COMPARATIVE ANTIBACTERIAL ACTIVITY AND QUALITY ASSESSMENT OF ESSENTIAL OILS FROM DIFFERENT PRODUCERS AGAINST ORAL PATHOGENS

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Abstract: The aim of the study is to evaluate antibacterial properties of commercial essential oils (Oregano, Black pepper, Coriander, Cinnamon) against oral pathogens (*Streptococcus mutans*, *Enterococcus faecalis*). Nevertheless, we want to assess if the price of the essential oils (EO) which were purchased from 2 different retailers, can influence the quality of essential oil and the antimicrobial effect. Materials and methods: Essential oils from two different Producers (A-high price range, respectively B-low price range) with two opposite price ranges were purchased from local retailers. The inhibitory effect was assessed by disk diffusion. Bacterial inoculums of 0.5 McFarland units ($\sim 1.5 \times 10^8$ CFU/ml) were prepared from *Streptococcus mutans* *Enterococcus faecalis*. Muller-Hinton agar plates were inoculated with bacterial inoculums. Sterile paper disks with the diameter of 6 mm (Bioanalyse Ltd, Turkey) were dispensed on the medium surface. Five μ l of essential oils were pipetted on corresponding disks. After 10 minutes (time needed for a proper diffusion of active components), the plates were incubated in normal atmosphere at 37°C. After 24 hours of incubation, the inhibition zone diameters (in millimeters) were read and noted in spreadsheet software, aside the average price in €/ml of essential oil. A cost-efficiency antibacterial activity index was also calculated. Results: With regard to EO from Producer A, the inhibition zone against *Enterococcus* ranged from the weakest 14mm (Black pepper), 28mm (Coriander) to the strongest over 40 mm (Oregano and Cinnamon). With regard to volatile essential oils from Producer B, against the *Enterococcus*, the smallest inhibition zone was observed for Coriander (10 mm), followed by Black pepper (11 mm); on the opposite side, the strongest inhibition zone presented both Oregano EO and Cinnamon EO (34 mm). Against *S. mutans*, the weakest inhibition zone showed Black pepper (6 mm), followed by Coriander EO (12 mm) and the strongest inhibition zone had Oregano EO (38 mm) and Cinnamon EO (40 mm). All the EO from the Producer A managed to successfully inhibit *S. mutans* growth. In both producers, *Origanum vulgare* EO was the most cost-efficient against *E. faecalis* (index=0.06 for Producer A and 0.03 for Producer B). In the case of Producer A, against *S. mutans* the best index was seen at *Coriandrum sativum* EO (0.05) and the weakest at *Cinnamomum zeylanicum* (0.14). In the case of Producer B, against *E. faecalis*, the lowest index was seen at *Piper nigrum* (0.13). The same pattern was seen against *S. mutans*, the highest index had *Origanum vulgare* (0.03) and the lowest *Piper nigrum* (0.23). Conclusions: Most of essential oils studied from both Producers, had better antibacterial effect against *S. mutans* compared to *E. faecalis*. All the EO from the Producer A managed to successfully inhibit *S. mutans* growth. The high price EO had a better antimicrobial effect compared to the low price EO. The strongest antimicrobial effect against *E. faecalis* had the high-price Oregano and Cinnamon EO and the weakest antimicrobial effect had low-price Coriander EO. Against *S. mutans*, the greatest efficacy had all high-price EO, and the lowest had the low-price Black pepper EO. The high price of some EO (*Piper nigrum*) does not make any difference regarding the antibacterial effect. Lower price EO may be still suitable in regards of antibacterial effect if considering the associated costs, even if the effect does not reach the potency of the higher-price alternatives.

INTRODUCTION

Essential oils (EO) are compounds which are extracted from plants, they are used in aromatherapy and also as a form of alternative medicine for a long time. Their characteristic essence is given by their aromatic compounds. The volatile oils can be extracted from different parts of the plants such as flowers,

roots, bark or leaves.(1,2). The antibacterial properties of volatile oils had been demonstrated in previous studies.(3,4,5) Oregano essential oil is a result from the distillation of *Origanum vulgare* (oregano) from Lamiaceae family. Oregano oil shows a variety of effects such as antiinflammatory, antifungal, antiallergic, antimicrobial and antioxidant. Its activity depends

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on the source of the oregano, the assay method and the bacteria isolates.(6,7,8) *Piper nigrum* (Black pepper), known as black pepper, is a member of Piperaceae family which includes over a 1000 of species. It is cultivated mainly in tropical areas, such as South India and Indonesia. It has been used in medicine for its antimicrobial effect and in food industries as herbal spice.(9) Piperine, which exist in the essential oil of the black pepper, is a bioactive constituent and it is effective in soothing muscular pains and improving respiratory infections.(10) Coriander (*Coriandrum sativum*) is a herbaceous annual from Apiaceae family. Its fresh or dried leaves are used as spice and as an ingredient in different type of food. The seeds of coriander are used as a condiment and in medicine for its expectorant role. Also it is used in different conditions such as stomach disorders and diarrhea. The essential oils is made from the distillation of the seeds.(11,12) Cinamon (*Cinnamomum zeylanicum*) is a member of Lauraceae family and it grows in South and South-East Asia. It is used in Ayurvedic medicine for its soothing effect, also for its antiinflammatory, antidiabetic, anticancer and antibacterial properties. The essential oil of cinnamon contains phenolic and poly-phenolic compounds such as cinnamaldehyde and eugenol in small amount which increases the antioxidant activity.(13,14,15,16,17,18)

Enterococci are bacteria which are present in oral cavity, vagina and gastrointestinal tract of humans and animals, are one of the most important cause of nosocomial infection. *Enterococcus faecalis* is the predominant human enterococcus which in dentistry have been found to be associated with oral diseases such as dental caries, periodontitis and endodontic infections.(19,20) In the etiology of dental caries, *Streptococcus mutans* plays an important role. It has been shown that *S.mutans* can be transmitted from mother to child which can open the early risk for dental caries.(21) *S. mutans* is a facultative anaerobic bacteria which is responsible for the dental plaque biofilm formation.(22)

Many antimicrobial agents have been use, during the last decades, for the prevention of dental caries. The major agent for prevention of dental caries is fluoride. Another agent, chlorhexidine has been used against *S. mutans* but its unpleasant taste determined the search for new alternative therapeutic agents against oral types of bacteria. Previous studies reported antibacterial activity of different EO against *E.faecalis* or *S. mutans*.(23,24,25,26,27)

PURPOSE

The aim of the study is to evaluate antibacterial properties of commercial essential oils (Oregano, Black pepper, Coriander, Cinnamon) against oral pathogens (*Streptococcus mutans*, *Enterococcus faecalis*). Nevertheless, we want to asses if the price of the essential oils which were purchased from 2 different retailers, can influence the quality of essential oil and the antimicrobial effect.

MATERIAL AND METHOD

Essential oils from two different Producers (A-high price range, respectively B-low price range) with two opposite price ranges were purchased from local retailers. Table no.1 shows the manufacture method and the provenance of each essential oil.

Table no. 1. Essential oils used in the study and their origin

Producer	Essential oil	Country of origin	Extraction method	Source
A	<i>Origanum vulgare</i>	Turkey	Steam Distillation	Leaf
	<i>Piper nigrum</i>	India	Steam Distillation	Fruit
	<i>Coriandrum</i>	U.S.A.	Steam	Seed

B	<i>sativum</i>		Distillation	
	<i>Cinnamomum zeylanicum</i>	Madagascar, Indonesia	Steam Distillation	Bark
	<i>Origanum vulgare</i>	Morocco	Steam Distillation	Leaf
	<i>Piper nigrum</i>	Madagascar	Steam Distillation	Fruit
	<i>Coriandrum sativum</i>	Hungary	Steam Distillation	Seed
	<i>Cinnamomum zeylanicum</i>	Sri Lanka	Steam Distillation	Bark

The chemical composition of the bioactive compounds was analysed from the official reports from the producers. The composition was detected by Gas Chromatography and was specific for the lot number of each used essential oil.

The inhibitory effect was assessed by disk diffusion. Bacterial inoculums of 0.5 McFarland units ($\sim 1.5 \times 10^8$ CFU/ml) were prepared from a clinical isolate of *Streptococcus mutans* (identified by Vitek2 Compact system) and from *Enterococcus faecalis* ATCC29212. Muller-Hinton and Muller-Hinton supplemented with 5% defibrinated sheep blood agar plates (Oxoid Ltd, UK) were inoculated with the bacterial inoculums. Sterile paper disks with the diameter of 6 mm (Bioanalyse Ltd, Turkey) were dispensed on the medium surface. Five μ l of essential oils were pipetted on corresponding disks. After 10 minutes (time needed for a proper diffusion of active components), the plates were incubated in normal atmosphere at 37°C. After 24 hours of incubation, the inhibition zone diameters (in millimeters) were read and noted in spreadsheet software, aside the average price in €/ml of essential oil. A cost-efficiency antibacterial activity index was also calculated, in order to offer a proper view on the quality-price relation regarding the antibacterial effect, using the formula:

$$\text{index} = \frac{\text{price (euro)}}{\text{effect (diameter in mm)}}$$

When the diameter exceeded 40 mm, the index was calculated using this maximum readable value. The lower the index, the cost-efficiency is better.

RESULTS

By following the official reports on the chemical composition of EO by GC/MS analysis, we identified the major components which are presented in table no. 2. Small variances were found between the same constituents of the EO of the two producers.

Table no. 2. GC/MS analysis of EO of *Pipper nigrum*, *Cinnamomum zeylanicum*, *Coriandrum sativum*, *Origanum vulgare*

<i>Pipper nigrum</i>	Producer A	Producer B
limonene	23.35 %	19.15 %
beta-caryophyllene	16.81 %	15.93 %
delta-3-carene	15.56 %	12.82 %
alpha-pinene	15.49 %	14.79 %
beta-pinene	14.89 %	13.17 %
	86.1 %	75.86 %
<i>Cinnamomum zeylanicum</i>	Producer A	Producer B
trans-cinnamaldehyde	59.01 %	52.82 %
trans-cinnamyl acetate	7.55 %	10.94 %
beta-caryophyllene	5.53 %	3.39 %
eugenol	3.97 %	3.1 %
linalool	2.17 %	2.4 %
camphor	1.76 %	0.99 %
	79.99%	73.64 %
<i>Coriandrum sativum</i>	Producer A	Producer B
linalool	77.47 %	75.11 %
geranyl acetate	3.79 %	1.44 %
camphor	3.28 %	4.5 %
alpha-pinene	4.97 %	0.48 %

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gamma-terpinene	3.47 %	4.53 %
	92.98 %	86.06 %
Origanum vulgare	Producer A	Producer B
carvacrol	71.99 %	70.6 %
beta-bisabolene	2.07 %	0 %
thymol	1.55 %	3.8 %
gamma-terpinene	4.11 %	6.19 %
para-cymene	3.48 %	8.53 %
	83.2 %	89.12 %

Table no. 3 presents the antibacterial efficacy of all oils studied from both producers. All the EO from the Producer A managed to successfully inhibit *S. mutans* growth. With regard to EO from Producer A, the inhibition zone against *E. faecalis* ranged from the weakest 14mm (Black pepper), 28mm (Coriander) to the strongest over 40 mm (Oregano and Cinnamon). With regard to volatile essential oils from Producer B, against *E. faecalis*, the smallest inhibition zone was observed for Coriander (10 mm), followed by Black pepper (11 mm); on the opposite side, the strongest inhibition zone presented both Oregano EO and Cinnamon EO (34 mm). Against *S. mutans*, the weakest inhibition zone showed Black pepper (6 mm), followed by Coriander EO (12 mm) and the strongest inhibition zone had Oregano EO (38 mm) and Cinnamon EO (40 mm). Intense hemolysis has also observed for all EO from producer A, and for Oregano EO and Cinnamon EO from producer B (figure no. 1).

Figure no. 1. Inhibition zone of the essential oils

Table no. 3. Antibacterial effects of the tested essential oils on *E. faecalis* and *S. mutans* and the cost-efficiency antibacterial activity index

	Inhibition zone diameters (mm)							
	Producer A (high price range)				Producer B (low price range)			
	Origanum vulgare	Piper nigrum	Coriandrum sativum	Cinnamomum zeylanicum	Origanum vulgare	Piper nigrum	Coriandrum sativum	Cinnamomum zeylanicum
<i>E. faecalis</i>	>40	14	28	>40	34	11	10	34
<i>S. mutans</i>	>40	>40	>40	>40	38	6	12	40
price in € / inhibition zone diameter ratio (index value)								
	Producer A (high price)				Producer B (low price)			

<i>E. faecalis</i>	range)				range)			
	0.06	0.39	0.08	0.14	0.03	0.13	0.12	0.04
<i>S. mutans</i>	0.07	0.13	0.05	0.14	0.03	0.23	0.10	0.04

The prices for 1 ml of each oil from Producer A varied between 2,27-5,70 €/ml and those of Producer B varied between 1,18-1.68 €/ml. As presented in table no. 3, the obtained indices (price ratio / inhibition zone diameter) of each essential oil, both from Producer A and Producer B, were compared.

In both producers, *Origanum vulgare* EO was the most cost-efficient against *E. faecalis* (index=0.06 for Producer A and 0.03 for Producer B). In the case of Producer A, against *S. mutans* the best index was seen at *Coriandrum sativum* EO (0,05) and the weakest at *Cinnamomum zeylanicum* (0,14). In the case of Producer B, against *E. faecalis*, the lowest index was seen at *Piper nigrum* (0,13). The same pattern was seen against *S. mutans*, the highest index had *Origanum vulgare* (0,03) and the lowest *Piper nigrum* (0,23).

DISCUSSIONS

The aim of this work was to evaluate the antimicrobial capacity of some essential oils against an oral *S. mutans* and *E. faecalis*, which are responsible for different oral inflammations. The study showed that from all the low price EO tested against *S. mutans*, the highest effect had Cinnamon EO (40 mm diameter) and the lowest had Black pepper EO (6mm). Also, our results revealed that all the high price EO showed excellent antibacterial effect against the *S. mutans* (>40 mm inhibition diameter). A similar study involving the effect of *Cinnamomum zeylanicum* EO against some gram positive and negative bacteria was carried by Al-fekaiki et al., and their inhibition diameter was less than ours, and ranged from 17.00-30.30 mm.(28) Although the antibacterial effect of Oregano EO from the Producer A against *E. faecalis* is the most intense (>40 mm) of all studied oils, the cost-efficiency antibacterial activity index was not so good (0,06) compared to the same oil (0,03) from the Producer B, because it has a price three times higher. Similarly, even if the Cinnamon EO from the Producer A had a very strong antibacterial effect against *E. faecalis* (> 40 mm) compared to the same EO of the producer B (34 mm), the cost-efficiency antibacterial activity index of Producer A was less (0,14) than the index of Producer B (0,04) because of the big price difference. The antibacterial effect of cinnamon may be explained due to its cinnamic aldehyde and eugenol compounds. Against group *D streptococcus*, a previous study showed that *Cinnamomum zeylanicum* EO had an inhibitory effect, where the inhibition diameter was 26mm, even was less than in our study.(29) The antimicrobial activity of *Cinnamomum zeylanicum* and Black pepper was tested against *S. mutans* in a previous study which reported an 32.17±1.32 mm inhibition zone for C zeylanicum and 14.00±0.63 for Black pepper, a weaker effect in comparison with our obtained effects.(30) P.V. Karsha et al. in their study revealed that Black pepper EO had a very good inhibitory effect on the growth of Gram positive bacteria like *Staphylococcus aureus*, *Bacillus cereus* and *E. faecalis* as the Black pepper altered the membrane permeability.(31) As presented in table no. 3, in case of Coriander EO from the Producer A, we noticed an 28 mm inhibition zone against *E. faecalis* as well as a better value for the cost-efficiency antibacterial activity index (0,08) than the effect of the same EO (10 mm)and the index (0,12) from the Producer B, due also because of the small price difference between the two producers.

This study reveals that the Black pepper EO of Producer A presented a much better effect against *S. mutans* (> 40 mm) than the same EO (6 mm) of Producer B, and also

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showed a better index (0,13 for producer A vs 0.23 for Producer B). According to the results of a previous study, the antimicrobial effect of *Origanum vulgare* EO against *Streptococcus epidermidis* was noticed using a disc diffusion assay, and was reported a 37 mm inhibition diameter.(32) Carvacrol and thymol, which are the main constituents of *Oregano* EO, are able to alter the permeability of the cell membrane that cause the death of the bacterial cells.(33) Hilal Yildiz (34) reported that the *Coriandrum sativum* EO inhibited a large spectrum of bacterias such as *E.faecalis* (with inhibition diameter 11 mm+- 0,60, *Str.pyogenes* (inhibition diameter 26 mm) which is nearly in harmony to our low-price EO results against the *S. mutans* (12 mm) and *E. faecalis* (10 mm). In this current study we noticed that from the all low-price EO tested against *E. faecalis*, the lowest effect had *Coriandrum sativum* (10 mm) and the highest antibacterial effect had *Oregano* and *Cinnamon* (34 mm). Also, this study demonstrates that from the all high- price EO tested against *E. faecalis* the lowest effect was seen in *Black Pepper* (14 mm) and the highest in *Oregano* and *Cinnamon*(>40 mm). Our results confirm that although the antibacterial effect of *Cinnamon* EO against *S.mutans* is nearly the same for both producers (A-> 40 mm and B- 40 mm) the cost-efficiency antibacterial activity index is better in Producer B (0,04) than in Producer A (0,14) due to the price advantage. While there is a very small difference between the Inhibition zone diameters of both *Oregano* EO tested against *S. mutans* in favor of Producer A , the cost-efficiency antibacterial activity index is in favor of Producer B (index 0,03) due to the price difference.

Goñi et al. reported in a previous study that the vapour phase of a combination of *Cinnamon* and *Clove* EO had an inhibition diameter of 17+-1mm against *E. faecalis*, less than our results presented in our study. The chemical constituents of EO are hydrophobic and the bacterial cell membrane can accumulate these constituents leading to structural damages.(35) According to Melo et al. (36), *E. faecalis* was sensitive by the disc diffusion method to *Oregano* EO where the inhibition zone in mm ranged from 31,8 +/- 1,01, nearly the same result we obtained with *Oregano* EO from the Producer B. As we can see in Table 3 the antibacterial effect of *Black pepper* EO against *E. faecalis* was weak (11-14 mm inhibition zone) but is in harmony with the findings of a similar study about the antimicrobial effect of the pepper oleoresin from Vietnam (9-10 mm Inhibition Zones).(10)

CONCLUSIONS

1. Most of essential oils studied from both Producers, had better antibacterial effect against *S. mutans* compared to *E. faecalis*.
2. All the EO from the Producer A managed to successfully inhibit *S. mutans* growth.
3. The high price EO had a better antimicrobial effect compared to the low price EO.
4. The strongest antimicrobial effect against *E. faecalis* had the high- price *Oregano* and *Cinnamon* EO and the weakest antimicrobial effect had low- price *Coriander* EO.
5. Against *S. mutans*, the greatest efficacy had all high-price EO, and the lowest had the low-price *Black pepper* EO.
6. The high price of some EO (*Piper nigrum*) does not make any difference regarding the antibacterial effect. Lower price EO may be still suitable in regards of antibacterial effect if considering the associated costs, even if the effect does not reach the potency of the higher-price alternatives.

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