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EVALUATION OF THE TEA TREE OIL ACTIVITY TO ANAEROBIC BACTERIA - *IN VITRO* STUDY

MARTA ZIÓŁKOWSKA-KLINKOSZ[1]*, ANNA KĘDZIA[1], HENRY O. MEISSNER[2] and ANDRZEJ W. KĘDZIA[3]

[1]Department of Oral Microbiology, Medical University of Gdańsk, 38 Do Studzienki St., 80-227 Gdańsk, Poland
[2]Health Science, Faculty of Health Studies, Charles Sturt University, Sydney, Australia
[3]Department of Pediatric Nursing, Poznań University of Medical Sciences, Poznań, Poland

Abstract: The study of the sensitivity to tea tree oil (Australian Company TTD International Pty. Ltd. Sydney) was carried out on 193 strains of anaerobic bacteria isolated from patients with various infections within the oral cavity and respiratory tracts. The susceptibility (MIC) of anaerobes was determined by means of plate dilution technique in Brucella agar supplemented with 5% defibrinated sheep blood, menadione and hemin. Inoculum contained 10^5 CFU per spot was cultured with Steers replicator upon the surface of agar with various tea tree oil concentrations or without oil (anaerobes growth control). Incubation the plates was performed in anaerobic jars under anaerobic conditions at 37°C for 48 h. MIC was defined as the lowest concentrations of the essential oil completely inhibiting growth of anaerobic bacteria. Test results indicate, that among Gram-negative bacteria the most sensitive to essential oil were strains of *Veillonella* and *Porphyromonas* species. Essential oil in low concentrations (MIC in the range of = 0.12 - 0.5 mg/mL) inhibited growth of accordingly 80% and 68% strains. The least sensitive were strains of the genus *Tannerella*, *Parabacteroides* and *Dialister* (MIC 1.0 - 2.0 mg/mL). In the case of Gram-positive anaerobic bacteria the tea tree oil was the most active to strains of cocci of the genus *Anaerococcus* and *Ruminococcus* (MIC in range = 0.12 - 0.5 mg/mL) or strains of rods of the genus *Eubacterium* and *Eggerthella* (MIC = 0.25 mg/mL). Among Gram-positive rods the least sensitive were the strains of the genus *Bifidobacterium* (MIC = 2.0 mg/mL). The tea tree oil was more active to Gram-positive than to Gram-negative anaerobic bacteria.

Keywords: anaerobic bacteria, tea tree oil, *Melaleuca alternifolia,* oral cavity

The interest in plant medicines is constantly increasing, particularly in antibacterial activity. It is conected with a common resistance of various pathogens on antibiotics and chemoterapeutic agents. Plant medicines used in the prevention and treatment of infections usually contain extracts of plants, essential oils or some of their ingredients. The tea tree oil from *Melaleuca alternifolia* has been used as treatment agent by aboriginal population in Australia in XVIII century (1, 2). The investigations carried out in XX century revealed that there are many possibilities of using antimicrobial properties in preparations for oral hygiene (toothpaste, rinsing liquids), decontamination wounds and inhalations (3-6). Nowadays the oil is added to soaps, the liquid disinfectants, shampoos, deodorants, powders and bath liquid designed for people and animals (2, 7). Moreover, herbal preparations containing tea tree oil are used in bacterial and mycotic infections of the skin, ginecology and urinary tract infections (2, 8, 9). Studies confirm good antiseptic and antipathogen activity in the microbial infections in the oral cavity (gingivae, periodontal disease) and upper respiratory tract (10-14). Blackwell (15) proved that tea tree oil does not show mutagenic activity. Essential oil is obtained from the leaves of plants *Melaleuka alternifolia* with using method of steam distillation. It is colorless or light yellow liquid with spicy smell, its compositions depends on the properties of plants and place of origin. Antibacterial activity is connected with the contents of trepinen-4-ol, which should be more than 30% composition the tea tree oil (3). A number of publications indicates that essential oil has antibacterial, antifungal and antiviral activity (1-3, 7-9, 12-14, 16-27). Studies suggest that the mechanism of action of the microorganism is damaging the cell wall and membrane, which leads to a leakage of cytoplasm

* Corresponding author: e-mail: martaz-k@gumed.edu.pl

(2, 16, 28). It was ascertained that essential oil stimulates escape from cells *Escherichia coli* potassium ions and sets back breathing process what contributes to the preservation of these bacteria (29). Antibacterial activity of the tea tree oil to aerobic bacteria or facultative anaerobes has been described in many publications. Few publications were about its activity to anaerobic bacteria.

The aim of investigations was evaluation of sensitivity to the tea tree oil of anaerobic bacteria causing infections in the field of oral cavity and respiratory tracts.

MATERIALS AND METHODS

Materials for the investigation have been collected from patients with different infections in the oral cavity (mucositis, periodontitis, ulcerations, abscesses) and respiratory tracts (chronic obstructive pulmonary disease, lung abscesses, pharyngitis,

Table 1. Sensitivity of Gram-negative anaerobic bacteria to tea tree oil.

Anaerobic bacteria	Number of strains	Minimal inhibitory concentration MIC mg/mL				
		≥ 2.0	1.0	0.5	0.25	≤ 0.12
Gram-negative rods						
Porphyromonas asaccharolytica	7	2	1	2	1	1
Porphyromonas gingivalis	15	2	2	2	5	4
Porphyromonas levii	2	2				
Prevotella bivia	7	2	2	1		2
Prevotella buccalis	2	2				
Prevotella denticola	6	3	1		1	1
Prevotella disiens	1	1				
Prevotella intermedia	9	6	1			2
Prevotella heparinolytica	2	1			1	
Prevotella loescheii	2	2				
Prevotella nigrescens	3	1	1	1		
Prevotella melaninogenica	2	1	1			
Prevotella oralis	7	1	1	1	2	2
Prevotella oris	6	1		2	3	
Tannerella forsythia	5	4	1			
Fusobacterium mortiferum	1	1				
Fusobacterium nucleatum	12	3	2	2	4	1
Bacteroides fragilis	10	2	3	3	2	
Bacteroides ovatus	2	1	1			
Bacteroides ureolyticus	4	4				
Bacteroides uniformis	2	2				
Bacteroides vulgatus	3			1	1	1
Parabacteroides distasonis	2	2				
Dialister pneumosintes	2	1	1			
Gram-negative rods Total	120	52	20	15	20	13
Gram-negative cocci *Veillonella parvula*	5	1			1	3
Gram-negative anaerobic bacteria Total	125	53	20	15	21	16

tonsillitis). Those have been placed in containers for anaerobic conditions and during 1 hour sent to the lab. Samples overlaid on a number adequate enriched and selective medium (30, 31). Incubation of inoculations has been carried out at 37°C in anaerobic jars containing 10% CO_2, 10% H_2 and 80% N_2, palladium catalyst and indicator of anaerobiosis, for 10 days. The tested strains of anaerobic bacteria have been identified by standard criteria (30, 31). Tea tree oil used for testing came from Australian Company TTD International Pty. Ltd., Sydney. Among ingredients of the essential oil dominated: terpinen-4-ol (37.2%), γ-terpinen (21.5%), α-terpinen (10.3%) and 1,8-cyneol (7.8%). Experiments were conducted with a total of 193 strains of anaerobic bacteria which were isolated from materials collected from patients. They belonged to the following genus: *Porphyromonas* (24 strains), *Prevotella* (47), *Tannerella* (5), *Fusobacterium* (19), *Bacteroides* (21), *Parabacteroides* (2), *Dialister* (2), moreover, 36 strains of Gram-positive cocci and 32 strains of Gram-positive rods. The research also included reference strains belonging to the following genus: *Porphyromonas levii* ATCC 29147, *Fusobacterium nucleatum* ATCC 25586, *Bacteroides fragilis* ATCC 25285,

Table 2. Sensitivity of Gram-positive anaerobic bacteria to tea tree oil.

Anaerobic bacteria	Number of strains	Minimal inhibitory concentration MIC mg/mL				
		≥ 2.0	1.0	0.5	0.25	≤ 0.12
Gram-positive cocci						
Anaerococcus prevotii	4	2			1	1
Atopobium parvulum	2			1	1	
Finegoldia magna	8	2	3	1	1	1
Parvimonas micra	11	5	2	2	2	
Peptostreptococcus anaerobius	5	2		1	2	
Ruminococcus products	6	1		1	2	2
Gram-positive cocci Total	36	12	5	6	9	4
Gram-positive rods						
Actinomyces israelii	3		1	1		1
Actinomyces meyeri	1			1		
Actinomyces naeslundii	1				1	
Actinomyces odontolyticus	1					1
Actinomyces viscosus	2		2			
Bifidobacterium breve	2	2				
Eubacterium brachy	1					
Eggerthella lenta	1				1	
Pseudoramibacter alactolyticus	2	1			1	
Propionibacterium acnes	12	5	2	1	1	3
Propionibacterium granulatum	2			1	1	
Propionibacterium propionicum	4		2	2		
Gram-positive rods Total	32	8	7	6	6	5
Gram-positive anaerobic bacteria Total	68	20	12	12	15	9
Anaerobic bacteria Total Percent	193 100%	73 38%	32 16%	27 14%	36 19%	25 13%

Table 3. Sensitivity of reference strains of anaerobic bacteria to tea tree oil.

Strains of anaerobic bacteria	Number of strains	Minimal inhibitory concentration MIC mg/mL				
		≥ 2.0	1.0	0.5	0.25	0.12
Bacteroides fragilis ATCC 25285	1	1				
Finegoldia magna ATCC 29328	1	1				
Fusobacterium nucleatum ATCC 25585	1		1			
Parabacteroides distasonis ATCC 8503	1	1				
Peptostreptococcus anaerobius ATCC 27337	1	1				
Porphyromonas levii ATCC 29147	1	1				

Parabacteroides distasonis ATCC 8503, *Finegoldia magna* ATCC 29328 , *Peptostreptococcus anaerobius* ATCC 27337 and *Propionibacterium acnes* ATCC 11827. Sensitivity of anaerobic bacteria strains has been determined by a serial dilution method of the oil in the Brucella agar supplemented with 5% defibrinated sheep blood, menadione and hemin (32). Tea tree oil directly before the test was dissolved in DMSO (Serva), to concentration of 100 mg/mL. Further dilutions of essential oil were performed in sterile distilled water. Essential oil concentration tested were: 0.12, 0.25, 0.5, 1.0, and 2.0 mg/mL. Inoculum contained 10^5 CFU (colony forming unit) per spot has been cultured with Steers replicator upon the surface of agar with various tea tree oil concentrations or without oil (anaerobes growth control). Incubation of plates has been performed in anaerobic jars under anaerobic conditions at 37°C for 48 h. MIC (minimal inhibitory concentration) has been defined as the lowest concentrations of the essential oil completely inhibiting growth of anaerobic bacteria.

RESULTS

The results of the sensitivity to the tea tree oil of 125 strains of Gram-negative anaerobic bacteria isolated from patients are collected in Table 1, 68 strains of Gram-positive bacteria isolated from materials are presented in Table 2 and reference strains in Table 3. From the evaluated Gram-negative anaerobic bacteria the most sensitive to tea tree oil showed cocci of genus *Veillonella* and rods of genus *Porphyromonas*. Low concentration of essential oil from ≤ 0.12 to 0.5 mg/mL inhibited 80% and 63% of these strains, recpectively. Lower activity of tested oil was shown to strains of genus *Prevotella* and *Fusobacterium*. In case of concentration ≤ 0.12 to 0.5 mg/mL 40% and 37% strains were sensitive, respectively. Moreover, tea tree oil showed activity in concentrations in the range ≤ 0.12 - 0.5 mg/mL only to 7 (33%) rods of genus *Bacteroides*. The least sensitive were the strains of genus *Tannerella*, *Parabacteroides* and *Dialister*, their growth was inhibited in concentration 1.0 - 2.0 ≥ mg/mL. Among the tested Gram-positive anaerobic bacteria on low concentration of essenial oil from tea tree, in the range ≤ 0.12 - 0.5 mg/mL, sensitive were 36 (53%) strains. From Gram-positive cocci, strains of the species *Anaerococcus prevotii* and *Ruminococcus parvula* proved to be the most sensitive onto essential oil. Growth of these strains was inhibited in the range of concentration from ≤ 0.12 to 0.5 mg/mL. However, among Gram-positive rods, the most sesitivity showed strains of the species *Eubacterium brachy* and *Eggertella lenta* (MIC = 0.25 mg/mL). The tea tree oil was most effective against strains of the species *Actinomyces*. MIC for 63% of this genus were in ≤ 0.12 - 0.5 mg/mL. Rods of the species *Propionibacterium* were characterized by lower sensitivity (MIC for 50% strains were ≤ 0.12-0.5 mg/mL). Growth of other strains was inhibited in concentrations 1.0 ≥ 2.0 mg/mL. Among the tested Gram-positive rods, the least sensitive were the strains of the species *Bifidobacterium breve*, for which to inhibit the upgrowth was required higher concentration of the essential oil (≥ 2.0 mg/mL).

DISCUSSION

Evaluation studies of antibacterial activity of tea tree oil have been carried out in many countries. Welsh and Longstaff (25) proved high sensitivity to this essential oil among some species of anaerobic bacteria isolated from oral cavity. Estimated essential oil in the range of concentration from 0.2 to 0.8 mg/mL inhibited growth of the species *Porphyromonas gingivalis* (1 strain), *Prevotella intermedia* (1

strain), *Fusobacterium nucleatum* (1 strain), *Bacteroides fragilis* (1 strain), and of the species *Actinomyces* (2 strains). In the other study, Hammer et al. (8) found that strains of the species *Bacteroides* (12 strains), *Prevotella* (20 strains), *Fusobacterium* (10 strains) and Gram-positive cocci (24 strains) were sensitive on concentration of the essential oil in the range of 0.3-0.5 mg/mL. In the following studies carried out by Shapiro et al. (26), Takarada et al. (11, 24) and Hammer et al. (12) it was proved sensitivity of the species *Porphyromonas gingivalis* in the range of concentrations 1.1 - >6.0 mg/mL, *Prevotella intermedia* 0.3 - 1.0 mg/mL, other species *Prevotella* and *Bacteroides spp*. 0.3 - 5.0 mg/mL, *Fusobacterium nucleatum* 0.6 - >6.0 mg/mL and *Veilonella spp*. 0.16 - 10.0 mg/mL. Genus of mentioned above anaerobic bacteria and estimated in our investigations, with the exception of *Porphyromonas gingivalis* which were more sensitive, characterized similar sensitivity (MIC ≤ 0.12 - 0.5 mg/mL). In other investigations, tea tree oil was active against rods of species *Actinomyces viscosus* and genus *Actinomyces* in concentrations from 0.5 to >6.0 mg/mL (26, 27, 33, 34). The tested strains of Gram-positive rods reported similar to the obtained sensitivity by above-mentioned authors (≤ 0.12 - ≥ 2.0 mg/mL). Moreover, the cocci tested by us also characterized similar sensitivity (MIC in the range ≤ 0.12 - 2.0 mg/mL) to tested by Hammer et al. (8), but they proved to be more sensitive than strains determined by Shapiro et al. (26) (MIC >6.0 mg/mL).

In summary, tea tree oil showed good activity against a lot of tested anaerobes. Low concentrations (MIC ≤ 0.12 - 0.5 mg/mL) inhibited upgrowth of more than 46% of all tested anaerobic bacteria. Gram-positive anaerobic bacteria proved more sensitive, especially in low concentrations to the essential oil than Gram-negative (53% and 42% sensitive strains, respectively). This is important information because preparations with tea tree oil are often used both in preventive treatment and therapy of infections.

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