



Preparation of an Antibacterial Herbal Mouthwash Containing Extract of *Erythrina Abyssinica*

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Abstract

Many plants derived medicines used in traditional medicinal system have been reported to treat infections against dental caries and periodontal diseases. The main aim of this research was to evaluate the antibacterial activity of herbal mouthwash formulation containing the extract of *Erythrina abyssinica* against *Staphylococcus aureus*. The bark of *Erythrina abyssinica* has been previously reported to have antibacterial activity. Thus, the bark of *E. abyssinica* was extracted using methanol and four base formulations were prepared using different volumes of water, glycerin, ethanol and polyethylene-glycol 400. Stocks of 10 % extract, 5 % tannin and 10 % thymol were prepared and these stock solutions and the four base formulations were used to prepare twelve 50 ml of herbal mouthwash formulations. The transmittance at 280 nm and pH of the herbal mouthwash formulations were determined as quality control tests. The herbal mouthwash formulations which showed best quality after the quality test were selected for antibacterial activity tests against *S. aureus*. All the selected mouthwash formulations containing *E. abyssinica* bark extract were found to show antibacterial activity against *S. aureus*. Therefore, this study shows that *Erythrina abyssinica* may be used to prepare a herbal mouthwash and hence playing a major role in controlling oral infections.

Keywords: oral infections, *Erythrina abyssinica*, herbal mouthwash, antibacterial, *Staphylococcus aureus*

INTRODUCTION

Oral infections are one of the most common chronic infections caused by colonization of healthy oral cavity by fungi, bacteria and viruses^[1]. Oral infections may occur either from an endogenous source or from an exogenous source. The most common microorganisms that are found within the oral cavity are *Staphylococcus aureus* and *Streptococcus mutans*^[2]. Mass of bacteria and their synthesized products adheres to the surface of the teeth and this is known as plaque. Some of the bacteria that are part of the plaque include *Streptococcus sp*, *Lactobacillus acidophilus*, *Campylobacter gracilis* and *concisus*, *Peptostreptococcus micros*, *Prevotella sp* and *Fusobacterium sp*.^[3] Plaque leads to gingival inflammation and periodontitis which may affect the oral health and also contribute to systemic diseases. The most common infectious oral diseases that are encountered by most individuals are dental caries and periodontal diseases^[4]. Dental caries is a great problem in developing countries, with poor living conditions and inadequate accessibility and affordability of oral health services.

The development of periodontal diseases can be prevented by dental plaque management^[5]. Various mechanical aids such as toothbrushes, interdental and chemotherapeutic cleansers such as mouthwashes, teeth and chewing gums are part of oral hygiene steps to prevent dental plaque accumulation^[6]. To improve the oral efficacy of the mechanical methods, antimicrobial agents, both chemical and herbal have been implemented. Mouthwashes are safe and efficient anti-plaque and antimicrobial agents that resist bacterial adhesion, invasion, metabolic activity and proliferation^[7]. However, in spite of the cost effectiveness of chemical mouthwashes commercially available, they tend to create undesirable side effects such as teeth discoloration and taste disturbance. Therefore, nontoxic herbal mouthwashes which are made from herbs and plant products have been implemented to overcome such side effects^[4].

Herbal mouthwash is a non-sterile solution which consists of natural ingredients known as phytochemicals that have anti-inflammatory and anti-microbial effects. The demand for natural mouthwash is high because they offer significant

advantages over chemical mouthwashes [8]. Herbal mouthwash rinses away bacteria while feeding and nourishing cells. Herbs in mouthwash also energize and create circulation for the gums and the surrounding mucous membranes [9]. Thus, this study focuses on preparing the antibacterial herbal mouthwash containing the bark extract of *E. abyssinica*. *Erythrina abyssinica* has previously been reported to have antibacterial activity against *S. aureus* [10].

Methodology

Plant Collection

The bark of the *E. abyssinica* plant was collected along Seke road in Chitungwiza and authentication was done at Harare Botanical Gardens. The bark was sun-dried for a couple of days until it was dry and free of moisture. A mortar and pestle were used to grind the bark into fine powder. Powder was placed in an airtight container, labeled and stored at room temperature.

Extraction of plant

A balance was used to measure 95 g of *E. abyssinica* powder and 380 ml of methanol was added to the powder. The mixture was placed in a shaker at 130 rpm for 48 hours. After 48 hours the mixture was filtered using Whatmann filter paper and the filtrate was set aside in an open beaker to dry.

Preparation of herbal mouthwash formulation

The different solvents mixtures that added up to 100 ml were prepared as mouthwash base formulations (B1-B4) using poly ethylene glycol 400, glycerin, ethanol and distilled water as shown in table 1.

Table-1: The different compositions of base formulations expressed as percentage.

Base formulation	PEG400	Glycerin	Ethanol	Water
B1	30	30	10	30
B2	40	45	5	10
B3	45	50	5	0
B4	30	30	0	40

Stocks of 10 % extract, 5% tannin and 10 % thymol were prepared then combined with base formulations (B1 – B4) to come up with twelve herbal mouthwash formulations (F1-F12) as shown in table 2. The concentration of tannin was varied whilst for extract and thymol were kept constant for all the formulations prepared.

Table-2: Preparation of antibacterial herbal mouthwash formulations using *E. abyssinica*

Mouthwash formulation	Base formulation	Volume (ml) added			
		Thymol	Extract	Tannin	Base formulation
F1	B1	0.25	2.5	1	46.25
F2	B2	0.25	2.5	1	46.25
F3	B3	0.25	2.5	1	46.25
F4	B4	0.25	2.5	1	46.25
F5	B1	0.25	2.5	5	42.25
F6	B2	0.25	2.5	5	42.25
F7	B3	0.25	2.5	5	42.25
F8	B4	0.25	2.5	5	42.25
F9	B1	0.25	2.5	20	27.25
F10	B2	0.25	2.5	20	27.25
F11	B3	0.25	2.5	20	27.25
F12	B4	0.25	2.5	20	27.25

Quality control tests

To select a suitable mouthwash base, quality control tests including pH and transmittance were determined in triplicate on 3 randomly chosen days, for two weeks. Transmittance was measured at 280 nm using a double beam UV spectrophotometer. pH of *E. abyssinica* mouthwash was measured using an Adwa (AD8000) pH meter and the buffers used for calibration were standardized buffer solutions for 7.4 and 4.0.

Antibacterial activity test

Streptococcus aureus was resuscitated in luria bertani broth overnight in a shaker at 37 °C at 120 rpm. Luria bertani agar was then prepared, autoclaved at 121 °C and then allowed to cool. The agar was poured into one plate which was labeled as negative control. To the remaining agar solution, *S. aureus* was inoculated, shaken to mix well and poured into one plate which was labeled as positive control. To the remaining mixture, herbal mouthwash formulation 6 (F6) was added and poured into three plates which were labeled as test samples. This same procedure was repeated with herbal mouthwash 7 (F7). The solutions were poured into agar plates. The plates were incubated at 37 °C overnight to investigate the effects of mouthwash formulation containing *E. abyssinica* extract against *S. aureus*.

RESULTS

Quality control test

The pH and transmittance at 280 nm of the mouthwash formulations were measured as quality control tests for 2 weeks on three randomly chosen days to select a suitable mouthwash. The results were reported as shown in table 3 and 4. The herbal mouthwashes 6 and 7 were selected for performance of antibacterial activity against *S. aureus* using pH and percentage transmittance values obtained. Herbal mouthwash formulations 6 and 7 showed that there were slight deviations of the triplicate values from their mean values. The mouthwash formulations F10 had a constant pH value however there was a significant change in percentage transmittance.

Table-3: Transmittance of different herbal mouthwash formulations, F1-F12 measured at 280 nm on three randomly chosen days during the 2 weeks.

Mouthwash Formulation (F)	Day 1	Day 2	Day 3
F1	92.5	93.8	85.5
F2	85.9	93.5	81.9
F3	89.3	93.0	82.2
F4	88.0	92.0	87.0
F5	92.0	96.7	87.5
F6	92.8	93.0	92.2
F7	94.0	94.5	95.0
F8	90.1	96.0	83.0
F9	87.9	91.0	68.8
F10	83.0	86.7	84.8
F11	91.8	94.0	90.0
F12	86.7	93.0	84.3

Table-4: The pH of the different herbal mouthwash formulations (F1-F12) that were measured for two weeks on 3 randomly chosen days

Mouthwash Formulation (F)	Day 1	Day 2	Day 3
F1	2.76	2.89	2.79
F2	2.95	3.42	3.27
F3	2.75	3.27	3.20
F4	2.58	2.89	2.81
F5	2.41	2.79	2.61
F6	2.97	2.93	3.15
F7	3.02	3.20	3.03
F8	2.58	2.75	2.70
F9	2.82	2.94	2.84
F10	2.99	3.00	2.97
F11	2.73	3.01	3.03
F12	2.73	2.89	2.82

Antibacterial activity of herbal mouthwash formulations

The mouthwash formulations 6 and 7 were selected for antibacterial activity tests against *S. aureus* as oral bacteria. The results obtained are as shown in figure 1 and 2. No growth was observed in any of the test samples, this confirms that the mouthwash formulation prepared had antibacterial activity against *S. aureus*.

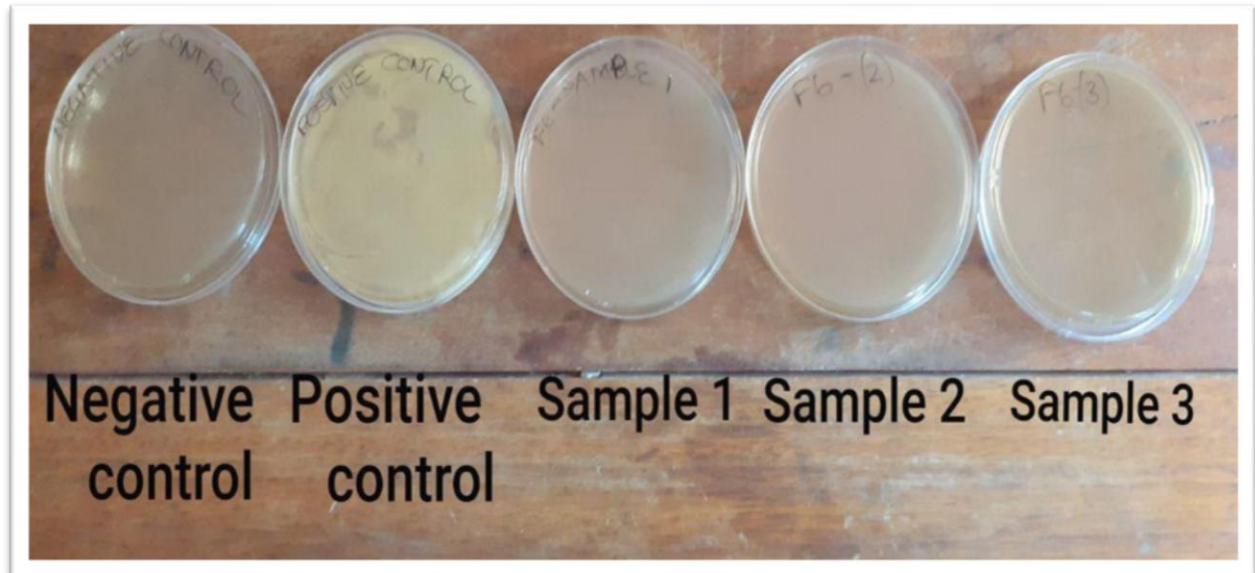


Figure-1: The effects of the herbal mouthwash formulation on the growth of *S. aureus*. Negative control contains agar only, positive control contains a mixture of agar and *S. aureus*, sample 1, sample 2 and sample 3 contain a mixture of agar, *S. aureus* and herbal mouthwash formulation 6 (F6). The herbal mouthwash formulation 6 contained 0.5 % extract of *E. abyssinica*, 0.5 % tannin, 0.05 % thymol and base formulation B2.

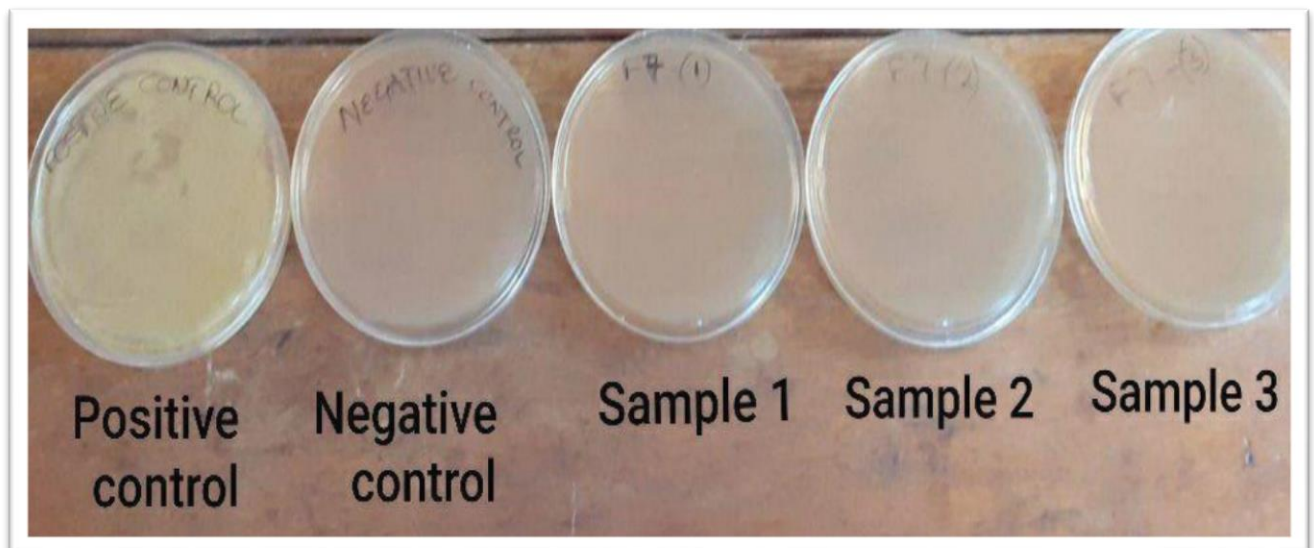


Figure-2: The effects of the herbal mouthwash formulation on the growth of *S. aureus*. Negative control contains agar only, positive control contains a mixture of agar and *S. aureus*, sample 1, sample 2 and sample 3 contain a mixture of agar, *S. aureus* and herbal mouthwash formulation 7 (F7). The herbal mouthwash formulation 6 contained 0.5 % extract of *E. abyssinica*, 0.5 % tannin, 0.05 % thymol and base formulation B3.

DISCUSSION

Previously, the bark of *Erythrina abyssinica* has been reported to have antibacterial activity, hence, this current study focused on mouthwash preparation. The results showed that the two selected prepared mouthwashes had the antibacterial activity as they inhibited the growth of the bacteria. These mouthwashes can play an effective role in the inhibition of the growth of infectious bacteria in the oral cavity thereby contributing to the prevention and control of the development of plaque and inflammation in periodontal disease.

To evaluate the herbal mouthwash, quality control tests were performed by measuring transmittance and pH as shown in table 3 and 4. The pH alone or the transmittance alone cannot justify the quality of the herbal mouthwash formulation; both pH and transmittance have to be used. The herbal mouthwash formulations 6 and 7 were selected for testing the antibacterial activity considering that both the pH and transmittance values had no significant differences. The constant pH and transmittance in herbal mouthwash formulations indicate that the organoleptic characteristics of the herbal mouthwashes such as the color and odor were not altered during the two weeks. Contamination or changes in the properties of the mouthwash formulation causes a shift in pH and transmittance values. The trend in pH is one of the reasons leading to teeth discolorations by some chemical mouthwashes therefore the pH of mouthwashes should be stable^[11]. The pH of the mouthwash in this study was acidic ranging from 2.41 to 3.42, and mouthwashes with acidic pH are regarded as more effective because they influence the metabolism of dental biofilm and hence reduce fermentation and production of extracellular polysaccharides^[12].

The herbal mouthwash formulations 6 and 7 that were selected for antibacterial activity contained 0.5 % of tannin concentration. These results are in line with previous work which reported that mouthwash formulations containing 0.2 % and 0.5 % tannin concentration were found to be the best formulations with adequate stability and antibacterial activity^[13].

Generally, results from this study show that the prepared mouthwash formulations may be recommended for use as they are stable and had antibacterial activity. Previous reports agree that herbal mouthwashes are as effective as chemical mouthwashes and can be recommended in place of the commercially available mouthwash^[14, 4]. Moreover, herbal mouthwashes offer significant advantages over chemical mouthwashes^[8]. A randomized trial was carried out on antiplaque control using chlorhexidine and herbal mouthwash containing *Aloe vera*. *A. vera* containing mouthwash statistically showed to have no significant difference with chlorhexidine in the reduction of oral plaque. However, the patients that were treated with chlorhexidine experienced mild brown staining of teeth that was not observed in *A. vera* patients. In addition to the positive side effects shown by mouthwash formulation containing *A. vera*, the mouthwash is recommended as an alternative of chlorhexidine because it is economically affordable and easy to prepare^[15].

Therefore, the herbal mouthwash overcomes the significant disadvantages of chemical by rinsing away bacteria while feeding and nourishing cells. Herbs in mouthwash also energize and create circulation for the gums and the surrounding mucous membranes^[9].

CONCLUSION

Based on the results obtained in this study, it could be concluded that *E. abyssinica* extract can be used in preparation of herbal mouthwash as the mouthwash had antibacterial activity against the oral pathogen. It can be attributed that the significant antibacterial efficacy of the herbal mouthwash in this study could have been due to the presence of bioactive compounds in the plant extract. Therefore, the significant inhibitory effects shown by herbal mouthwash containing *E. abyssinica* against *S. aureus* proved that herbal products can be used to replace the use of chemical mouthwash in oral hygiene. As a result, this will help in controlling oral diseases.

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