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ORIGINAL ARTICLE

THE ANTIMICROBIAL ACTIVITY OF DIFFERENT MOUTHWASHES IN MALAYSIA

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Seven different brands of mouthwashes were assessed for the inhibition of growth of oral micro-organisms. The results showed wide variations in their effectiveness: Those containing cationic surfactants and complex organic nitrogenous compounds were more active than the older formulations based on phenols. A list was compiled ranking the mouthwashes according to their antimicrobial activity, which did not always agree with the manufacturer's claims or indication for use.

Key words : Mouthwashes, Micro-organism, Antimicrobial activity

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Introduction

The widespread use of mouthwashes as an aid to oral hygiene is a relatively recent phenomenon in Malaysia. This is mainly because the awareness of the public for the need of oral hygiene and healthier life as resulted in an increase in sales and number of brands coming onto the market.

This major change in the nation's oral hygiene habits seems to have gone largely unnoticed by the dental profession. Development work on the mouthwashes (apart from chlorhexidine) has been done mostly by the manufacturers, and little has been reported in the scientific literature (1-5). Most of the work that has been published relates to the individual antiseptic ingredients they contain rather than to complete formulation of the mouthwashes (2).

While their primary appeal is as an aid to breath freshness and cleansing the mouth, the majority of the newer mouthwashes also claim to have antiseptic or anti-infective properties. Thus, the purpose of this investigation was, to examine the various preparations that were on the market and to compare their antimicrobial effectiveness.

Materials and Methods

Three different techniques were used to assess anti-microbial effectiveness:

1. Microbial Growth in Liquid Nutrient Media

One per cent of nutrient broth (Oxoid Ltd.) was prepared containing a 10% concentration of the mouthwashes, according to the manufacturer's instruction on dilution. example, Dettol, dilute 2.5 ml mouthwash with 50 ml distilled water). After autoclaving, the broth and mouthwashes were inoculated with a standardised volumes (0.5 ml or 2 drops) of pooled, fresh saliva and were incubated at 37°C for 24 hours aerobically. Finally the inoculated broth were mixed and their optical density was measured by spectrophotometer at a wavelength of 490 nm as a guide to microbial growth. Acid production (pH) were also recorded at the same time. The whole set of experiments was performed in triplicate, taking the means to get reliable results, and each set included a control broth containing no mouthwashes.

2. Microbial Growth on Solid Media

Several agar media were prepared: trypton soya agar, TSA (Oxoid Ltd) (a general purpose medium supporting the growth of a wide variety of micro-organisms), and Lab M TYC agar (for the growth of oral streptococci). One part of each of the mouthwashes was added to 15 parts of the molten agar, and plates were poured, on which were spread standardised amounts of pooled saliva as inoculum. After 24 to 48 hours incubation at 37°C microbial growth was compared with that on non-inoculated controls by colony counts and expressed the results as percentage, again with repetition to measure consistency.

3. Determination of the prevention of growth by the zone inhibition method.

Phenol red (0.01%) was added to tryptone soya agar as an acid-based indicator. After autoclaving and just before solidification, a standard inoculum of pooled, fresh saliva (in triplicate) and *Streptococcus mitior* as a control was added, and plates were poured. After they had set, cut 4 mm in diameter circular wells in the agar and fill with standard volumes of mouthwashes. The area of the zone inhibition (colour change of indicator from purple to yellow) was determined and recorded after incubation at 37°C for 24 hours aerobically.

Results

1. Microbial growth in liquid nutrient media.

Figure 1 shows a histogram of the optical density and table II gives the pH data. It can be seen (Fig.1) that when the mouthwashes were used at the concentration recommended by the manufacturer's compared with the control (no mouthwash), Mouth Guard, Dettol, Search and Listerine were most efficient in curbing microbial growth while Plax, Listermint and Fresh & White had no effect.

Least acid was produced in the media containing Listermint, Fresh & White, Search and Plax. The final pH after incubation was lowest (that is, highest acid production) in the Dettol, Mouth guard and Listerine media (Table II).

Table 1. The mouthwashes.

Name	Manufacturer or distributor.	Purpose proposed by manufacturer.	Active principle	Mode of use
Dettol	Reckitt & Colman	Antiseptic,prevent plaque, tartar and gum disease. Freshening the breath.	Cetylpyridinium chloride Bp. 1.0%	Dilute 1:20.Daily. (Night &Morning)
Mouth Guard	Beecham	Antiplaque, cleansing and freshening the breath.	Cetylpyridium chloride & Sod.flouride 0.5 %	Neat, twice daily.
Search	Stafford & Miller	Antiseptic, prevent plaque.	Cetylpyridium chloride, 0.5%	Neat, twice daily
Plax	Colgate & Palmolive	Antiplaque, cleaning and healthier the mouth.	Trclosan, 0.03%, Sod.flouride 05%	Neat, twice daily (Night &Morning)
Listerine	Warner - Lambert	Prevent plaque and bad breath.	Benzoic acid,Menthol eucalypton, thymol methyl salicylate.	Neat, twice daily. (Night &Morning)
Listermint	Warner - Lambert	For fresh breath and to clean the whole mouth.	Cetylpyridium chloride.	Neat, twice daily.
Fresh & White	Lion Cop. (Japan)	To help protect teeth and for fresh breath.	Cetylpyridium chloride.	Neat, twice daily.

Table 2. Summary of pH data in liquid nutrient media.

Mouthwashes	pH
Control (no mouthwashes)	7.0
Dettol	4.7
Mouth Guard	6.0
Listamint	7.0
Fresh & White	7.0
Search	6.7
Plax	6.9
Listerine	5.2

Figure 1. Microbial growth in liquid media : Summary of optical density data (5 sets of experiments).

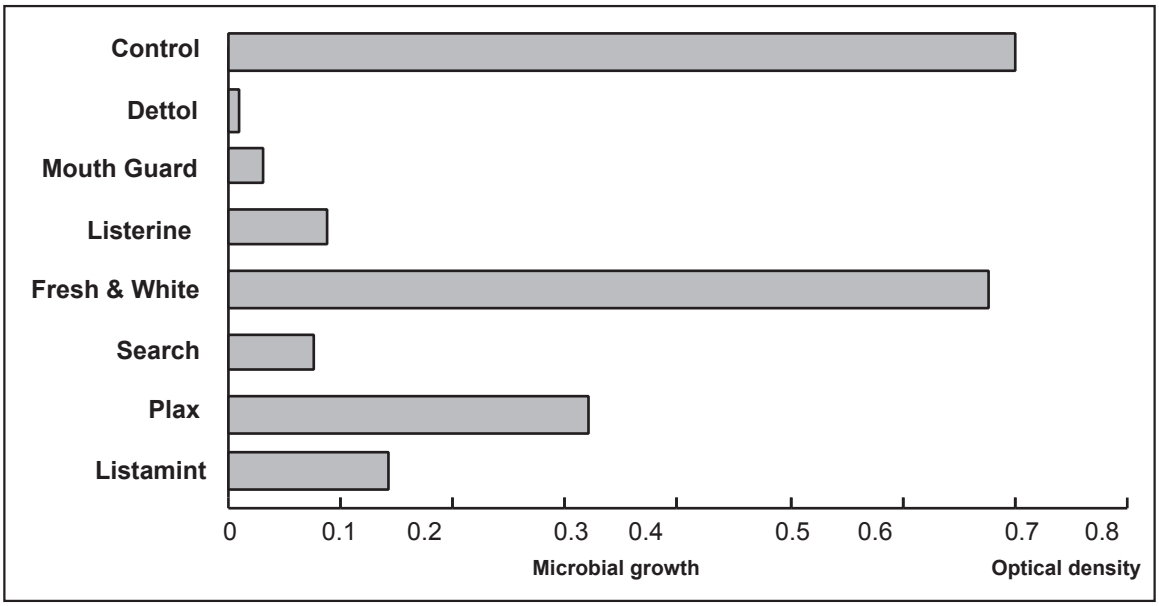


Figure 2. Microbial growth on agar media : colony counts expressed as percentages of the control (no mouth wash) figures. Means of 10 sets of data.

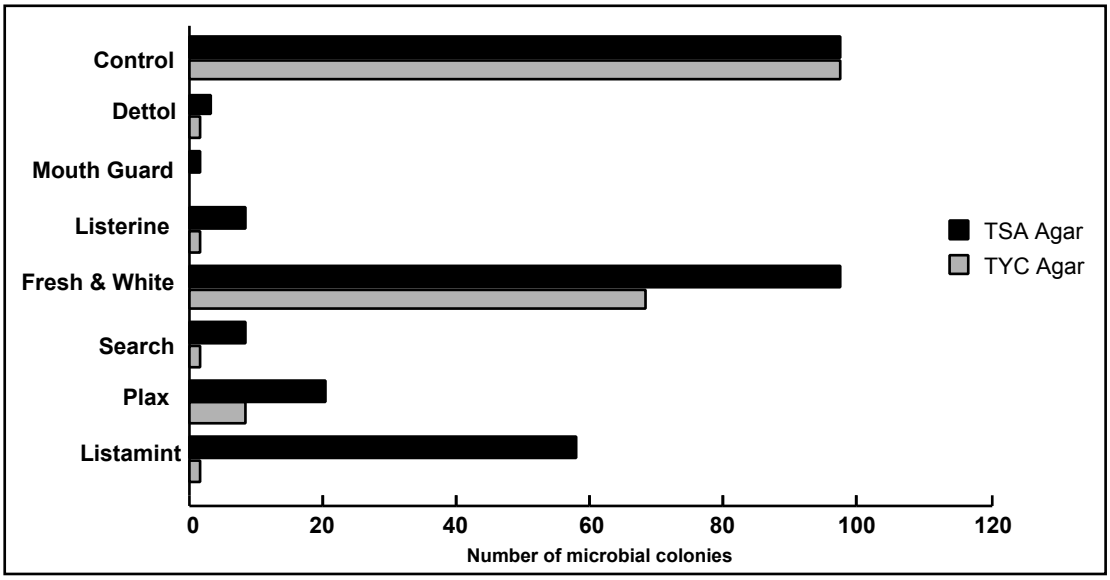
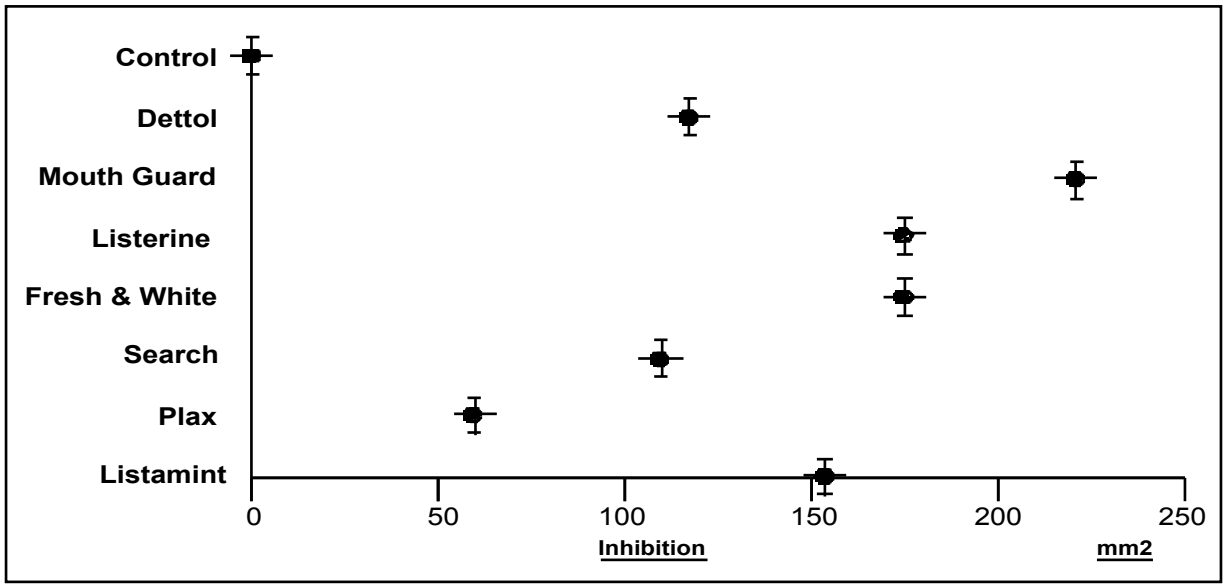


Figure 3. Areas of zones of microbial growth inhibition : means and standard errors of 8 sets of data.



2. Microbial growth on solid media.

The number of colonies growing on the agar plates are shown in figure 2 as a percentage of the total number on the control (no mouthwashes) plates. The five most effective mouthwashes in inhibiting microbial growth on both medium were Dettol, Mouth Guard, Search, Listerine and Listermine. As before, Plax and Fresh & White were among the least effective.

3. Zone inhibition method.

The areas of zone of microbial growth inhibition for Dettol and six other mouthwashes are shown in figure 3. This time Mouth Guard emerged as by far the most effective preparation followed by Search, Dettol and Listerine, While Plax, Fresh & White and Listermint was the least effective.

Discussion

Following the completion of the three sets of experiments, statistical ranking procedures were used to place the seven different mouthwashes in order of antimicrobial effectiveness. In general, Mouth Guard emerged as the most effective antibacterial preparation, followed by Dettol and Search, all of which had about the same level of activity. In the next group, a long way behind the first three in effectiveness, were Listerine and Plax, all with similar rankings. Finally, displaying very little antimicrobial activity, were Listermint and Fresh & White.

The result justify the antimicrobial claims made by them, however, Dettol and Listerine do not inhibit microbial growth completely on TSA (Fig.2). This revealed that those mouthwashes inhibit only oral *Streptococci* rather than other oral flora.

All mouthwashes examined contain one or more different active agents into their ingredient as seen in table 1. Most of them contains cetylpyridinium chloride, but in different concentration, except Plax and Listerine where they contain Triclosan and Thymol respectively. The use of cetylpyridinium chloride and chlorhaxidine gluconate are known to inhibit the accumulation of dental plaque (3-4). The mechanism of action of these diverse agents are not completely known , but one site of activity is at the cell surface by interference with carbohydrate transport into micro-organism (1). Phenolic compound such as thymol in Listerine also have the ability to inhibit the accumulation of dental plaque, however, it has been reported as potentially irritant (1).

How important are these findings in relation to what is expected of the mouthwashes by consumer? This depend on how and what purpose the products are marketed. As can be seen from Table 1, antibacterial or anti-infective claims are made by all except Listermint and Fresh & White, although the labels of both Listermint and Fresh & White describe them as antiseptic mouthwashes. These two are therefore likely to be used for breath freshness and cleansing the mouth rather than for any antimicrobial or medicinal purposes.

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