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Original scientific paper

The potential of ragweed (*Ambrosia artemisiifolia* L.) essential oil in the suppression of bacterial growth

Abstract

Plant essential oils have antimicrobial properties that are widely used in food preservation and aromatherapy and also have various medicinal uses. The way essential oil will affect the microorganisms widely depends on the type of microorganism and the type of oil used. Gram-positive bacteria are more sensitive to essential oil antimicrobial traits than gram-negative bacteria. Ragweed (Ambrosia artemisiifolia L.) essential oil has a wide range of antimicrobial applications; this oil is recommended for potential health benefits because of its antioxidant activity. The aim of this study was to examine the antibacterial potential of the ragweed essential oils against Escherichia coli, Bacillus subtilis and Salmonella spp. The antimicrobial effect of the oil was examined using a diffusion method test. Before the experiment, the inhibition zone of bacterial growth was determined using the antibiotic gentamicin. The results of the study confirmed the antimicrobial effect of ragweed oil on the growth of Salmonella spp. and Bacillus subtilis growth, while this oil had negligible effect on Escherichia coli growth. Moderate susceptibility of Salmonella spp. and Bacillus subtilis and resistance of E. coli was observed after ragweed treatment compared to the standard inhibition zone values proposed by EUCAST. This research confirms the potential application of ragweed essential oil in the inhibition of Salmonella spp. and Bacillus subtilis growth.

Keywords: *essential oil, antimicrobial activity, Escherichia coli, Bacillus subtilis, Salmonella spp.*

Introduction

Agriculture production is one of the most developed fundamental activities (Rafael, 2023), which provides products for human nutrition (Beckman and Countryman, 2021). As human civilization continues to grow, the production of safe and healthy food is a primary challenge in food production (Garcia et al., 2020). However, food contains various organic compounds, such as carbohydrates, proteins, and lipids (Amit et al., 2017) and undergoes deterioration due to chemical, physical and/or microbiological impacts (Rahman, 2007). Thus, Sherawat et al. (2021) suggest the necessity of the preservation of food and prevention of spoilage to retain food quality and prolong the product shelf-life. Although food spoilage occurs due to various actions (Amit et al., 2017), Aung and Chang (2014) indicate the importance of pathogenic microbes' presence in food deterioration in all stages of food production and distribution.

Spoilage microbes often include spore-forming Gram-positive and facultative anaerobic Gram-negative bacteria that belong to the *Enterobacteriaceae* family (Rawat, 2015). Among the spore-forming Gram-positive bacteria, the genus *Bacillus* is an important food deterioration agent for various agricultural products (Pacher et al., 2022). Moreover, *Bacillus subtilis* is described as one of the most present spoilage agents belonging to *Bacillus* spp. (Marangoz et al., 2018) found in bread (Cauvain, 2015), dairy desserts (Mochonas et al., 2021) and meat (Daszczuk et al., 2014). However, some studies revealed that members of *Enterobacteriaceae*, e.

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g. *Escherichia coli* or *Salmonella* spp. were frequently isolated from fresh fruits and vegetables (Amrutha et al., 2017; Luna-Guevara et al., 2019; Possas et al., 2021). Those bacteria are involved in several health complications (Momtaz et al., 2013), suggesting the need to remove and/or inactivate them from food. For this purpose, several approaches were proposed; one of them being an application of essential oils (EO) obtained from medicinal and aromatic plants, spices and herbs (Puvača et al., 2021), which express remarkable antimicrobial impact (Mohsen et al., 2020) and strong antioxidant activity (Pateiro et al., 2018). Nevertheless, apart from these popular plants, EOs from some exotic and endemic plants (Jugreet and Mahomoodally, 2020) and weeds, such are *Conyza canadensis* (Veres et al., 2012) and *Bidens pilosa* (Wahjudi et al., 2023) were used in the suppression of various microbes. One of the genera of interest is *Ambrosia* L., which consists of 40 species (Dahl et al., 1999; Wang et al., 2006), originating from North America (Han et al., 2021; Voća et al., 2020), but widespread in different countries of the world (Montagnani et al., 2017). Although ragweed (*A. artemiisifolia* L.) EO is not examined frequently, Han et al. (2021) and Chalchat et al. (2004) suggest that specific chemical content may contribute to the antimicrobial properties of this EO.

The objective of this paper was to determine the antibacterial properties of the ragweed EO.

Material and methods

For this research, a dry shoot of ragweed collected in the full bloom phase was used for the EO extraction. Prior to EO extraction, milling of dry shoot (100 g) and subsequently dilution in distilled water (1000 ml) was performed. After homogenization, hydro-distillation using a Neo Clevenger-type apparatus according to ISO 8571-1984 (E) was performed.

The impact of ragweed EO was estimated using pure cultures of *Escherichia coli, Bacillus* subtilis, and *Salmonella spp.* belonging to the Laboratory of Microbiology (Faculty of Agricultural and Food Sciences Sarajevo, Bosnia, and Herzegovina). Bacterial inoculum was prepared according to EUCAST (2017). Overnight bacterial cultures on nutrient agar were diluted in saline (5 ml) up to 0.5 McFarland turbidity standard, which corresponds to an inoculum density of 10^8 cells/ml. Before the experiments, the inoculum was incubated for 24 hours at 30°C.

The antimicrobial activity of ragweed EO was determined using the disc diffusion method. Mueller-Hinton agar, previously autoclaved at 120°C for 20 minutes, was inoculated with bacterial inoculum. After 15 minutes of agar plate inoculation, 6 filter paper disks (6 mm diameter) impregnated with ragweed EO were placed on each agar surface. Incubation in the Petri dish was performed at 37°C and 24 hours for *E. coli* and *Salmonella* spp., while at 28°C and 72 hours for *Bacillus subtilis*. The diameter of the zone of inhibition was measured and expressed in millimeters (mm). The interpretation of the obtained results was performed according to EUCAST (2017), where the effect of the extracts is classified as resistant (R), susceptible - increased exposure (I) and susceptible - standard dose (S) based on standard values for gentamicin.

After determining the zone of inhibition, the sensitivity of bacterial strains to ragweed EO treatment was estimated using the PAST statistical program (v. 4.10), Levene´s, Kruskal-Wallis, and Mann - Whitney test.

Results and discussion

The results of this research showed that ragweed EO has had an impact on the suppression of bacterial growth. The variations among inhibition zone diameter were noticed, depending on bacterial species (Table 1). The results presented in Table 1 showed a weak inhibition effect of ragweed essential oil against *Escherichia coli*; the average diameter of the inhibition zone was 5.0 mm. On the other hand, a more pronounced effect of selected essential oil was noticed against *Bacillus subtilis* and *Salmonella* spp., where inhibition zone diameters were 16.2 and 15.0, respectively.

Table 1. The diameter of the inhibition zone (mm) of tested bacteria using ragweed essential oil
Tablica 1. Promjer zone inhibicije (mm) testiranih bakterija uslijed primjene esencijalnog ulja ambrozije

Diameter of inhibition zone (mm)	*Esherichia coli*	*Bacillus subtilis*	*Salmonella* spp.
Disc 1	9.0	16.0	13.0
Disc 2	9.0	16.0	15.0
Disc 3	1.0	18.0	16.0
Disc 4	2.0	14.0	16.0
Disc 5	2.0	15.0	15.0
Disc 6	7.0	18.0	15.0
Average	5.0	16.2	15.0

Compared with standard values (EUCAST, 2011) for the antibiotic gentamicin (Table 2), *Bacillus subtilis* and *Salmonella* spp. were susceptible to increased exposure to ragweed essential oil, while *E. coli* was resistant.

Table 2. Diameter of inhibition zone (mm) for tested bacteria using gentamicin
Tablica 2. Promjer zone inhibicije (mm) za testirane bakterije primjenom gentamicina

Diameter of inhibition zone (mm)			
EO effect			
Bacteria	S	I	R
Escherichia coli	≥15	13-14	≤12
Bacillus subtilis	≥20	15-19	≤14
Salmonella spp.	≥17	13-16	≤14

Legend: S – susceptible (standard dose); I – susceptible (increased exposure); R – resistant

Table 3. Analysis of bacterial growth suppression using Levene´s and Kruskal-Wallis tests
Tablica 3. Analiza supresije bakterijskog rasta primjenom Levene i Kruskal-Wallis testa

	Escherichia coli	*Bacillus subtilis*	*Salmonella* spp.
N	6	6	6
Min	1	14	13
Max	9	18	16
Sum	30	97	90
Mean	5	16.16667	15
Std. error	1.527525	0.6540472	0.4472136
Variance	14	2.566667	1.2
Stand. dev	3.741657	1.602082	1.095445
Median	4.5	16	15
25 prcntil	1.75	14.75	14.5
75 prcntil	9	18	16
Skewness	0.1030865	0.04053167	-1.369306
Kurtosis	-2.871429	-1.310508	2.5
Geom. mean	3.624715	16.1003	14.96498
Coeff. var	74.83315	9.909785	7.302967
Levene´s test for homogeneity of variance, from means p (same)			**0.0001434**
Levene´s test, from medians p (same)			**0.0003831**
		Kruskal-Wallis test	
H (chi2)	12.03	Hc (tie corrected)	12.32
p (same)			**0.002114**

The inhomogeneity of variance was detected with the Levene´ test (Table 3) due to a low p-value (0.0003831), suggesting the need to use a non-parametric test.

Using the Kruskal-Wallis test (Table 3), the obtained p-value (0.002114) suggests the statistically significant impact of ragweed EO on the inhibition zone diameter of selected bacterial strains. For further comparison between the treatments, the Mann-Whitney test was used (Table 4).

Table 4. Impact of ragweed EO on inhibition zone diameter using Mann-Whitney test
Tablica 4. Utjecaj esencijalnog ulja ambrozije na promjer zone inhibicije primjenom Mann-Whitney testa

	E. coli	*B. subtilis*	*Salmonella* spp.
E. coli		0.004772	0.004551
B. subtilis	0.004772		0.2442
Salmonella spp.	0.004551	0.2442	

The obtained data using the Mann-Whitney test (Table 4) showed that significant differences among the inhibition zone diameter of *E. coli*, and *B. subtilis* and *Salmonella* spp., were noticed. On the other hand, a non-significant impact of ragweed EO between the inhibition zone diameter in *B. subtilis* and *Salmonella* spp. was observed.

Our results showed the negligible impact of ragweed EO against *E. coli* compared to other bacteria. Selim (2011) found that ragweed EO had no effect on the growth of *E. coli* strains, which is similar to our observations. Molinaro et al. (2018) noticed the absence of *E. coli* inhibition using isabelin, a sesquiterpene lactone isolated from ragweed.

According to the obtained results, *Bacillus subtilis* and *Salmonella* spp. were susceptible to increased exposure to ragweed essential oil. When *Artemisia trifida* EO was used, Wang et al. (2006) found that *B. subtilis* was less sensitive to EO treatment compared to our results. These authors revealed that the inhibition zone diameter of *B. subtilis* was 12.5 to 14 mm, depending on the EO concentration. In our research, the average diameter of the zone was 16.2 mm. However, Chalchat et al. (2004) noticed inhibition zone diameters of 26, and 23 mm against *Bacillus subtilis*, and *Salmonella enteritidis*, respectively, suggesting the sensitivity of these bacteria to ragweed EO treatment.

The efficacy of ragweed EO treatment against *Bacillus subtilis* and *Salmonella* spp. could be related to the chemical composition. Han et al. (2021) and Chalchat et al. (2004) showed that monoterpenes and sesquiterpenes are the most abundant compounds responsible for antimicrobial activity (Vaou et al., 2021). In addition, Brückner et al. (2003) demonstrated an algicidal effect and a reduction in chlorophyll content by ragweed EO. Wang et al. (2006) described the antifungal activity of *Ambrosia trifida* EO against *Aspergillus niger* and *Candida albicans*, confirming the potential of ragweed species to inhibit microbial growth.

Conclusion

According to results of this research, ragweed EO showed negligible effect on the growth of *E. coli*, while *Bacillus subtilis* and *Salmonella* spp. showed moderate susceptibility. Using statistical tests, significant differences were found between the inhibition zone diameter of E. coli, and *Bacillus subtilis* and *Salmonella* spp., indicating the potential of ragweed EO in suppression of *Bacillus subtilis* and *Salmonella* spp. growth. Further research will be focused on the chemical characterization of the ragweed EO and its effects against other bacterial genera.

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Izvorni znanstveni rad

Potencijal esencijalnog ulja ambrozije (Ambrosia artemisiifolia L.) u supresiji bakterijskog rasta

Sažetak

Biljna esencijalna ulja imaju antimikrobne karakteristike, zbog čega su ulja našla svoju primenu i široko su zastupljena u očuvanju hrane i aromaterapiji, ali svoju primjenu nalaze i u medicini. Utjecaj esencijalnog ulja na mikroorganizme ovisi o tipu mikroorganizama i tipu ulja. Gram-pozitivne bakterije su osjetljivije na antimikrobno djelovanje ulja u usporedbi sa Gram-negativnim bakterijama. Esencijalno ulje ambrozije (Ambrosia artemisiifolia L.) ima širok spektar antimikrobnih aplikacija pa se njegova primjena preporučuje zbog potencijalnog pozitivnog učinka na zdravlje, odnosno antioksidativne aktivnosti. Cilj ovog rada bio je ispitati antibakterijski potencijal esencijalnog ulja ambrozije na rast bakterija Escherichia coli, Bacillus subtilis i Salmonella spp. Antimikrobni učinak ulja je ispitan difuzijskom metodom. Prije eksperimenta, zona inhibicije bakterijskog rasta je određena primjenom antibiotika gentamicina. Rezultati istraživanja potvrđuju antimikrobni učinak ulja ambrozije na rast bakterija Salmonella spp. i Bacillus subtilis, dok je na rast bakterije Escherichia coli utjecaj ulja bio neznatan. Usporedbom sa standardnim vrijednostima zone inhibicije preporučenim od EUCAST-a, utvrđena je osrednja osjetljivost bakterija Salmonella spp. i Bacillus subtilis i otpornost bakterije E. coli nakon tretmana uljem ambrozije. Ova istraživanja potvrđuju potencijal aplikacije esencijalnog ulja ambrozije u cilju inhibicije rasta bakterija Salmonella spp. i Bacillus subtilis.

Ključne reči: *esencijalno ulje, antimikrobna aktivnost, Escherichia coli, Bacillus subtilis, Salmonella spp.*