


COMPREHENSIVE REVIEW

In vitro antimicrobial activity of extracts and essential oils of *Cinnamomum*, *Salvia*, and *Mentha* spp. against foodborne pathogens: A meta-analysis study

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Abstract

Essential oils (EOs) are a class of natural products that exhibit potent antimicrobial properties against a broad spectrum of bacteria. Inhibition diameters (IDs) and minimum inhibitory concentrations (MICs) are the typical measures of antimicrobial activity for extracts and EOs obtained from *Cinnamomum*, *Salvia*, and *Mentha* species. This study used a meta-analytical regression analysis to investigate the correlation between ID and MIC measurements and the variability in antimicrobial susceptibility tests. By utilizing pooled ID models, this study revealed significant differences in foodborne pathogens' susceptibility to extracts, which were dependent on both the plant species and the methodology employed ($p < .05$). Cassia showed the highest efficacy against *Salmonella* spp., exhibiting a pooled ID of 26.24 mm, while cinnamon demonstrated the highest efficacy against *Bacillus cereus*, with a pooled ID of 23.35 mm. Mint extract showed the greatest efficacy against *Escherichia coli* and *Staphylococcus aureus*. Interestingly, cinnamon extract demonstrated the lowest effect against Shiga toxin-producing *E. coli*, with a pooled ID of only 8.07 mm, whereas its EOs were the most effective against this bacterial strain. The study found that plant species influenced the MIC, while the methodology did not affect MIC measurements ($p > .05$). An inverse correlation between ID and MIC measurements was identified ($p < .0001$). These findings suggest that extracts and EOs obtained from *Cinnamomum*, *Salvia*, and *Mentha* spp. have the potential to inhibit bacterial growth. The study highlights the importance of considering various factors that

may influence ID and MIC measurements when assessing the effectiveness of antimicrobial agents.

KEYWORDS

Cinnamomum, inhibition diameter, *Mentha*, meta-regression, minimum inhibitory concentration, *Salvia*

1 | INTRODUCTION

Foodborne illnesses pose a significant threat to public health, resulting in a substantial number of cases, hospitalizations, and deaths. According to the Centers for Disease Control and Prevention (CDC, 2023), it is estimated that these illnesses account for approximately 9.4 million cases of illness, 56,000 hospitalizations, and 1350 deaths. Furthermore, recent data from the European Food Safety Authority and the European Centre for Disease Prevention and Control (EFSA & ECDC, 2022) reveal a concerning trend in the European Union. In 2021, there were 4005 foodborne outbreaks reported, representing a significant increase of 29.8% compared to the previous year. Bacterial pathogens such as *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., *Staphylococcus aureus*, and *Escherichia coli* are responsible for the majority of these cases (WHO, 2015). Moreover, the emergence and spread of antimicrobial resistance (AMR), particularly in clinically significant bacterial species, have exacerbated the critical issue of AMR, which is considered a global health threat (CDC, 2019). In 2019, bacterial AMR was associated with approximately 4.95 million deaths globally (Murray et al., 2022), with an annual treatment cost of around US\$4.6 billion in the United States alone (Nelson et al., 2021).

Consequently, there exists a pressing need to explore alternative antimicrobial agents for controlling foodborne pathogens. Plants are a promising source of secondary metabolites with medicinal properties, including essential oils (EOs), alkaloids, polyacetylenes, phenolic compounds, and lectins/polypeptides (Da Silva et al., 2021; Istúriz-Zapata et al., 2020). EOs, which contain a wide variety of terpenes and their derivatives, are typically extracted using steam distillation, hydrodistillation, or mechanical methods (Tongnuanchan & Benjakul, 2014). In addition to their antibacterial, antiviral, antifungal, anti-toxicogenic, antiparasitic, and insecticidal activities (Burt, 2004; Jackson-Davis et al., 2023), these compounds may also have potential health benefits such as reducing the risk of diabetes, cancer, and cardiovascular diseases (Hejna et al., 2021; Rezaie et al., 2015; Wu et al., 2019). Due to their numerous applications in the pharmaceutical, food, agricultural, and cosmetic industries, research into EOs and

other plant secondary metabolites has increased in recent years.

Cinnamomum, *Salvia*, and *Mentha* are plant species that have been investigated for their antimicrobial properties. In vitro studies have demonstrated significant antimicrobial activity of plant extracts and EOs derived from these plants against a spectrum of foodborne pathogens, including *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., and *E. coli* (Hatab et al., 2016; Huang et al., 2014; Kerekes et al., 2019; Kobus-Cisowska et al., 2019; Liang et al., 2012, 2019; Lorenzo-Leal et al., 2019; Vihanova et al., 2021). However, factors such as extraction method, microbial strain, and chemical composition can influence their effectiveness (Burt, 2004; Gonelimali et al., 2018; Mostafa et al., 2018). Thus, a synthesis of the available evidence is necessary to identify key factors that modulate the in vitro antimicrobial activity of these natural products.

In vitro assays, including diffusion and dilution methods, are used to evaluate the antimicrobial activity of natural products (Balouiri et al., 2016). Standards from International Organization for Standardization (ISO) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) ensure reliable and accurate results by providing guidance on culture media, incubation conditions, and quality control measures (ISO, 2019; Matuschek et al., 2014). While disk and well diffusion methods do not provide minimum inhibitory concentration (MIC) values or differentiate between bactericidal and bacteriostatic effects, dilution methods such as agar and broth dilution determine MIC values by incorporating varying concentrations of the antimicrobial agent into liquid agar or using serial dilutions in tubes or 96-well trays (Balouiri et al., 2016; Wiegand et al., 2008).

Therefore, this study aims to investigate the relationship between inhibition diameters (IDs) and MIC values obtained via diverse in vitro methods, as well as the impact of methodological variations in diffusion and dilution protocols. For that, a meta-analysis was conducted on publicly accessible findings of the antibacterial effects of *Cinnamomum*, *Salvia*, and *Mentha* spp. extracts and EOs in vitro, with the goals of summarizing outcomes, assessing sources of heterogeneity, and evaluating the likelihood of publication bias.

Previous studies have employed regression analysis to compare and correlate outcomes obtained through various methods (Bruin et al., 2013; DeCross et al., 1993; Gaudreau & Gilbert, 1997; Steward et al., 1999). However, to the best of our knowledge, only one study by Silva et al. (2023) utilized meta-analysis to investigate the correlation between ID and MIC measurements of extracts and EOs derived from *Syzygium aromaticum*, *Citrus* L. and *Origanum* L. Therefore, there is a critical need to fill the gap in knowledge regarding the lack of studies that have explored the relationship between ID and MIC. The findings of this research will contribute to a better understanding of the antimicrobial susceptibility testing (AST) and aid in the interpretation of the results obtained from ID and MIC measurements mainly for natural/or plant antimicrobials.

2 | MATERIALS AND METHODS

The following sections outline the methodology employed to accomplish the objectives of this meta-analysis (Gonzales-Barron et al., 2021).

2.1 | Inclusion and exclusion factors for articles

The criteria for including and excluding articles in this review were selected to narrow the search for primary articles relevant to the main research question. Inclusion criteria were predetermined to include (i) extracts from *Cinnamomum*, *Salvia*, and *Mentha* species; (ii) outcomes for both MIC and ID; (iii) antimicrobial activity against *Listeria monocytogenes*, *S. aureus*, *Salmonella* spp., *Bacillus cereus*, *E. coli*, and Shiga toxin-producing *E. coli* (STEC); (iv) information on the extract's dose and pathogen's inoculum size; and (v) publications containing primary data published since 2000 in peer-reviewed journals

The exclusion criteria for articles in this review were studies presenting incomplete or secondary data. This systematic review excluded primary articles with insufficient data. In addition, gray literature was excluded from consideration due to concerns about the validity of data and duplication issues. Typically, peer-reviewed journals publish high-quality theses and reports. Moreover, other meta-analysis studies and systematic reviews were excluded.

2.2 | Articles search

The databases searched for this study were PubMed, Web of Science, Scopus, and SciELO. To identify rel-

evant articles, search terms were combined using the “AND” and “OR” logical connectors to match keywords related to pathogens, biopreservatives, and antimicrobial susceptibility methods as follows: (*Salmonella* OR *Listeria* OR *Campylobacter* OR *Escherichia coli* OR *Staphylococcus aureus*) AND (antimicrobial* OR extract* OR “essential oil”) AND (“agar diffusion” OR “inhibition” OR “minimum inhibitory concentration” OR MIC OR “minimum bactericidal concentration” OR MBC OR “halo” OR “zone”) AND food. The search was limited to the title, keywords, and abstract fields, and any duplicate articles were removed. The literature search was conducted for English, French, Portuguese, and Spanish languages.

In the second stage of the literature screening, the titles and abstracts of the collected articles were carefully examined, applying a predefined set of inclusion and exclusion criteria. This process eliminated studies that did not align with the research objectives, while identifying potential articles for inclusion. The third stage involved a comprehensive reading of the filtered articles to confirm they adhered to the specified criteria. Subsequently, relevant variables for the study were extracted from the selected articles.

2.3 | Variables extracted from primary articles

After a rigorous assessment of relevant publications, 91 studies published since 2000 were identified as eligible for inclusion in this investigation (Abu-Darwish et al., 2012; Akarca et al., 2019; Akdemir Evrendilek, 2015; Aliakbarlu et al., 2013; Al-Nabulsi et al., 2020; Al-Saghir, 2009; Alizadeh Sani et al., 2017; Andleeb et al., 2014; Awaisheh, 2013; Ayala-Zavala et al., 2013; Baali et al., 2019; Bahadori et al., 2016; Bayoub et al., 2010; Bhavya et al., 2020; Bonilla & Sobral, 2016; Boukhebtbi et al., 2011; Bouyahya et al., 2019; Butkhup & Samappito, 2011; Campana et al., 2017; Cansian et al., 2010; Celikel & Kavas, 2008; Ceylan et al., 2014; de Oliveira et al., 2012; Deka et al., 2016; Djenane et al., 2012; Dobre et al., 2011; Eissa et al., 2012; El Abdouni Khayari et al., 2016; Elgayyar et al., 2001; Elshafie et al., 2016; El-Shenawy et al., 2015; Feng et al., 2017; Fernández-López et al., 2005; Ferreira et al., 2019; Fidan et al., 2019; Frank et al., 2018; Ghabraie et al., 2016; Golestani et al., 2015; Gonelimali et al., 2018; Goñi et al., 2009; Gupta et al., 2008; György, 2010; Hayouni et al., 2008; Huang et al., 2014; Hussein et al., 2018; Ibrahim, 2014; Chobba et al., 2012; Irkin & Korukluoglu, 2009; Iseppi et al., 2019; Iturriaga et al., 2012; Keskin et al., 2010; Kim et al., 2004, 2017; Kobus-Cisowska et al., 2019; Kulaksiz et al., 2018; Kumaravel & Martina, 2011; Li et al., 2019; Liang et al., 2019; Liaqat et al., 2017; López et al., 2005; Lv et al., 2011; Maidment

et al., 2006; Martac & Podea, 2012; Mathlouthi et al., 2012; Mau et al., 2001; Melo et al., 2015; Mihaly Cozmuta et al., 2015; Mishra & Behal, 2010; Mith et al., 2014; Moosavi-Nasab et al., 2016; Moreira et al., 2005; Nimje et al., 2013; Olaimat et al., 2019; Özkan et al., 2003; Ozogul et al., 2015; Park et al., 2016; Patil & Shanmgam, 2016; Pesavento et al., 2015; Pl'uchtová et al., 2018; Prabuseenivasan et al., 2006; Ramdan et al., 2018; Rana et al., 2014; Ribeiro-Santos et al., 2018, 2017; Shahbazi, 2015; Silveira et al., 2012, 2019; Sofia et al., 2007; Thanissery et al., 2014; Zhang et al., 2019, 2016). The information collected from the chosen studies includes article identification, plant species, plant portion used, extraction method including its parameters such as temperature and solvent, antimicrobial susceptibility test, extract or EO dosage applied ("LogDose"; %w/v or %v/v), bacterium, strain, inoculum size, inhibition diameter value (ID [mm]), and MIC value ("LogMIC"; mg/mL for extracts and $\mu\text{L}/\text{mL}$ for EOs). The comprehensive meta-analytical data derived from each study are available upon request.

2.4 | Meta-regression modeling

The pertinent data subsets were subjected to fitting of weighted mixed-effects linear models to evaluate the pooled ID or MIC values of EOs or extracts derived from *Cinnamomum*, *Salvia*, and *Mentha* species, against different bacterium.

Relevant study parameters, chosen from primary studies to elucidate between-study variation in effect size, were extracted for each data set. These comprised plant category, extract or EO dosage examined, volume of extract or EO (absorbed by the disk or poured into the well), method used to determine ID, inoculum level, and number of replicates utilized for ID testing. Pooled models of MIC were codified based on the plant species, method of MIC determination, minimum bactericidal concentration, antimicrobial type (extract or EO), standard errors, and/or number of replicates utilized for the test. In some adjusted models, interactions between factors were examined to determine whether the impact of one term was conditional on the level of one or more terms. More than 30 meta-regression models were adjusted to synthesize ID and MIC, with the following general forms:

$$ID_{ij} = \beta_1 \text{LogDose} + (\beta_{2j} + u_i) \text{Plant}_j + \varepsilon_{ij}, \quad (1)$$

$$\begin{aligned} \text{Log MIC}_{ijmn} = & (\beta_{1j} + u_i) \text{Plant}_j + \beta_{2m} \text{Method}_m \\ & + \beta_{3n} \text{AntimicrobialType}_n + \varepsilon_{ijmn}. \quad (2) \end{aligned}$$

Equation (1) depicts the ID observation (ID_{ij}) acquired from the j th plant and the i th study. β_1 represents the effect of a 1-log increase in extract dose (%v/v or %w/v) on the ID, whereas β_{2j} signifies the set of fixed effects of the j types of plant. Similarly, Equation (2) represents the MIC observation (MIC_{ijmn}) obtained from the i th study, j th plant, the m th method, and the n th antimicrobial type. In this equation, β_{1j} , β_{2m} , and β_{3n} represent the set of fixed effects of the j types of plant, m types of MIC determination method (class variable consisting of the levels: agar dilution and broth microdilution), and n types of antimicrobial type (class variable consisting of the levels: extract and EO), respectively.

Equations (1) and (2) include the model residuals, represented by the terms ε_{ij} and ε_{ijmn} , respectively. To account for the remaining unexplained variability, random-effects u_i were incorporated into β_{2j} and β_{1j} (set of fixed effects of the j types of plant in Equations 1 and 2, respectively). In both models, u_i are presumed to follow a normal distribution with zero mean and between-study variability of τ^2 .

To assess the relationship between pathogen susceptibility and the use of plant extracts or EOs, a weighted mixed-effects linear model was applied to the relevant data set, examining the correlation between ID and MIC. The moderators for the model included the logarithm of the extract dose, logarithm of MIC, and the specific bacterium being studied. To account for these factors, a meta-regression model was adjusted with the following format:

$$\begin{aligned} ID_{ik} = & (\beta_0 + u_i) + \beta_1 \text{LogDose} + \beta_2 \text{LogMIC} \\ & + \beta_{3k} \text{Bacterium}_k + \varepsilon_{ik}. \quad (3) \end{aligned}$$

Equation (3) is structured as follows: β_0 represents the intercept; β_1 and β_2 represent the effect of a 1-log increase in extract dose (%v/v or %w/v) and a 1-log increase in MIC, respectively, on the ID; and β_{3k} represents the set of fixed effects of the k types of bacteria. The error term ε_{ik} accounts for the variation between studies i and pathogens k . The random effects (u_i) introduced in the intercept (β_0) were used to account for the between-study heterogeneity that could not be explained by other factors.

To ensure a normalized data distribution and reduce heteroscedasticity, all models were adjusted by logarithmically transforming (with a base of 10) the extract or EO dose tested, as well as the MIC values. Furthermore, to ensure accurate estimates of the antimicrobial effect on pathogen inactivation and account for the quality of the research design, varying weights were assigned to each primary study based on its size ($n \geq 2$).

TABLE 1 Pooled inhibition diameters (mean and standard error) produced by extracts of *Cinnamomum* species by method of determination, as estimated by meta-analysis models separately adjusted by bacterium.

Bacterium	Plant	Method	Pooled inhibition diameter (mm) [SE]	n	N	Publication bias (p-value)
<i>E. coli</i> ^B	Cinnamon	Disk and well	16.00 (2.146)	28	7	ND
<i>B. cereus</i> ^A	Cinnamon	Disk and well	23.35 (2.853)	14	7	.409
<i>S. aureus</i> ^B	Cassia	Disk	23.99a (0.882)	3	20	.071
		Well	16.29b (0.691)	44		
	Cinnamon	Disk	16.29b (0.691)	44		
		Well	16.69b (0.745)	22		
<i>Salmonella</i> spp. ^C	Cassia	Disk	26.24a (2.678)	14	16	.558
		Well	21.38a (0.847)	4		
	Cinnamon	Disk	13.16b (2.672)	56		
<i>L. monocytogenes</i> ^B	Cassia	Disk	20.83a (2.539)	11	10	.925
	Cinnamon	Disk	14.87b (2.536)	55		
STEC ^C	Cassia	Disk	20.89a (2.649)	18	6	.622
	Cinnamon	Disk	8.066b (2.652)	8		
	Others*	Well	8.549b (0.224)	4		

Note: The number of observations (*n*), number of primary studies (*N*), and *p*-value of the publication bias test are shown per meta-analysis model. Different superscript uppercase letters indicate significant differences in the pooled inhibition diameter produced by extracts of cinnamon only at a dose of 100 mg/mL; A to C: highest to lowest. Different superscript lowercase letters indicate significant differences in the pooled inhibition diameter against a given bacterium produced by the extracts of *Cinnamomum* species at a dose of 100 mg/mL.

Abbreviation: STEC, Shiga toxin-producing *E. coli*.

*Category that encompasses *Cinnamomum camphora* (L.), *Cinnamomum burmannii* (Nees & T.Nees) Blume, *Cinnamomum loureiroi* Nees, and *Cinnamomum wilsonii* Gamble.

The fitted meta-regressions were used to calculate model parameters affected by moderators, and the significance of these moderators was assessed by analysis of variance ($\alpha = .05$). Publication bias was evaluated using two methods: assessment of the funnel plot and analysis of the effect of the total sample size (*n*) on the pooled ID/MIC (Borenstein et al., 2009; Xavier et al., 2014). All meta-regression models were fitted using the *rma.mv* function in the *metafor* package of R software (version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria) (Viechtbauer, 2010).

3 | RESULTS AND DISCUSSION

3.1 | Inhibition diameter

The results of the meta-analysis investigating the pooled ID of extracts from *Cinnamomum*, *Mentha*, and *Salvia* species, as estimated by separate meta-regression models, are presented in Tables 1, 2, and 3, respectively. The models were adjusted for six common foodborne pathogens, namely, *L. monocytogenes*, *S. aureus*, *Salmonella* spp., *B. cereus*, *E. coli*, and STEC, whenever data were available.

In terms of the effects of *Cinnamomum* extracts, *B. cereus* was the most susceptible bacterium, followed by *S.*

aureus, *L. monocytogenes*, and *E. coli*, while *Salmonella* spp. and STEC displayed the least susceptibility at a concentration of 100 mg/mL ($p < .05$). Similarly, the susceptibility of bacteria to cinnamon extracts varied, with *E. coli*, *Salmonella* spp., *B. cereus*, *S. aureus*, and *L. monocytogenes* being the most affected, and STEC being less susceptible. More specifically, cinnamon showed notable antibacterial activity against *E. coli* and *B. cereus*, with pooled ID of 16.00 mm (± 2.146) and 23.35 mm (± 2.853), respectively. On the other hand, cassia was more effective against *S. aureus*, yielding a larger pooled ID of 23.99 mm (± 0.882) compared to the 16.29 mm (disc method) and 16.69 mm (well method) achieved by cinnamon. This trend continued with *Salmonella* spp., *L. monocytogenes*, and STEC, where cassia consistently showed the greatest antibacterial efficacy, as demonstrated by a larger pooled ID (Table 1).

Mentha spp. extracts caused the highest antimicrobial effects toward *B. cereus*, followed by *S. aureus*, whereas *Salmonella* spp. and *E. coli* showed less vulnerability (Table 2). Conversely, the antimicrobial efficacy of *Salvia* extracts varied with plant species for most bacteria. Among the microorganisms tested, *B. cereus*, *S. aureus*, and *L. monocytogenes* were found to be most susceptible to the inhibitory effects of both rosemary and sage extracts, while *E. coli* and *Salmonella* spp. exhibited comparatively greater resistance to these extracts (Table 3).

TABLE 2 Pooled inhibition diameters (mean and standard error) produced by extracts of *Mentha* species (nonspecific mint, apple mint, horsemint, pennyroyal, peppermint, and spearmint), as estimated by meta-analysis models separately adjusted by bacterium.

Bacterium	Plant	Method	Pooled inhibition diameter (mm) [SE]	n	N	Publication bias (p-value)
<i>E. coli</i> ^B	All	Disk and well*	10.84 (0.512)	32	16	.162
<i>B. cereus</i> ^A	All	Disk	14.77b (1.043)	13	17	.741
		Well	19.38a (0.649)	5		
<i>S. aureus</i> ^{AB}	All	Disk	9.577b (1.102)	12	12	.004
		Well	16.28a (1.642)	7		
<i>Salmonella</i> spp. ^B	All	Disk and well*	12.15 (1.157)	22	7	.432

Note: The number of observations (*n*), number of primary studies (*N*), and *p*-value of the publication bias test are shown per meta-analysis model. Different superscript uppercase letters indicate significant differences in the pooled inhibition diameter produced by the extracts of *Mentha* species at a dose of 100 mg/mL; A to B: high to low. Different superscript lowercase letters indicate significant differences in the pooled inhibition diameter against a given bacterium produced by the extracts of *Mentha* species at a dose of 100 mg/mL. No significant differences were found between *Mentha* species.

*The inhibition diameters obtained from both the disk and well methods were combined due to the lack of statistical significance in the effect of the method of determination (*p* > .10).

TABLE 3 Pooled inhibition diameters (mean and standard error) produced by extracts of *Salvia* species using different determination methods were estimated through meta-analysis models adjusted by bacterium.

Bacterium	Plant	Method	Pooled inhibition diameter (mm) [SE]	n	N	Publication bias (p-value)
<i>E. coli</i> ^B	Rosemary	Disk and well*	11.01b (1.075)	20	22	.372
	Sage	Disk and well*	13.66a (0.900)	26		
<i>B. cereus</i> ^A	Rosemary	Disk	14.63b (1.308)	6	11	.374
		Well	23.59a (2.749)	4		
	Sage	Disk	15.64b (1.472)	5		
<i>S. aureus</i> ^A	Rosemary	Disk and well*	15.93a (1.984)	24	24	.276
	Sage	Disk and well*	13.33a (1.544)	40		
<i>Salmonella</i> spp. ^B	Rosemary	Disk	11.99a (1.147)	22	15	.013
	Sage	Disk	9.973a (0.727)	26		
<i>L. monocytogenes</i> ^A	Others**	Disk	18.74a (0.369)	4	17	.286
	Rosemary	Disk and well*	9.826b (1.533)	14		
	Sage	Disk and well*	16.19a (1.755)	44		
STEC ^B	Rosemary	Disk	14.54a (1.002)	4	6	<.0001
	Sage	Disk	8.598b (1.203)	4		

Note: The number of observations (*n*), number of primary studies (*N*), and *p*-value of the publication bias test are presented for each meta-analysis model. Different superscript uppercase letters indicate significant differences in the pooled inhibition diameter produced by extracts of sage and rosemary at a dose of 100 mg/mL; A to C: highest to lowest. Different superscript lowercase letters indicate significant differences in the pooled inhibition diameter against a given bacterium produced by the extracts of *Salvia* species at a dose of 100 mg/mL.

*The inhibition diameters obtained from both the disk and well methods were combined due to the lack of statistical significance in the effect of the method of determination (*p* > .10).

**Category that encompasses *Salvia fruticosa* Mill, *Salvia sclarea* L., and *Salvia aucheri* Benth.

Plant extracts have attracted considerable attention in recent years for their remarkable broad-spectrum antimicrobial activity, which has been attributed to their complex and diverse chemical composition (Álvarez-Martínez et al., 2021; Gillings et al., 2019). The literature suggests that these extracts often exhibit more significant antimicrobial activities against Gram-positive than Gram-negative bacteria. Moreover, plant extracts are well known for their

diverse modes of action against their target organisms. Probably due to their wide variety of bioactive constituents. *Cinnamomum* spp., for instance, exhibit their antimicrobial efficacy through compounds such as cinnamaldehyde that demonstrate activity against *E. coli*, *Salmonella typhimurium*, and *L. monocytogenes* (Husain et al., 2018; Kim et al., 2004; Vihanova et al., 2021). Conversely, *Mentha* spp., which are enriched with bioactive constituents such

as eriocitrin, hesperidin, narirutin, luteolin, isorhoifolin, rosmarinic acid, and caffeic acid, show broad-spectrum activity against a variety of bacteria including *E. coli*, *S. typhimurium*, *Salmonella arizona*, *L. monocytogenes*, *B. cereus*, and *S. aureus* (Alharbi et al., 2022). *Salvia* spp., however, contains a diverse group of active compounds such as phenolic acids, flavonoids, and terpenes like camphene, 1,8-cineole, and camphor. These compounds exhibit significant antimicrobial activity against pathogens such as *E. coli*, *P. aeruginosa* (Ozkan et al., 2010), and *S. aureus* (Veličković et al., 2003). Therefore, while each genus exhibits potent antimicrobial activity, the specific bioactive compounds and their spectrum of activity vary, pointing toward their unique potential for developing novel natural antimicrobial agents.

The influence of determination method (disk diffusion and well diffusion methods) on the inhibitory activity of *Cinnamomum*, *Salvia*, and *Mentha* extracts against various bacterial strains was investigated. The results revealed no significant differences in *Salvia* spp. extracts except for the model adjusted for *B. cereus* upon exposure to rosemary extracts (Table 3). Conversely, *Mentha* spp. extracts exhibited significant discrepancies in ID between the disk and well diffusion methods for *B. cereus* and *S. aureus* ($p < .05$); however, no such differences were observed for *E. coli* and *Salmonella* spp. in the adjusted model (Table 2). Our findings are consistent with the results of the study conducted by Silva et al. (2023), which also showed that the determination method can affect the inhibitory activity of plant extracts against specific bacterial strains. These results suggest that the choice of determination method should be carefully considered when assessing the inhibitory activity of plant extracts against specific bacterial strains to ensure accurate and reliable results. Furthermore, the findings highlight the need for further research in this area to optimize the determination methods for evaluating the antimicrobial properties of plant extracts.

Comparative analysis of the three studied plant species revealed that the antimicrobial activity of their extracts is contingent upon the specific bacterial strain targeted. For instance, cinnamon exhibited the highest efficacy against *B. cereus*, with a pooled ID of 23.35 mm, while cassia was most effective against *Salmonella* spp., displaying a pooled ID of 26.24 mm. Interestingly, the cinnamon extract exhibited the least effect against STEC, with a pooled ID of only 8.07 mm. These results underscore the importance of considering the targeted bacteria when assessing the antimicrobial activity of plant extracts.

Most of the meta-analytical models generated did not show significant publication bias above 5%. However, there is potential publication bias in the models adjusted for *S. aureus* in the case of *Mentha* spp. extracts (Table 2) and *Salmonella* spp. and STEC for *Salvia* spp. extracts (Table 3).

Funnel plots were also used to visually assess publication bias, which are available in Figures S1–S3.

The results obtained from this study suggest that the antimicrobial activity of plant extracts is highly dependent on the species of the plant utilized, which is consistent with prior research (Gonelimali et al., 2018; Hemeg et al., 2020; Khameneh et al., 2019; Nascimento et al., 2000; Vaou et al., 2021). Furthermore, our findings emphasize the importance of selecting appropriate plant extracts with demonstrated antimicrobial activity for the treatment of microbial infections or foodborne pathogens.

The bioactivity of plant extracts can be influenced by several factors, including the part of the plant from which the extract was obtained, the method of extraction, and the concentration of the extract used. For instance, studies have shown that extracts obtained from certain plant parts, such as leaves or stems, may exhibit greater antimicrobial activity compared to extracts obtained from other plant parts such as flowers or roots (Ghavam et al., 2020; Mohamed et al., 2020; Mostafa et al., 2018). Similarly, the method of extraction can impact the bioactivity of the extract, with some methods such as ultrasonic producing extracts with higher antimicrobial activity compared to other methods like maceration (Farahmandfar et al., 2019).

Furthermore, it is essential to note that not all plant extracts possess antimicrobial properties. Hence, selecting plant extracts with demonstrated efficacy against the specific microbe of interest is crucial. In this regard, prior research can provide valuable insight into the antimicrobial potential of various plant extracts, allowing researchers and clinicians to make informed decisions regarding the selection of appropriate extracts for treatment and/or application as food preservatives.

3.2 | Minimum inhibitory concentration

Tables 4, 5, and 6 depict the outcomes of the meta-analysis carried out on the MICs generated by extracts and EOs of *Cinnamomum*, *Mentha*, and *Salvia* species, respectively. Distinct models were established to account for various foodborne pathogens, which included *L. monocytogenes*, *S. aureus*, *Salmonella* spp., *B. cereus*, *E. coli*, and STEC. Notably, the *Cinnamomum* model was adjusted only for observations on cinnamon extracts and EOs, hence the effect of different *Cinnamomum* species on pathogens could not be assessed.

The results showed that the efficacy of plant extracts varied among different species of plants, which is consistent with prior research (Didehdar et al., 2022; Gourich et al., 2022; Huang et al., 2014; Hussein et al., 2018; Park et al., 2016; Pl'uchtová et al., 2018). Notably, the meta-analysis revealed significant differences in the MIC produced by

TABLE 4 Pooled minimum inhibitory concentrations (MICs) (mean and 95% confidence intervals [CIs]) produced by extracts (mg/mL) or essential oils ($\mu\text{L/mL}$) of cinnamon (*Cinnamomum* spp.), by determination method (agar dilution [AD], broth macrodilution [BMaD], and broth microdilution [BMiD]), were estimated separately adjusted by bacterium using meta-analysis models.

Plant	Bacterium	Type	Method	MIC [95% CI] (mg/mL or $\mu\text{L/mL}$)	n	N	Publication bias (p-value)
Cinnamon	<i>E. coli</i>	Extract	BMaD and BMiD*	0.341a [0.060–1.948]	24	14	.140
		EO	BMiD	4.893a [0.753–31.78]	5		
	<i>S. aureus</i>	Extract	BMaD	0.788ab [0.219–2.835]	13	15	.489
			BMiD	0.598a [0.235–1.521]	14		
	<i>Salmonella</i> spp.	Extract	EO	2.602b [0.974–6.947]	37		
			BMaD	0.716a [0.373–1.374]	16	16	.241
	<i>L. monocytogenes</i>	Extract	BMiD	0.665a [0.265–1.672]	8		
			EO	2.186b [1.474–3.242]	42		
	STEC	Extract	BMaD and BMiD*	0.237a [0.040–1.378]	6	12	.858
			EO	1.577a [0.340–7.238]	42		
	STEC	Extract	BMiD	0.731a [0.162–3.290]	3	4	<.0001
			EO	0.354a [0.072–1.743]	3		

Note: The number of observations (n), number of primary studies (N), and p-value of the publication bias test are presented for each meta-analysis model. Within a given combination plant \times bacterium, where a meta-analysis model was fitted, different superscript lowercase letters indicate significant differences in MIC against a given bacterium produced by extracts and EOs.

*MICs from AD, BMaD, or BMiD were combined, since the effect of method of determination was not statistically significant ($p > .10$).

TABLE 5 Pooled minimum inhibitory concentrations (MICs) (mean and 95% confidence intervals [CIs]) produced by extracts (mg/mL) or essential oils (EOs) ($\mu\text{L/mL}$) of *Mentha* species, by determination method (agar dilution [AD], broth macrodilution [BMaD], and broth microdilution [BMiD]), were estimated separately adjusted by bacterium using meta-analysis models.

Bacterium	Plant	Type	Method	MIC [95% CI] (mg/mL or $\mu\text{L/mL}$)	n	N	Publication bias (p-value)
<i>E. coli</i>	Mint	Extract	BMiD	0.031a [0.003–0.280]	6	15	.794
	Pennyroyal	Extract	BMiD	2.352b [0.410–13.47]	4		
	Peppermint	Extract	BMiD	1.414b [0.295–6.769]	6		
EO		BMiD	3.148b [0.551–17.98]	5			
<i>B. cereus</i>	All*	Extract	BMiD	6.278a [1.507–26.15]	4	6	.589
		EO	BMiD	1.418a [0.364–5.528]	4		
<i>S. aureus</i>	Mint	Extract	BMiD	0.327a [0.017–5.420]	5	19	.863
	Pennyroyal	Extract	BMiD	1.463a [0.508–4.218]	6		
	Peppermint	Extract	BMiD	1.268a [0.500–3.201]	7		
		EO	BMiD	3.498a [1.385–8.832]	9		
	Spearmint	Extract	BMiD	2.753a [0.542–13.97]	3		
<i>Salmonella</i> spp.	All*	Extract	BMiD	0.994a [0.082–12.11]	7	8	.748
		EO	BMiD	7.447a [0.233–23.03]	3		
<i>L. monocytogenes</i>	All*	Extract	BMiD	2.455a [0.917–6.573]	7	11	.116
		EO	BMiD	4.854a [2.003–11.76]	11		
STEC	All*	EO	BMiD	3.017 [0.401–22.70]	3	3	.902

Note: The number of observations (n), number of primary studies (N), and p-value of the publication bias test are presented for each meta-analysis model. Within a given bacterium, different superscript lowercase letters indicate significant differences in MIC produced by extracts and EOs of *Mentha* species.

*Extracts or EOs of all plants were combined since the effect of species was not statistically significant ($p > .10$).

TABLE 6 Pooled minimum inhibitory concentrations (MICs) (mean and 95% confidence intervals) produced by extracts (mg/mL) or essential oils (EOs) ($\mu\text{L}/\text{mL}$) of *Salvia* species, by determination method (agar dilution [AD], broth macrodilution [BMaD], and broth microdilution [BMiD]), were estimated separately adjusted by bacterium using meta-analysis models.

Bacterium	Plant	Type	Method	MIC [95% CI] (mg/mL or $\mu\text{L}/\text{mL}$)	<i>n</i>	<i>N</i>	Publication bias (<i>p</i> -value)
<i>E. coli</i>	Rosemary and sage*	Extract	AD	1.344a [0.272–6.627]	3	16	0.678
			BMiD	4.728a [2.357–9.487]	11		
		EO	BMiD	9.504a [3.375–26.76]	8		
<i>B. cereus</i>	Rosemary	Extract	BMiD	6.431a [1.618–12.75]	4	12	0.310
		EO	BMiD	1.489a [0.270–8.196]	3		
	Sage	Extract	BMiD	1.230a [0.288–5.254]	5		
<i>S. aureus</i>	Rosemary and sage*	Extract	BMiD	2.035a [1.682–2.462]	104	20	0.657
		EO	BMiD	4.746a [0.993–22.68]	21		
	Others	Extract	BMiD	3.725a [1.162–11.94]	5		
<i>Salmonella</i> spp.	Rosemary	Extract	AD and BMiD**	3.243a [0.653–16.10]	10	17	0.318
		EO	BMaD	18.11a [5.422–60.51]	3		
	Sage	Extract	BMiD	6.381a [1.151–35.37]	6		
		EO	BMiD	14.15a [4.237–47.28]	5		
<i>L. monocytogenes</i>	Rosemary	Extract	BMiD	1.735a [0.781–3.853]	8	10	0.710
		EO	BMiD	4.980a [1.553–15.96]	7		
	Sage	Extract	BMiD	6.300a [1.144–34.68]	3		
		EO	BMiD	6.201a [0.982–39.14]	3		

Note: The number of observations (*n*), number of primary studies (*N*), and *p*-value of the publication bias test are presented for each meta-analysis model. Within a given bacterium, where a meta-analysis model was fitted, different superscript lowercase letters indicate significant differences ($p < .10$) in MIC produced by extracts and EOs of *Salvia* species.

*MIC values for rosemary and sage were combined since the effect of species was not statistically significant ($p > .10$).

**MIC values measured by AD and BMiD were combined since the effect of method of determination was not statistically significant ($p > .10$).

extracts or EOs of various *Mentha* spp., but only for *E. coli*, while no significant differences were observed in MIC produced by different *Salvia* spp. for all bacteria, as shown in Table 6. Additionally, *Mentha* spp., specifically the mint plant, demonstrated the lowest MIC in the models adjusted for *S. aureus* and *E. coli* (Table 5). These findings are consistent with previous studies reporting the antimicrobial activity of *Mentha* spp. against these foodborne pathogens (Baali et al., 2019; Gourich et al., 2022; Pluchtová et al., 2018; Shahbazi, 2015).

The impact of different types of antimicrobials applied (extract or EO) derived from *Cinnamomum*, *Salvia*, and *Mentha* species was also examined. The results showed that *Cinnamomum* EO was particularly effective against *S. aureus* and *Salmonella* spp. (Table 4, $p < .05$). For *Salvia* spp., both extracts and EOs did not show significant differences in their antimicrobial activity against the tested bacteria (Table 6, $p > .05$). The type of antimicrobial from different *Mentha* spp. could only be evaluated for peppermint against *E. coli* and *S. aureus*. The outcomes showed no significant difference between extracts and EOs, indicating comparable antimicrobial effects (Table 5). These results underscore the importance of carefully selecting type of

plant extracts for their specific antimicrobial properties to ensure effective treatment against specific bacterial pathogens.

The suggestion put forth is that when appraising the antimicrobial potential of natural products, mixtures containing extracts and EOs with MICs measuring below 0.1 mg/mL should be recognized as having significant effectiveness and a promising outlook. Conversely, samples with MICs exceeding 1 mg/mL should be strictly considered as ineffective (Kokoska et al., 2019). In light of this, the findings indicate that among the analyzed plant extracts, mint extract shows a good activity against the Gram-negative *E. coli*, with MIC of 0.031 mg/mL (Table 5). Cinnamon extract, on the other hand, presents the highest efficacy against *Salmonella* spp. and *L. monocytogenes* (Table 4). Of particular interest, the cinnamon EO exhibits a remarkably low MIC value of 0.354 $\mu\text{L}/\text{mL}$ against STEC, aligning closely with the threshold for high activity. The observed variation in the effectiveness of cinnamon EO and extract against STEC is of particular interest and may be attributed to differences in the extraction process and chemical composition of the EO compared to the extract (Nwanade et al., 2021). Steam distillation is typically used

to extract EO, which preserves the volatile compounds responsible for the cinnamon's antimicrobial activity. In contrast, cinnamon extract is obtained using different extraction methods that may not retain these volatile compounds to the same extent (Nabavi et al., 2015; Wong et al., 2014).

None of the models generated in the study showed any evidence of publication bias, except for the model adjusted for STEC ($p < .0001$) for cinnamon. Furthermore, a visual representation using funnel plots of these findings is presented in Figures S3–S5.

3.3 | Assessing the antimicrobial efficacy of medicinal and aromatic plant extracts: Considerations for standard testing methods

When examining the possible antimicrobial properties of newly discovered medicinal plant extracts, a diverse range of assessment tests are commonly utilized (EUCAST, 2003). It is crucial to acknowledge that employing different evaluation methods may lead to variations in the observed results. The outcomes of the assessment tests may be influenced by various factors, such as the research methodology implemented for the selection of plant material, the extraction system and solvent utilized, the techniques applied, and the microorganisms chosen for testing (Rios et al., 1988; Ross et al., 2001; Zhang et al., 2018). While standard AST methods are traditionally classified into diffusion and dilution techniques, their direct suitability for evaluating plant extracts may be limited. Consequently, it may be necessary to make modifications to the testing protocol to ensure precise and reliable outcomes (Balouiri et al., 2016). The principal challenge in utilizing diffusion and dilution-based AST methods when evaluating plant extracts is linked to the availability of active principles, which may differ depending on the solubility of the test compound (Ncube et al., 2008).

Diffusion methods are qualitative techniques that are utilized to establish the existence or absence of antimicrobial substances. Although diffusion methods are straightforward and uncomplicated, they may produce results that are unreliable and nonreproducible due to the absence of standardization (Ncube et al., 2008). In contrast, dilution methods are quantitative assays that determine the MIC of antimicrobial agents (Wiegand et al., 2008). Compared to diffusion techniques, these methods offer several advantages, including heightened sensitivity to small extract volumes, the ability to distinguish between the bacteriostatic and bactericidal effects of the extracts, and the capability for quantitative analysis (Langfield et al., 2004).

The broth microdilution method is a fast and accurate assay that utilizes small volumes of test antimicrobial and allows for rapid testing of bacteria. Nonetheless, the process of manually handling solutions of antimicrobial agents during the preparation stage may increase the possibility of errors (Salam et al., 2023). Agar dilution methods offer several advantages when compared to diffusion techniques, including the ability to test multiple biological isolates simultaneously, the capacity to observe heterogeneous populations or mixed cultures, and the flexibility to select a range of sample concentrations for testing (Salam et al., 2023).

The impact of the used methods for determining the MIC (agar dilution and broth microdilution) on the efficacy of plant extracts and EOs against different microorganisms was investigated. The results showed that there were no significant differences ($p > .05$) in the pooled MIC values obtained using either method for all plant species studied (Tables 4–6). This finding suggests that the antimicrobial effects of these extracts and EOs were comparable regardless of the determination method used. A recent study by Silva et al. (2023) revealed that the method employed to determine the MIC had a substantial impact on the results obtained for oregano extracts and EOs in models adjusted for *L. monocytogenes* and *S. aureus*. In contrast, no noteworthy variation in the results was observed for *Syzygium aromaticum* and *Citrus* spp. extracts and EOs, irrespective of the determination method utilized.

These findings highlight the importance of standardizing the methods used for determining the antimicrobial activity of plant extracts and EOs. The standardization of methods can greatly facilitate the selection of appropriate plant extracts with demonstrated antimicrobial activity. Currently, there is a lack of standard criteria and evaluation methods for assessing the antimicrobial activity of plant extracts, leading to variations in results between different studies (Burt, 2004; Ncube et al., 2008). This lack of standardization makes it difficult to compare and interpret the antimicrobial activity of plant extracts across different studies (Anyanwu & Okoye, 2017; Leouifoudi et al., 2015). By using standardized methods, researchers can overcome the challenges associated with variability and obtain more accurate and meaningful results.

3.4 | Relationship between ID, MIC, extract dose, and bacterium

The estimated model parameters for a meta-regression analysis exploring the influence of MIC, extract dose, and bacterium on the ID induced by extracts of *Cinnamomum*, *Salvia*, and *Mentha* species are presented in Table 7. The

TABLE 7 Meta-regression model on inhibition diameter produced by extracts of *Cinnamomum* ($n = 86$), *Salvia* ($n = 16$), and *Mentha* ($n = 6$) plants, as a function of the minimum inhibitory concentration (MIC) (mL/mg for extracts and $\mu\text{m}/\text{mL}$ for essential oils), extract dose (%), and bacterium.

Parameter	Estimate	SE	p-value	n	Heterogeneity analysis
Log MIC	-5.603	0.178	<.0001		$s^2 = 24.7$
Log dose	11.32	0.306	<.0001		$\tau^2 = 61.76$
Bacterium					$I^2 = 64.5\%$
<i>C. jejuni</i>	9.565 ^c	3.928	.015	22	$\tau^2_{\text{res}} = 45.02$
<i>E. coli</i>	12.72 ^b	3.928	.001	7	$R^2 = 27.1\%$
<i>L. monocytogenes</i>	15.67 ^a	3.930	<.0001	5	Publication bias
<i>S. aureus</i>	11.47 ^b	3.921	.003	36	p = .035
<i>Salmonella</i> spp.	9.705 ^c	3.919	.013	35	
STEC	9.887 ^c	3.939	.012	3	

Note: The number of observations (n) per factor level, heterogeneity analysis, and p -value of the publication bias test are shown. Different superscript letters indicate significant differences in the estimates between bacteria. Heterogeneity analysis includes within-study variability (s^2), between-study variability of the null model (τ^2), intraclass correlation (I^2), residual between-study variability (τ^2_{res}), and between-study variability explained by significant moderators (R^2). Abbreviation: STEC, Shiga toxin-producing *E. coli*.

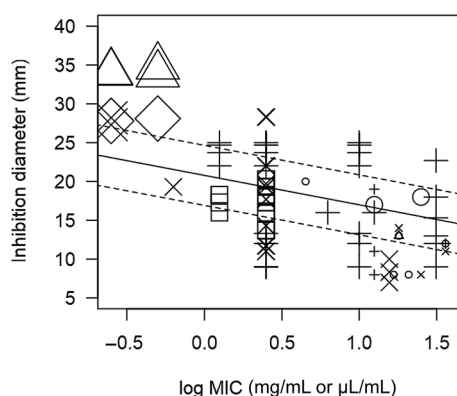


FIGURE 1 Scatter plot depicting the effect ($p < .001$) of the logarithm of the minimum inhibitory concentration of extracts of *Cinnamomum* ($n = 86$), *Salvia* ($n = 16$), and *Mentha* ($n = 6$) plants on inhibition diameters for each bacterium. Markers symbolize bacterium: $\square = C. jejuni$, $\circ = E. coli$, $\Delta = L. monocytogenes$, $+ = S. aureus$, $\times = Salmonella$ spp., $\diamond =$ Shiga toxin-producing *E. coli*; and marker size is proportional to study size.

statistical analysis indicated a tendency for an inverse correlation between the ID and MIC, as evidenced by the negative coefficient of “Log MIC” (-5.60 ± 0.18 , $p < .0001$). This implies that a higher MIC indicates less effective inhibition of bacterial growth by plant extracts applied, leading to smaller ID when testing the plant extract at a specific concentration using any methods used to determine the MIC (diffusion or dilution method). This relationship is further depicted in Figure 1, which shows a negative slope.

In contrast, the positive estimate of “Log Dose” (11.32 ± 0.31 , $p < .0001$) suggested a tendency for larger ID as the dose of the extract applied increases. This means that the effectiveness of the extracts depends on the dose

(Table 7). This information can be useful in selecting biopreservatives for food products or packaging to control pathogens, in line with the latest trends in the food industry (Pandey et al., 2016; Sharifi-Rad et al., 2018).

Table 7 also demonstrates that distinct pathogens exhibit different ID when treated with the same concentration of a plant extract, as shown by the different mean values of the moderating variable “Bacterium.” *Listeria monocytogenes* displayed the highest ID when challenged with a specific plant extract at a certain dose (15.67 ± 3.93), followed by *E. coli* (12.72 ± 3.93) and *S. aureus* (11.47 ± 3.93). In contrast, *Campylobacter jejuni*, *Salmonella* spp., and STEC exhibit the least sensitivity to the application of antimicrobial plant extracts, as indicated by the lower ID (Table 7).

Extracts and EOs have gained popularity as an effective means of inhibiting bacterial growth due to their unique composition, which allows for a synergistic effect of their various components (Hyltdgaard et al., 2012). This makes them a promising alternative for inactivating drug-resistant bacterial strains. The antimicrobial action of EOs is based on their hydrophobic nature, which enables them to interact with the microbial cell membrane. This interaction can increase the permeability of the membrane, leading to eventual rupture and release of ions and genetic material contained within the cell, ultimately resulting in cell death (Hyltdgaard et al., 2012; Nazzaro et al., 2013).

The complex mixture of compounds found in EOs, including terpenes, phenolic compounds, and fatty acids, allows for multiple routes of antimicrobial action, providing an advantage over traditional antibiotics that typically target a single pathway. Furthermore, EOs have been shown to be effective against a broad range of microorganisms, including Gram-positive and Gram-negative bacteria (Álvarez-Martínez et al., 2021; Vaou et al., 2022).

Observations showed inconsistent patterns of susceptibility between Gram-negative and Gram-positive bacteria. For example, while some demonstrated no significant distinctions, certain *Mentha* and *Salvia* species exhibited less antibacterial activity against *Salmonella* spp. and *E. coli* (Tables 2 and 3). Furthermore, there is an extensive body of research documenting the in vitro antimicrobial activity of plant extracts and EOs against both Gram-positive and Gram-negative bacteria. These studies suggest that the effectiveness of such antimicrobial agents can vary significantly depending on the bacterial strain tested, with some strains being more susceptible than others (Abers et al., 2021; Ghavam et al., 2022; Semeniuc et al., 2017). The differential antimicrobial activity of EOs against Gram-positive and Gram-negative bacteria has important implications for the development of new antimicrobial agents. The peptidoglycan layer, which is present in the cell wall of Gram-positive bacteria, plays a key role in the susceptibility of these bacteria to EOs (Semeniuc et al., 2017). This layer is responsible for maintaining the structural integrity of the bacterial cell and is an essential component of the cell wall (Hsouna et al., 2011). When EOs are applied to Gram-positive bacteria, they interact with the peptidoglycan layer and disrupt its structure, leading to increased permeability and eventual cell death (Semeniuc et al., 2017).

In contrast, Gram-negative bacteria have a more complex cell wall structure, which includes an outer membrane composed of a double layer of phospholipids and lipopolysaccharides (Koohsari et al., 2015; Vikram et al., 2007). This outer membrane acts as a barrier, preventing the entry of many antimicrobial agents, including EOs. The lipopolysaccharides on the outer membrane are responsible for the Gram-negative bacteria's resistance to EOs, as they create a negatively charged barrier that repels the hydrophobic molecules present in the EOs. However, some EOs have shown broad-spectrum activity against both Gram-positive and Gram-negative bacteria, indicating that they may have mechanisms of action beyond simply disrupting the cell wall (Abers et al., 2021; Chouhan et al., 2017; Galgano et al., 2022; Patterson et al., 2019; Semeniuc et al., 2017).

Despite the inherent resistance of Gram-negative bacteria to EOs, researchers are actively exploring ways to enhance the efficacy of EOs against these organisms. One promising approach is the use of nanoparticles to improve the delivery of EOs to the bacterial cell wall (Bagheri et al., 2021; Hadidi et al., 2020; Liakos et al., 2018). Another strategy involves the combination of EOs with other antimicrobial agents to create a synergistic effect (Basavegowda & Baek, 2022).

For the meta-regression model reported in Table 7, the statistical tests indicated the absence of potential publica-

tion bias. Following same previous proceeding, the funnel plot (with symmetry) for this model is given in the Supporting Information to visually assess the publication bias (Figure S7). Moreover, the intraclass correlation I^2 was below the value considered as indicative of high heterogeneity (<75%), with a moderate level of heterogeneity (64.5%), thus suggesting that about two thirds of the variation in the outcome measures may be attributed to other moderating variables explaining the remaining between-study variability that were not codified in the present meta-analysis study.

Although the meta-regression model introduced by the moderators has shown some correlation between the predicted and observed values, it only accounts for a modest proportion of the variability between studies ($R^2 = 27.1\%$). This suggests that other sources of variation in the antimicrobial activity of extracts from different plant species remain unexplained. A number of possible sources of variability have been identified in the literature. For instance, the source, stage of development, and seasonality of the plants used to obtain the extracts have been shown to impact their antimicrobial properties (Chouhan et al., 2017; Costa et al., 2022; Gourich et al., 2022). Furthermore, the composition of these extracts, specifically the presence of phenolic compounds and their derivatives, fatty acids, terpenes, and other secondary metabolites, can also play a role in their antimicrobial activity (Altun & Yapici, 2022; Alves et al., 2022). Moreover, the volatility of plant derivatives, particularly EOs, can significantly influence the results of standard microplate-based assays, as reported by Houdkova et al. (2020). Vapor transition can affect the assay results, potentially leading to false positives in nonsealed microtiter plates. As a result, careful and considered assay design is critical when investigating volatile antimicrobial agents.

Environmental factors, such as temperature and humidity, have also been shown to impact the antimicrobial activity of plant extracts. These variables can affect the growth rate and metabolic activity of microorganisms, thereby influencing their susceptibility to the extracts (de Macêdo et al., 2020). Additionally, the strain and inoculum size of the microorganisms being tested can affect the results of antimicrobial susceptibility assays. Some microorganisms may be inherently more resistant or sensitive to specific plant extracts due to genetic differences, while different inoculum sizes can lead to varying degrees of growth inhibition (Bidlas et al., 2008). It is important to consider these potential sources of variability when interpreting the results of studies investigating the antimicrobial properties of plant extracts. By accounting for these factors in experimental design and data analysis, researchers may be able to more accurately identify the active compounds and optimal conditions for the use of

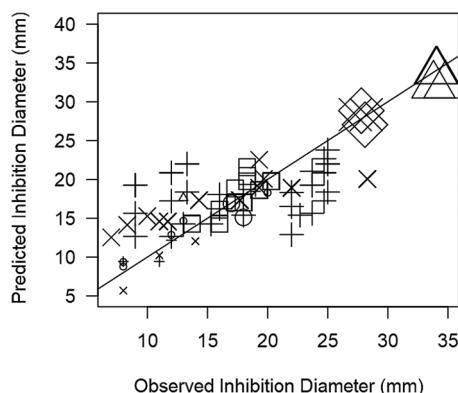


FIGURE 2 Scatter plot of the observed inhibition diameters produced by extracts of *Cinnamomum* ($n = 86$), *Salvia* ($n = 16$), and *Mentha* ($n = 6$) plants versus values predicted by the meta-regression model ($R^2 = .788$), with 45° reference line. Markers symbolize bacterium: $\square = C. jejuni$, $\circ = E. coli$, $\Delta = L. monocytogenes$, $+$ = *S. aureus*, \times = *Salmonella* spp., \diamond = Shiga toxin-producing *E. coli*; and marker size is proportional to study size.

these natural products in the treatment and prevention of microbial infections.

The meta-regression model has shown a satisfactory correlation between predicted and observed values ($R^2 = .788$), indicating that there is a significant underlying correlation between the two antimicrobial susceptibility determinations. This is further supported by the findings presented in Figure 2, which demonstrate the effectiveness of extracts from *Cinnamomum*, *Salvia*, and *Mentha* species in inhibiting the growth of various microorganisms.

Despite the limitations of the model in fully capturing all the sources of variability reported in the literature, the insight it provides into the effectiveness of these extracts should not be underestimated. In fact, other authors have also demonstrated the antimicrobial activity of these extracts against a range of organisms (Al-Mariri & Safi, 2014; Parham et al., 2020; Stan et al., 2021), further highlighting their potential as natural alternatives to conventional antimicrobial agents. These findings have important implications for the development of new antimicrobial agents and the fight against AMR. By identifying and harnessing the antimicrobial properties of these plant extracts, we may be able to develop new treatments that are both effective and sustainable. Additionally, these findings may contribute to a growing body of evidence supporting the use of natural products in the fight against foodborne pathogen.

4 | CONCLUSIONS

This meta-analytical study employed literature data to develop regression models with the aim of providing a

comprehensive understanding of the antimicrobial activity of *Cinnamomum*, *Salvia*, and *Mentha* species extracts and EOs, as well as the relationship between ID and MIC against various pathogens. The meta-regression models revealed distinct susceptibilities of bacterial strains, with *B. cereus* being the most sensitive to cinnamon extracts, while *Salvia* and *Mentha* species extracts showed effectiveness against *B. cereus*, *S. aureus*, and *L. monocytogenes*. In general, the pooled MIC models did not show any significant impact of the methodology used or discernible differences between the efficacy of extracts and EOs. The study also demonstrated an inverse correlation between MIC and ID and provided a summary of inhibitory effectiveness and the impact of extract dose, highlighting the importance of considering variables that affect these measurements.

Overall, this meta-analysis provides evidence supporting the potential of natural extracts and EOs from *Cinnamomum*, *Salvia*, and *Mentha* species as effective antibacterial agents. However, further research is necessary to fully explore their potential and consider factors affecting interpretation of antimicrobial studies. Such research could contribute to the expanding literature on natural products for managing infectious diseases and selecting biopreservatives to control pathogenic microorganisms in food.

AUTHOR CONTRIBUTIONS

Youssef Ezzaky: Investigation; data curation; writing—original draft. **Abdelkhaleq Elmoslih:** Investigation; data curation; writing—original draft. **Beatriz Nunes Silva:** Investigation; methodology; data curation; writing—review and editing. **Olga María Bonilla-Luque:** Investigation; data curation; visualization; writing—review and editing. **Aricia Possas:** Investigation; validation; writing—review and editing. **Antonio Valero:** Resources; writing—review and editing. **Vasco Cadavez:** Conceptualization; resources; investigation; software; supervision; formal analysis; writing—review and editing. **Ursula Gonzales-Barron:** Conceptualization; resources; investigation; software; formal analysis; writing—review and editing; supervision. **Fouad Achemchem:** Conceptualization; resources; investigation; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

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