ECRONICON OPEN ACCESS

MICROBIOLOGY
Research Article

Comparative Study of Antimicrobial, Toxicity and Physicochemical Properties of the New Generation Mouthwash "*Oralnet*" with Common Mouthwashes

Drasko D Pekovic*, Hassane Kacimi and Zana Zarkovic

Head of Environmental Laboratory of the Institute of BioMedical Research, Canada

***Corresponding Author:** Drasko D Pekovic, Head of Environmental Laboratory of the Institute of BioMedical Research, (Quebec) Canada.

Received: September 18, 2015; **Published:** November 13, 2015

Abstract

Recently, a new generation of mouthwash, called "*OralNet*", became commercially available in the province of Quebec, Canada. According to the technical specifications, *OralNet* is based on ultrapure Arctic Ocean water supplemented with colloidal silver.

In the present study the Microbicidal efficacy, toxicity, endogenous pH and physicochemical content of *OralNet* have been evaluated in comparison with the following standard over-the-counter (OTC) mouthwashes: Biotene with Calcium, Colgate-Peroxyl, Life-Citrus, Listerine Anti-Tartar, Listerine Original, Scope-Mint and Tetrabreath.

The obtained results show that *OralNet* displayed the highest microbicidal activities *in vitro* exceding 99.9% after 30 seconds of contact with test organisms and the lowest toxicity *in vitro* (3.69%), as compared to the standard mouthwashes studied. In addition, *OralNet* has a well balanced endogenous pH (7.3), as well as a simple chemical structure composed of two natural active ingredients.

The standard mouthwashes are also characterized by relatively high *in vitro* microbicidal activities, higher toxicity levels than *OralNet*, and more complex chemical structures, involving up to 18 components. Five of the seven displayed low endogenous pH, below the critical value for tooth structure dissolution (pH < 5.5). Contrarily, Tetrabreath displayed a high alkaline level (pH 9.52).

The present study shows microbicidal and biocompatibility superiority of *OralNet*, over all other mouthwashes studied. In addition, *OralNet* is a natural compound with a simple structure compared to the other products, which are composed of numerous chemical ingredients with potential side effects.

Keywords: *Mouth wash; Oral hygiene; Ocean water; Colloidal silver*

Abbreviations: CFU: Colony Forming Unit; OTC: Over-The-Counter;

Introduction

Mouthwashes are solutions used for rinsing the mouth, to remove or destroy oral bacteria, to act as an astringent, to deodorize and to have a therapeutic effect by preventing or relieving oral diseases, mainly dental caries, gingivitis and periodontitis. However, these relative therapeutic benefits are not clearly defined [1].

The earliest report on mouthwash use, as a formal treatment of oral health conditions, is attributed to Chinese medicine in approximately 2700 B.C. when mouth rinsing was recommended with the urine of a child. This practice spread across many ancient civilizations and persisted until the early 1700s. In the latter half of the 18th century, Miller advanced the knowledge on oral rinsing by distinguishing between their bacteriostatic and bactericidal effects [2]. The modern era of mouthwashes was introduced by the release of Listerine as an OTC remedy for bad breath in 1914 [3]. Since then oral antiseptic and germicidal claims for mouthwashes have been abundant. However, few of these claims had supportive clinical data, and those that did were in the form of *in-vitro* testing [2].

Current knowledge in dental medicine states that maintaining good oral hygiene is a preventive measure for a variety of oral diseases, as well as for health conditions in general [4]. Tooth brushing alone is the most common way of maintaining oral health by removing as much as 65% to 75% of the total plaque [5].

Since this mechanical method of plaque control remains insufficient to prevent oral diseases chemical agents may add relevant benefits when used in addition to tooth brushing and flossing. For this reason antibacterial agents are widely used in a variety of mouthwash preparations [6].

Many scientific reports show that the use of antiseptic mouthwash significantly decreases the total number of oral bacteria for up to 2 hours after rinsing [7-10].

Although mouthwashes have demonstrated the ability to inhibit the formation of dental plaque, data about their side effects, such as unpleasant taste, oral mucosa ulceration, toxicity and genotoxicity, mouth irritation and drying, tooth staining and other oral complications are emerging slowly [11-18].

Recently, a new generation of mouthwash, called *OralNet*, has become commercially available in the Canadian province of Quebec. As declared by the manufacturer, *OralNet* is composed of two active ingredients: ultra pure water from the Arctic Ocean and colloidal silver.

The ocean water is known as the richest, most complete natural source of minerals and metals [19]. It is recognized for its nutritional qualities, oxygenation of somatic cells, stimulation of blood circulation, and restoration of tonicity and dynamism to the tissues [19,20]. Ocean water is also known as a powerful antibiotic. It kills many kinds of microbes and prevents their proliferation [21-23].

It is important to point out that the beneficial action of the salted solutions in stomatologic practice has been appreciated for a long time. The oral crenotherapy finds in natural ocean water a preferential field of application, without iatrogenic risks [20].

OralNet is also supplemented with colloidal silver, recognized for its general anti-microbial effect, without known resistance, which has been used therapeutically since the old civilizations. Colloidal silver releases silver ions which penetrate into the cellular walls of the microbes and disable the enzymes that all bacteria and fungi use for their oxygen metabolism, resulting in death of the cells [24-26].

This paper describes a comparative study of a new generation mouthwash, *OralNet*, with 7 other OTC mouthwashes on the basis of the following studies:

a. Evaluation *in vitro* of antimicrobial effectiveness,
b. Determination *in vitro* of toxicity,
c. Measuring of endogenous pH, and
d. Comparison of the physiochemical content by compiling the ingredients presented on the product labels.

Material and Methods

Mouthwashes

For the present study, all 8 brands of OTC mouthwashes (Illustrated by Figure 1), containing various ingredients, have been obtained from local commercial sources in Montreal, Canada. Table 1 shows their trade name, in alphabetical order, the name of producer, lot number, expiration date and code used during testing for blinding purposes.

Name	Name of Producer	Lot#	Expiration Date	Code
Biotene	Glaxo Smith Klein, Missisagua, Ontario	5C20N1	02/2018	A
Colgate Peroxyl	Colgate-Palmolive Toronto, Canada	444US11H	11/2016	B
Life Citrus Flavour	VI –Jon Inc St .Louis, USA	0012684BB	05/2016	C
Listerine anti-tartar	Johnson & Johnson Inc. Markham, Canada	30024277	03/2017	D
Listerine Original	Johnson & Johnson Inc. Markham, Canada	3084LZ	09/2016	E
OralNet	BioMedco Services Inc., Montreal, Canada	200615	*	F
Scope Smooth Mint	Procter & Gamble Inc., Toronto, Canada	51315395TD	*	G
Tetrabreath	Manufactured in USA Dv. Harold Kats, Los Angeles, CA	061492	09/2017	H

Table 1: *Name of mouthwashes, producer, lot and expiration date.*

**Not indicated*

At the beginning of the study all mouthwashes were assigned a code and then all testing was carried out on coded samples.

Figure 1: *OTC mouthwashes studied.*

All mouthwashes were analyzed for:

a. Bactericidal activity *in vitro* against salivary flora of healthy individuals [27].

b. Toxicity *in vitro* by Toxi-Chromotest [28].

c. Endogenous pH [29,30]

d. Content of ingredients, according to the declarations of each respective manufacturer.

Bactericidal activity

Saliva collection

The anti-bacterial activity of the mouthwashes was assessed on salivary flora. Whole saliva was collected from 5 healthy adult subjects who were asked first to brush teeth and rinse the mouth with 40 ml of water for 15 seconds. Following oral hygiene measures, saliva was collected. Spitting method was employed into a 20 ml sterile specimen bottle for 5 minutes [31,32].

Evaluation of microbial load

The individual whole saliva samples were mixed with PBS (1:1 v/v) for 2 min sonication. Sonicated saliva was then serially diluted in 10-fold dilutions in PBS, and aliquots plated on 5% sheep blood agar. Plates were incubated under aerobic conditions at 35ºC, with the number of colony forming units (CFU) reported for each dilution. The bacterial test suspension was prepared and adjusted at 5.1 x 10^6 CFU/ml and used for in vitro testing in this study [33].

Determination of bactericidal activity

The anti-bacterial effects of all mouthwash samples have been analyzed according to a standard protocol [27,34].

The cell suspension of human saliva, containing 5.1 x 10^6 CFU/ml was used for this purpose. One ml of the cell suspension was inoculated in 30 ml of each mouthwash sample. The antibacterial effectiveness was determined by the number of CFU/ml, of the test suspension after 30s, 1 and 5 min of contact with tested mouthwashes and compared to the number of CFU/ml of the bacterial control suspension. After each contact time, 10 ml was taken from each mouthwash test suspension. Microbes were removed by membrane filtration and harvested cells resuspended and cultured as previously described [35,36].

Determination of toxicity

The toxicity level of each mouthwash was determined by EBPI Toxi-Chromotest (Brampton, Ontario) [28,37]. Briefly, Toxi-Chromotest is a rapid bacterial-based colorimetric bioassay generally used for determination of toxicity of a broad spectrum of liquid and solid toxic substances. The assay determines the ability of substances (toxicants) to inhibit the de novo synthesis of an inducible enzyme –*β-galactosidase* in a highly permeable mutant of *Escherichia coli.*

The procedure exposes the bacteria to the test sample for a short incubation period after which a chromogenic substance is added. If the sample is toxic, no color will develop but if the sample is not toxic a distinctive blue color quickly develops. The test provides a clear, completely objective measurement of the toxicity of the sample by a simple visual appreciation of the color obtained or by spectrophotometry using a micro-plate-reader.

In the present study a DMS 300 spectrophotometer (Varian) was used at 615 nm, for evaluation of enzyme activity.

Toxi-Chromotest includes control standards: Mercury chloryde ($Hg\ CL_2$) was used as the positive control, displaying a standard toxicity of 95%, and distilled water (H_2O) was used as the negative control having toxicity value of 0%.

Toxicity of the tested samples was calculated using the following equation:

% Toxicity = [0.1–(OD treated cells/OD control cells)] x 100

Measuring of endogenous pH values

The pH is an important parameter in oral microbiological ecology.

The endogenous pH of each mouthwash was measured at room temperature immediately after the package was opened, using a digital pH meter (Hanna Instruments, USA) [29,30].

Evaluation of the content of the mouthwashes

The content of each mouthwash sample was evaluated on the basis of ingredients declared on the product label. No specific analyses were carried out on any of the mouthwashes studied.

Results

Bactericidal activity

The results of the comparative studies of antimicrobial effectiveness of *OralNet* and seven other OTC available mouthwashes are reported in Table 2 and Figure 2.

Mouth Washes	Initial Concentration (*CFU/ml) 0 min	Results: % of microbial killings after contact times		
		30s	1 min	5 min
PBS (control)	1.7 x 10^5	0.2	0.2	0.3
Biotene with Calcium		97.6	99.9	99.9
Colgate-Peroxyl		94.1	99.9	99.9
Life-Citrus		95.4	99.9	99.9
Listerine Anti-Tartar	1.7 x 10^5	93.9	98.4	99.9
Listerine Original		93.9	98.1	99.9
Scope-Mint		98.6	99.9	99.9
Tetrabreath		96.4	98.9	99.9
Oralnet	1.7 x 10^5	99.9	99.9	99.9

Table 2: *Results of comparative studies of antimicrobial effectiveness.*
**CFU = Colony Forming Unit*

Results of this study show that mouthwash solutions displayed variable antibacterial activity depending on their chemical composition. However, only *OralNet* killed 99.9% of test bacteria after 30 seconds of contact. Biotine with Calcium, Colgate Peroxyl, Life-Citrus, Scope-Mint and Tetrabreath destroyed 99.9% of the test bacteria after 1 min of contact. All studied mouthwashes achieved killing of 99.9% of tested bacteria following 5 min of contact.

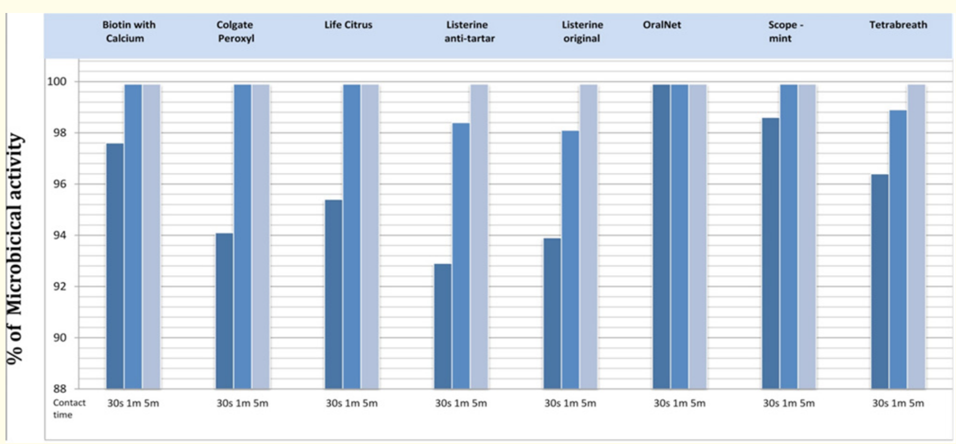

Figure 2: *Microbicidal activities of tested mouthwashes.*

Toxicity

The toxicity levels of tested mouthwashes are specified in Table 3.

The positive ($HgCL_2$) and negative controls (H_2O) gave values according to the specification of test-kit manufacturer [37].

All toxicity levels were low with a range from 3.6% to 5.1% with *OralNet* the lowest and Life Citrus the highest.

Endogenous pH measurement

Endogenous pH values are specified in Table 3.

OralNet showed a neutral pH value of 7.30, Colgate Peroxyl, Life Citrus, Listerine Anti-Tartar and Scope Smooth Mint displayed low endogenous pH, even below the critical value (pH 5.5) for tooth structure dissolution [29,30]. Contrarily, Tetrabreath showed a very high alkaline endogenous pH of 9.52.

Tested samples	% of Toxicity*	Endogenous pH	Total Number of ingredients
Biotene with Calcium	4.92	6.80	13
Colgate-Peroxyl	3.84	4.17	9
Life-Citrus	5.10	4.23	18
Listerine Anti-Tartar	4.52	4.09	11
Listerine Original	4.56	4.36	10
OralNet	3.69	7.30	5
Scope-Mint	4.60	5.43	12
Tetrabreath	4.88	9.52	9

Table 3: *Toxicity, endogenous pH and total number of ingredients.*

**Test technical specification values:*

%of Toxicity of distilled water (negative control) < 0.1.

%of Toxicity of Mercury chloride (positive control) = 95.0.

Ingredients	Biotene with calcium	Colgate Peroxyl	Life Citrus	Listerine Anti-Tartar	Listerine Original	Scope Classic	Tetrabreath	OralNet
Alcohol			x	x	x	x		
Aroma	x							
Benzoic acid			x	x	x	x		
Blue 1		x				x		x
Brilliant F.C.F						x		
Caramel					x			
Calcium lactate								
Cetylpyridinium chloride						x		
Citric acid								
Colorant								x
Colloidal silver								x
Dissodium phosphate	x							
Domiphen bromure								
Eucalyptol			x		x			
F.D. & C. Red No.40			x					
F.D. & C. Green No. 3				x				
F.D. & C. Yellow No.6			x					
Flavor			x	x		x		

Glucose oxydase								
Glycerine	x					x		
Hydrogenated Castor Oil			x				x	
Hydrogen peroxide								
Hydroxyethyl cellulose	x							
Lactoferrine								
Lactoperoxydase								
Lysozyme								
Mentha piperita oil							x	
Menthol		x	x		x			
Methil paraben	x							
Methyl salicylate		x	x	x	x			
Ocean water								x
PEG-40			x				x	
Poloxamer 338		x						
Poloxamer 407	x		x	x	x			
Polysorbate 20		x						
Polysorbate 80						x		
Propanol								
Propyleneglycol	x	x						
Propylparaben	x							
Sodium benzoate	x		x	x	x	x	x	
Sodium bicarbonate							x	
Sodium citrate								
Sodium chloride							x	
Sodium hydroxide							x	
Sodium phosphate	x							
Sodium saccharine		x	x	x		x		
Sorbitol	x	x	x	x				
Sucralose			x	x				
Tetrasodium EDTA							x	
Thymol			x		x			
Water	x	x	x	x	x	x	x	x
Xylitol	x							
Yellow 5						x		
Zinc chlorure			x					
Zinc gluconate								
Total number of ingredients	13	9	18	11	10	12	9	5

Table 4: *Structural content of mouthwashes as declared by their manufacturers.*

These comparative studies have shown that the commercially available standard mouthwashes are complex in their structure and contain up to 18 chemical ingredients. *OralNet* mouthwash displayed a simple structure containing no chemical considered potentially harmful for human health.

Discussion

Despite almost ubiquitous use of toothbrushes with fluorinated toothpaste, and tooth flossing, epidemiological data indicates that these methods do not achieve their theoretical potential for controlling plaque formation and preventing oral diseases [5].

As it is impossible to eliminate all oral flora, which is composed of almost 500 types of microorganisms, it is important to achieve plaque control by limiting growth of harmful bacteria causing oral infections [3,38].

This situation provided the impetus for the use of antimicrobial mouthwashes, which are reported to be favored by the public due to their ease of use and breath freshening effect, with the aim of better controlling dental plaque and gingivitis [39-42]. Current statistics indicate that almost 50% of the population uses mouthwash; however half of these rinses are not therapeutic preparations [43].

Mouthwashes are typically used as adjunctive to tooth brushing regiments. They encompass antimicrobial activity that ensures elimination of harmful oral bacteria, which aids in preventing dental caries, gingivitis and periodontitis. However, almost all of these preventive effects have been studied *in vitro* or in animal models [43].

When selecting the appropriate mouthwash, in addition to its antimicrobial effect, its biocompatibility has also to be given due consideration [44]. Mouthwashes are complex mixtures and can include various types of antimicrobials, such as alcohol, metal ions, essential oil, chlorhexidine, quaternary ammonium compounds and many other chemicals. Few of these mouthwashes, however, have undergone rigorous testing, as evidenced by the limited information on their safety and clinical efficacy [45-47].

An increasing number of scientific publications indicate occurrence of numerous side effects associated with the use of mouthwashes. With this perspective it is important to point out that almost all mouthwashes contain microbiocidal active ingredients which are generally recognized, more or less, as toxic substances.

The use of mouthwashes with high ethanol content, as a microbicidal agent has frequently been linked to an increased risk of oral mucosal irritation and ulceration [16,17]. The ethanol concentration of numerous commercially available mouthwashes is situated between 5 and 27% [3]. The regular use of mouthwashes with high alcohol content may contribute to oral cancer risk. A causal interpretation seems biologically plausible because drinking alcoholic beverages has been associated with oral cancer [48-50].

It is likely that in addition to ethanol various other constituents of mouthwashes are candidates to interact, for example, with plasma membrane oral squamous cells and to cause irritation [18]. Other types of irritants, e.g. terpenes, terpenoids and surfactants are additionally presents in many mouthwashes as effective ingredients [3].

Many brands of mouthwashes contain chlorhexidine as an active ingredient. It has been shown that long-term use of chlorhexidine-containing mouthwashes can cause local calculus formation, staining of teeth and delayed mouth healing [11-14]. In addition, it has been shown that chlorhexidine is highly cytotoxic *in vitro* and that more caution has to be applied when using this antiseptic in oral surgery procedures [15].

Numerous OTC mouthwashes contain a significant number of chemicals, as declared in the product labels. However, the toxicity of these ingredients remains scientifically poorly documented.

The literature review claims that mouthwashes are often evaluated by *in-vitro* tests on lab animals. The tests give inadequate understanding of the side effects of this toxicity, for the short or long term, to the oral or general health of the users.

The administration of antimicrobials to the mouth may result in some, or even all, of the administered dose being swallowed. Therefore it is appropriate to consider the possibilities of gastric irritation and/or systemic toxicity.

Increasing numbers of studies indicate that pH is an important physic-chemical condition in oral ecology, and that the endogenous pH value is an important property of mouthwashes. The measurement of the pH is a practical method to assess the erosive potential of a mouthwash [29,30]. Low pH mouthwashes (≤ 5.5) can cause dental demineralization, erosion, and significant loss of enamel within the first few minutes of contact. Although pH values ≤ 5.5 are considered critical for enamel dissolution, mineral loss may begin even at a higher pH i.e. 6. Therefore, the prolonged use of mouthwashes with pH < 5.5 may be potentially harmful to dental structure [29].

The low pH of oral care products increases the chemical stability of some fluoride compounds and favors the incorporation of fluoride ions into the lattice of hydroxyapatite and the precipitation of calcium fluoride onto the tooth surface. Based on this statement, product labels were examined to identify mouthwashes containing fluoride. Among the six with a pH < 5.5, three mouthwashes contain fluoride (0.05% NaF). The label of the other three mouthwashes with pH below the critical value for enamel dissolution did not list fluoride in their ingredients.

The aim of this study was to determine the antimicrobial properties, toxicity level, endogenous pH and the content of ingredients of seven commercially available mouthwashes as compared to *OralNet*, a new generation mouthwash.

Of the eight mouthwashes tested, *OralNet* emerged as the most effective with 99.9% microbial killing after 30 seconds of contact time in experimental saliva-bacterial suspension. *OralNet* has also shown the lowest toxicity level as evaluated by Toxi-Chromotest, neutral pH value for better prevention of mineral dissolution from the teeth, as well as the simplest structure in number of ingredients and its natural content.

Results of this *in vitro* study cannot be directly extrapolated to the clinical situation. However, obtained results indicate significant differences in test-microbes killing and pH level among the mouthwashes studied.

There is clearly a great need for more *in vivo* studies to understand the possible microbial and toxicity effects of mouthwashes in order to balance formulations more efficiently for benefits provided by them.

Conclusion

OralNet, a new generation of mouthwash has shown better qualities, as compared to conventional mouthwashes by:

1. Rapid killing of oral flora,
2. Maintaining physiological conditions of oral tissues,
3. The lowest toxicity level,
4. Neutral pH, and
5. The simplest structure composed of two natural active ingredients

Bibliography

1. Akande O., et al. "Efficacy of different brands of mouth rinses on oral bacterial load count in healthy adults". *American Journal of Biomedical Research* 7.3 (2004): 125-128.
2. Mandel ID. "Chemotherapeutic agents for controlling plaque and gingivitis". *Journal of Clinical Periodontology* 15.8 (1988): 488-498.
3. Van Zyk AW and Van Heerdem WFP. "Mouthwashes: A review for South African health care workers". *South African Family Practice* 52.2 (2010): 121-127.
4. Wikipedia Oral hygiene.

5. Hansen F and Gjermo P. "The plaque removing effect of four tooth brushing methods". *Scandinavian Journal of Dental Research* 79.7 (1971): 502-506.
6. Ayad F., *et al*. "A comparative investigation to evaluate the clinical efficacy of an alcohol-free CPC containing mouthwash as compared to a control mouthwash in controlling dental plaque and gingivitis: A six months clinical study on adults in San Jose, Costa Rica". *Journal of Clinical Dentistry* 22.6 (2011): 204-212.
7. Pianotti R andPitts G. "Effects of an antiseptic mouthwash on odorigenic microbes in the human gingival crevice". *Journal of Dental Research* 57.2 (1978):175-179.
8. Pianotti R and Pitts G. "A method for in situ evaluation of antiseptic mouthrinses". *Journal of Dental Research* 48.1 (2015): 42-47.
9. Schegg H K and Lebek G. "Test model to stimulate the healing effect of antibacterial moutwashes". *Schweizerische Monatschrift fuer Zahnheilkunde* 3.3 (1970): 80-175.
10. Wynder EL., *et al*. "Oral cancer and mouthwash use". *Journal of the National Cancer Institute* 113.3 (1995): 253-2611.
11. Yates R., *et al*. "A 6-month home usage trial of a 1% chlorhexidine toothpaste: effects on plaque, gingivitis, calculus and tooth staining". *Journal of Clinical Periodontology* 20.2 (1993): 130-138.
12. Albandar JM., *et al*. "Chlorhexidine use after two decades of over-the-counter availability". *Journal of Periodontology Online* 65.2 (1994): 109-112.
13. Saatman RA., *et al*. "A wound healing study of chlorhexidine digluconate in guinea pigs". *Fundam Appl Toxicol* 6.1 (1986): 1-6.
14. Bassetti C and Kallenberger A. "Influence of chlorhexidine rinsing on the healing of oral mucosa and osseous lesions". *Journal of Periodontology Online* 7.6 (1980): 443-456.
15. Blot WJ., *et al*. "Smoking and drinking in relation with oral and pharyngeal cancer". *Cancer Research* 48.11 (1988): 3282-3287.
16. Moghadam BK., *et al*. "Extensive oral mucosal ulceration caused by misuse of a commercial mouthwash". *Cutis* 64.2 (1999): 131-134.
17. Smigel K. "High alcohol mouthwashes are under scrutiny". *Journal of the National Cancer Institute* 83.11 (1991): 751.
18. Mondal S., *et al*. "Two stage chemical oncogenisis in culture of C3H/10T1/2 cells". Cancer Research 36.7 (1976): 2254-2260.
19. Wikipedia Eau de mer
20. Jean-Louis Poirier, Ph.D. Eau de Mer Cristalline du Labrador -O de mer propulsion, 2005.
21. Saz AK., *et al*. "Antimicrobial activity of marine waters I. Macromolecular nature of antistaphylococcal factor". *Limnology and Oceanography* 8.1 (1963): 63-67.
22. Eau de mer
23. William J., *et al*. "Survival of certain pathogenic microorganisms in sea water". *Hydrophobiologia* 50.2 (1976): 117-21.
24. Goldman F. "Une arme secrète contre la maladie". Le lotus d'or (2004).
25. La médecine est dans la nature.
26. Natural Health and Longevity Resource Center. "The Rediscovery of a Super Antibiotic".
27. Clinical and Laboratory Standard Institute M26-A, Method for determining bacterial activity of antimicrobial agents; Approved Guidelines (1999) : 1-29.
28. Environmental Bio-Detection Products Inc. Toxi-chromotest, version 3.2.
29. Vivek S., Shwetha R. "Endogenous pH, titratable acidity of commercial available mouthwashes in Indian market". *Int J Clin Trials* 2015; 2 (1):20-24.
30. Cavalcanti AL., *et al*. "Endogenous pH, titratable acidity and total soluble solid content of mouthwashes available in the Brazilian market". *European Journal of Dentistry* 4.2 (2010): 156-159.
31. Saliva collection and handling advice Salimetrics, LLC, PA 16803, USA. Copiright (2009) : pp14.
32. Linke HA., *et al*. "Microbiological composition of whole saliva and caries experience in minority populations". *Dental Clinics of North America* 47.1 (2003): 67-85.

33. Socransky SS, and Haffaje AD. "Periodontal microbial ecology". *Periodontology* 2000 38 (2005) : 135-187.
34. Nunez L, and D'Aguino M. "Microbicide activity of clove essential oil". *Brazilian Journal of Microbiology* 43.4 (2012): 1255-1260.
35. Paster BJ., *et al*. "Bacterial diversity in human subgingival plaque". *Journal of Bacteriology* 183.12 (2001): 3770-3783.
36. Nicole V., *et al*. "The essential of United States Pharmacopeia chapter < 51>. Antimicrobial Effectiveness Testing". *International Journal of Pharmaceutical Compounding* 18.2 (2014): 123-130.
37. D'Haese E., *et al*. "Inhibition of β-galactosidase biosynthesis in Escherichia coli by tetracycline residues in milk". *Applied and Environmental Microbiology* 63.10 (1997): 4116-4119.
38. Theilade E. "Advances in oral microbiology". *Annals of the Royal Australasian College of Dental Surgeons* 10 (1989): 62-71.
39. Witt J., et al. "Antibacterial and antiplaque effects of a novel, alcohol-free oral rinse with cethylpyridinium chloride". *The Journal of Contemporary Dental Practice* 6.1 (2005): 1-9.
40. Yengopal V. "Essential oils and interdental hygiene". *SADJ: journal of the South African Dental Association* 59. 4 (2004): 155, 157, 170.
41. Charles C., *et al*. "Comparative antiplaque and antigingivitis effectiveness of chlorhexidine and an essential oil mouthrinse: 6-month clinical trial". *Journal of Clinical Periodontology* 31.10 (2004): 87-884.
42. Well A., *et al*. "The effect of a polyhexamethylene biguanide mouthrinse compared with a triclosan rinse and a chlorhexidine rinse on bacterial counts and 4-day plaque re-growth". *Journal of Clinical Periodontology* 32.5 (2005): 499-505.
43. White D. "An alcohol therapeutic mouthrinse with cetylpyridinium Chloride (CPC) – The latest advance in preventive care: Crest Pro-Health Rinse". *American journal of dentistry* 18 (2005): 3A-8A.
44. Tatnall FM., *et al*. "Assay of antiseptic agent in cell culture: conditions affecting cytotoxicity". *Journal of Hospital Infection* 17.4 (1991): 287-296.
45. Marsh PD. "Controlling the oral biofilm with antimicrobials". *Journal of Dentistry* 38 (2010): S11-S15.
46. Marsh PD. "Microbiological aspects of the chemical control of plaque and gingivitis". *Journal of Dental Research* 71.7 (992): 1431-1438.
47. Walker CB. "Microbiological effects of mouthrises containing antimicrobials". *Journal of Clinical Periodontology* 15.8 (1998): 499-505.
48. International Agency for Research on Cancer. "Monographs on the evaluation of carcinogenic risks to human". *Alcohol drinking* 44 (1988) 13-20.
49. Winn DM., *et al.* "Mouthwash and oral cancer condition in the risk of oral and pharyngeal cancer". *Cancer Research* 51.11(1991): 3044-3047.
50. Pianotti R and Pitts G. "Effects of an antiseptic mouthrinse on crevicular anaerobes". *Journal of Dental Research* 56:560 (1978).

Volume 2 Issue 3 November 2015