})	
C	0.625	0.625
JA1	0.625	0.625
JA2	0.625	0.625
JA3	0.312	0.156
	MBC (μL mL^{-1})	
C	1.250	0.625
JA1	0.625	0.625
JA2	1.250	0.625
JA3	0.625	0.312

*–values are expressed as mean ± SD; different letters in the same column indicate significant differences ($p < 0.05$); AMP–ampicillin (10 μg/disk); NE–neomycin (30 μg/disk); OX–oxytetracycline (30 μg/disk); E–erythromycin (15 μg/disk); MIC–minimum inhibitory concentration; MBC–minimum bactericidal concentrations; C, control; JA1, 0.01 μM jasmonic acid; JA2, 1 μM jasmonic acid; JA3, 100 μM jasmonic acid.

4. Discussion

Elicitation is a natural method for the enhancement in plant resistance to biotic and abiotic stresses and improvement in the quality of edible plants, especially in relation to their health-promoting phytochemicals [15]. As indicated in many studies, jasmonic acid used as an elicitor can influence the biosynthesis of some plant secondary metabolites [28,29]. It is well known that some plant metabolites, e.g., phenolic compounds or essential oil compounds, possess many biological activities; therefore, increased biosynthesis of the secondary metabolites has an effect on the biological activity of plants [18,29]. Our previous publication indicated that elicitation with 0.01 μM jasmonic acid (JA1), 1 μM jasmonic acid (JA2), and 100 μM jasmonic acid (JA3) influenced the composition of essential oils from basil and increased the antioxidant and anti-inflammatory potential of these EOs (especially the JA2 and JA3 sample) [22].

Another very important activity attributed to essential oils is the ability to inhibit the acetylcholinesterase enzyme. Acetylcholinesterase (AChE) is a key enzyme involved in the hydrolysis of acetylcholine in the nervous system of animals and insects. Acetylcholine deficiency in the human cerebral cortex is associated with neurodegenerative disorders, such as Alzheimer's

disease (AD). Nowadays, commercial drugs acting as selective acetylcholinesterase inhibitors are used in the treatment of Alzheimer's disease. Yet, synthetic compounds exhibit some toxicity during prolonged use; hence, many researchers are looking for compounds of natural origin that can be a potential alternative for the treatment of AD patients. There are some reports on the ability of essential oils from some plants to inhibit acetylcholinesterase activity, but there is no information about the effect of plant elicitation on these properties [7,30]. Literature data prove that EOs from medicinal plants, especially from *Melissa officinalis*, *Citrus aurantifolia*, *Ocimum gratissimum*, *Citrus aurantium*, *Cupressus sempervirens*, *Eucalyptus globulus*, *Foeniculum vulgare*, *Thymus vulgaris*, *Eucalyptus camaldulensis*, and *Ocimum canum*, have high acetylcholinesterase inhibitory activity [7,30]. As shown in the present study, the essential oil from lettuce leaf basil showed moderate AChE inhibition (IC_{50} value from 38.31 ± 3.30 to 16.89 ± 1.08 µL/mL)–Table 1. These results correspond to the findings reported by Kiendrebeogo et al. [7] and Phrompittayarat, Hongratanaworakit, Tadtong, Sareedenchai, and Ingkaninan [8]. It should also be noted that the elicitation with JA in our study significantly improved the ability of lettuce leaf basil EOs to inhibit AChE, and the best result was achieved in the case of the JA2 sample (Table 1). There are no reports on the influence of jasmonic acid elicitation on the ability of plant essential oils to inhibit AChE in the available literature. Other reports on AChE inhibition by some compounds of essential oils indicated that the anticholinesterase inhibition activity of essential oils could especially be associated with monoterpenes, e.g., 1,8-cineole, camphor, α-pinene, β-pinene, borneol, linalool, menthone, carvone, anethole, anisole, and geraniol [7,8]. The analysis of the composition of essential oil from basil elicited with jasmonic acid compared to the control basil essential oil presented in our earlier publication emphasizes a partial correlation between the content of essential oil components indicated above (responsible for the inhibition of acetylcholinesterase) and the ability of the tested essential oils to inhibit this enzyme. It should be noted that the elicitation with jasmonic acid increased the content of 1,8-cineole (JA1 and JA2 sample), α-pinene (JA1 sample), and β-pinene (JA1 sample). Additionally, geraniol was identified in the essential oil from basil elicited with 1 µm jasmonic acid (JA2 sample), which was not detected in the control sample [22]. However, the correlation analysis revealed no significant correlation between the content of these compounds and anti-acetylcholinesterase activity (data not published).

The very high ability of 1,8-cineole to inhibit acetylcholinesterase activity was confirmed in a study conducted by Picollo et al. [21]. Furthermore, some in vivo studies confirmed the properties of *Ocimmu basilicum* L. essential oil that may be helpful in the treatment of Alzheimer's disease and other conditions [14]. The results obtained in the current study indicate that the elicitation with jasmonic acid can be an effective method for the enhancement in these properties of basil essential oil (Table 1).

The treatment of neurodegenerative diseases that most often affect elderly people is now an enormous challenge for healthcare. These problems are often related to such metabolic disturbances as obesity and type 2 diabetes mellitus, which are part of metabolic syndrome (MS) [31]. MS has become a public health problem, as it may influence the health status both in adults and in children and increase the risk of development of neurodegenerative diseases in the future [32]. There are some enzymes involved in these diseases, and inhibition of their activity could help in the prevention or treatment of metabolic syndrome-related conditions. Some secondary metabolites, e.g., phenolic compounds or compounds of herbal essential oils, were reported to modulate the activity of some digestive enzymes, such as α-glucosidase, α-amylase, and lipase [9,33].

Obesity is associated with an imbalance between the organism's demand for calories and their supply with food. This leads to the disproportional accumulation of adipose tissue, its excessive activity, and disorders in fat metabolism [34]. One of the methods for prevention and treatment of this disease is to reduce lipid hydrolysis in the gastrointestinal tract. To achieve this goal, the activity of pancreatic lipase (triacylglycerol lipase, EC 3.1.1.3) must be inhibited. It is the main enzyme involved in lipid hydrolysis and responsible for the digestion of 50–70% of fats consumed with diet [35]. A method for the treatment of obesity is the application of Orlistat, i.e., a drug that inhibits the activity of pancreatic lipase, which, however, can cause serious side effects such as the development of kidney

diseases [36]. Compounds that inhibit pancreatic lipase include peptides [37], polyphenols [38], and essential oils [39]. In our study, we investigated the effect of elicitation on the lipase inhibitory activity of essential oils obtained from basil. The current data on oils as pancreatic lipase inhibitors mainly relate to plant extracts, and the main goal of the research is to investigate the influence of various solvents on the acquisition of extracts with high inhibitory properties [40]; however, there is no information on the effect of elicitation on their activity. As demonstrated by the results obtained in our experiment, the greatest effect on the potential anti-obesity activity was exerted by JA2, with an IC_{50} value of 0.09 µL/mL (Table 1). It should be noted that all the tested samples exhibited lipase inhibitory activity, but the elicitation enhanced this property. The results indicate that lettuce leaf basil is a good source of essential oils with lipase inhibitory properties, compared to the results obtained by [10] in investigations of essential oils obtained from *Cinnamomum chemungianum* originating from different locations in the southern Western Ghats. The plants collected from Chemungi, Kannikatti, and Athirumala were tested and their ability to inhibit lipase was determined, with IC_{50} of 919.75, 923.17, and 838.46 µg/mL, respectively.

The inhibition of α-amylase or α-glucosidase, i.e., enzymes involved in the digestion of carbohydrates, is considered a strategy for the treatment of type 2 diabetes and obesity. There are some reports documenting the antidiabetic potential of essential oils from some medicinal plants in both in vitro (via inhibition of α-amylase or α-glucosidase) and in vivo studies [35,36]. Our study confirmed these reports, as the EOs from lettuce leaf basil also acted as α-amylase or α-glucosidase inhibitors, with a greater observed inhibitory activity against α-glucosidase–Table 2. It should also be noted that the JA elicitation improved the anti-α-amylase potential of the analyzed EOs. The contribution to the antidiabetic potential of essential oils, based on literature data, is attributed to such compounds as D-limonene and linalool [41], which are present in the basil EOs analyzed in the present study [11]. As indicated in our previous publication, these activities may be connected with the increased content of some components of essential oils. For example, all JA-elicited samples have much better α-amylase-inhibiting properties, which may be partially correlated with the higher content of D-limonene (JA3 sample) or linalool (JA1 and JA3 sample) in the studied essential oil [22]. However, the correlation analysis confirmed only a moderate positive correlation between the anti-α-amylase activity and the linalool content in the essential oils tested, $R^2 = 0.44$ (data not published). The in vitro antidiabetic and anti-obesity properties of basil essential oils were also confirmed in a study conducted by Noor et al. [20]. Their ability to suppress endogenous glucose release was found to contribute to the inhibition of glycogenolysis and/or stimulation of glycogenesis. The antidiabetic properties of basil were also investigated in an in vivo study conducted by Ezeani et al. [11]. Therefore, in light of the literature, basil is a very valuable herb for use in such health conditions as diabetes and Alzheimer's disease [14,21]. The research obtained in this work confirms these observations. The elicitation (which is a new method in this type of research) makes the essential oil obtained from the elicited basil an even more valuable ingredient for use in the above-mentioned diseases. Extraction of essential oils from elicited basil plants may set a new direction in the acquisition of substances of natural origin for use in civilization diseases.

There are some studies indicating that essential oils are able to act as antimicrobial agents as well [28], but there is no information in the available literature about the influence of plant elicitation on these properties of essential oils. For this reason, in the present study, we analyzed the essential oils from jasmonic acid-elicited and control basil (studied previously to assess their composition and antioxidative and anti-inflammatory potential) [11] in terms of the effect of elicitation on the additional biological properties of these oils, i.e., the antimicrobial, anticholinesterase, and potential antidiabetic activity. The antibacterial activity of essential oils from some herbs including *Ocimum basilicum* has been documented [37–39].

As suggested by many researchers, the characteristic hydrophobicity of EO components is involved in their antibacterial action. It allows EOs to separate the lipids of the bacterial cell membrane and mitochondria, disrupting the cell structures, which become more permeable. This mechanism of the

antibacterial action of essential oil is associated with the inhibition of Gram-positive and Gram-negative bacterial growth [8,37]. Some studies have indicated that Gram-negative bacteria are more susceptible to essential oils than Gram-negative species [28]. However, our results indicated that the basil EOs demonstrated similar activity against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). The agar disk diffusion method demonstrated that the inhibition zones were from 15.70 ± 0.37 (control sample) to 23.60 ± 1.91 mm (JA1 sample) in the case of *Staphylococcus aureus* and from 14.50 ± 1.12 mm (JA3 sample) to 22.20 ± 3.34 mm (JA2 sample) for *Escherichia coli*–Table 3. These results correspond perfectly to the results obtained by [39], in which basil EO showed antimicrobial activity with a similar inhibition zone diameter, i.e., 23.3 ± 0.4 mm against *Staphylococcus aureus* and 15.2 ± 0.5 mm against *Escherichia coli*. Additionally, the MIC values of the basil EO studied in the present publication were similar to those obtained by Lv et al. [40]. On the contrary, in the work conducted by [38], it was observed that the EO from basil (*Ocimum basilicum var. Grant verte*) had antibacterial activity against *Staphylococcus aureus* (MIC = 0.53 µL/mL) but no activity against *Escherichia coli*. As suggested by some researchers, this difference may be associated with the fact that *O. basilicum* has a big number of chemotypes [41]. Essential oils are thought to be produced by plants in response to some environmental conditions, and, therefore, growth conditions, stressors, or elicitation may affect the yield and composition of EOs [28,42]. As indicated in our previous study [11], jasmonic acid elicitation influenced the composition, as well as the antioxidant and anti-inflammatory activities, of lettuce leaf basil EOs. As shown by the present results, the antimicrobial activity of basil EO was also determined by the JA elicitation–Table 3. This may be associated with differences in the content of some compounds in EO from the control and JA-elicited basil leaves [11]. As demonstrated by the present results, the best antimicrobial activity based on the MIC and MBC values was observed for JA2 and JA3–Table 3. Based on literature data, limonene, carvacrol, geraniol, α-pinene, β-pinene, eugenol, cinnamaldehyde, thymol, and menthol are the main compounds with a significant contribution to the antimicrobial activity of EOs [8,39]. Some of these compounds (limonene, α-pinene, β-pinene, eugenol) are contained in the basil EOs studied by us [11]. Additionally, the elicitation with jasmonic acid increased the level of some of these EO compounds; specifically, the JA3 elicitation increased the levels of eugenol (from 17.59 to 24.88%) and limonene (from 0.64 to 0.88%). The elicitation with jasmonic acid also increased the level of α-pinene (in the JA1 sample) and β-pinene (in the JA1 and JA2 sample) in the basil essential oil [11]. Based on the previously published information discussed above, it may be supposed that the changes in the composition of basil EOs resulting from the JA elicitation may have been the cause of the increased antibacterial activity of the tested oils observed in the present study.

The studied bioactivities of essential oils probably depend on the composition of the oil (concentration of individual components of the oil). As indicated in our previous study, the induction of individual components of essential oil was not dependent on the elicitor dose [22]. The different responses of basil plants (i.e., the lower content of some compounds in plants treated with the higher elicitor concentrations) are difficult to explain. Therefore, as assumed by many researchers, the effect of elicitation depends largely on such factors as the type and doses of elicitors and the species of the elicited plant [15,43]. In many other studies, it was also observed that plants did not respond in a dose–response manner in terms of production of secondary metabolites. For example, elicitation with 10 µM salicylic acid was more efficient for the induction of the phenolic content in purple coneflower tops (*E. purpurea* L. Moench.) than 100 µM salicylic acid [44]. Additionally, in a study conducted by Złotek et al. [16], 1 µM jasmonic acid was more effective in induction of the biosynthesis of phenolic compounds in lettuce leaves than 100 µM jasmonic acid. Similarly, in our previous research concerning elicitation with 1, 10, and 100 µM jasmonic acid, only the 10 µM elicitor dose caused a statistically significant increase in the sum of phenolic compounds [17].

5. Conclusions

In conclusion, our results indicate that elicitation with jasmonic acid may increase some potential biological activity of basil essential oils, e.g., the potential anti-diabetic and anti-Alzheimer's

disease activities. The treatment with 1 µM jasmonic acid (JA2) was found to be most effective. Additionally, the essential oils from basil elicited with 1 µM jasmonic acid (JA2) and 100 µM jasmonic acid (JA3) were characterized by the best antimicrobial activity.

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