A novel formulation of dentrifice paste from extracts of chewing sticks used in Nigeria

By
*MENDIE, U.E.A. IGWILO, C., NASIPURI,R.N.,
ADEOYE, A.O., +ROTIMI, V.O; AND BISONG, S.D.
School of Pharmacy
College of Medicine, University of Lagos, Lagos.

+ Department of Medical Microbiology
College of Medicine, University of Lagos, Lagos.

* Address for Correspondence*

SUMMARY

Using a mixed aqueous extract of ***Zanthoxylum zanthoxyloides*** (10% $^{w}/_{w}$), Massularia acuminata (20% $^{w}/_{w}$) and Veronia amygdalina (10% $^{w}/_{w}$) which are widely used chewing sticks in Nigeria a dentrifice paste of high antimicrobial activity and frothing ability was formulated.

The product was found to have a satisfactory shelf-life when stored at room temperature, and low minimum inhibitory concentration of 0.25% $^{w}/_{v}$, and minimum bactericidal concentration of 2.5% $^{w}/_{v}$ against resistant ***Staph. aureus.*** It was observed that the formulation of the combined chewing sticks extract had an increased activity against ***Staph. aureus,*** while the individual extracts did not show any appreciable activity against ***Staph. aureus*** suggesting a synergistic effect in the formulation.

INTRODUCTION

The use of chewing stick particularly in West Africa for dental hygiene is an age-long practice which has been observed to lead to lower cases of dental caries and gingivities (Odeblyi and Sofowora, 1979). This may be related to improve cleansing action of the chewing stick and/or the inhibitory activity of its extract on oral flora associated with oral and dental diseases (Rotimi and Mosadomi 1987). Most workers have shown that chewing sticks contain substances with antimicrobial activity particularly against pathogenic oral flora (Wolinsky and Sote, 1987). Lewis (1974) has reported that some chewing sticks contain cariogenic substances which prevent the development of dental caries.

The aim of the study is to formulate a dentrifice paste using the local chewing stick extracts in order to investigate if the anti-bacterial and frothing properties of the chewing sticks are retained in the formulated paste. It is hoped that the formulated paste will have an added advantage over the conventional tooth paste by possessing anti-bacterial activity and also enhancing the aesthetic use of chewing sticks.

MATERIALS AND METHODS

In the collection, preparation and standardization of chewing sticks extracts, the following chewing sticks were obtained. The roots of Zanthoxylum zanthoxyloides (Za), Veronia anygdalina (Va) and stem of Massularia acuminata (Ma) were obtained locally, but were authenticated in the Department of Pharmacognosy, School of Pharmacy, University of Lagos, Nigeria.

Each chewing stick was washed with water, then cut into pieces for drying in an oven at 60°c to a constant weight. It was then powdered using a roller mill.

200g of each powdered chewing stick was weighed into a clean cotton cloth bag and exhausted in soxhlet extractor using ethanol 70% (1) distilled water (2) and phosphate buffer (pH 7.4) (3) respectively. The extract was evaporated in vacuo to dryness and further dried at 45°c to a constant weight and stored at 4°c. Each extract was then subjected to the following tests:

Frothing test

1g of the extract was dissolved in 20ml of water in a 100ml conical flask and boiled gently for 3 mins, filtered hot and allowed to cool. About 0.5ml of water was added to 2ml of the filtrate in a test tube and shaken vigorously.

Anti-microbial tests

All the test micro-organisms used were hospital isolates which included ***Staphylococcus aureus, Staph. albus, Streptococcus mutans*** and ***Strept mitis.*** These organisms were maintained on blood agar (Oxoid) slopes at 4°c.

The test organisms were cultured overnight in Brain Heart Infusion (BHI) broth (BBL, USA) and diluted to 1×10^{6} cells ml with sterile water.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The method used to determine the MIC and MBC of the extracts against the bacterial isolates was a modification of Petersdorf and Sherris (1965).

The weighed amount of each extract was redissolved in phosphate-saline solution (pH 7.4) to obtain a 30% w/v solution which was centrifuged at 3000g for 10 mins. The supernatant was filtered through 0.54 micron millipore membrane filter and used immediately or stored in aliquots of 10mls at 4°c.

Using broth dilution method, double strength BHI broth was used to prepare the following concentrations of 24, 21, 18, 15, 12, 9, 6, 3% w/v of the extract. 0.1ml of the standard inoculum was then added to each broth concentration for the extract in a test tube. The 0.1ml of test organism was inoculated into BHI broth and phosphate saline solution respectively to serve as the positive controls.

All the test tubes were incubated at 37°c for 24h and examined for growth. The MIC of each extract against the test organism was determined as the least concentration required to inhibit its growth. No standard anti-microbial agent was included, since the aim was use of extracts in formulation of the paste as the anti-bacterial activity of the extracts have been established by other workers (Wolinsky and Sote, 1987).

In the MBC determination, a 3mm loopful sample was taken from tubes showing no growth and subcultured unto blood agar plates. They were incubated at 37°c for 24h. The lowest concentration which yielded no growth on the plate was recorded as the MBC of the extract against the test organism.

The mixed water extracts of the chewing stick in the ratio of their individual MIC, was used to determine the MIC of combined extracts against the test organism as earlier discussed. The choice of the water extract was due to the fact that they gave the lower MIC and MBC values of the 3 different extracts against each organism; and the availability of this solvent for extraction necessitated such choice.

The minimum bactericidal concentration (MBC) of the extracts Ma_2, Za_2 and Va_2 against ***Staph. aureus*** (most resistant) were 21, 12 and 12% w/v respectively (Table 2). From calculation 20ml of each will contain 4.20g Ma, 2.40g Za and 2.40g Va extracts respectively with a combined weight of 9.0g. A combination of the extracts at this stage was necessary since all contained saponins, had anti-microbial activities and Za extract causes a peppery sensation in the mouth (deodourizing effect). All these properties are necessary for a toothpaste formulation. Thus, 4.20g Ma, 2.40g Za and 2.40g Va extracts were dissolved in 20ml of phosphate buffer solution to give a 45% w/v stock solution. MIC and MBC of this stock solution were determined on all four bacterial species by broth dilution method as in the previous cases. In each case one test tube containing extract free-broth and the other containing only extract were also inoculated with the test organisms as controls.

Formulation of the toothpaste:

Based on the MIC and MBC determinations, the excellent frothing ability of ***Massularia acuminata*** as well as peppery sensation for the ***Zanthoxylum zanthoxyloides*** extract in the mouth; a mixed water extract containing 20g Ma, 10g Za, and 10g Va obtained from the three chewing sticks was prepared.

In this method, 20g of ***Massularia acuminata*** was weighed into a beaker containing 10g of ***Zanthoxylum zanthoxyloides*** extract in a water bath at 100°c. Both extracts were stirred continuously using a glass rod. Then, 10g of ***Veronia amygdalina*** extract was added and stirred to a uniform mixture without the formation of air bubbles. The final mixture was allowed to cool to room temperature and then transferred to a porcelain mortar. 12.10ml of deionized water was added to the extract mixture and triturated to form a homogenous paste. Then, 35g of calcium carbonate was then added in aliquot amounts and with trituration after each addition for even distribution.

Other ingredients which were added sequentially included sorbitol, 6g, sodium monofluoro phosphate 2.8g and saccharine powder 3.6g.

Finally, peppermint oil 0.5g was added, and triturated into a smooth tooth-paste.

Quality Assurance Tests:

These included the anti-microbial and the frothing tests which were similar to the ones earlier described. Other parameters examined included appearance and taste of the paste.

The anti-microbial test to examine whether the anti-bacterial activity of the extracts was retained in the paste formulated involved the following. 10g of the paste containing 4.0g of the extract was dissolved in 20ml of phosphate buffer solution to give 20.0% w/v solution of the extract. MIC and MBC were determined on ***Staphylococcus aureus*** which was the most resistant test organism. The control was a formulation of the paste without the extract.

Results and Discussions:

Table 1 shows the yield of the extracts obtained using different solvents such as 70% alcohol, distilled water, and phosphate buffer solutions. The alcoholic extracts of the chewing sticks gave slightly higher yield.

Table 2 shows the MIC and MBC of the extracts against the est organisms. Extracts with 70% alcohol as solvent had the lowest MIC values of 8 - 18% w/v compared to those using water or phosphate buffer solutions with a range of 10 - 21% w/v. The ***Veronia amygdalina*** alcoholic extract exhibited the highest activity against Staphylococci but gave similar activity to that obtained from ***M. Acuminata*** against the Streptoccal isolates. The alcoholic extract of ***Z. zanthoxyloides*** exhibited the least activity on all the test organisms.

The MIC of the combined extracts was observed to be lower than those of the individual aqueous extracts (Table 3). Generally the Streptococci gave lower MICs with the least value of 0.025% w/v against ***Strept. mitis***. The enhanced activity of the mixed extracts may be attributed to a synergistic activity of the individual extracts. This was observed from the proportion of individual extracts' contribution to the combined extracts' MIC (Table 3), which was much lower than the MIC of the individual extracts in water (Table 2).

The choice of a particular chewing stick depends largely on traditional preferences rather than clinical effectiveness. Such attributes may include the frothing ability, taste or the cleansing effect of a plant species. The lower incidence of dental carries among users of chewing sticks (compared to non-users) has been attributed to the superior mechanical cleasing action on the teeth (Enwonwu, 1985), while other workers have attributed it to anti-microbial properties of the extracts of these plants (Akpata and Akinrimisi, 1977).

Thus, the formulation of the chewing stick extracts into a dentrific paste was intended to enhance the aesthetics of its usage. The presence of the combined extract will impart the desired properties of the chewing sticks, and the incorporation of fluoride should significantly improve the therapuetic value of the paste against dental caries and other mouth infections. The low MIC and MBC values of 0.25% w/v and 2.5% w/v respectively obtained from the formulated paste against resistant ***Staph. aureus*** is a manifestation of its efficacy against oral infection.

The slight peppery taste and light brownish appearance of the paste were all acceptable indices, and the frothing ability was satisfactory for adequate cleaning of the teeth.

Conclusion:

Our study has established that incorporated extracts of chewing sticks in dentrifice formulation can impart the desirable properties of chewing stick and may go a long way to preventing oral and dental infections.

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Table 1: The yield of chewing stick extracts obtained from different solvents

Chewing Stick	Wt. of Powdered Plant (g)	Wt. of Extract (g)	% Yield
	Using 70% Alcohol (1)		
Z. zanthoxyloides (Za)	200	52.40	26.20
V. amygdalina (Va)	200	40.60	20.30
M. acuminata (Ma)	200	38.70	19.35
	Using distilled water (2)		
Z. zanthoxyloides (Za)	200	56.10	28.05
V. amygdalina (Va)	200	42.30	21.15
M. acuminata (Ma)	200	44.15	22.08
	Using Phosphate buffer (3)		
Z. zanthoxyloides (Za)	200	54.40	27.20
V. amygdalina (Va)	200	28.25	14.13
M. acuminata (Ma)	200	28.30	14.15

Table 2: The Anti-microbial activities of the individual chewing sticks extracts in different solvents against test organisms.

Chewing Stick extract	*Staph. aureus*		*Staph. albus*		*Strept. mutans*		*Strept. mitis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	(%w/v)		(% w/v)		(% w/v)		(% w/v)	
Za_1	9.0	12.0	9.0	9.0	5.0	6.0	4.0	5.0
Za_2	12.0	12.0	12.0	12.0	6.0	7.0	5.0	6.0
Za_3	12.0	12.0	12.0	12.0	6.0	7.0	5.0	7.0
Ma_1	18.0	21.0	12.0	15.0	0.1	1.0	4.0	5.0
Ma_2	21.0	21.0	15.0	18.0	2.0	2.0	5.0	6.0
Ma_3	21.0	21.0	18.0	21.0	2.0	4.0	5.0	6.0
Va_1	8.0	10.0	5.0	5.0	0.1	1.0	1.0	2.0
Va_2	10.0	12.0	6.0	6.0	2.0	4.0	2.0	5.0
Va_3	10.0	12.0	6.0	6.0	2.0	4.0	4.0	4.0

N.B (1) 70% alcohol
(2) Distilled water
(3) Phosphate buffer p^H (7.4)

Table 3: The Anti-microbial activities of the combined aqueous extracts against the test organisms

Test Organism	MIC of combine extracts (% w/v)	Proportion of individual extracts to combined MIC (% w/v)			MBC of combined extracts (% w/v)	Proportion of individual extracts to combined MBC (% w/v)		
		Za	Ma	Va		Za	Ma	Va
Staph. aureus	5.0	1.33	2.33	1.33	7.5	2.0	3.50	2.0
Staph. albus	2.50	0.67	1.17	0.67	5.0	1.33	2.33	1.33
Strept. mutans	0.25	0.07	0.12	0.07	0.25	0.07	0.12	0.17
Strept. mitis	0.025	0.07	0.01	0.007	0.25	0.07	0.12	0.07