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Meta-analysis of antimicrobial activity of *Allium*, *Ocimum*, and *Thymus* spp. confirms their promising application for increasing food safety

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ABSTRACT

Biopreservation strategies such as the use of Mediterranean plant extracts to ensure food safety are promising to deal with the emergence of antimicrobial resistances and the overreliance on food chemical additives. In the last few decades, antimicrobial susceptibility testing (AST) for evaluating the *in vitro* antibacterial potential of plant extracts against the most relevant foodborne pathogens has been widely reported in the literature. The current meta-analysis aimed to summarise and analyse the extensive evidence available in the literature regarding the *in vitro* antimicrobial capability of *Allium*, *Ocimum* and *Thymus* spp. extracts against foodborne pathogens. A systematic review was carried out to gather data on AST results of these extracts against *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and *Bacillus cereus*, including inhibition diameters (ID) and minimum inhibitory concentrations (MIC). A total of 742 records were gathered from a raw collection of 2,065 articles. Weighted mixed-effect linear models were adjusted to data to obtain pooled ID, pooled MIC and the relationship between both model estimations and observations. The pooled results revealed *B. cereus* as the most susceptible bacteria to *Allium sativum* (pooled ID = 20.64 ± 0.61 mm) by diffusion methods and *S. aureus* (pooled MIC = 0.146 mg/mL) by dilution methods. Diffusion methods did not yield conclusive results for *Ocimum* spp. extracts; however, the lowest pooled MIC was obtained for *S. aureus* (0.263 mg/mL). Among the foodborne pathogens evaluated, *B. cereus* showed the highest sensitivity to *Thymus* spp. extracts by both diffusion and dilution methods (pooled ID = 28.90 ± 2.34 mm and MIC = 0.075 mg/mL). The methodology used for plant extraction was found to not significantly affect MIC values ($p > 0.05$). Overall, the antimicrobial effectiveness of the studied extracts against Gram-positive and Gram-negative bacteria was demonstrated. Finally, the robustness of the meta-regression model was confirmed, also revealing an inversely proportional correlation between the ID and MIC measurements ($p < 0.0001$). These results provide a robust scientific basis on the factors affecting the *in vitro* antimicrobial efficacy of extracts from Mediterranean plants. They also provide valuable information for stakeholders involved in their industrial application in food, including producers, regulatory agencies and consumers which demand green-labelled foods.

1. Introduction

Multidrug-resistance (MDR) emerging in bacterial pathogens poses a significant global health threat (Algammal et al., 2023a, 2023b; Elbehiry et al., 2022). Foodborne pathogens, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella* spp., and *Bacillus cereus*, are commonly associated with human diseases due to different pathogenicity and virulence factors (Bintsis, 2017; Castro et al.,

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2018; Dougnon et al., 2017; Thakur et al., 2018). A wide variety of mechanisms have been described for those microrganisms to effectively resist antimicrobials, such as changes in the cell membrane permeability and the efflux pump mechanism (Abdelhamid & El-Dougdoug, 2020; Álvarez-Martínez et al., 2020; Vaou et al., 2021; Yuan & Yuk, 2019). The antimicrobial susceptibility testing (AST) results crucial to identify suitable antimicrobials and screen for emerging MDR strains.

The application of natural compounds in food production as alternative solutions to MDR has steadily increased, aligning with current consumer preferences for "green label foods" (Bondi et al., 2017; Settanni & Moschetti, 2014). Plant extracts and essential oils (EO), classified as Generally Recognized as Safe (GRAS), represent a natural substitute to synthetic antimicrobial agents (Laranjo et al., 2017). Their incorporation into food products offers the opportunity to enhance their nutritional and organoleptic properties while improving their shelf-life and microbiological safety (Nikmaram et al., 2018). The broad-spectrum antimicrobial activity of plant-derived extracts, attributed to their complex compositions of bioactive compounds, cultural and traditional uses, low toxicity and high availability, represents advantages for their use as biopreservatives (Bhavaniramya et al., 2019). Regulation (EU) 1129/2011 sets out criteria for selection, allowances, and limits of their use in food products (Ojeu, 2011).

The Mediterranean area represents a rich source of plant species and culinary traditions, wherein aromatic herbs and spices are used in a wide range of food products (Bower et al., 2016; Delgado et al., 2023; Stefanaki & van Andel, 2021). The large variety of bioactive compounds present in autochthonous plants, such as polyphenols, terpenoids, organosulfurs and alkaloids, has been widely described for their antioxidant, antimicrobial and health-related potential (Alirezalu et al., 2020; Awad et al., 2022). The antimicrobial effects of various formulations of Mediterranean plant extracts have been evaluated to enhance the hygiene standards in diverse food matrices, such as "Mediterranean burgers" (Albergamo et al., 2021), dry fermented sausages (El Adab & Hassouna, 2016), fresh chicken sausages (Sharma et al., 2017), minced pork (Krisch et al., 2010), fresh Mediterranean swordfish fillets (Kykkidou et al., 2009), bonito fish (Guran et al., 2015) and dairy products (Kaptan & Sivri, 2018). Cheeses and fermented sausages have been traditionally regarded as the products of highest interest for the application of EOs. Nevertheless, most of the studies evaluating the antimicrobial potential of plant extracts and EOs available in literature have been carried out *in vitro* (Awad et al., 2022).

The *in vitro* AST has been extensively applied over the last decades for antimicrobial screening of plant extracts and EOs (Ríos et al., 1987). In this context, numerous studies have been conducted using non-standardized AST techniques and varying parameters, such as microbial and extract concentrations and culture media used, raising concerns about divergent findings and the risk of inaccurate conclusions (Balouiri et al., 2016; Othman et al., 2011). To address this, AST methods have adhered to well-described guidelines and standardised methodologies, mainly designed by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (CLSI, 2018; International Organization for Standardization, 2019; Matuschek et al., 2013).

The most common AST techniques comprehend both diffusion tests (agar disk diffusion [DD] or well diffusion [WD]) and dilution tests (agar dilution [AD] and broth macro- [BMaD] or micro-dilution [BMiD]) (Silva et al., 2023). In diffusion methods, paper disks impregnated with the antimicrobial agent (for the DD method), or agar holes filled with the antimicrobial agent (WD) are placed on agar plates previously spread with a bacterial culture. In contrast, dilution methods involve testing different serial dilutions of the antimicrobial agent incorporated into a molten agar medium (AD), in a liquid growth medium (BMaD) or a 96-well microtitration plate (BMiD). After microbial inoculation, these media are later incubated under optimal conditions according to the tested microorganism (Balouiri et al., 2016; Chiu et al., 2021).

Diffusion methods, such as WD, are commonly used in AST of plant-derived extracts due to their simplicity (Balouiri et al., 2016). However, they are neither suitable for distinguishing bactericidal from bacteriostatic effects, nor for testing all microorganisms (i.e., some fastidious bacteria or fungi). In contrast, dilution methods, particularly the BMiD according to EUCAST and CLSI criteria, are preferred for estimating the minimum inhibitory concentration (MIC) or the lowest antimicrobial concentration required to halt visible microorganism growth following overnight incubation (Andrews, 2001). These methods are described as quantitative, reproducible and suitable for a range of bacteria, yeasts and fungi. Several studies have compared results among different AST techniques and parameters, aiming to define accurate techniques for specific purposes (Gaudreau & Gilbert, 1997; Chiu et al., 2021).

The available information pertaining to the AST of plant extracts continue to exhibit disparity in data (Balouiri et al., 2016; Chiu et al., 2021; Gaudreau & Gilbert, 1997; Othman et al., 2011; Sana et al., 2019). Apart from the selection of methodology, other factors may contribute to variability in *in vitro* AST results (Panda, 2012). Some of them are related to the inoculum preparation procedures, such as selection of strains, physiological state, growth medium, initial colony forming units (e.g., standardized by turbidimetry) or incubation conditions. Other sources are associated to the plant extract, such as plant origin, extraction method, samples presentation (e.g., EO, encapsulation, dried extracts, etc.) and loading (i.e., volume and concentration of extracts in paper disks/wells), among others (Alirezalu et al., 2020; Balouiri et al., 2016). Particularly, variations in the extract's chemical composition have been pointed out as the primary limiting factor in the introduction of natural preservatives in the food industry context (Alirezalu et al., 2020).

From this perspective, obtaining conclusive *in vitro* results from all previously published studies, while embracing their inherent variability, may enhance the applicability of plant-derived extracts in the food context. The meta-analysis represents a valuable statistical tool for analysing and synthesising information to obtain concise, applicable and science-based outcomes with an enhanced statistical power (Gonzales-Barron & Butler, 2011). Tools such as systematic reviews have been used for food safety applications (EFSA, 2010). However, the meta-analysis allows for linking information from different primary studies, by accurately defining specific moderating variables to the research parameters with the aim of reducing the between-study heterogeneity (Gonzales-Barron & Butler, 2011). The relationship between these variables and their probability distributions may accurately estimate the effect size of certain interventions or treatments (factors) on the outcomes obtained.

This study aimed to conduct a comprehensive meta-analysis of the *in vitro* antimicrobial activity exhibited by Mediterranean extracts of *Allium*, *Ocimum* and *Thymus* spp. For this purpose, the following objectives have been addressed i) conducting a systematic review and compilation of existing literature regarding the *in vitro* AST of *Allium*, *Ocimum* and *Thymus* spp. extracts; ii) generating independent pooled outcomes both for inhibition diameter (ID) and MIC; iii) quantifying the magnitude of the effects' size or influences exerted by various factors and interventions; and iv) evaluating sources of heterogeneity among AST outcomes and exploring correlations between ID and MIC values.

2. Material and methods

2.1. Selection of plant extracts with antimicrobial properties

Firstly, the selection of plant-derived extracts of *Allium*, *Ocimum* and *Thymus* spp. was conducted based on their traditional popularity in the Mediterranean area, culinary uses, chemical composition, antimicrobial properties and food industry applications (Fig. 1). This work was part of a more extensive initiative that encompassed a broader framework of common Mediterranean plants, including *Cinnamomum*, *Salvia*, *Mentha* (Silva et al., 2023) and *Syzygium aromaticum*, *Citrus*, and *Origanum* spp. (Ezzaky et al., 2023).

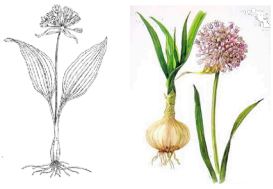
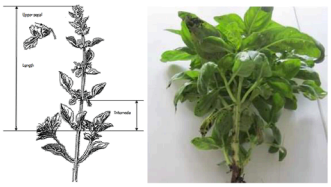
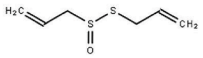
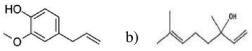
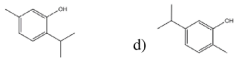
	Allium spp.	*Ocimum* spp.	*Thymus* spp.
Plant morphology			
Most Common Mediterranean species	Garlic (*Allium sativum*), onion (*Allium cepa*), leeks (*Allium ampeloprasum* var. *porrum*), chives (*Allium schoenoprasum*) and shallots (*Allium ascalonicum*)	Sweet basil (*Ocimum basilicum*), clove basil (*Ocimum gratissimum*) and holy basil (*Ocimum sanctum*)	Spanish thyme (*Thymus vulgaris*), white thyme (*Thymus zygis*) and other (*Thymus hyemalis, Thymus mastichina, Thymus citriodotus*…)
Plant part used	Bulbs, whole plant, essential oils	Whole plant, seeds, essential oils	Whole plant, seeds, essential oils
Traditional uses/ Biological properties	Culinary (food and flavouring) and medicinal Antimicrobial, antiviral, antidiabetic, antioxidant, anticarcinogenic, antithrombotic, antihypertensive, hypolipidemic, hypocholesterolemic, antimutagenic, anti-inflammatory, hepatoprotective, neuroprotective, hypotensive, hypoglycemic, immunomodulatory, urease/xanthine oxidase inhibitory and prebiotic properties	Culinary (flavouring), perfumery and medicinal Antimicrobial, antifungal, insecticidal, antiparasitic, antioxidant, immunomodulatory, anti-inflammatory, hepatoprotective, antiosteoporosis, cardioprotective, neuroprotective, anticancer, and other beneficial health effects.	Culinary (flavouring), perfumery and medicinal Antirheumatic, antiseptic, antispasmodic, antimicrobial, anti-inflammatory, carminative, diuretic, and expectorant activities antioxidant, antimicrobial and anticancer antibacterial, antifungal, antiviral, and antiparasitic
Bioactive compounds	Flavonoids, steroidal saponins, phytosterol, phenolic acids, cepaenes, anthocyanins and organosulfur compounds (allicin in garlic; methiin in garlic, onions, leeks, and shallots; propiin in shallots and isoalliinin in onions and shallots)	Alkaloids, flavonoids, phenolic compounds, tannins, saponins, quinones, carbohydrates, and proteins.	Monoterpenes (e.g., thymol, carvacrol 1,8-cineole, linalool, α-terpineol, geraniol, borneol, and thujanol), monoterpenes (e.g., γ-terpinene and p-cymene), sesquiterpenes (caryophyllene) and oxygenated sesquiterpenes (caryophyllene oxide)
Antimicrobial activity	Allyl group; e.g., allicin 	Eugenol (a), linalool (b), Estragole, 1,8-cineole and α-terpineol 	Monoterpenes; e.g., thymol (c), carvacrol (d), 1,8-cineole 
Prospects uses	Food preservation, animal feed and supplementation, aquaculture	Food industry, animal feed and supplementation, aquaculture	Ethnomedicinal, pharmaceutical, food preservation, pesticides, cosmetics, aquaculture, animal production

Fig. 1. Comparative overview of the principal characteristics of *Allium*, *Ocimum*, and *Thymus* spp. in the Mediterranean cultural context.

2.2. Selection of primary articles and criteria for data extraction

A systematic review was carried out using the Scopus, PubMed, Web of Science and SciELO databases. The most used classical AST techniques, most prevalent foodborne pathogens associated with human diseases, and minimum quality criteria for articles, were considered for the information search. The minimum quality criteria encompassed the exclusion of grey literature, articles lacking complete or primary information, articles published before 2000, as well as other meta-analyses and systematic reviews. Therefore, the sources of information were selected according to the following inclusion criteria: (i) articles dealing with the assessment of AST of *Allium*, *Ocimum* and *Thymus* spp. Mediterranean extracts, (ii) ID and MIC results, (iii) AST against the pathogens *E. coli* or Shiga toxin-producing *E. coli*, *L. monocytogenes*, *S. aureus*, *B. cereus* and *Salmonella* spp., (iv) minimum information requirements regarding AST parameters.

For this purpose, a search strategy was defined using a combination of keywords formulated as follows: the tested pathogen separated by "OR" (i.e., *Listeria* OR *Salmonella* OR "*Staphylococcus aureus*" OR "*Escherichia coli*" OR *Campylobacter*); followed by the connector "AND" to incorporate the treatment applied (i.e., extract OR antimicrobial OR "essential oil") obtained from the plant under study (i.e., *Allium* OR *Ocimum* OR *Thymus*); continued by "AND" and the method of determination (MIC OR "agar diffusion" OR halo OR inhibition OR zone OR "minimum inhibitory concentration"). The different microbial groups were selected based on their relevance to foodborne outbreaks. For the optimal execution of the meta-analysis, the grey literature, articles with incomplete information, as well as other meta-analyses and systematic reviews were excluded. Titles, keywords and abstracts were primarily considered in the pre-selection of publications. The references were managed with the software JabRef®.

From the articles chosen for inclusion in the study, the collection of information was feasible for the following parameters: literature source (authors, year of publication and country), plant extract (scientific and common name of the plant species, source or plant part used, extraction method, temperature and solvent used, and dose applied expressed as "LogDose" in %w/v or %v/v), the microbial group (strain and source, such as collection number, and inoculum concentration expressed as "Log cfu/mL"), ID (method distinguishing between DD and WD; values expressed in mm) and MIC (method distinguishing between AD, BMaD and BMiD; values expressed as "LogMIC" in mg/mL for extracts, and in μL/mL for EOs). The data were compiled and subsequently formatted for statistical analysis.

2.3. Meta-regression modelling

Weighted fixed-effect linear models were adjusted to the selected dataset to estimate the pooled ID or MIC produced by the extracts of *Allium*, *Ocimum* and *Thymus* spp. against the selected pathogens (Ezzaky et al., 2023; Silva et al., 2023). The meta-regression fitting was performed using the 'rma.mv' function from the *metafor* package integrated in R software (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria) (Viechtbauer, 2022). Model fitting was performed individually by microbial group, thereby considering the different statistical partitions as an independent meta-analysis for each of them (i.e., multilevel regression). For all the adjusted models, the tested extract dose, inoculum concentration, as well as the MIC values, were logarithm transformed (base 10) to normalise data distribution and reduce heteroscedasticity.

To explain the existing between-study variability embraced in effect size, the following variables were extracted from the primary studies: plant type, extract or EO dose tested, volume of extract or EO absorbed or poured, inoculum level, ID determination method, and number of replicates used for the test. Pooled MIC models were codified based on plant type, MIC determination method, standard errors, antimicrobial type (extract or EO), and number of replicates used for the test (Silva et al., 2023). Additionally, the possibility of occurring interactions between these factors were considered in certain adjusted models, assessing whether the effect depended solely on one-term level or involved multiple terms. Over thirty meta-regression models were

adjusted. The factors were displayed as follows in the general form of the equations (Eq. (1) and (2), to synthesise ID and MIC, respectively:

$$ID_{ij} = \beta_1 LogDose + (\beta_{2j} + u_i)Plant_j + \varepsilon_{ij} \quad (1)$$

$$LogMIC_{ijmn} = (\beta_{1j} + u_i)Plant_j + \beta_{2m}Method_m + \beta_{3n}AntimicrobialType_n + \varepsilon_{ijmn} \quad (2)$$

where β_1 represents the impact on the ID of a one-unit increase in extract dose. Other components of the statistical equation, such as β_{2j}, β_{1j}, β_{2m}, and β_{3n}, denote the fixed effects of different categories of plants, types of MIC determination methods, and types of antimicrobial tests. The terms ε_{ij} and ε_{ijmn} represent the model residuals, or discrepancies between the model's predictions and observations. To account for the remaining variability that cannot be explained, random effects u_i are introduced for each study i in β_{2j} and β_{1j} (which are sets of fixed effects representing different types of plants in Eq. [1] and [2], respectively) (Silva et al., 2023; Ezzaky et al., 2023).

Eq. (1) has been used to determine the ID observation from the j^{th} plant taken from the i^{th} study; whereas Eq. (2) was used to determine MIC observations based on the j^{th} plant, the m^{th} method, the n^{th} antimicrobial type and the i^{th} study. In general, the terms u_i were postulated to follow a normal distribution with a mean of zero and a between-study variability τ^2.

Furthermore, the relationship between the ID and MIC values produced by extracts of *Allium*, *Ocimum* and *Thymus* spp. was modelled for the tested pathogens. Similarly, a weighted mixed-effect linear model was adjusted to observations, considering the extract dose, MIC and the microbial group as moderators (Eq. [3])

$$ID_{ik} = (\beta_0 + u_i) + \beta_1 LogDose + \beta_2 LogMIC + \beta_{3k}MicrobialGroup_k + \varepsilon_{ik} \quad (3)$$

where β_0, β_1, β_2 and β_{3k} represent the mean intercept, and the effects of the logarithm of dose, the logarithm of MIC and the microbial group, respectively. The variability between studies i and pathogens k was considered into the error term ε_{ik}. The intercept u_i was used to explain the remaining unexplained variability in ID, also assuming a normal distribution with mean zero and between-study variability τ^2.

The parameters of the models, as affected by moderators, were calculated from the fitted meta-regressions, and the significance of moderators was evaluated by analysis of variance (ANOVA, $\alpha = 0.05$). Additionally, the quality of the research design was assessed by assigning different weights to each primary study according to the total study sample size (n). The existence of publication bias was also considered. For its assessment, the primary method chosen was the examination of significance by including n as moderator variable. When p-values were found to be lower than 0.05, publication bias was notably affecting the estimations. This indicates that the studies examined in the analysis had insufficient sample sizes or included results for extracts with very low or no inhibition activity. On the other hand, funnel plots were created as a qualitative method for assessing publication bias (included as Supplementary Material). As normally interpreted, larger studies positioned closer to the average in the plot suggest reduced bias, while unconfigured data points or empty areas may be indicative of heterogeneity. However, relying solely on this visual inspection for concluding, especially in cases of unreported sample sizes, may introduce inconsistencies.

3. Results and discussion

3.1. Data collection

In this study, we analysed the available evidence in scientific literature about how extracts from *Allium*, *Ocimum* and *Thymus* spp. affect foodborne pathogens, more specifically *L. monocytogenes*, *S. aureus*, *E. coli*, *B. cereus* and *Salmonella* spp.. By means of a systematic review, a total of 82 primary articles published during the 2000–2021 period were selected from a former database composed of 2,065 articles. The references included in the meta-analysis study, after the selection detailed in Section 2.2., are listed in Supplementary Material. From studies meeting the specified criteria and duplicates removed, 742 data records were extracted. These data included ID and MIC values of *Allium*, *Ocimum* and *Thymus* spp. extracts against the relevant foodborne pathogens.

3.2. Meta-analytical modelling of data obtained by diffusion methods

For the evaluation of the information available in the literature for AST of plant extracts applied through diffusion methods, the ID was the outcome considered in this meta-analytical approach (most reported for diffusion methods). Pooled ID produced by *Allium*, *Ocimum* and *Thymus* spp. extracts were estimated through meta-analysis models separately adjusted for the different pathogens studied (Tables 1-3). In those cases where data were available, evaluation was conducted by using different diffusion methods.

Table 1
Model estimations for the inhibition diameters produced by extracts of *Allium* spp., pooled through the adjustment of the meta-analytical model separately by microbial group and considering disk diffusion or well diffusion as determination methods.

Microbial group[1]	Plant variety	Method	Pooled ID[2] (SE)	n	N	Pub. bias[4] (p-value)
Escherichia coli[B]	Garlic	DD	15.09[a] (1.341)	10	10	0.372
		WD	16.33[a] (1.345)	14		
	Onion	DD	11.63[b] (1.450)	10		
Bacillus cereus[A]	Garlic	DD	19.35[a] (0.609)	5	5	0.200
		WD	20.64[a] (0.608)	3		
	Onion	DD	17.15[b] (0.810)	4		
Staphylococcus aureus[B]	Garlic	DD	15.15[a] (1.862)	16	14	0.661
		WD	12.20[b] (1.903)	11		
	Onion	DD	6.865[c] (1.990)	9		
Salmonella spp.[B]	Garlic	WD	16.27[a] (1.746)	32	8	0.004
	Onion	DD & WD[3]	14.07[b] (1.758)	5		
Listeria monocytogenes[C]	Garlic	WD	13.51[a] (1.803)	20	7	0.895
	Onion	DD	12.00[b] (1.837)	6		

Pooled ID = model-estimated inhibition diameter, expressed as mean (mm) and standard error (SE); DD = disk diffusion; WD = well diffusion; n = number of observations; N = number of primary studies.

[1] Significant differences ($p \leq 0.05$) obtained between the estimated pooled inhibition diameters across different microbial groups are indicated with superscript uppercase letters (A to C, from highest to lowest inhibition) by only considering estimations for garlic (*Allium sativum*, dose of 100 mg/ml).

[2] Significant differences ($p \leq 0.05$) obtained between the estimated pooled inhibition diameters within the same microbial group are indicated with superscript lowercase letters (a to c) by considering both garlic and onion extracts (*Allium sativum* and *Allium cepa*, respectively, dose of 100 mg/ml).

[3] Inhibition diameters from DD and WD method were combined since the effect of the determination method was not significant ($p > 0.10$).

[4] The publication bias (p-value) is obtained considering each individual meta-analysis model.

Table 2
Model estimations for the inhibition diameters produced by extracts of *Ocimum* spp., pooled through the adjustment of the meta-analytical model separately by microbial group and considering disk diffusion or well diffusion as determination methods.

Microbial group[1]	Plant variety	Method	Pooled ID[2] (SE)	n	N	Pub. bias[4] (*p*-value)
Escherichia coli[A]	All[3]	DD	12.51[b] (1.403)	33	18	0.370
		WD	18.25[a] (1.296)	5		
Bacillus cereus[A]	All[3]	DD	17.04 (1.119)	8	5	0.495
Staphylococcus aureus[A]	All[3]	DD	15.39[b] (1.198)	22	14	0.375
		WD	26.39[a] (3.676)	4		
Salmonella spp.[A]	Basil	DD	11.19[b] (0.446)	5	6	0.949
	Holy basil	DD	18.21[a] (0.408)	3		
Listeria monocytogenes[A]	All[3]	DD	14.80 (1.129)	17	10	0.017

Pooled ID = model-estimated inhibition diameter, expressed as mean (mm) and standard error (SE); DD = disk diffusion; WD = well diffusion; n = number of observations; N = number of primary studies.

[1] Significant differences ($p \leq 0.05$) obtained between the estimated pooled inhibition diameters across different microbial groups are indicated with superscript uppercase letters (A to C, from highest to lowest inhibition) by only considering estimations for basil and holy basil (*Ocimum basilicum* and *Ocimum sanctum*, respectively, dose of 100 mg/ml) as measured by DD method.

[2] Significant differences ($p \leq 0.05$) obtained between the estimated pooled inhibition diameters within the same microbial group are indicated with superscript lowercase letters (a to c) by considering both basil and holy basil (dose of 100 mg/ml).

[3] No significant differences ($p > 0.10$) were found between species (i.e., basil and holy basil), therefore observations were pooled.

[4] The publication bias (*p*-value) is obtained considering each individual meta-analysis model.

3.2.1. *Allium spp*

A meta-analytical modelling was separately displayed for each of the different microbial groups selected against *Allium* spp. extracts (Table 1). For all the partitions, a recurrent significant effect of extract doses (%w/v or %v/v) was found. In this regard, an extract dose of 100 mg/mL was fixed to facilitate the comparisons between the effects observed among different microbial groups. Thus, the significant differences ($p \leq 0.05$) in the pooled ID revealed a reasonable agreement in *B. cereus* as the most sensitive bacteria to the different *Allium* spp. plant extracts. Specifically, *B. cereus* was highlighted as the most susceptible bacteria to garlic extracts (*Allium sativum*) applied by WD (20.64 ± 0.61 mm). The potential of garlic extracts controlling microbial growth has been widely reported (El-Azzouny et al., 2018; Yasin et al., 2022). In detail, the major compound of garlic EO, diallyl disulfide (constituting 20–30 % of its content), has been shown to affect *B. cereus* by increasing the lag phase, decreasing maximum concentrations, inhibiting virulence factors and reducing the toxin and biofilm-production capacity, even at sub-inhibitory concentrations (≤60 μg/mL) (Chen et al., 2018). Contrarily, at a garlic dose concentration of 100 mg/mL, the lowest inhibitory activity was observed on *L. monocytogenes*. Under this scenario, enhancing the effectiveness of the tested extract could be achieved by implementing strategies such as synergising with other extracts that contain nisin (Jin et al., 2021).

The inoculum level (log cfu/mL), at concentrations of 100 mg/mL of *Allium* spp. extracts, resulted as a significant continuous variable ($p \leq 0.05$) both in the cases of extracts tested against *Salmonella* spp. and *L. monocytogenes* (Figure S1). In relation to the AST methodology, data was concurrently available for the methods of DD and WD in testing assays of garlic extracts against *E. coli*, *B. cereus* and *S. aureus*. In this case, the obtained pooled ID showed significant differences between both methodologies only in the case of *S. aureus* (significant effect of the method, $p \leq 0.10$, Table 1). In the case of onion extracts (*Allium cepa*), data availability for both methodologies were limited to AST against *Salmonella* spp, although not resulting significant ($p \leq 0.10$), thus they were combined as "DD & WD". Regardless of the methodology used, the pooled ID obtained for the application of garlic extracts and onion extracts showed significant differences between them ($p \leq 0.05$) in all the evaluated microbial groups.

Among partitions, statistical significance revealed the presence of publication bias solely in *Salmonella* spp. testing against *Allium* spp. extracts by diffusion methods ($p = 0.004$), elucidating the possible lack of valuable data in the literature compared to the other analysed microbial groups. Figure S2 illustrates funnel plots for studies analysing AST of *Allium* spp. extracts against the studied pathogens.

Table 3
Model estimations for the inhibition diameters produced by extracts of *Thymus* spp., pooled through the adjustment of the meta-analytical model separately by microbial group and considering disk diffusion or well diffusion as determination methods.

Microbial group[1]	Plant variety	Method	Pooled ID[2] (SE)	n	N	Pub. bias[5] (*p*-value)
Escherichia coli[B]	Moroccan & Tunisian[3]	DD	14.92[a] (1.854)	5	19	0.691
	Thyme	DD	23.74[a] (1.653)	13		
		WD	17.15[b] (1.244)	68		
Bacillus cereus[A]	Moroccan	DD	18.88[b] (2.605)	4	15	0.995
	Thyme	DD	28.90[a] (2.389)	16		
		WD	17.67[b] (2.345)	8		
Staphylococcus aureus[B]	Moroccan	DD	14.79[b] (0.337)	3	21	0.792
	Thyme	DD & WD[4]	20.80[a] (2.518)	109		
Salmonella spp.[B]	Thyme	DD & WD[4]	17.77 (1.805)	103	22	0.376
Listeria monocytogenes[A]	Moroccan	DD	12.64[b] (0.342)	3	17	0.403
	Thyme	DD & WD[4]	23.14[a] (2.823)	99		

Pooled ID = model-estimated inhibition diameter, expressed as mean (mm) and standard error (SE); DD = disk diffusion; WD = well diffusion; n = number of observations; N = number of primary studies.

[1] Significant differences ($p \leq 0.05$) obtained between the estimated pooled inhibition diameters across different microbial groups are indicated with superscript uppercase letters (A to C, from highest to lowest inhibition) by only considering estimations for thyme (*Thymus vulgaris*, dose of 100 mg/ml).

[2] Significant differences ($p \leq 0.05$) obtained between the estimated pooled inhibition diameters within the same microbial group are indicated with superscript lowercase letters (a to c) by considering *Thymus* spp. (dose of 100 mg/ml).

[3] *Thymus saturejoides* and *Thymus capitatus* are commonly referred to as Moroccan and Tunisian thyme, respectively.

[4] Inhibition diameters from DD and WD method were combined since the effect of the determination method was not significant ($p > 0.10$).

[5] The publication bias (*p*-value) is obtained considering each individual meta-analysis model.

3.2.2. *Ocimum spp*

As occurred for *Allium* spp., the effect of the extract dose (%w/v or % v/v) resulted significant ($p \leq 0.05$) for all the partitions. The ID outcomes obtained across the different microbial groups for the application of *Ocimum* spp. extracts did not reveal significant differences ($p > 0.05$, at doses of 100 mg/mL) (Table 2). Similarly, significant differences were not found ($p > 0.10$) between the pooled ID obtained for basil (*Ocimum basilicum*) and holy basil (*Ocimum sanctum*) extracts within the same microbial group (therefore observations were grouped as "all"), apart from *Salmonella* spp. testing. The absence of conclusive findings for AST of *Ocimum* spp. using diffusion methodologies emphasises the need for further testing employing alternative methods to evaluate the antimicrobial efficacy of their extracts.

The influence of the methodology selected could be assessed on *Ocimum* spp. AST against *E. coli* and *S. aureus*. Statistically significant differences ($p \leq 0.10$) were observed when comparing DD and WD observations, with the WD method producing higher pooled ID values. Furthermore, the volume of extract in the disk (μL), together with the extract dose, resulted significant in AST of *B. cereus* against *Ocimum* spp. extracts ($p \leq 0.05$) (Figure S1).

Funnel plots of the meta-regression on ID produced by *Ocimum* spp. plant extracts are shown in Figure S3. The existence of publication bias was only significant in the case of *L. monocytogenes* testing ($p = 0.017$). This observation probably underscores the need for further investigation in AST of *Ocimum* spp. extracts against *L. monocytogenes* using diffusion methods.

3.2.3. *Thymus spp*

The *in vitro* effectiveness of *Thymus* spp. extracts in inhibiting a wide range of foodborne pathogens has been extensively reported (De Carvalho et al., 2015, Salehi et al., 2019). Potential antimicrobial compounds, such as thymol and carvacrol, have been demonstrated to have a synergistic effect with other extract's compounds as 1,8-cineole, linalool, geraniol, camphor and α-pinene (Nieto, 2020).

In this analysis, observations obtained through DD and WD methods were available for all the cases of AST for thyme extracts (*Thymus vulgaris*) (Table 3). The selected method only resulted significant ($p \leq 0.10$) for AST against *E. coli* and *B. cereus*, in both cases obtaining higher pooled ID values in observations determined by DD. In the case of Moroccan thyme extracts (*Thymus saturejoides)*, only observations determined by DD were available for the data set. Our results indicated *B. cereus* and *L. monocytogenes* as the most susceptible pathogens to 100 mg/mL doses of thyme extract, with average pooled ID values of 28.90 ± 2.34 mm and 23.14 ± 2.82 mm, respectively ($p \leq 0.05$). The dose-dependent effect (%) of *Thymus* spp. extracts was demonstrated, elucidating once again the importance of testing the effects and interactions of different doses in food matrices.

The publication bias was not significant ($p > 0.05$) for any of the microbial groups evaluated against *Thymus* spp. extracts. The absence of publication bias in the analysis of *Thymus* spp. AST using diffusion methods suggested that our study included a balanced representation of the extensive body of available research in the literature. Additionally, Figure S4 displays funnel plots from meta-regression analysis on ID generated by *Thymus* spp. plant extracts.

3.3. *Meta-analytical modelling of data obtained by dilution methods*

The MIC (most reported for dilution methods) was chosen as the primary outcome to assess the information gathered from the literature regarding the AST of plant extracts using dilution methods. The pooled MICs were estimated for AST of *Allium*, *Ocimum* and *Thymus* spp. extracts through different microbial-adjusted meta-analytical models (Table 4). It is important to note that models for *Allium* and *Ocimum* spp. were adjusted only for observations of garlic (*A. sativum)* and basil

Table 4
Model estimations for the minimum inhibitory concentrations produced by extracts or essential oils of *Allium*, *Ocimum* and *Thymus* spp., pooled through the adjustment of the meta-analytical model separately by microbial group and considering agar dilution, broth macro-dilution and broth micro-dilution as determination methods.

Genus plant	Microbial group	Plant variety	Type	Method	Pooled MIC[1] [95 % CI]	n	N	Pub. bias[3] (*p*-value)
Allium spp.	*Escherichia coli*	Garlic	Extract	AD & BMaD[2]	0.238 [0.016 – 3.651]	3	3	0.001
	Staphylococcus aureus	Garlic	Extract	AD & BMiD[2]	0.146 [0.012 – 1.735]	21	6	<.0001
	Salmonella spp.	Garlic	Extract	BMaD	12.13 [4.420 – 33.26]	7	3	0.746
Ocimum spp.	*Escherichia coli*	Basil	Extract	BMiD	0.555[a] [0.069 – 4.433]	8	6	0.760
			EO	BMiD	4.022[a] [0.169 – 9.562]	3		
	Staphylococcus aureus	Basil	Extract	BMiD	0.263[a] [0.014 – 4.745]	9	7	0.354
			EO	BMiD	1.772[a] [0.015 – 21.56]	3		
	Salmonella spp.	Basil	Extract	BMiD	1.307 [0.451 – 3.786]	4	3	0.513
	Listeria monocytogenes	Basil	Extract	BMiD	0.808 [0.115 – 5.644]	4	3	0.018
Thymus spp.	*Escherichia coli*	Moroccan	Extract	BMiD	8.976[b] [2.687 – 30.00]	5	19	0.727
		Thyme	Extract	BMiD	0.361[a] [0.118 – 1.104]	18		
			EO	AD	1.553[ab] [0.292 – 8.278]	8		
	Bacillus cereus	Moroccan	Extract	BMiD	1.654[b] [0.300 – 9.149]	4	14	0.424
		Thyme	Extract	AD	0.075[a] [0.015 – 0.900]	6		
				BMiD	0.919[ab] [0.271 – 3.117]	7		
			EO	BMiD	0.956[ab] [0.212 – 4.303]	3		
	Staphylococcus aureus	Moroccan	Extract	BMiD	2.666[a] [0.798 – 8.906]	5	22	0.894
		Thyme	Extract	AD	1.726[a] [0.347 – 8.592]	7		
				BMiD	0.908[a] [0.286 – 2.885]	15		
			EO	BMiD	1.278[a] [0.473 – 3.459]	41		
	Salmonella spp.	Thyme	Extract	AD	1.898[a] [0.516 – 6.984]	4	16	0.197
				BMiD	1.538[a] [0.779 – 3.038]	14		
			EO	BMiD	1.189[a] [0.804 – 1.758]	44		
	Listeria monocytogenes	Moroccan	Extract	BMiD	3.014[b] [0.902 – 10.07]	5	17	0.454
		Thyme	Extract	BMiD	0.205[a] [0.023 – 1.207]	6		
			EO	BMiD	1.875[b] [0.910 – 3.863]	43		

Pooled MIC = model-estimated minimum inhibitory concentration, expressed as mean, in mg/mL or μL/mL, and 95 % confidence interval (CI); EO = essential oil; AD = agar dilution, BMaD = broth macro-dilution; BMiD = broth micro-dilution; n = number of observations; N = number of primary studies.

[1] Within a given genus plant and microbial group, different superscript lowercase letters indicate significant differences ($p \leq 0.05$) in MIC produced by extracts and EO.

[2] MIC values measured by two different methods were combined since the effect of the determination method was not significant ($p > 0.10$).

[3] The publication bias (*p*-value) is obtained considering each individual meta-analysis model.

(*O. basilicum*) extracts, respectively.

Regarding *A. sativum*, the observations obtained by dilution methods (AD and BMaD) were combined since the effect of the methodology was not significant ($p > 0.10$). In this case, the highest antimicrobial capacity of garlic extracts was found against *S. aureus* (pooled MIC = 0.146 mg/mL). Considering the extensive reported antimicrobial spectrum of garlic extracts, this study highlights their notable effectiveness against *S. aureus*. This approach is especially advantageous for future applications, such as garlic peels utilization, in products susceptible to *S. aureus* risks (Hernández-Montesinos et al., 2023). At present, industries producing milk and meat fermented products are assessing the addition of commercial unflavoured garlic products to enhance food safety. On the other hand, the results demonstrated significant publication bias in *E. coli* and *S. aureus* testing ($p = 0.001$ and $p < 0.001$, respectively). In these cases, the results indicate the need for more comprehensive studies with larger sample sizes. Indeed, this finding emphasizes the necessity for additional comprehensive research into the efficacy of *Allium* spp. extracts against these pathogens.

Concerning *O. basilicum*, AST data using the BMiD method were available for assessment. In cases where observations for both extracts and EO were available (*E. coli* and *S. aureus*), no significant differences were observed between the two applications ($p > 0.05$). The highest *Ocimum* spp. antimicrobial capacity was shown against *S. aureus* (pooled MIC = 0.263 mg/mL), followed by *E. coli* (pooled MIC = 0.555 mg/mL). In this context, the effects of adding basil extracts to control the growth rate of *S. aureus* and *E. coli* have been tested in food matrices such as boiled sausages (Macari et al., 2021). The publication bias was only significant in *Ocimum* spp. AST by dilution methods against *L. monocytogenes*, revealing the need for more in-depth research.

Further assessment of *Thymus* spp. was feasible due to the availability of AST data obtained from different methods (BMiD or AD) and extracts sourced from different plant species. Comparing pooled MIC within each microbial group, no significant differences ($p > 0.05$) were obtained between MIC values for *S. aureus* and *Salmonella* spp., inferring similar effects regardless of the plant applied (Moroccan or thyme), type of extract or method. However, for the remaining cases for which significant differences ($p \leq 0.05$) were obtained, pooled MIC values were higher in all cases for the application of Moroccan *Thymus*. The reduced antimicrobial activity of Moroccan *Thymus* aligns with the findings obtained through diffusion methods detailed in Section 3.1.3. In relation to *T. vulgaris*, data obtained using the methods of AD and BMiD were simultaneously available for extracts tested against *B. cereus*, *S. aureus* and *Salmonella* spp. In all these cases, the effect of the applied method did not show significant differences ($p > 0.10$). On the other hand, MIC data for thyme extracts in both dried and EO forms were accessible for all evaluations. However, the thyme extract presentation did not result significant ($p > 0.10$), apart from *L. monocytogenes*. In this particular case, the highest antimicrobial activity was shown by the dried extracts (pooled MIC = 0.205 mg/mL).

Regardless the level of significance, the extract form of *T. vulgaris* demonstrated high antimicrobial capacity against all the microbial groups evaluated. In particular, the lowest pooled MIC value (0.075 mg/mL) was achieved for *B. cereus* partition. Based on the meta-analytical models generated from data obtained through diffusion and dilution methods, *B. cereus* emerged as the most sensitive foodborne pathogen to *Thymus* spp. extracts in this study. *B. cereus* holds significant importance for the food industry due to its capacity to cause foodborne intoxications given its production capacity of emetic toxins, virulence proteins (hemolysins, proteases and cytotoxin K, among others), spores and biofilms (Ayari et al., 2020). In this sense, recent studies have shown the high inhibitory activity of thyme EO on *B. cereus* biofilm cells. Sateriale et al. (2023) delineated the impact of thyme EO on inhibiting biofilm formation in *B. cereus* strains isolated from minced pig meat. Similarly, Kang et al. (2018) outlined the changes produced by the same extract on the biofilm-ability formation and cell morphology of *B. cereus*.

3.4. Meta-regression relationship between MIC and ID

Based on the selected moderating factors (MIC, extract dose and microbial group), a meta-regression model was constructed to assess their importance as predictors on the ID produced by the effects of *Thymus* and *Ocimum* spp. plant extracts. The influence of these factors and the obtained estimations are depicted in Table 5.

For the interpretation of the meta-regression model, the impact of different moderating factors on the antimicrobial effect, or the estimated ID, was evaluated. Considering that the intercept is a statistical product obtained from the meta-regression analysis (7.525 ± 9.103, $p = 0.408$), the only effect revealing a positive correlation with the ID estimation was the factor "Log Dose" (31.750 ± 0.244, $p < 0.001$). The positive relationship can be elucidated by noting that higher ID values are achieved with an increase in the extract dose. This observation underscores the importance of conducting meticulous investigations into the optimal treatment conditions with plant extracts in food applications. This involves determining the precise dosage needed for the diverse food matrices and antimicrobial purposes, as emphasised by Tajani and Bisha (2023). Moreover, it is essential to ensure that this dosage effectively reaches the food matrix and maintains its stability over time to guarantee effectiveness, while preserving the desirable sensorial and organoleptic features of the product. For instance, current advancements in the meat industry actively explore the integration of plant extracts through emulsion, gelling, encapsulation, and active packaging (Alirezalu et al., 2020). Nonetheless, optimising plant extraction, preserving the desired phenolic composition, ensuring long-term stability and sustainability, while addressing factors such as extraction scope, volatilisation time and potential loss of effectiveness, is continuing to be challenging (Maurya et al., 2021).

Conversely, the remaining factors evaluated were inversely correlated with ID in the meta-regression model. The "Log MIC" obtained an estimate of -3.858 ± 0.281 ($p < 0.001$), producing a negative effect on the predicted ID values. This outcome was expected, since high MIC values are associated with low antimicrobial effects in AST and hence, low ID values. Fig. 2 illustrates the inversely proportional relationship observed between the logarithm of the MIC and ID values produced by

Table 5
Meta-regression model on the inhibition diameter produced by extracts of *Thymus* (n = 145) and *Ocimum* (n = 5) spp. plants as a function of the minimum inhibitory concentration, extract dose and microbial group.

Parameter	Estimate[1]	SE	*p*-value[2]	n	Heterogeneity analysis[3]
Intercept	7.525	9.103	0.408		
Log MIC	−3.858	0.281	<.0001		$s^2 = 54.15$
Log Dose	31.750	0.244	<.0001		$\tau^2 = 47.87$
Microbial group					$I^2 = 46.9$ %
Campilobacter	-9.757^b	0.442	<.0001	26	$\tau^2_{res} = 16.00$
jejuni	-11.29^c	0.553	<.0001	6	$R^2 = 66.5$ %
Escherichia coli	-7.509^a	0.439	<.0001	41	
Listeria	-8.102^a	0.443	<.0001	44	Publication bias
monocytogenes	-8.303^a	0.439	<.0001	33	$p = 0.283$
Staphylococcus aureus					
Salmonella spp.					

Log MIC = logarithm of the minimum inhibitory concentration, expressed in ml/mg and μL/mL for extracts and essential oils, respectively; Log Dose = logarithm of the extract dose, expressed in %w/v and %v/v for extracts and essential oils, respectively; SE = standard error; n = number of observations.

[1] Different superscript letters indicate significant differences ($p \leq 0.05$) in the estimates between microbial groups.

[2] *P*-value obtained for the significance test of the publication bias per factor level.

[3] Heterogeneity analysis encompasses within-study variability (s^2), between-study variability of the null model (τ^2), intra-class correlation (I^2), residual between-study variability (τ^2_{res}), and between-study variability explained by significant moderators (R^2).

Thymus and *Ocimum* spp. plant extracts against the different pathogens studied. It's noteworthy that the negative relationship remained consistent at a specific extract dosage, unaffected by other factors incorporated. For instance, the selection of the determination method (diffusion or dilution methods) did not exert any influence on the predictions of the meta-regression model. This observation aligns with Silva et al. (2023) regarding *Syzygium aromaticum*, *Citrus* spp. and *Origanum* spp. extracts, as well as with Ezzaky et al. (2023) for *Cinnamomum*, *Salvia* and *Mentha* spp. extracts.

Considering the variable microbial group, the calculation of an intercept did not yield significant results ($p = 0.408$, Table 5). All the tested microbial groups showed the same effect, at different levels of significance, on the estimated ID at a specific extract dose applied. The negative outputs generated by the meta-regression model indicated that the most negative values (or the largest magnitudes when considering absolute values) are associated with smaller ID within the mathematical operations of the model. Therefore, *L. monocytogenes* was estimated as the most sensitive foodborne pathogen to the effects of *Thymus* and *Ocimum* spp. extracts (-7.509 ± 0.439 mm); *S. aureus* and *Salmonella* spp. were also susceptible at a same level of significance (-8.102 ± 0.443 and -8.303 ± 0.439 mm, respectively) and *E. coli* was predicted as the most resistant pathogen to the antimicrobial effects of *Thymus* and *Ocimum* spp. (-11.290 ± 0.553 mm). Traditionally, the potential effects of plant-derived extracts, particularly in the case of EO, have been primarily associated with Gram-positive (G +) bacteria, due to the presence of an outer layer of lipopolysaccharides (LPS) in Gram negative (G-) bacteria (Maurya et al., 2021). However, our model demonstrated an inhibitory impact on both G + and G- bacteria, with values consistently exhibiting statistical significance across all evaluated scenarios. These findings notably highlight the multi-mechanism of action involved in the antimicrobial effects exerted by plant-derived extracts, elucidating their broad-spectrum utility across various applications (Álvarez-Martínez et al., 2021; Bhavaniramya et al., 2019; Vaou et al., 2021).

The influence of the heterogeneity on the ID estimations in this meta-regression model was also assessed. As first step, the parameter I^2 was used as indicator of intra-class correlation. In essence, it was utilized to ascertain the proportion of heterogeneity explained by the variability obtained between the studies used for the model construction. In this case, the I^2 resulted in a value of 46.9 % (Table 5). This explains that nearly half of the overall variability in the effect size can be attributed to the inherent between-study heterogeneity. According to Higgins et al. (2003), the level of heterogeneity in this study can be classified as low-moderate (Gonzales-Barron et al., 2021).

Furthermore, R^2 was calculated to assess the impact of moderators on the between-study variability. This was performed by comparing the residual between-study variability of the model (with the moderator τ^2_{res}) with that obtained for the null model (τ^2) (Gonzales-Barron & Butler, 2011). The parameter R^2 presented a value of 66.5 %. This value was deemed acceptable, as the modulating factors included into the model accounted for two-thirds of the observed between-study variability. The remaining non-explained or residual heterogeneity may originate from other factors inherent to each specific study included in this meta-analysis, such as the plant part utilized, extraction method employed, or the composition of the final extract.

Concerning the publication bias, while an initial presumption may be drawn from the absence of data in the lower-left region of Figure S5, it was ultimately determined that this bias did not achieve statistical significance ($p = 0.283$). Finally, the model's validity was assessed by determining the Pearson correlation coefficient. The model exhibited a strong goodness-of-fit when the predicted ID were plotted against the observed values (Fig. 3). Notably, a correlation coefficient of 0.906 underscored the model's adjustment and robustness.

The models' limitations in comprehensively capturing the varied sources of variability within the literature can be addressed by supplementing data, especially in cases where publication bias has been identified. However, it is important not to undermine the value of the studied extracts as sustainable alternatives to conventional antimicrobial agents. The model may assist in synthesising data of the *in vitro* antimicrobial efficacy of extracts of *Allium*, *Ocimum* and *Thymus* spp., potentially elucidating the importance of factors such as the extract dose. Future research may aim to integrate these commonly used Mediterranean plant extracts into high-risk food matrices, particularly focusing on ready-to-eat (RTE) products traditionally manufactured in the Mediterranean basin. Furthermore, this study and similar research methodologies can be expanded to explore spoilage microorganisms, aiming to discover innovative strategies to extend product shelf-life and implement efficient technological solutions. The design of novel biopreservatives applied to food products could be a valuable contribution to the development of effective and sustainable measures for preventing foodborne outbreaks, economic losses and emerging MDR strains.

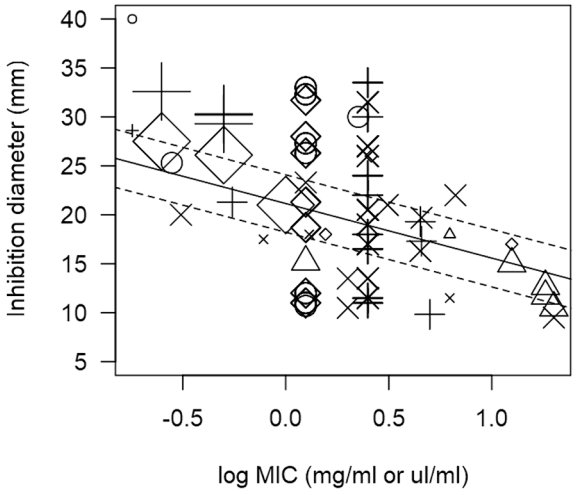


Fig. 2. Scatter plot depicting the effect ($p \leq 0.001$) of the logarithm of the minimum inhibitory concentration (MIC) of extracts of *Thymus* (n = 145) and *Ocimum* (n = 5) spp. plants on inhibition diameters (ID) for each microgial group (○= *Campilobacter jejuni*, Δ= *Escherichia coli*, += *Listeria monocytogenes*, ×= *Staphylococcus aureus*, ◊= *Salmonella* spp., with marker sizes proportional to the study size).

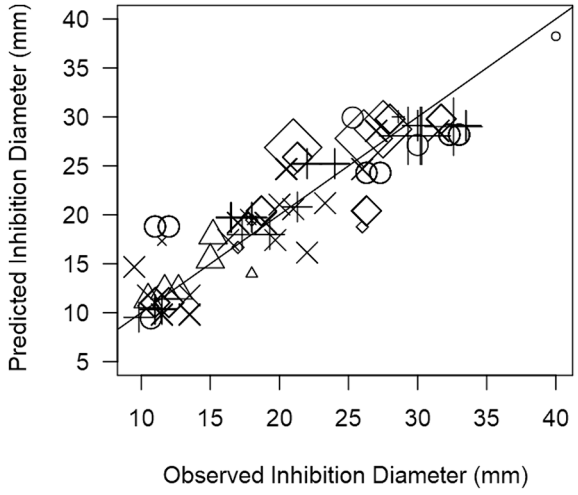


Fig. 3. Scatter plot of the observed inhibition diameters (ID) produced by extracts of *Thymus* (n = 145) and *Ocimum* (n = 5) spp. plants versus values predicted by the meta-regression model (R = 0.906) for each microbial group (○= *Campilobacter jejuni*, Δ= *Escherichia coli*, += *Listeria monocytogenes*, ×= *Staphylococcus aureus*, ◊= *Salmonella* spp.; with marker sizes proportional to the study size).

4. Conclusions

The meta-analysis of available literature on the *in vitro* antimicrobial effects of *Allium*, *Ocimum* and *Thymus* spp. revealed variations in microbial susceptibilities among foodborne pathogens and the significant influence of testing variables, especially extract dosage, on these activities. Particularly, *Allium* and *Thymus* spp. extracts showed notable efficacy against *B. cereus*, while *Ocimum* spp. extracts exhibited significant antimicrobial capacity against *S. aureus*. The developed meta-regression model revealed a positive correlation between the extract dose and the inhibition zones recorded in disk and well diffusion methods, whereas minimum inhibitory concentrations, determined through agar dilution, broth macro- and micro- dilution techniques, were negatively correlated with these inhibition zones. These results aid on the design and/or adoption of biopreservation strategies by food producers to increase the microbiological safety of their products, while reducing the use of chemical preservatives. Regulatory agencies may also consider the results of this study when evaluating the safety and efficacy of these plant-derived antimicrobial agents for food applications. Finally, consumers may benefit from enhanced food safety and quality resulting from the use of the evaluated extracts as natural biopreservatives.

CRediT authorship contribution statement

Olga María Bonilla-Luque: Data curation, Investigation, Visualization, Writing – original draft. **Beatriz Nunes Silva:** Writing – review & editing, Methodology, Investigation, Data curation. **Youssef Ezzaky:** Writing – review & editing, Visualization, Investigation, Data curation. **Arícia Possas:** Writing – review & editing, Validation, Investigation. **Fouad Achemchem:** Writing – review & editing, Resources. **Vasco Cadavez:** Writing – review & editing, Supervision, Software, Resources, Investigation, Formal analysis, Conceptualization. **Úrsula Gonzales-Barron:** Writing – review & editing, Supervision, Software, Resources, Investigation, Formal analysis, Conceptualization. **Antonio Valero:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2024.114408.

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