Antibacterial properties of "Accmus" mouth wash

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Abstract

Antimicrobial herbs can be used individually or in combination to prepare mouth wash which is healthier and safer than the synthetic ones. In this study a new "Accmus" herbal mouth wash was prepared and its antibacterial properties were evaluated. Alcoholic, boiled alcoholic and aqueous extracts of "Accmus" mouth wash were prepared from the bark of *Acacia arabica, Acacia speciosa* and root of *Calamus rotang* in combination by tincture and hot extract methods respectively. Alcohol content and pH were also determined. Antibacterial properties of the above extracts were also studied against *Staphylococcus aureus*, *Bacillus* sp of Gram (+)ve and *Pseudomonas aeruginosa*, *Klebsiella* sp of Gram (-) ve *in vitro* by using agar well diffusion method. This study showed that the alcohol content and pH of mouth wash preparations were in acceptable levels. Aqueous extract exhibited better antibacterial activity compared with alcoholic extract and had maximum sensitivity towards *Bacillus* sp and low towards *Klebsiella* sp. *Staphylococcus aureus* was only inhibited by all preparations of mouth wash. So the hot extraction method was efficient than the alcoholic extraction and this could be recommended with antibacterial properties rather than the alcoholic extract of mouth wash. Further study is needed for further purification and characterization of active constituents from various solvent extracts of mouth wash against oral diseases.

Introduction

Mouth wash or mouth rinse is a product used to enhance oral hygiene. Commercial brands of mouth wash contain synthetic and semisynthetic chemical substances such as thymol, methyl salicylate, menthol, chlorhexidine gluconate, methylparaben, hydrogen peroxide etc [1] and also include water and sweetness such as sorbitol, sodium saccharin [2]. Sometimes a significant amount of alcohol is added as the carrier for the flavour. Sodium benzoate is a common preservative in commercial mouth washes [3]. The risk of acquiring cancer rises almost five times for users of alcohol containing mouth wash who neither smoke nor drink [4]. Mouth washes containing cetylpyridinium chloride are also associated with loss of taste sensation and brown discoloration of teeth [4]. To overcome such harmful effect natural mouth washes are available in markets and are produced from plant based healthy ingredients such as organic aloe vera, peppermint, clove bud essential oils, perilla seed extract etc. The present study is to prepare a new "Accmus" mouth wash from the bark of *Acacia arabica*, bark of *Acacia speciosa* and root of *Calamus rotang*. *Acacia arabica* (Karuvel-"T") is a tree, becomes under family leguminosae. Its bark has medicinal properties, mainly used in oral diseases. Hence, it has 24-42% of tannin. *Acacia speciosa* (Kadduvakai – "T") becomes under family mimosaceae. Its bark decoction is being used in orodental diseases for gargle. Powder of root bark is used for bleeding. *Calamus rotang* is a climber one and it is classified under family palmae. In traditional medicine the root of *Calamus rotang* has been used against many oral diseases such as gum bleeding and aphthous ulcer in form of decoction for gargling [5,6]. The objective of this study is to prepare a natural new "Accmus" mouth wash and test its antibacterial activity against Gram (+) ve and Gram (-)ve bacteria.

Materials and Methods

Collection of plant materials

The plant *Acacia speciosa* was collected by the Unit of Siddha Medicine, University of Jaffna, Sri Lanka and it was identified based on herbarium records in the Department of Botany, University of Jaffna and other relevant materials [7,8]. And healthy bark was obtained, washed under running tap water, dried in sun shade for three weeks. Then ground into fine powder. Bark of *Acacia arabica* and root of *Calamus rotang* were also collected from local market and their characters were compared with herbarium records [7,8]. The above parts were washed under running tap water, dried in sun shade for five days and then ground into fine powder, by using electric blender. The powder was stored in air tight dark bottles at room temperature.

Preparation of mouth wash

"Accmus" mouth wash was prepared by two methods.

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Tincture method

25 g of each of the above herbal powder was mixed and mixture was soaked in 93.75 ml of 25% ethanol and 281.25 ml distilled water for two weeks under direct sun light with occasional shaking. The mixture was filtered through double layered muslin cloth and the filtrate (355 ml) was collected into a clean dried dark bottle.

Half of the above volume of the filtrate was boiled at 85°C for 30 minutes and poured into a clean dried dark bottle as boiled alcoholic extract [9].

Hot extract method

25 g of each of the above herbal powder mixture was mixed with 250 ml distilled water in a sterile beaker. It was heated at 50°C on hot plate for 6 hours continuously till the final volume of extracts reached as 150 ml. Then extracts were filtered through double layered muslin cloth and the filtrate was concentrated by heating. It was kept at 4°C until used for assay [10].

Determination of pH was determined by pH meter.

Determination of alcohol content

Alcohol content of mouth wash was determined by ebuliometer. Durability of mouth wash also noted based on its characters such as color change, (odour) smell formation, turbidity and change in viscosity.

Antibacterial assay

Culture preparation.

The bacterial isolates of *Staphylococcus aureus*, *Bacillus* sp from Gram positives and Gram negative *Pseudomonas aeruginosa*, *Klebsiella* sp were obtained from bacterial culture collection, Department of Botany, University of Jaffna for this study. Test organisms were stored on nutrient agar slants at 4°C and these were sub cultured before 24 hours of the experiment and incubated at 37°C. After the incubation a loop full of young bacterial inoculum was transferred into the 10 ml of sterile saline water (0.85%) in an aseptic condition. Inoculum concentration was estimated by haemocytometer and the number of cells per ml was adjusted to 10^6 cells by using tenfold dilution [11].

Determination of antibacterial activity

Nutrient agar medium was autoclaved and cooled to 40°C. The antibacterial assay was performed by agar well diffusion method [12]. 1 ml of test culture (10^6 CFU/ml) was inoculated into a sterile petridish with 20 ml sterile nutrient agar and mixed well and allowed to solidify. Then wells were made by using sterile cork borer (8 mm in diameter) on the surfaces of agar plates and were filled with 100 µl of each extracts using sterile Pasteur pipette. 100 µl of commercially available "Chlorhexidine digluconate" mouth wash was used as standard and alcohol and water were used as control. Then plates were incubated at 37°C for 24-48 hours. Antibacterial activity was determined by measuring the diameter of the clear zone around the well. The above experiment was repeated five times and the mean diameter of the zone of inhibition was calculated.

Results and Discussion

Table 1: Antibacterial activity of mouth wash extracts on test bacteria

Mouthwash extracts	*Mean zone of inhibition (mm)*			
	S. aureus (+)ve	*Bacillus sp (+)ve*	*P. aeruginosa (-)ve*	*Klebsiella sp (-)ve*
Alcoholic extract of mouth wash (Tincture)	12	-	-	-
Alcoholic extract of mouthwash after boiling (Tincture)	10	-	-	-
Aqueous extract of mouthwash	15	16	12	10
Chlorhexidine digluconate (Standard)	16	20	15	13

Zone of inhibition includes the diameter of the well (8mm in diameter). (-) No activity.

Table 2: pH and alcohol content of mouth wash extracts

Mouth wash extracts	*pH*	*Alcohol content (%)*
Alcoholic extract of mouth wash	4.5	18
Alcoholic extract of mouthwash after boiling	5.1	3
Aqueous extract of mouthwash	5.8	-

Out of five samples of alcoholic mouth wash, turbidity was observed after 8 months in two samples and 11 months in other three samples. Whereas in aqueous mouth wash, cloudiness and colour change were observed after 3 days. This indicated that the durability period of alcoholic mouth wash was higher (8-11 months) than that of aqueous mouth wash (2-3days) at room temperature. But aqueous mouth wash could be kept safe at 4°C for 6-8 months.

In commercially available mouth wash, alcohol content goes up to 27% and the pH ranges from 5-7 [13]. These two parameters were in acceptable ranges in newly prepared mouth wash (Table 2). Results also showed that aqueous extract of mouth wash containing natural ingredients, exhibited better antibacterial activity when compared to alcoholic extract. It had maximum sensitivity towards *Bacillus* sp, while it had low sensitivity towards the *Klebsiella* sp. Among the tested bacterial growth, *Staphylococcus aureus* was only inhibited by both preparations of mouth wash. All tested bacterial growth was inhibited by the aqueous extract of mouth wash and the positive control "Chlorhexidine digluconate". But alcohol alone (control) didn't inhibit the growth of any tested bacteria (Table 1). This is due to less alcoholic concentrations and the tolerance of test bacteria. Aqueous natural mouth wash showed greater antibacterial activity than alcoholic extracts of mouth wash. Hot extract method was highly efficient for the extraction of antibacterial compounds rather than tincture method. Long term use of alcoholic mouth wash is not preferable, because of the hazardous effects especially for children and causes dehydration in mouth [14]. Recently the possibility that the alcohol used in mouth wash acts as a carcinogen has been raised [15]. Even though the durability period of aqueous mouth wash was low at room temperature, it showed greater range of antibacterial activity against test bacteria and absence of alcohol. So this could be recommended rather than the alcoholic extract of mouth wash.

Further studies should be done clinically and test the effectiveness of this "Accmus" aqueous extract of mouth wash against oral diseases.

Conclusion

In both preparations of mouth wash pH and alcohol content were in acceptable level. *Staphylococcus aureus* growth was only inhibited by both mouth wash preparations. Hot extraction method was efficient than that of alcoholic extraction. Aqueous mouth wash showed greater antibacterial activity against test bacteria and it could be recommended with antibacterial activity rather than the alcoholic extract of mouth wash.

References

1. Goldberg S, Konis Y, Rosenberg. Effect of Cetylpyridinium chloride on microbialadhesion to hexadecane and polystyrene. *Appl Environ Microbiol* 1990: 1678-82.
2. Giertsen E, Emberland H, Scheie AA. Effects of mouth rinses with xylitol and fluoride on dental plaque and saliva. *Caries Res* (1999); **33**(1): 23-31.
3. Lachenmeier DW, Keck WA, Sauermann A, Mildau G. Safety assessment of alcohol containing mouth washes and oral rinses. *SOFWJ* 2008; **134**: 70-8.
4. Farah C, Mclntosh L, McCullough M. Mouth washes. *Australian Prescriber* 2009; **32**: 162-4.
5. National Institute of Science Communications. The Wealth of India, Council of Scientific and Industrial Research, Vol II, New Delhi 2001; 97.
6. Muthaliyar M. Kunapadam (Moolikaivakuppu). 1936; **I**: 621-3.
7. Pandey BP. Taxonomy of Angiosperms, S. Chand and Company Ltd, 6th edition, Ram Nagar, New Delhi, 1997; ISBN: 81-219-0932-5.
8. Kugathasan KS. Check List of Some Plants of Botanical and General Interest With Brief Descriptions, Field Workcentre, Thondaimannaru, Jaffna. 2004; Bulletin no :16.
9. Melntyre A. Herbs For Common Ailment, Gaia Books Limited, UK. 1992; 13.
10. Chikitsai S. Sarabenthiravaiththiyamuraikal, Saraswathi-Mahal, Thanjavour. 1951; 89.
11. Nester W, Evans Roberts C, Pearsall N, Anderson G, Nester T. Microbiology. A human perspective, WCB/Mc Graw-Hill, 2nd edition, 1998; ISBN-0-697-28602-9: 86-96.
12. Lino A, Deogracious O. The in vitro antibacterial activity of *Annona senegalensis, Securidacca longipendiculata* and *Steanotaenia araliacea* – Ugandan medicinal plants. *Afr Health Sci* 2006; **6**: 31-5.
13. Lachenmeier DW, Keck-Wilhelm A, Sauermann A, Mildau G. Safety assessment of alcohol-containing mouthwashes and oral rinses. *SOFW J* 2008; **134**: 70-8.
14. Cole P, Rodu B, Mathisen A. Alcohol-containing mouthwash and oropharyngeal cancer: a review of the epidemiology. *J Am Dent Assoc* 2003; **134**(8): 1079-87.
15. Weaver C. Mouthwash linked to cancer. The Daily Telegraph (News Ltd). 2009; http://www.news.com.au/dailytelegraph/story/0,22049,24896583-5001021,00.html. (retrieved 12 January 2009).