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ENHANCING THE SHELF LIFE OF FRESH MINCED MEAT WITH *THYMUS PALLESCENS* AND *ORIGANUM FLORIBUNDUM* ESSENTIAL OILS: ANTIMICROBIAL ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* AND *SALMONELLA TYPHIMURIUM*

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Abstract: The use of natural antimicrobial substances enhances food shelf life, safety, and marketability. This study evaluates the antimicrobial efficacy of essential oils (EOs) from two Algerian plants, *Thymus pallescens* (TP) and *Origanum floribundum* (OF), against *Staphylococcus aureus* and *Salmonella Typhimurium* and their potential as natural preservatives in minced meat. The chemical composition of TP and OF EOs was analyzed by GC-MS, revealing thymol (26.5%) and carvacrol (26.6%) as major compounds. Antimicrobial activity was assessed using disk and agar well-diffusion methods, with MIC and MBC values determined. Applied to minced meat, EOs significantly reduced total plate count and *S. Typhimurium* levels, especially under refrigeration. OF showed slightly higher efficacy. These findings highlight the potential of *T. pallescens* and *O. floribundum* EOs as natural food preservatives, providing an alternative to synthetic preservatives in the food industry.

Keywords: minced meat preservation, *Thymus pallescens*, *Origanum floribundum*, essential oils, antimicrobial activity.

INTRODUCTION

Over the past decade, significant transformations in consumer lifestyles and eating habits have been emerging as a consequence of rapid urbanization, technological advancements, globalization, and economic growth. One of the most profound shifts in consumer behavior today is the increasing recognition of the integral role food plays in maintaining and enhancing human well-being. This awareness has fueled a growing demand for healthier food options, free of artificial additives and rich in natural ingredients that promote well-being (Salanță *et al.*, 2020; Farcas *et al.*, 2021). This trend is particularly noticeable in the meat industry, where consumers are increasingly seeking products that not only satisfy their dietary needs but also align with their health-conscious lifestyles.

Meat, a staple in many diets, is one of the most perishable food items due to its susceptibility to oxidative processes and microbiological spoilage. This spoilage not only diminishes the sensory qualities of meat, such as taste, color, and texture, but also reduces its essential nutrient content. The perishable nature of meat is a major concern for both consumers and the food industry, as it results in significant economic losses and raises health concerns. Among the pathogens commonly associated with raw and processed meats, *Salmonella*, *Staphylococcus aureus*, *Campylobacter*, and *Escherichia coli* are particularly concerning. These bacteria are responsible for a range of foodborne illnesses that pose serious public health risks (Hennekinne *et al.*, 2015).

To combat these challenges, the food industry has traditionally relied on synthetic preservatives. These additives are used during processing to extend the shelf life of meat products, prevent spoilage, and ensure safety. However, the prolonged use of synthetic preservatives has raised significant concerns regarding their safety. Studies suggest that while effective in preventing spoilage, these compounds may cause adverse health effects, including gastrointestinal issues, allergies, and even cancer (Lourenço *et al.*, 2019). Additionally, high concentrations of nitrites and benzoates, commonly used in meat preservation, have been linked to toxicity and carcinogenicity (Basavegowda *et al.*, 2021). As a result, these health concerns have driven a growing demand for natural alternatives to synthetic preservatives.

In response to this demand, interest in naturally derived antimicrobials for food preservation has been increasing. Among these, essential oils (EOs) and phenolic compounds have emerged as the most promising bioactive substances. EOs, in particular, have

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been extensively studied for their antimicrobial, antioxidant, and preservative properties. Extracted from various plant species, these oils have proven highly effective against a wide range of microbial contaminants, including foodborne pathogens. Their natural origin, coupled with their recognized safety and efficacy, makes them attractive alternatives to synthetic preservatives (Perricone *et al.*, 2015).

Essential oils have a long history of use in traditional medicine and are well-known for their aromatic properties. Historically, they were primarily valued for their fragrance and flavoring potential in foods, beverages, and other products (Dima *et al.*, 2015). However, recent research has highlighted their potential as natural preservatives in the food industry, expanding their application beyond flavoring. The United States Food and Drug Administration (FDA) has classified many EOs as generally recognized as safe (GRAS) for use in food products, enhancing their appeal as natural food protectants (Maurya *et al.*, 2021).

Among the essential oils gaining attention for their antimicrobial properties are those derived from species of the *Origanum* and *Thymus* genera, both in the Lamiaceae family. These plants, widespread in the Mediterranean region, produce essential oils rich in bioactive compounds with potent antimicrobial and antioxidant activities. In Algeria, *Origanum floribundum* Munby and *Thymus pallescens* de Noé are two notable species that have been the subject of scientific investigation due to their distinctive chemical compositions and potential health benefits. *Origanum floribundum*, endemic to Algeria, and *Thymus pallescens*, one of eleven *Thymus* species recorded in the Algerian flora, have long been used in traditional medicine for their medicinal properties (Quezel *et al.*, 1963).

Research has shown that the essential oils of *Origanum* and *Thymus* species possess significant antimicrobial activity against various foodborne pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Hazzit *et al.*, 2006; Kerbouche *et al.*, 2015). These findings suggest that essential oils could be crucial in enhancing food safety and extending the shelf life of perishable products like meat. However, the application of essential oils in food preservation is not without challenges. A primary limitation is their strong sensory impact, which can alter the flavor and aroma of food. Therefore, it is essential to determine the minimum effective concentration of essential oils that can inhibit the growth of pathogenic bacteria without compromising the food's sensory quality (Agrimonti *et al.*, 2019).

In this context, the present study aims to evaluate the *in vitro* antimicrobial activity of essential oils from *Origanum floribundum* and *Thymus pallescens* against two foodborne pathogens: *Salmonella* Typhimurium ATCC 14028 and *Staphylococcus aureus* ATCC 12228. After analyzing the GC-MS profiles of the two essential oils, the study will determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to assess their potential as natural preservatives. Additionally, the study will explore the use of these essential oils to extend the shelf life of fresh minced meat inoculated with *S.* Typhimurium. By doing so, the research seeks to contribute to the growing body of knowledge on natural food preservation and provide valuable insights into the practical application of essential oils in the food industry.

MATERIALS AND METHODS

Plant material

The two plants were harvested during their flowering phase from the Bouira region in Kadiria (85 km east of Algiers, coordinates: 36° 32’ 00” N, 3° 41’ 00” E, altitude: 1005 m). Their taxonomic identity was confirmed by Professor H. Abdelkrim of the National Superior School of Agronomy (ENSA) in Algiers, Algeria.

Extraction of the essential oils

Essential oils (EOs) were extracted from two endemic Algerian species, *Thymus pallescens* de Noé (TP) and *Origanum floribundum* Munby (OF). The dried aerial parts of each plant, consisting of leaves and flowers (100 g), were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus, as recommended by the European Pharmacopoeia (Council of 1997). The extracted oils were then dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4°C in sealed brown flasks until further analysis.

Gas chromatography mass spectrometry analysis

Gas chromatography (GC) analyses were performed on a Hewlett-Packard 6890 GC-FID chromatograph equipped with an HP-5 MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The column temperature was programmed at 60°C for 8 minutes, then increased at a rate of 2°C min^{-1} to 280°C, and held at 280°C for 15 minutes; nitrogen was used as the carrier gas at a flow rate of 0.5 mL min^{-1}. The injection was performed in split mode (split ratio 1:25) with an injection volume of 0.2 µL at 250°C. Flame ionization detection (FID) was performed at 320°C. The percentages of compounds were determined from their peak areas in the GC-FID profiles.

A Hewlett-Packard computerized system, consisting of a 6890 gas chromatograph coupled with a quadrupole mass spectrometer (HP 5973) and equipped with an HP-5 MS capillary column, was used to perform the gas chromatography-mass spectrometry (GC-MS) analysis. Helium was used as the carrier gas with a flow rate of 0.5 mL min^{-1}; the injection volume was 0.2 µL in split mode (1:25) with an injection temperature of 250°C. The oven temperature program was the same as described for the GC analysis. Electron impact ionization mode at 70 eV with a scan range of 30-550 atomic mass units was employed for detection.

Identification of essential oil components

The identification of essential oil components was performed by comparing the retention indices of the EOs components relative to C8-C22 n-alkanes with those reported in the literature (Adams 2007; Babushok *et al.*, 2011). Additionally, their mass spectra were compared with those in the Wiley 7N and NIST 2005

data libraries, as well as with the spectral data provided by Adams (Adams 2007). For further confirmation, the identification of some components was validated using available authentic standards, which were analyzed under the same conditions as the EOs.

Bacterial strains

Salmonella Typhimurium ATCC 14028 and *Staphylococcus aureus* ATCC 12228 were used to determine the minimum inhibitory concentration and minimum bactericidal concentration (MBC) of OF and TP essential oils.

Media and reagents

The media and chemical compounds used during the experiment included nutrient agar and nutrient broth (TM Media, India), bacteriological peptone water (Merck, Germany), brain heart infusion broth (BHI: Merck, Germany), plate count agar (PCA: Merck, Germany), xylose lysine deoxycholate agar (XLD agar: Oxoid, UK), Tween 80, anhydrous sodium sulfate, and n-alkanes C8-C22 (Sigma-Aldrich, St. Louis, MO, USA).

In vitro antibacterial assays

Preparation of bacterial strains

Stock cultures of *Salmonella* Typhimurium and *Staphylococcus aureus* previously grown on nutrient agar were used. A loopful of the overnight culture was transferred to 10 mL of fresh nutrient broth. The mixture was then shaken for 5 minutes using a vortex and incubated for 24 hours at 37°C to allow the bacteria to reach the exponential growth phase.

Disk diffusion method

The solid medium diffusion technique using filter paper discs was employed to screen the antibacterial activity of *Origanum* and *Thymus* essential oils. In this method, 1 mL of bacterial suspension (approximately 10^6 CFU mL^{-1}) was uniformly spread onto sterile nutrient agar Petri dishes. Filter paper discs (Whatman No. 1, diameter 6 mm) were then soaked with 20 µL of the essential oil (100%) or its dilutions (50, 25, and 10%) prepared with Tween 80 (0.2% in water) and placed on the inoculated agar (Souza *et al.*, 2006). After 24 hours of incubation at 37°C, the diameters of the bacterial growth inhibition zones were measured using calipers and expressed in millimeters.

Agar well-diffusion test

This method was performed as previously described by Gutierrez *et al.* (2009), with some modifications. A volume of 20 mL of brain heart infusion agar (BHI Agar) was inoculated with 10^6 CFU mL^{-1} of *Salmonella* Typhimurium or *Staphylococcus aureus* strains and then poured into a Petri dish. After the agar solidified, wells of 6 mm diameter were aseptically formed, and 50 µL of serially diluted essential oil solutions (100, 50, 25, and 10%) in water-Tween 80 (0.2%) were added to the wells. The plates were kept at 4°C for 2 hours and then incubated under optimal conditions for the growth of the target strains (37°C for 24 hours). The antibacterial activity was visually assessed by observing the inhibition zones surrounding the wells. The zone of inhibition for each well was measured from the underside of the plate with calipers in millimeters. Tween 80 (0.2%) was used as a negative control in both assays.

Determination of MIC and MBC

The minimum inhibitory concentration was defined as the lowest concentration of essential oil that prevented visible bacterial growth, indicated by the absence of turbidity, which corresponded to a 90% reduction of the initial inoculum. The MBC was determined as the concentration at which 99.9% or more of the initial inoculum was killed.

The MIC and MBC of the tested volatile oils were determined according to the procedure described by Rasooli *et al.* (2006), with some modifications. All tests were performed using the broth dilution method in sterile test tubes as follows: 5 mL of each of the various oil dilutions (0.05 - 0.25%) was added to 0.5 mL of BHI broth containing 10^6 cells mL^{-1}. The tubes were then incubated at 37°C for 24 hours in a shaking incubator to ensure even dispersion of the oil throughout the broth. The highest dilution (lowest concentration) showing no visible growth was regarded as the MIC. In tubes where the concentration of the essential oils was below the inhibitory level, bacterial growth was observed, and the broth became turbid (cloudy). The MBC was determined as the highest dilution (lowest concentration) at which no growth occurred on agar plates.

From the tubes showing no visible growth, 0.1 mL of the bacterial suspension was spread on BHI agar plates in duplicate to determine whether the inhibition was reversible or permanent. The negative control consisted of BHI broth with only Tween 80 (0.2%) inoculated with the diluted bacterial culture.

Antimicrobial activity of essential oils in minced meat

To evaluate the antimicrobial activity of oregano and thyme essential oils in a food system, a sufficient amount of minced meat was purchased from a local supermarket.

In this study, two antimicrobial tests were conducted on minced meat: (1) evaluation of the antimicrobial activity of essential oils against the natural mesophilic microbiota of the meat, assessed by total plate count (TPC), and (2) assessment of the antimicrobial activity against the pathogenic bacterial strain *Salmonella* Typhimurium. Meat samples were treated with four different concentrations of essential oils, corresponding to 5, 10, 15, and 20 times the Minimum Inhibitory Concentration (MIC) previously determined for the tested strain (*S. Typhimurium*) (Burt 2004).

This experiment was conducted in commercially sterile polystyrene cups (50 cm^3). A total of 45 g of fresh ground meat was uniformly spread onto the bottom of each cup (Souza *et al.*, 2006). Following this, solutions of *O. floribundum* and *T. pallescens* essential oils at different concentrations were uniformly poured over the ground meat, which had been previously inoculated with 10^6 CFU g^{-1} of *Salmonella* Typhimurium. The cups were

then aseptically sealed and stored at two different temperatures: under refrigeration (4°C) for 24 and 48 hours, and at room temperature (25 to 30°C) for 6 hours. In addition to these sample tests, a reference control test without the essential oil was also conducted.

After the incubation periods, the TPC was determined using the pour plate method with serial dilutions on Plate Count Agar (PCA), followed by incubation at 30°C for 48 hours (ISO 2003). The colony-forming units per gram (CFU g^{-1}) of *Salmonella* Typhimurium were enumerated on XLT agar, with plates incubated for 24 hours at 37°C. All tests were performed in triplicate.

Statistical analysis

All experiments were conducted in triplicate, and the results are presented as mean values ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to determine significant differences between groups. A significance level of $p < 0.05$ was considered for all tests. Data analysis was carried out using STATISTICA software, version 7.0.61.0 (StatSoft, Inc., USA, 2004; *www.statsoft.com*).

RESULTS AND DISCUSSION

Yields and chemical composition of essential oils

The chemical classes, GC-MS data, yields, and chromatogram profiles of the constituents of essential oils from OF and TP are presented in Table 1 and Fig. 1. The essential oil yields were 4.6 and 4.3% (*v/w* dry matter) for *O. floribundum* and *T. pallescens*, respectively, indicating no significant difference. These results fall within the range reported by other authors for the same species: 2.0-5.8% for *O. floribundum* (Hazzit *et al.*, 2009; Daoudi-Merbah *et al.*, 2016) and 1.7-6.2% for *T. pallescens* (Hazzit *et al.*, 2009; Alloun *et al.*, 2019).

Table 1.

Essential oils composition (%) of *T. pallescens* and *O. floribundum*

N°	Compounds	Retention index		*Origanum floribundum*	*Thymus pallescens*	Identification
		ERI	LRI			
1	Tricyclene	924	925	-	0.1	RI, MS, Std
2	α-Thujene	928	930	4.0	3.4	RI, MS
3	α-Pinene	936	937	2.6	7.9	RI, MS, Std
4	Camphene	949	950	0.2	0.3	RI, MS, Std
5	Sabinene	973	973	0.3	0.3	RI, MS, Std
6	β-Pinene	978	979	0.5	0.7	RI, MS, Std
7	1-Octene-3-ol	980	980	1.3	0.4	RI, MS
8	β-Myrcene	989	991	5.1	4.9	RI, MS, Std
9	3-Octanol	993	993	-	0.1	RI, MS
10	α-Phellandrene	1004	1004	0.6	0.6	RI, MS, Std
11	δ-3-Carene	1009	1011	0.1	0.1	RI, MS
12	α-Terpinene	1019	1017	6.8	3.6	RI, MS, Std
13	p-Cymene	1028	1025	**16.0**	**16.5**	RI, MS, Std
14	β-Phellandrene	1030	1030	1.0	2.2	RI, MS
15	1,8-Cineole	1033	1032	0.1	0.6	RI, MS, Std
16	cis- β-Ocimene	1038	1038	0.2	0.1	RI, MS
17	trans- β-Ocimene	1047	1049	0.1	0.6	RI, MS
18	γ-Terpinene	1060	1060	**17.0**	**16.8**	RI, MS, Std
19	cis-Sabinene hydrate	1066	1068	0.8	0.7	RI, MS
20	α-Terpinolene	1086	1088	0.1	0.1	RI, MS, Std
21	trans-Sabinene hydrate	1097	1098	0.1	0.1	RI, MS
22	Linalool	1099	1099	2.4	5.0	RI, MS, Std
23	Borneol	1162	1166	0.1	0.2	RI, MS, Std
24	4-Terpineol	1176	1177	0.1	0.3	RI, MS, Std
25	α-Terpineol	1189	1189	0.1	-	RI, MS, Std
26	Thymol methyl ether	1233	1235	0.1	-	RI, MS
27	Carvacrol methyl ether	1244	1244	0.3	0.2	RI, MS
28	Thymol	1293	1291	**26.5**	0.2	RI, MS, Std
29	Carvacrol	1301	1299	10	**26.6**	RI, MS, Std
30	α-Gurjunene	1409	1409	0.1	0.6	RI, MS
31	trans-β-Caryphyllene	1421	1419	1.1	2.3	RI, MS
32	α-Humulene	1454	1454	0.1	0.1	RI, MS
33	Germacrene D	1479	1480	0.1	0.7	RI, MS
34	Bicyclogermacrene	1495	1495	0.1	0.5	RI, MS
35	β-Bisabolene	1510	1509	0.1	0.4	RI, MS
36	γ-Cadinene	1513	1513	0.1	0.1	RI, MS
37	δ-Cadinene	1535	1534	0.1	0.2	RI, MS
38	trans-α-Bisabolene	1543	1541	-	0.1	RI, MS
39	Spathulenol	1576	1576	0.2	0.3	RI, MS
40	Caryophyllene oxide	1581	1581	0.4	0.4	RI, MS
Total (%)				98.8	97.7	

N°	Compounds	Retention index		*Origanum floribundum*	*Thymus pallescens*	Identification
		ERI	LRI			
Monoterpenes hydrocarbons				69.6	58.2	
Oxygenated monoterpenes				25.6	33.9	
Sesquiterpenes hydrocarbons				1.8	4.4	
Oxygenated sesquiterpenes				0.6	0.7	
Others				1.2	0.5	
Yield (%, *v/w*)				4.6	4.3	

ERI, Experimental retention index relative to C8-C22 n-alkanes on an HP5MS column, LRI, Literature retention index, RI, comparison of retention indices with those found in the literature, MS, comparison of mass spectra with MS libraries and literature data, Std, standard (comparison with authentic compounds; Components marked in bold correspond to main constituents (≥10%).

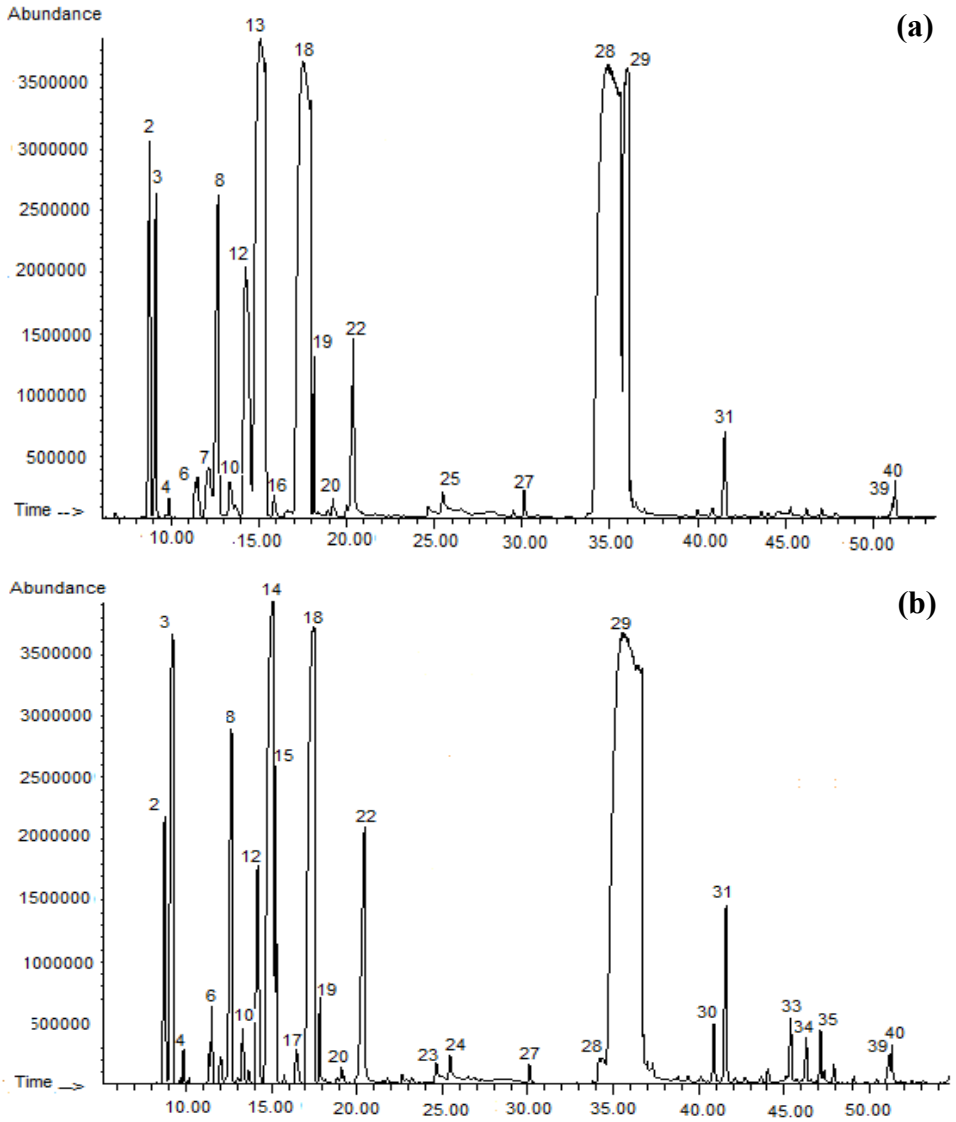

Fig. 1. Essential oil chromatograms of *O. floribundum* (a) and *T. pallescens* (b). *The number on the peak matches the number of the compound in Table 1.*

Thirty-seven compounds were identified in the OF essential oil, accounting for 98.8% of the total oil, while thirty-eight compounds were identified in the TP essential oil, representing 97.7% of the total oil. Both oils exhibited nearly the same qualitative composition, with similar major components, except for the two predominant constituents: thymol (26.5% in OF) and carvacrol (26.6% in TP). The oils were primarily composed of monoterpene hydrocarbons (69.6% in OF and 58.2% in TP) and oxygenated monoterpenes (25.6% in OF and 33.9% in TP). The compositions of the two oils were qualitatively similar to those previously reported for the same species from the same region (Hazzit *et al.*, 2009; Kerbouche *et al.*, 2015).

Various studies have indicated that thyme essential oil is rich in phenolic monoterpenes, with the chemotype of thyme typically being either thymol or carvacrol (Babushok *et al.*, 2011). Regarding the EO of *T.*

pallescens, it has been consistently reported to be rich in carvacrol (Benchabane *et al.*, 2015; Alloun *et al.*, 2019).

Regarding OF, its composition was qualitatively and quantitatively different from samples from other locations reported in the literature, where *p*-cymene was identified as the most significant compound, with levels reported at 42.6% (Daoudi-Merbah *et al.*, 2016) and 60.7-73.4% (Hadjadj *et al.*, 2020).

In vitro antibacterial assays

Disk diffusion method

The results presented in Table 2 represent the net zones of inhibition, including the 6 mm diameter of the paper disk. The data shows that both *Thymus pallescens* and *Origanum floribundum* essential oils exhibit antimicrobial activity against *Staphylococcus aureus* and *Salmonella* Typhimurium, with *O. floribundum* consistently producing larger inhibition zones, indicating stronger antimicrobial properties. *St. aureus* generally shows greater susceptibility to the oils compared to *S.* Typhimurium. The effectiveness of both oils is concentration-dependent, with higher concentrations leading to larger inhibition zones. Even at the lowest concentration, the oils still demonstrate antimicrobial activity, though reduced. *O. floribundum* is more potent than *T. pallescens* at all concentrations, especially at 100% and 50%.

Table 2.

Diameters of microbial inhibition zones (mm) determined by the disk diffusion method

Plant	Oil Concentration (%)	Inhibition zone (mm)	
		St. aureus	***S.* Typhimurium**
T. pallescens	100	29.5	26.5
	50	22.5	21.5
	25	14.0	13.0
	10	11.5	11.5
O. floribundum	100	37.0	35.0
	50	26.5	27.5
	25	20.0	20.0
	10	12.0	10.0

Agar well-diffusion test

The antibacterial activity of the essential oils of *O. floribundum* and *T. pallescens* against the two strains, as determined by the agar well-diffusion test, is shown in Table 3. The results indicate that both essential oils are effective against the two microbial strains, with *O. floribundum* showing markedly higher antimicrobial activity. *St. aureus* is generally more sensitive to the oils, especially at higher concentrations. The inhibition zones decrease with lower concentrations, highlighting a clear dose-dependent response. Notably, *O. floribundum* retains strong antimicrobial properties even at reduced concentrations, making it more potent overall than *T. pallescens*. At the highest concentration, *O. floribundum* produces inhibition zones that are more than twice the size of those produced by *T. pallescens*, underscoring its superior efficacy.

From the results in Tables 2 and 3, it is clear that the growth inhibition zones are larger with the agar well-diffusion method compared to the disk diffusion method for both essential oils. This difference likely results from the faster and more efficient diffusion of the oil in the well compared to the paper disc.

Table 3.

Diameters of microbial inhibition zones (mm) determined by the agar well-diffusion test

Plant	Oil Concentration (%)	Inhibition zone (mm)	
		St. aureus	***S.* Typhimurium**
T. pallescens	100	29.0	26.0
	50	19.0	24.0
	25	14.0	20.0
	10	9.0	6.0
O. floribundum	100	75.0	60.0
	50	39.0	42.0
	25	29.0	26.0
	10	19.0	7.0

The Gram-positive bacterium *St. aureus* was slightly more sensitive to both essential oils compared to *S.* Typhimurium, as evidenced by larger inhibition zones. In contrast, the Gram-negative bacterium *S.* Typhimurium exhibited smaller inhibition zones for the same essential oil concentrations. This difference in sensitivity can be attributed to the complex cell envelope of Gram-negative bacteria, which contains lipopolysaccharides that hinder the accumulation of essential oils on the cell membrane, contributing to their resistance (Cosentino *et al.*, 1999).

Results of minimum inhibitory and minimum bactericidal concentrations

The MIC and MBC values of the studied essential oils, determined using the broth dilution method, are shown in Table 4. The data shows that both *T. pallescens* and *O. floribundum* essential oils exhibit effective

antimicrobial activity against the tested strain, with slightly lower MIC and MBC values for *S.* Typhimurium compared to *St. aureus*. The MBC/MIC ratios, all close to 1, indicate that both oils have strong bactericidal properties against these strains, with *O. floribundum* showing marginally higher efficacy.

Table 4.

MIC and MBC values determined using the broth dilution method

	Thymus pallescens		*Origanum floribundum*	
	St. aureus	*S.* Typhimurium	*St. aureus*	*S.* Typhimurium
MIC (%)	0.12	0.10	0.15	0.16
MBC (%)	0.15	0.11	0.16	0.17
MBC/MIC	1.250	1.100	1.066	1.062

Bacteriostatic activity is observed when a compound inhibits bacterial growth in broth, but the bacteria can still be cultured when transferred to an agar plate. In contrast, bactericidal activity is characterized by the complete elimination of the microbial inoculum (Burt 2004; Souza *et al.*, 2006). The study found that both essential oils demonstrated bactericidal effects against the two tested strains, as indicated by MBC/MIC ratios ranging from 1.06 to 1.25. Essential oils are considered bactericidal when their MBC values are less than four times the minimum inhibitory concentration (MBC/MIC ratio ≤ 4) (Pankey *et al.*, 2004). Additionally, the results indicate that both strains were more susceptible to the action of thyme essential oil than oregano essential oil.

The antibacterial effect of the essential oils observed in this study can likely be attributed to their specific components. In particular, the primary constituents of *O. floribundum* and *T. pallescens* essential oils, thymol (26.5%) and carvacrol (26.6%), respectively, are known for their potent antibacterial properties. The higher concentration of thymol in *O. floribundum*'s essential oil, a compound frequently cited in the literature as a key contributor to antibacterial activity (Memar *et al.*, 2017), may explain its stronger antibacterial effect compared to *T. pallescens* oil. These findings are consistent with previous research highlighting the significant antibacterial properties of essential oils rich in thymol and/or carvacrol (Laghmouchi *et al.*, 2018; Chroho *et al.*, 2024).

For example, among 21 compounds investigated for antibacterial action against 25 pathogens, phenolic monoterpenes such as thymol and carvacrol were found to be particularly effective against *E. coli* (Dorman *et al.*, 2000; Kerbouche *et al.*, 2021). Additionally, like many other essential oils, oregano oil exhibits several potential mechanisms of action against microbial cells (Sakkas *et al.*, 2017). According to Al-Mijalli *et al.* (2022), *Origanum compactum* essential oil obtained from the Boulemane region in Morocco showed higher antibacterial activity against *E. coli*, *B. subtilis*, *St. aureus*, and *L. innocua* strains than samples collected from Taounate, due to their higher concentration of carvacrol (45.80%).

The antibacterial activity of phenolic compounds like carvacrol and thymol is primarily due to their ability to disrupt bacterial membranes by increasing permeability and causing depolarization, leading to bacterial cell death (Cristani *et al.*, 2007).

Treatment with these two compounds destroys various microorganisms, including *Shigella sonnei*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella enterica* subsp. *enterica* serovar Typhimurium. With their hydroxyl, methyl, and isopropyl groups, thymol and carvacrol feature a system of delocalized electrons that is essential to their antibacterial properties. These compounds act as proton exchangers due to the presence of double bonds between electrons, which reduces the gradient across the cytoplasmic membrane, collapses the proton motive force, depletes the ATP pool, and ultimately leads to cell death (Kachur *et al.*, 2020).

However, an essential oil's antibacterial properties should not be attributed solely to its major components but also to its complex molecular composition and the potential interactions between its various constituents. These interactions can be antagonistic, synergistic, or additive. It is important to note that the antibacterial activity of the entire essential oil may be significantly enhanced by less common components, such as γ-terpinene, which is considered to exhibit relatively strong activity due to its possible synergistic or antagonistic effects (Badia *et al.*, 2020). This aligns with our findings, which suggest that the considerable amount of γ-terpinene in oregano essential oil (17.0%) may contribute to its high antimicrobial activity. Indeed, several studies have demonstrated that the entire essential oil exhibits greater biological efficiency than the combined effect of its individual main constituents, with even trace amounts of certain components being crucial for its biological activity. Moreover, when thymol and carvacrol are present together in one essential oil, they exert an additive impact (Laghmouchi *et al.*, 2018; Nieto 2020).

Antimicrobial activity of essential oils in minced meat assay

Effect of essential oils on the survival of TPC

This section of the study investigates the inhibitory effects of thyme and oregano essential oils at various concentrations (5, 10, 15, and 20 times MIC) on the survival of total aerobic plate count and *Salmonella* Typhimurium in fresh minced meat samples. The samples were stored at room temperature for 6 hours and at refrigeration temperature for 24 and 48 hours (Fig. 2).

During storage at room temperature, the TPC in samples treated with thyme essential oil at doses of 10, 15, and 20 × MIC, or oregano essential oil at all concentrations, was significantly lower than that of the

untreated control samples. Specifically, bacterial counts were reduced by 1.17 and 1.07 log CFU g^{-1} in samples treated with the highest concentrations of thyme and oregano essential oils, respectively. However, the sample treated with 5 times MIC of thyme essential oil showed only a marginal increase in TPC, which remained statistically insignificant.

During storage at refrigeration temperature, all samples exhibited a significant reduction in the TPC. As expected, this reduction was more pronounced in samples with higher concentrations of essential oil. The samples treated with *Thymus* essential oil and stored for 48 hours showed the highest log reduction in TPC, with a reduction of more than 1.98 log reduction observed at a concentration of 20 × MIC. In contrast, the highest log reduction in TPC for samples treated with oregano essential oil was noted after 24 hours of storage.

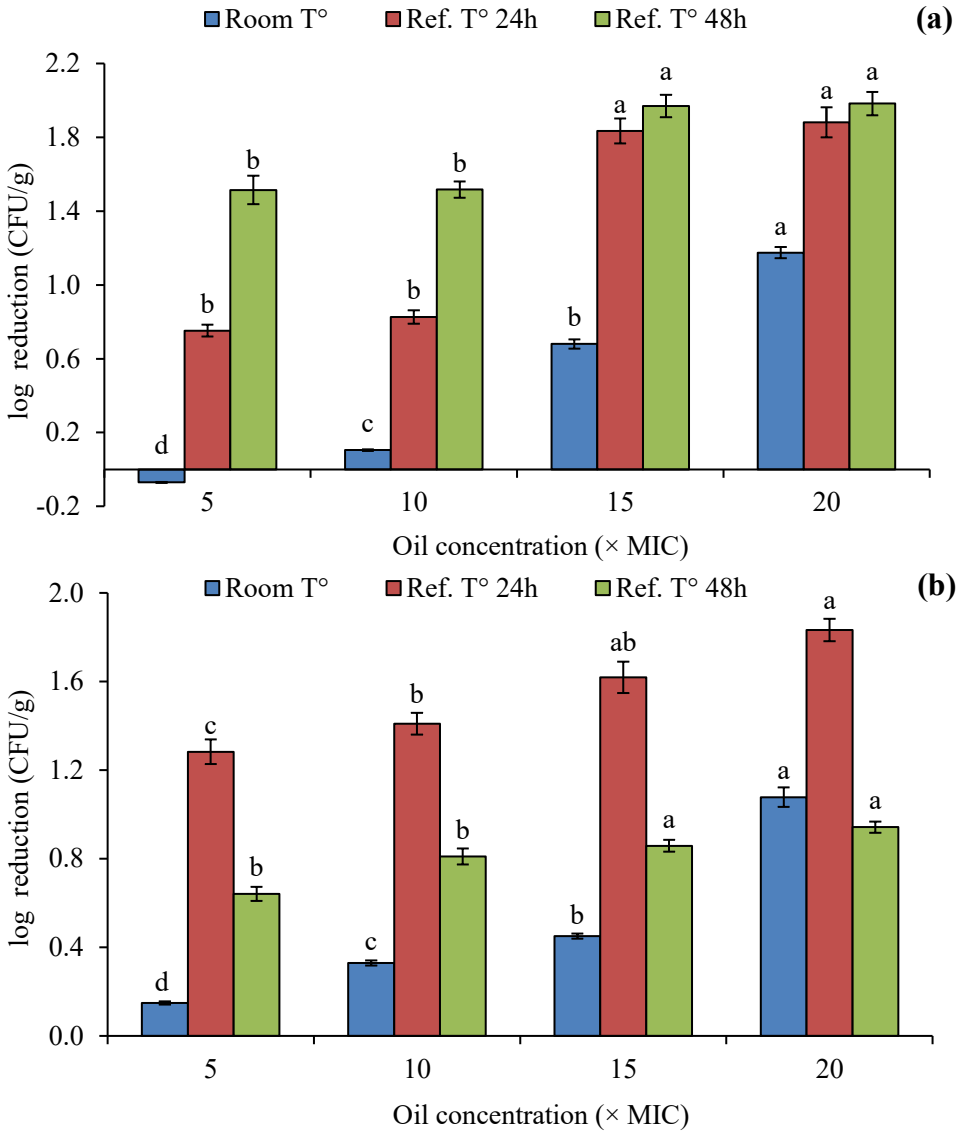

Fig. 2. Log reduction of TPC in fresh minced meat stored at room and refrigeration temperatures, and treated with *T. pallescens* (a) and *O. floribundum* (b) essential oil. *Results of each storage temperature with different letters are significantly different (LSD, p<0.05, with a>b>c>d).*

Effect of essential oils on the survival of Salmonella Typhimurium

The inhibitory effects of *Thymus* and *Origanum* essential oils at various concentrations (5, 10, 15, and 20 times MIC) on the survival of *Salmonella* Typhimurium in fresh minced meat samples stored at room temperature for 6 hours and at refrigeration temperature for 24 and 48 hours are depicted in Fig. 3.

During storage at room temperature, the population of the pathogen in samples treated with 5 times MIC of thyme and oregano oils presented populations of *Salmonella* Typhimurium significantly higher than the non-treated sample (control). However, preparations containing more than 10 × MIC of thyme or oregano essential oils exhibited a different profile, the number of colony forming units of the pathogen showed an evident decrease.

At the end of the storage period at 4°C, all samples expressed a very significant reduction in the population of *Salmonella* Typhimurium. Moreover, the inhibitory effect of both essential oils was proportional to their concentration, with higher levels (15 and 20 times MIC) demonstrating a more pronounced effect.

The applied *Thymus* essential oil had a significantly stronger inhibition effect on *S.* Typhimurium in that the control samples with the inoculation level of 6.64 log

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CFU mL^{-1} were reduced by 2.91 log after the application of the dose of 20 × MIC and stored for 24 hours at 4°C. In response to the dose of 20 × MIC of *Origanum* essential oil, the initial number of *S.* Typhimurium was reduced by 2.13 and 1.94 log compared with the control, respectively, after 24 and 48 hours of storage. No significant reduction of the population of the pathogen was observed in samples with *Origanum* oil stored for 24 and 48 hours. In contrast, the reduction of *S.* Typhimurium was more pronounced after 24 hours of storage in preparations treated with high doses of *Thymus pallescens* essential oil.

Several studies have investigated the *in vitro* efficacy of thyme essential oil against key foodborne pathogens and other food-related microorganisms. Other research has demonstrated the effectiveness of these compounds under *in vivo* conditions against various microorganisms. For example, Aureli *et al.* (1992) reported a reduction in the number of viable *Listeria monocytogenes* by 2 logs during the first week of storage after adding thyme EO to minced pork meat contaminated with this pathogenic strain. Similarly, Barbosa *et al.* (2009) found that thyme EO, when added to irradiated minced meat and stored at 5°C, had a bacteriostatic effect on *Salmonella enteritidis*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*.

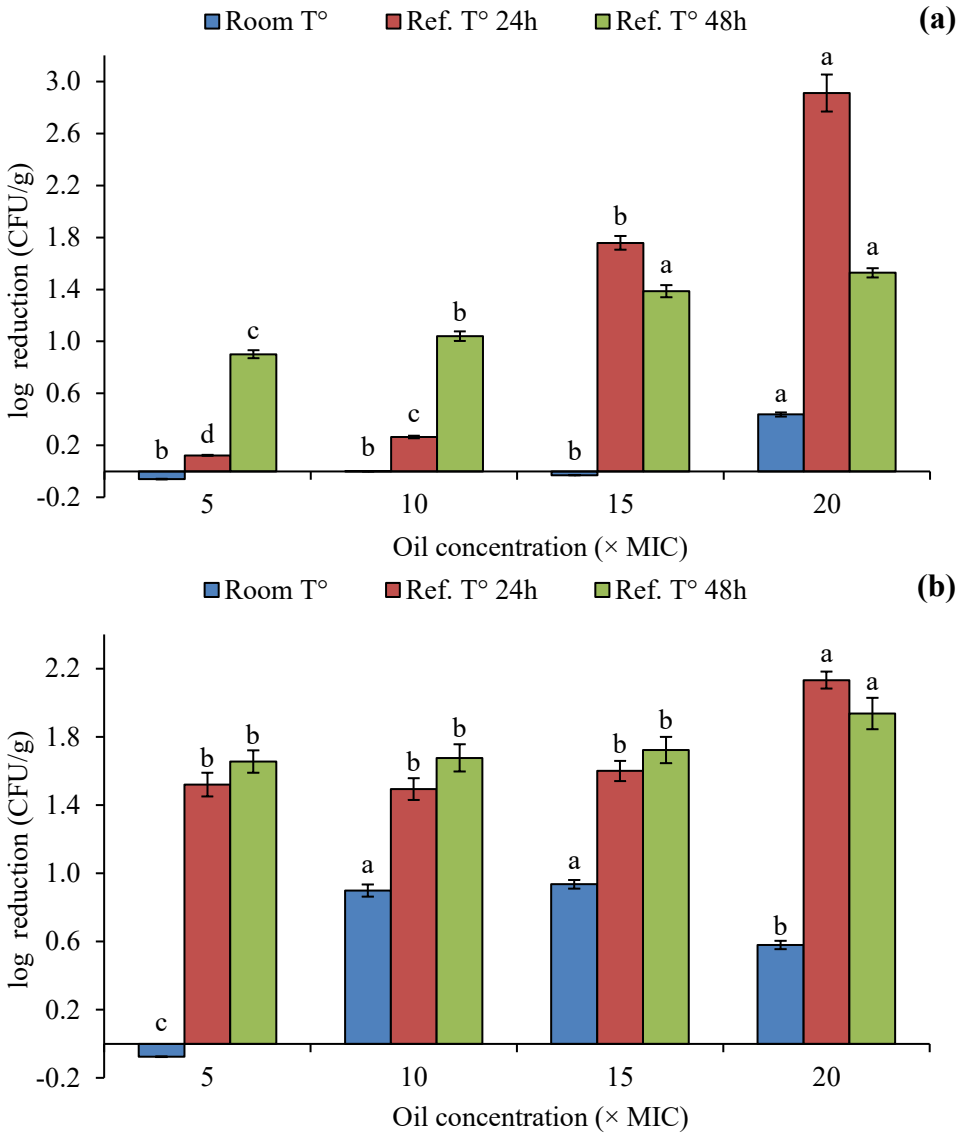

Fig. 3. Log reduction of *Salmonella* Typhimurium inoculated in minced meat stored at room and refrigeration temperatures, and treated with *T. pallescens* (a) and *O. floribundum* (b) essential oil. *Results of each storage temperature with different letters are significantly different (LSD, p<0.05, with a>b>c>d).*

Furthermore, Pesavento *et al.* (2015) found that the thyme oil supplemented in beef meatballs at a concentration of 0.5% was bacteriostatic against *L. monocytogenes* at 4°C, whereas the samples' microbial load decreased at 1 and 2% concentrations of this EO. Amariei *et al.* (2016) also noted that the microbiological stability of minced beef was similarly affected by essential oils (0.5–1.5%) of oregano, thyme, and rosemary.

In a study by Saad *et al.*, (2019), minced meat samples treated with 1% of thyme, cinnamon, and garlic essential oils showed a reduction in *St. aureus* and *E. coli* counts after 3 hours, as well as on the 1st, 2nd, 3rd, 4th, and 5th days of cold storage at 4°C. The treated

samples also had improved sensory properties compared to untreated control samples. Notably, thyme oil at 1% was more effective than cinnamon and garlic EOs.

Lastly, Huang *et al.* (2021) reported that while the number of lactic acid bacteria (LAB) did not significantly change with treatment, the application of thyme EO decreased the growth of *Enterobacteriaceae* family members in Chinese smoked horsemeat sausages.

This study demonstrated that *Salmonella* Typhimurium survived and multiplied in minced meat under refrigeration conditions (4°C) and ambient temperature (25 to 30°C). These findings were similar to those reported for the Gram-negative bacteria *E. coli* O157:H7 using ground beef (Tamplin 2002).

On the other hand, the relatively high initial numbers of TPC in minced beef (7 to 8 log CFU g^{-1}) may be attributed to the grinding process, which contributed to the increase of total viable counts of meat, including yeasts (Amariei *et al.,* 2016).

The results also showed that while the essential oil of thyme and oregano had significant inhibitory effects against *Salmonella* Typhimurium in broth culture (MIC: 0.1 and 0.16%, respectively), their inhibitory effect decreased under the experimental conditions used in the minced meat. For example, to reduce one (1) log CFU g^{-1} of the pathogen population from the minced meat at refrigeration temperature, more than 10 times MIC of thyme essential oil was needed. In this regard, it is interesting to note that most of the published work pointed out the need to use a high concentration of essential oil in food systems, typically from 2 to 100 times the determined *in vitro* MIC value, depending on the food characteristics. The antimicrobial efficiency of essential oils was not the same once they were added to meat. This discrepancy, also observed by other authors, has been attributed to the greater availability of nutrients in meat that could allow a more rapid repair of bacterial damaged structures (Burt 2004) or to a binding between meat proteins or fatty acids with EO components, which hinders access of the bioactive molecules to the microbial targets (Gutierrez *et al.*, 2008). De Oliveira et al. (2013) observed that the EOs of oregano are particularly active against *S. enterica* during the first 1–2 days of minced meat storage. Similarly, Amariei *et al.* (2016) observed a reduced count of lactic bacteria, yeast, and molds after one day of storage, but after four days, CFU g^{-1} were similar to those in the control. It is possible that during the early storage phase, these active molecules are more available for the bacterial targets; then, upon dissolution in the lipid phase of the meat or when reacting with proteins, the compounds become less available for bactericidal action (de Oliveira *et al.*, 2013). According to Farbood *et al.* (1976), high lipid fractions may absorb the spice oil and thus decrease its concentration in the aqueous phase and, consequently, its bactericidal effect. It is also possible that a fat coat is formed on the bacterial cells, thereby decreasing spice oil penetration and antimicrobial activity. Proteins in the food product could bind to the spices' active components and reduce the antibacterial effect (Uhart *et al.*, 2006).

In addition to the different antimicrobial capacity of EOs in culture media and food matrices discussed in the previous paragraph, the reasons for this difference can be attributed to microbial species that were not necessarily the same in culture and in meat which contains a complex microbiome; here, some species may not be cultivable in synthetic media or their relative abundance may be different from that used in microbiological cultures (Agrimonti *et al.*, 2019).

Shelef (1983) noted that while much of the early *in vitro* work with essential oils and their components showed they had substantial activity, when used in food systems amounts required were high (1–3%), and these levels were often higher than would normally be organoleptically acceptable (Lis-Balchin *et al.*, 1998). Firouzi *et al.* (2007) found that oregano and nutmeg oils were effective against *E. coli* O157:H7 in a broth system, but had no effect in ready-to-cook chicken.

As a comparison between the action of both essential oils to reduce the total aerobic plate count and the population of the pathogen, it was noted that the highest level of reduction was observed in the case of *Salmonella* Typhimurium. Reduction of Salmonella spp. in lamb meat with oregano EO was previously reported (Govaris *et al.*, 2010). The diversity of microorganisms constituting the TPC (bacteria, yeast, and mold) can explain the low reduction in their number in minced meat samples treated with thyme and oregano essential oils.

CONCLUSION

This study highlights the significant antimicrobial potential of *Thymus pallescens* and *Origanum floribundum* essential oils, particularly against *Staphylococcus aureus* and *Salmonella* Typhimurium. The oils demonstrated strong bactericidal effects, with *Origanum floribundum* showing slightly higher efficacy. When applied to fresh minced meat, these essential oils effectively reduced total plate counts and pathogen levels, especially under refrigeration, indicating their potential as natural preservatives for minced meat. The findings suggest that these essential oils could serve as viable alternatives to synthetic preservatives, offering a natural solution to enhance food safety and extend shelf life in minced meat products. Their application could be particularly valuable in the food industry, where there is an increasing demand for natural preservation methods. However, further research is needed to explore their effectiveness in different food matrices, optimize their concentrations for commercial use, and evaluate their impact on sensory properties to ensure consumer acceptance.

AUTHORS CONTRIBUTIONS

Conceptualization: K.I., A.C., and M.H.; methodology, A.C. and K.I.; data collection, S.S. and M.H.; data validation, S.S. and M.H.; data processing, Y.K.K. and M.B.; writing—original draft preparation, K.I. and A.C.; writing—review and editing, M.B. and Y.K.K.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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