*Anna Kędzia[1], Alina Mścisz[2], Henry O. Meissner[3]

The effect of Tamanu oil (*Calophyllum inophyllum*) on anaerobic bacteria isolated from respiratory tract

[1]Department of Oral Microbiology, Medical University of Gdańsk, Poland
Head of Department: dr hab. Anna Kędzia
[2]Herba Studio, Mścisz and Co., Poland
[3]Faculty of Health Studies, Charles Sturt University & TTD International Pty Ltd, Australia

DZIAŁANIE OLEJU Z TAMANU (CALOPHYLLUM INOPHYLLUM) NA BAKTERIE BEZTLENOWE WYODRĘBNIONE Z DRÓG ODDECHOWYCH

SUMMARY

The effect of cold-pressed oil from Tamanu (Calophyllum inophyllum) on total 30 strains (18 strains Gram-negative and 12 Gram-positive) of anaerobic bacteria isolated from respiratory tract of 12 patients. The degree of bacteriostatic property of tested Tamanu oil obtained from Vanuatu (Oceania) on bacteria was determined by means of plate dilution technique in Brucella agar with 5% sheep blood. Incubation was performed during 48 hours in anaerostats at temperature of 37°C in the presence of 10% CO_2, 10% N_2 and 80% N_2, palladic catalyst and anaerobic indicator. The MIC was interpreted as the lowest concentrations of the essential oil inhibiting the growth of bacteria. The highest sensitivity to oil showed Gram-positive anaerobic bacteria, strains Propionibacterium and Actinomyces (MIC between 0.6 and 5 mg/ml) micromonas and Peptostreptococcus (MIC 1.2-5 mg/ml). The lowest sensitivity to Tamanu oil showed Gram-negative strains of anaerobic bacteria: Dialister pneumosintes (MIC 2.5 mg/ml). The remaining strains: Bacteroides, Prevotella, Porphyromonas, Fusobacterium and Veilonella required Tamanu oil concentrations between 5->20 mg/ml to inhibit growth of these bacteria. It appears that Gram-positive anaerobic bacteria are generally more susceptible to Tamanu oil than Gram-negative anaerobic strains. Observed susceptibility of Gram-positive anaerobic bacteria to Tamanu oil may extend its current topical use to application in antimicrobial preparations for oral hygiene such as toothpastes, gargles, mouthwash etc.

KEY WORDS: TAMANU OIL – ANAEROBIC BACTERIA – RESPIRATORY TRACT – ANTIBACTERIAL PROPERTIES

There is a growing concern that anaerobic bacteria which show growing resistance to number of commonly-used antibiotics, may present difficulties for medical practitioners in treating patients with chronic pulmonary ailments or cases requiring long-term preventive or curative medication. During last few years a search for new effective pharmaceutical preparations intensified to treat chronic bacterial infections and pulmonary conditions including conventional medicines. This also includes preparations based on traditionally-used medicinal herbs and extracts from various plants and more are less refined plant derivatives, which tend do not caused side effects observed so often when various effective pharmaceuticals are used, particularly during extended period of time. Also, more and more attention is being given to application of herbal preparations with medicinal, preventive properties in products designed for oral hygiene such as toothpastes, mouth rinses, fresheners and gurgles.

Various volatile oils, which have been shown as having strong antibacterial properties (1-7) and been used traditionally in alleviation of pulmonary symptoms and infections of respiratory tract as well as in maintaining oral hygiene. During last decade, one of such oils – Australian Tea Tree Oil (*Melaleuca alternifolia* Cheel – *Myrtaceae* family) gained worldwide acceptance as a component of commercial therapeutic preparations, after its strong antimicroorganisms properties linked to main active constituents identified as terpinen-4-ol, γ-terpinene, α-terpinene, α-terpineol, 1,8-cyneol, p-cymene, α-pinene and limonene have been scientifically and clinically proven in a large number of independently-conducted study internationally as well as in one of the previous work from this series (2). As a result, a great many commercial products in variety forms of delivery have

been developed and are available on the market as Over the Counter (OTC) therapeutic and preventive preparations.

In a search for new sources of pure and environmentally non-polluted therapeutic oils which may have potential in application to treat chronic pulmonary ailments and/or as curative medications, an attention has been given to a therapeutic oil expressed from kernels separated from fruits of native Polynesian tree Tamanu (*Calophyllum inophyllum*). An oil derived from Tamanu seeds has been traditionally used for centuries in the South Pacific region as a local medicine to treat skin diseases and a variety of purposes ranging from treatment of scars, cuts, burns, rashes, stings to psoriasis, eczema, sores to neuralgia and sciatica and recently has found application in cosmetics to regenerate damaged skin and preventing skin aging as well as in therapeutics helping to heal and regenerate scarred tissues (8).

Tamanu tree, which grows primarily in the coral sands and on the sea shore, is 3 to 15 meters high and has a thick trunk covered with rugged and cracked bark. It has elliptical, shiny and tough leaves and flowers twice a year. Its flowers, arranged in auxiliary cymes, have a sweet, lime-like fragrance. The numerous fruits, arranged in dusters, are spherical, orange shaped. Once ripe, their smooth, yellow epidermis discloses a thin layer of pulp, with a flavour and taste reminiscent of an apple. The grey, ligneous and rather soft nut contains a pale yellow kernel, which is odourless when fresh. When chewed, it coats the mouth, emulsifies saliva and its insipid taste becomes bitter (8). Tamanu kernels have very high oil content (75%). Oil is mechanically extracted from seeds by cold-pressure system after the mature fruit is harvested and stored to dry. It yields refined, greenish yellow oil, similar to olive oil, with an aromatic odour and an insipid taste. Once grown, a Tamanu tree produces up to 100 kg fruits and about 18 kg oil (8).

The tree is known under botanical names: *Calophyllum inophyllum* Linn. (syn. *Calophyllum bintagor* Roxb.) (*Guttiferae*). It is a member of the mangosteen family. *Mesua ferrea* Linn. has also been seen as an alternative Latin name (8). English common names describe the tree as Alexandrian Laurel, Tamanu, Pannay Tree, Sweet Scented Calophyllum.

Tamanu oil activity was studied in numerous clinical cases showing that it can be applied on skins as well as in the case of mucous membrane lesions (8). It heals small wounds such as cracks and chaps, but is also efficient on more serious cutaneous problems and post-surgical wounds (9). Those healing, anti-inflammatory and antibiotic properties make Tamanu oil an excellent raw material for cosmetics, in regenerating and protective formulations (10). The oil has been reported as useful for dermal problems and was an ancient treatment for leprosy (11).

Apart of growing literature on use of the Tamau oil as an active stimulant of collagen synthesis to prevent skin aging, there is no publication available so far on bacteriostatic properties of Tamanu oil. However, it has been reported by Dweck and Meadowsy (8) that two essential components of the *Calophyllum* oil have been isolated by the French scientist Lederer, one of them being a lactone showing antibiotic properties, which, together with the other – a new fatty acid, Calophyllic acid are responsible for Tamanu oil's cicatrizing power. In order to assess to what degree reported antibiotic properties of the Tamanu oil may be of value in application to bacteriostatic preparations and specifically assess its potential for application in oral hygiene products, an attempt has been made to empirically evaluate a degree to which anaerobic bacteria isolated from respiratory tract of patients with pulmonary infections are sensitive to various concentrations of Tamanu oil of South Pacific origin.

Materials and methods

Product's origin and process used: the Tamanu oil, used in this study was supplied from Vanuatu, Oceania (South Pacific region). The fruit was harvested from trees grown in villages located in Santo island and processed in local manufacturing facility under quality controlled environment before being exported by air in a tight sealed glass containers for laboratory work reported in this paper. The received sample of oil (donated for study by an Australian company NatureCorp Pty Ltd, Sydney), represented 100% pure commercial batch of 550 l of oil obtained from kernels of naturally-matured Tamanu fruit. After being dried in a controlled low temperature oven (maximum 65°C), kernels were cracked and then pressed using cold extraction process in specially-modified high pressure copra screw press. Extracted batch of oil after coarse filtration through the cloth filter was then stored in sealed plastic containers, away from strong direct light in a cool dry area for transport (without freezing). The sample's characteristics and specification is presented in Table 1.

Experimental microbiological procedure

The antibacterial effect of Tamanu oil was investigated on total 30 strains (18 strains Gram-negative and 12 Gram-positive) of anaerobic bacteria isolated

Table 1. Specification of Tamanu Oil (*Calophyllum inophyllum*).

	Specifications	**Results**
Description: Appearance Smell	dark semi-viscous oil nutty odour and taste	complies complies
Fatty Acid Profile: Palmitic acid C16: 0 Stearic acid C18: 0 Oleic acid C18: 1 Linoleic acid C18: 2 Arachidic acid C20: 0	 more than 13% more than 13% more than 36% more than 20% more than 0,7%	 14.13% 13.78% 40.95% 22.37% 0,86%
Sterols: Campesterol Stigmasterol Beta-Sitosterol	non-saponified fraction: 0.91% 3.91% 3.26%	in oil: 0.014% 0.062% 0.052%
Microbiology: Yeasts and mold in 1 g Mesophyllic bacteria in 1 g *E. coli* bacteria group in 1 g Salmonella in 10 g *Staphylococcus aureus* in 1 g	 <102 <104 negative negative negative	 conforms conforms conforms conforms conforms

1. According to the supplier, NatureCorp Pty Ltd (Australia), the oil properly stored in sealed plastic or glass containers, away from strong direct light in a dry, cool area (without freezing) maintains its therapeutic properties for the period of minimum three years.
2. Crystallization and sedimentation may occur during longer storage of Tamanu oil, with the degree of solidifying depending on how low storage temperate is. This has no detrimental effect on the product.

from respiratory tract of 12 patients with respiratory tract infections. The materials were inoculated on the surface of enriched and selective media (12). The degree of bacteriostatic property was determined by means of plate dilution technique in Brucella agar with 5% sheep blood. Incubation was performed during 48 hours in anaerostats at temperature of 37°C in the presence of 10% CO_2, 10% H_2 and 80% N_2, palladic catalyst and an indicator of anaerobiosis (12). The MIC was interpreted as the lowest concentrations of the Tamanu oil inhibiting the growth of bacteria. Isolated strains of anaerobic bacteria were identified according to schemes of Virginia Anaerobe Laboratory Manual (12) and Bergey's Manual (13), taking into account the latest changes in taxonomy (14, 15).

The classification of microorganisms was based according to morphological, physiological and biochemical features according to API 20A Merieux.

Analysis of conversion of glucose into C1 to C6 fatty acids, succinic acid and lactic acid were determined using gas-liquid chromatography and ability of strains to produce natural fluorescence was also observed at a spectrum of ultra-violet radiation (UV) with the use of HBO-200 lamp.

The susceptibility of the following 30 strains of anaerobic bacteria were tested: *Bacteroides fragilis* (4 strains), *Dialister pneumosintes* (1), *Tannerella forsythia* (1), *Prevotella intermedia* (3), *P. denticola* (1), *P. oralis* (1), *P. bivia* (2), *Porphyromonas asaccharolytica* (1), *Fusobacterium nucleatum* (3), *Veillonella parvula* (1), *Propionibacterium acnes* (4), *Propionibacterium granulosum* (1), *Actinomyces israelii* (2), *Peptostreptococcus anaerobius* (1), *Micromonas micros* (4), and one reference strain of *Bacteroides fragilis* ATCC 25285.

The susceptibility of anaerobic bacteria to graded concentration of Tamanu oil was performed by means of plate dilution techniques in agar. The oil was diluted in the DMSO (Serva) to obtain dilution 200 mg in 1 ml. Futher dilutions were performed in sterile distilled water. The graded concentrations of diluted Tamanu oil were added into Brucella agar with 5% sheep blood, 1% Tween 80 and 0.1% lecithin. Inoculum containing 10^5 CFU per drop was seed with Steers inoculator upon the surface of the agar. Incubation of inoculated agar with various concentrations of Tamanu oil as well as control with no oil added (strains growth control with isopropyl alcohol only without alcohol and oil) was performed at 37°C for 48 h in anaerostats under anaerobic conditions. MIC was defined as the lowest oil concentration that completely inhibited growth of strains of anaerobic bacteria.

Sterols (campesterol, stigmasterol, β-sitosterol) in Tamanu oil and in non-saponified oil fraction were determined using HPLC procedure and fatty acids using liquid gas chromatography technique.

Results

While the oleic and linoleic acids (41% and 22% respectively) were major fat constituents in the Tamanu oil, then stigmasterol and β-sitosterol (3.91% and 3.26% respectively) were major sterols present in the oil (Table 1).

Results summarised in Table 2 demonstrate, that anaerobic Gram-negative microorganisms tested in this study displayed an overall moderate sensitivity to graded concentrations of Tamanu oil. The strain *Dialister pneumosintes* was the most sensitive Gram-negative strain (MIC 2.5 mg/ml). The strains of *Bacteroides* required 20 mg or more of oil in 1 ml of agar medium to inhibiting the growth. The MIC value for the reference strain of *Bacteroides fragilis* ATCC 25285 was also above the level of 20 mg/ml.

In comparison to *Bacteroides*, the strains from *Prevotella* group showed higher sensitivity to Tamanu oil with MIC values ranging between 5 mg/ml to 20 mg/ml.

Out of the all strains of bacteria tested in this study, the lowest sensitivity to Tamanu oil were recorded in the *Fusobacterium* and *Veillonella* group which required between 5 and above 20 mg Tamanu oil concentrations per ml medium to inhibit their growth.

In general, Gram-positive anaerobic bacteria showed higher sensitivity to Tamanu oil as compared to Gram-negative ones, with five out of twelve tested strains requiring concentrations below 5.0 mg oil per ml to inhibit growth. The highest sensitivity to Tamanu oil showed strains of Gram-positive bacteria *Propionibacterium* and *Actinomyces* (MIC between 0.6 and 5 mg/ml) *Micrococcus* and *Peptostreptococcus* (MIC 1.2-5 mg/ml).

Discussion

Mechanism of therapeutic action of therapeutic oils extracted from trees, shrubs, herbs and cultivated plants has not been yet scientifically explained in depth. One of the accepted explanations may be a lipophyllic action of oils on cell membranes and cytoplasmic membrane in microorganisms, casing damage to cells and leakage of cytoplasm from cells (16). It has also been shown that the bacteriostatic potency of therapeutic oils may be attributed to slowing down synthesis of nucleic acids and proteins in bacterial cells (16). Although the chemistry of Tamanu oil is complex and its physiological action is far from being fully understood (8), it is stipulated that a wide diversity of is uses may be ascribed to unique absorption properties of Tamanu oil and its cicatrizing, antibacterial, antioxidant and anti-inflammatory action. In addition to three common classes of lipids, Tamanu oil contains a group of non-steroidal anti-inflammatory agents such as calophyllolids, xanthones, a number of antibacterial and antifungal phyto-chemical agents such as friedelin and a novel antibiotic lactone – all of them contributing synergistically to the Tamanu oil's skin healing, cicatrizing and antibacterial properties.

Table 2. Sensitivity of anaerobic bacteria from respiratory tract to Tamanu Oil (*Calophyllum inophyllum*).

Microorganisms	Number of Strains	Minimum inhibitory concentration (MIC) (mg/ml)						
		>20.0	20.0	10.0	5.0	2.5	1.2	0.6
Gram-negative:								
Bacteroides fragilis	4	1	3					
Dialister pneumosintes	1					1		
Tannerella forsythia	1	1						
Prevotella intermedia	3		2	1	1			
Prevotella denticola	1		1					
Prevotella oralis	1		1					
Prevotella bivia	2		1					
Porphyromonas asaccharolytica	1	1						
Fusobacterium nucleatum	3	3						
Veillonella parvula	1	1						
Gram-positive:								
Propionibacterium acnes	4				3		1	
Propionibacterium granulosum	1						1	1
Actinomyces israelii	2			1				
Peptostreptococcus anaerobius	1				1	1		
Micromonas micros	4				2		1	
Total	30	7	8	2	7	2	3	1
Percentage participation (%)	100	23	27	7	23	7	10	3
Reference: *Bacteroides fragilis* ATCC 25285	1	1						

The active constituents of Tamanu oil were identified as coumarine derivatives (4): – calophyllolide ($C_{25}H_{22}O_5$) the molecule of which contains a lactonic and amethoxyl group and – calophyllic acid ($C_{25}H_{24}O_6$), which results from the saponification of the calophyllolide. A great number of compounds which have beed determined in Tamanu oil and importance of which is not yet fully understood, appears to act synergistically. The most pronounced groups of components are (i) free fatty acids, glycerides, and sterols, (ii) terpenoids and steroids (canophyllal, canophyllol, canophyllic acid) and (iii) coumarinic derivatives: calophyllolids (natural neo-flavonoids with antibacterial, anti-inflammatory and blood anti-coagulation properties), inophyllolids (natural neo-flavonoids with antiviral properties), calophyllic acid (natural neo-flavonoid with antimolluscidal and healing activities) (17). The ED_{50} of Tamanu oil was determine as 140 mg/kg when taken orally and for ulcerogenic activity it was twice the ED_{50} dose. Anti-inflammatory and anti-arthritic activity determined on formaldehyde-induced arthritis and adjuvant arthritis in rats showed LD_{50} at the level of 2.5 g per kg body weight when taken orally.

Observed susceptibility of Gram-positive anaerobic bacteria to Tamanu oil may extend its current applications in topical cosmetic and therapeutic skin preparations to its use in products designed for maintaining oral hygiene. Combined bacteriostatic and healing effect of Tamanu oil could be explored further in treating mouth ulcerations and various gums conditions, utilising this type of oral preparations for simultaneous prevention against common respiratory tract infections caused by Gram-positive anaerobic bacteria which have been shown in this work as the most susceptible to the presence of Tamanu oil in concentrations between 0.6 and 5 mg/ml of substrate.

Conclusions

1. In general, the Gram-positive anaerobic bacteria were more sensitive to Tamanu oil with five out of twelve tested strains requiring concentrations below 5.0 mg oil per ml to inhibit growth while majority of Gram-negative strains required more than 2.5 to well above 20 mg oil per ml to inhibit growth.
2. The highest sensitivity to Tamanu oil showed strains of Gram-positive bacteria *Propionibacterium* and *Actinomyces* (MIC between 0.6 and 5 mg/ml) *Micrococcus* and *Peptostreptococcus* (MIC 1.2-5 mg/ml).
3. Observed susceptibility of Gram-positive anaerobic bacteria to Tamanu oil may extend its current applications in topical cosmetic and therapeutic skin preparations to its use in designing antimicrobial products for oral hygiene.

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Adres/address:
*dr hab. Anna Kędzia
Department of Oral Microbiology
Medical University of Gdańsk
Do Studzienki Str. 38, 80-227 Gdańsk, Poland
tel. (58) 349-21-85
e-mail: zmju@amg.gda.pl