

# ANTIMICROBIAL EFFECT OF *ESCHERICHIA COLI* ON ESSENTIAL OILS DERIVED FROM ROMANIAN AROMATIC PLANTS

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**Abstract:** This paper investigates the antimicrobial action of *Escherichia coli* ATCC<sup>R</sup> CRM-8739<sup>TM</sup> on the following essential oils: *Teucrium marum*, *Pinus sylvestris*, *Thymus vulgaris*, *Salviae aethedaroleum*, *Cinnamomum aromaticum*, *Hippophae rhamnoides*, *Lavandula angustifolia*, *Abies alba*, *Zingiber officinale*, *Anethum graveolens*, *Coriandrum sativum*, *Origanum vulgare*, extracted industrially from Romanian plants, using the diffusion disc method. The most intense activity was observed at the essential oil of *Cinnamomum aromaticum* (cinnamon) and the mildest activity was observed at *Zingiber officinale* (ginger). Many of the essential oils tested exhibited moderate antimicrobial activity, as *Teucrium marum*, *Thymus vulgaris*, *Hippophae rhamnoides*, *Lavandula angustifolia*, *Coriandrum sativum*. The lowest antibacterial activity was exhibited on *Pinus sylvestris*, *Salviae aethedaroleum*, *Zingiber officinale* and *Anethum graveolens*.

**Keywords:** essential oils, *Escherichia coli*, fountain water, antimicrobial effects

## INTRODUCTION

The beneficial effects of some plant extracts in healing or treating diseases were known from ancient times, and they can be found in popular medicine (Tabata et al., 1988). Later on, people attempted to understand these phenomena and to identify the compounds that confer these properties. Currently, there are over 30.000 plants with medicinal applications known

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worldwide (Koshy et al., 2009; Martinez-Valverde et al., 2002). The properties of these plants are based on their composition that is rich in bioactive compounds such as essential oils, tannins, flavonoids or polyphenols. The essential oils extracted from plants contain terpenes and its subclasses: monoterpenes, diterpenes, sesquiterpenes, which have anti-bacterial, anti-septic, anti-inflammatory or anti-viral properties (Wallace, 2004; Georgescu and Mironescu, 2011). The antimicrobial activity of the essential oils that can be found in the plants is due to compounds such as carvacrol, camphor, terpinen-4-ol or thymol, and their activity is beneficial for the human organism, with more and more applications in the pharmaceutical or food industry (Hadizadeh et al., 2009; Ali-Shtayeh et al., 2008; Bhaskara et al., 1998).

Most of the time, infections emerge in the organism due to external factors, like the consumption of contaminated food, infested water or other affected sources as buildings (Mironescu and Georgescu, 2010). In Romanian villages, the consumption of water from fountains is a problem, as these waters are frequently contaminated with coliform bacteria that can appear in groundwater from fecal sources. The coliform bacteria *Escherichia coli* dominates both surface water and groundwater, especially water originating from areas contaminated with animal sewage or where hygiene is deficient (Oprean et al., 2013; Iancu et al., 2013; Stegăruș et al., 2013).

Using the aromatic plants in the daily life under various forms, the organism acquires natural immunity to contaminating factors, such as bacteria *Escherichia coli*. This paper aims to analyse the antimicrobial effect on the following essential oils: *Teucrium marum*, *Pinus sylvestris*, *Thymus vulgaris*, *Salviae aethedaroleum*, *Cinnamomum aromaticum*, *Hippophae rhamnoides*, *Lavandula angustifolia*, *Abies alba*, *Zingiber officinale*, *Anethum graveolens*, *Coriandrum sativum*, *Origanum vulgare*, extracted from industrial plants, of the contamination microorganism *Escherichia coli*.

## MATERIALS AND METHODS

Essential oils from *Teucrium marum*, *Pinus sylvestris*, *Thymus vulgaris*, *Salviae aethedaroleum*, *Cinnamomum aromaticum*, *Hippophae rhamnoides*, *Lavandula angustifolia*, *Abies alba*, *Zingiber officinale*, *Anethum graveolens*, *Coriandrum sativum*, *Origanum vulgare* were used.

The microorganism *Escherichia coli* ATCC<sup>R</sup> CRM-8739<sup>TM</sup> (for research only) was used (concentration 10<sup>6</sup> cells /mL).

Culture medium Eosin methylene blue agar (EMB) (Scharlau Chemie S.A., Barcelona, Spain) with the following composition: peptone 10g, lactose 10g,

Dipotassium hydrogen phosphate 2g, Eosin Y 0.4g, Methylene blue 0.065g, Agar 15g was used. The medium was prepared according to the producer's recipe, autoclaved and cooled.

Other materials were: 6 mm diameter sterile paper filter discs, 90mm diameter Petri dishes.

The antibiogram method was used, consisting in the following steps: 0.1 ml droplets of bacterial culture (*Escherichia coli* EC 218, concentration  $10^6$  cells/mL) were added in each of the 36 Petri dishes, over which the melted culture medium containing *Eosin methylene blue agar* was added and cooled at 40°C. The culture was homogenized with the culture medium and then it was cooled at room temperature. After solidification, it was added the discs impregnated with essential oils in each dish (100 µl/disc) as follows: 1. *Teucrium marum*, 2. *Pinus sylvestris*, 3. *Thymus vulgaris*, 4. *Salviae aethedaroleum*, 5. *Cinnamomum aromaticum*, 6. *Hippophae rhamnoides*, 7. *Lavandula angustifolia*, 8. *Abies alba*, 9. *Zingiber officinale*, 10. *Anethum graveolens*, 11. *Coriandrum sativum*, 12. *Origanum vulgare* (4 discs/dish). The dishes were incubated at 37°C for 48 h. The anti-microbial effect of the 12 essential oils was monitored by measuring the diameter of inhibition around the discs after 48 h of incubation at 37°C, then after 72 h and after 90 h. Finally, the average was determined for the same lots of essential oils.

## RESULTS AND DISCUSSIONS

The results obtained experimentally are presented in Table 1. The antimicrobial activity of the essential oils has been proven by the inhibition of bacteria growth in the impact zone of the discs with the culture of microorganisms. It can be seen that each oil has a different action, the inhibition zone ranging from 6.1 mm for *Zingiber officinales* and 20.2 mm for *Cinnamomum aromaticum*, after the first measurement at 48 h. Similar values are exhibited by *Thymus bulgaris* and *Hippophae rhamnoides*, of 8.2 mm, and *Pinus sylvestris*, *Silviae aethedaroleum* and *Anethum graveolens* of 7.1-7.2 mm. A 30% higher percentage can be observed in the case of *Coriandrum sativum*, whereas *Origanum vulgare* and *Abies alba* produce an inhibition of the reference zone of 17.3 mm. *Teucrium marum* has an average reference zone with a value of 9.2 mm.

After 72 h, the most powerful action is noticeable in *Cinnamomum aromaticum* where measured values reached 21.1 mm, the difference between the first measurement and the second being 3.8%. *Zingiber officinale* has the weakest antimicrobial activity, where measured values do not exceed 6,5 mm. Modest values are exhibited also by *Pinus sylvestris*, with a value of 7.3 mm,

*Salviae aethedaroleum* with 7.4 mm, *Anethum graveolens*, with 7.3 mm. The power of *Teucrium marum* is 50% lower than that of *Abies alba* or 51.2 % lower than *Origanum vulgare*.

Table 1. Antimicrobial activity of essential oils on the growth of the *Escherichia coli* EC 218 measured in mm after 48 h, 72h, and 96h.

No.	Name of the essential oil	Measured values of the inhibition diameter, mm		
		after 48 h	after 72 h	after 96 h
1	<i>Teucrium marum</i>	9.2	9.7	9.8
2	<i>Pinus sylvestris</i>	7.1	7.3	7.4
3	<i>Thymus vulgaris</i>	8.2	8.4	8.4
4	<i>Salviae aethedaroleum</i>	7.1	7.4	7.5
5	<i>Cinnamomum aromaticum</i>	20.2	21.1	21.2
6	<i>Hippophae rhamnoides</i>	8.2	8.5	8.7
7	<i>Lavandula angustifolia</i>	12.5	13.2	13.5
8	<i>Abies alba</i>	17.3	18.1	18.1
9	<i>Zingiber officinale</i>	6.1	6.5	6.7
10	<i>Anethum graveolens</i>	7.2	7.3	7.3
11	<i>Coriandrum sativum</i>	10.7	11.1	11.3
12	<i>Origanum vulgare</i>	17.3	19.1	19.3

It can be seen that the action of oils weakens, and it is slightly higher or even equal with the previous one. The same can be said of *Thymus vulgaris* with a value of 8.4 mm measured after 72 h and after 96 h. *Cinnamomum aromaticum* maintains its powerful antimicrobial character managing to inhibit the growth of bacteria after 96 h up to 21.2 mm. *Teucrium marum* has a moderate strength of inhibition, its values not surpassing 9.8 mm. *Salviae aethedaroleum* has a modest evolution, considering that the antibacterial action is limited, and so do *Zingiber officinale* and *Anethum graveolens*. *Abies alba* and *Origanum vulgare* feature an intense activity of inhibition of the growth of the *Escherichia coli*, the final results ranging from 18.1 mm to 19.3 mm.

These results are in accordance with other similar researches on volatile oils extracted from romanian plants (Mironescu et al., 2009).

## CONCLUSION

From the obtained results it can be concluded that the selected essential oils feature antimicrobial activity. The essential oils of *Cinnamomum aromaticum*, *Origanum vulgare* and *Abies alba* exhibited superior antimicrobial effects. The following essential oils exhibited moderate

antimicrobial activity: *Teucrium marum*, *Thymus vulgaris*, *Hippophae rhamnoides*, *Lavandula angustifolia*, *Coriandrum sativum*, and the lowest such activity was exhibited by *Pinus sylvestris*, *Salviae aethedaroleum*, *Zingiber officinale* and *Anethum graveolens*. The antimicrobial properties of these essential oils can lead to their utilization in the treatment of various disease caused by *Escherichia coli*.

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