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Original Article

Antifungal Screening of Bridelia ferruginea Benth (Euphorbiaceae) Stem Bark Extract in Mouthwash Formulations

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Abstract

The plant Bridelia ferruginea Benth (Euphorbiaceae) has been known for its use in the management of oral thrush ethnomedicinally in various parts of Africa, a practice which has been justified by results of certain scientific studies. The aim of this study was to develop an appropriate dosage formulation, a mouthwash and evaluate the antifungal potential of this dosage formulation against a major causative organism of oral thrush, Candida albicans. Extraction of the stem bark was carried out with boiled distilled water, the extract was formulated into mouthwashes at concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5%w/v. All formulations contained viscosity imparting agent, a sweetener, and a preservative. Physical characterisation, viscosity, pH and palatability of the mouthwash formulations were determined. Agar-well diffusion method was used to assess the antifungal activity of the formulations against Candida albicans and Nystatin oral suspension was used as reference compound. The results showed that Bridelia ferruginea stem bark extract mouthwash solutions were brown in color, had an agreeable odor and sweet astringent taste. The pH for all concentrations was in the range of 5.41-5.63. The viscosity at spindle No 2, 60 rpm range between 0.226-0.238 Pa.S for all concentrations studied. The formulations had antifungal activity against Candida albicans. The highest concentration (2.5%w/v) gave mean zone of inhibition of 25.50±0.71mm that was comparable with Nystatin oral suspension 28.00±1.41mm, a reference compound. The foregoing suggests that with little modification in the formulation especially the adjustment of the pH, Bridellia ferruginea mouthwash solutions may be developed into commercially useful preparations.

Keywords: Bridelia ferruginea, Stem bark, Oral thrush, Mouthwash, Antifungal

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Introduction

Drug-resistant micro-organism strains are causing severe problems in many infections including oral candidiasis also known as oral thrush which is candidiasis in the mouth, in which the use of azole antifungals in recurrent cases risks selection and enrichment of drug-resistant strains of candida organisms and in persons who are immune-compromised, drug resistance is increasingly more common and presents a serious problem (Rautemma and Ramage 2011; Scully 2013). Candida albicans is one of the most important micro-organisms often implicated in oral diseases, it is the most common yeast isolated from the oral cavity and a common cause of oral thrush. Individuals heavily colonized by this organism and other cariogenic bacteria are considered to be at high risk for dental caries. Hence eradication of these microorganisms is important for dental treatment. However, the prevention of oral diseases is easier than a cure (Aneja et al. 2010). Various products such as toothpaste, gels, pastes for application, mouthwash, lozenges, etc. have been available for years. However, in recent years, use of mouthwash has been on the increase as it is relatively easy to use for maintaining oral hygiene (Atul et al. 2011). While their primary appeal is as an aid to breath freshness and cleansing the mouth, they could also be used as antimicrobial solutions for the prevention and treatment of oral diseases by reducing microbial load in the oral cavity (Aneja et al. 2010). Commercially available mouthwash solutions containing synthetic and semi-synthetic active agents have several disadvantages like staining the teeth, irritation during use, a high degree of alcohol content. To overcome these disadvantages, naturally occurring antimicrobial herbs can be used individually or in combination (Atul et al. 2011). Bridelia ferruginea has been in use in ethnomedicine for the treatment of various ailments and is a popular herbal remedy in many parts of Africa. The bark, roots, fruits and leaves, leafy twigs are all employed, being used mainly as decoctions. The medicinal activity is believed to be mainly due to the presence of tannins (which are astringent) and saponoside. The extract of the bark, leaves and roots of Bridelia ferruginea has been used for oral thrush 'Efu' in many parts of Nigeria where some people use it as gargle or mouthwash for oral candidiasis or orally to clean the infection that may be in the gastrointestinal tract and in blood, (the 'internal Efu') this may be assumed, in medical term to mean systemic fungal infection or candidaemia. The bark and the bright red infusion from it are commonly sold in Nigerian markets and shops for use as a mouthwash and remedy for thrush in children. Similar use is made of a root decoction in Ivory Coast (Dada-Adegbola et al. 2010). However, despite the popular ethnomedicinal use in oral thrush, there is still a dearth of information on the use of Bridelia ferruginea in herbal mouthwash formulations. This present study involves the preparation and evaluation of a mouthwash for antifungal action against Candida albicans commonly implicated in oral thrush.

Materials and Methods:

Collection and Treatment of stem bark of Bridelia ferruginea Benth.

The plant material was collected from the botanical gardens (and surroundings) of Pharmacognosy Department, Faculty of Pharmaceutical Sciences, University of Ilorin. It was identified and authenticated at the herbarium section of the Department of Plant Biology, the University of Ilorin, Nigeria by a botanist and assigned no UILH/001/987. The international plant name index is Euphorbiaceae Bridelia ferruginea Niger Fl. [W. J. Hooker]. 511. 1849[Nov– Dec 1849] (IK) (Mbah et al. 2012). The bark was cleaned and

scales removed. The clean bark was cut into pieces and air-dried at room temperature for 2 weeks, then oven dried at 45°C for 24 hours. The dried bark was pulverized using mortar and pestle, then milled to a fine powder.

Extract Preparation.

According to the method described by Mbah et al. (2012), 500g of the ground stem bark was soaked in boiled distilled water overnight and filtered. The filtrate was then freeze-dried to obtain the dry extract. The dried extract was stored in the refrigerator until it was needed. The bark extract was subsequently reconstituted in water at appropriate concentrations for the experiment. The concentrations were 0.5, 1.0, 1.5, 2.0, and 2.5%w/v for the BF stem bark mouthwash formulation.

Preparation of Bridelia ferruginea (BF) Stem Bark Extract Mouthwash Solutions.

The mouthwash was prepared by dissolving saccharin and methylparaben in a small amount of water. Different amount of Bridelia ferruginea stem bark extract to achieve concentrations of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5%w/v respectively were dissolved in sorbitol and then incorporated into already prepared solutions and finally made up to required volume (Table 1).

Table 1: Bridelia ferruginea Stem Bark Extract Mouthwash Formulations

Ingredients		**Quantity of ingredient in each formulation**				
	A	B	C	D	E	F
Sorbitol	5.0g	5.0g	5.0g	5.0g	5.0g	5.0g
Methylparaben	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g
Saccharin	1.0g	1.0g	1.0g	1.0g	1.0g	.0g
B ferruginea stem bark extract	0.0g	0.5g	1.0g	1.5g	2.0g	2.5g
Purified water to	100mL	100mL	100mL	100mL	100mL	100mL

Characterization of the BF Stem Bark Extract Mouthwash Solutions.

Physical Characterization: The appearance, color, odor and taste of the different concentrations of mouthwash solutions prepared were observed and recorded.

pH: The pH of each mouthwash formulation was determined by a digital pH meter. The pH meter was immersed into the mouthwash sample, stirred a little and left for five minutes and the reading was taken. The process was repeated for all the other samples. All measurements were an average of three determinations and expressed as mean ± S.D.

Viscosity: The measurement of viscosity of the mouthwash solutions using spindle no. 2 at 60 rpm was carried out with an NDJ-5S viscometer.

Antifungal Screening of BF Stem Bark Extract Mouthwash Solutions

The antifungal activity of the formulated mouthwashes was screened by Agar well diffusion method in Petri dishes containing Saboraud dextrose agar (Biomark, India) medium.Cylindrical holes were bored into the agar medium using a sterile number 6 cork borer. The holes were sealed with 2 drops of molten agar and then filled with 0.1mL of different concentrations (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5% w/v) of mouthwash formulations. The plates were allowed to stand on the laboratory bench for 1hour to allow for

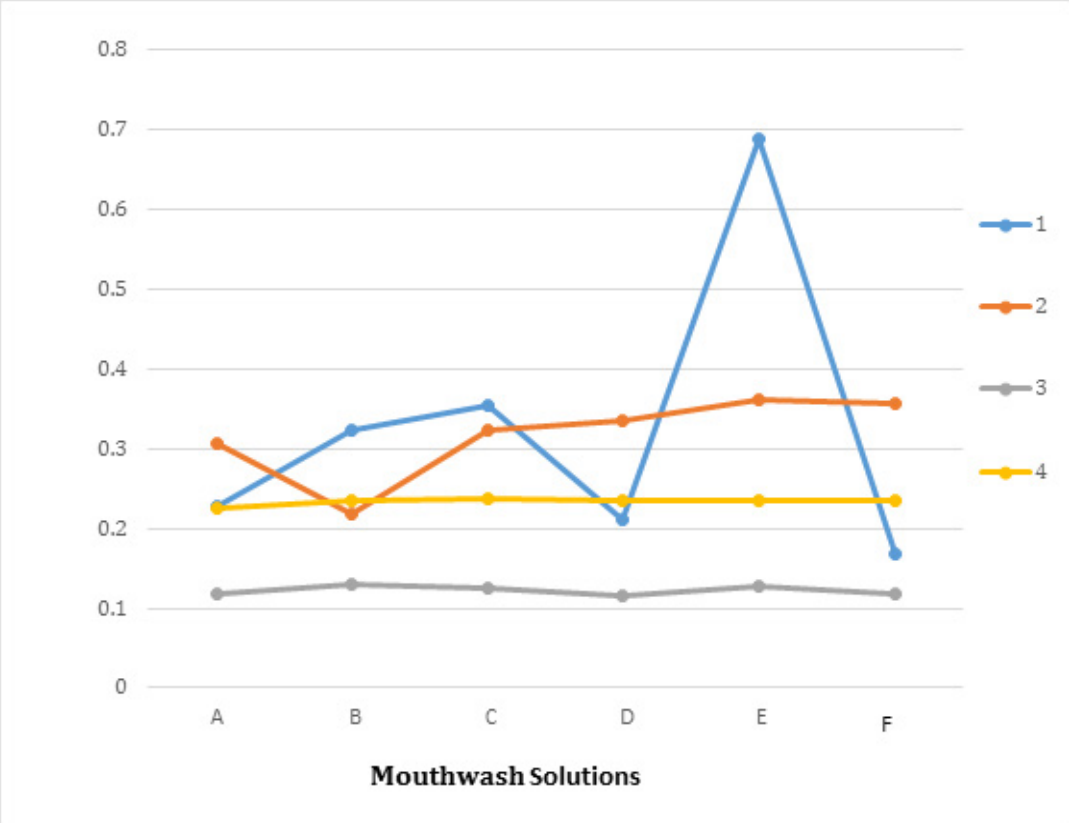

Figure 1: Graph of the viscosity of Bridelia ferruginea Stem Bark Extract Mouthwash Solutions at different rotations with spindle number 2.

Key:
A – 0%w/v B – 0.5%w/v C – 1.0%w/v D – 1.5%w/v E – 2.0%w/v F- 2.5%w/v
1 – 6rpm 2 – 12rpm 3 – 30rpm 4 – 60rpm

diffusion of the solutions into the medium. The plates were subsequently incubated at 27˚C for 3days and observed for the zone of inhibition of growth each day. The zones were measured with a meter rule and the result recorded in millimeters. The screening was done in triplicates. Nystatin oral suspension was used as a positive control and formulation without extract was used as negative control.

Statistical Analysis

One-way ANOVA was used to determine if there was a significant difference in the mean zones of inhibition of the different concentrations of Bridelia ferruginea stem bark extract mouthwash solutions. Student's t-Test (unpaired, one-tailed) was used to establish if there was a significant difference in the mean zones of inhibition of the highest concentration (2.5%w/v) of the formulation and Nystatin Oral Suspension, a reference compound. Differences between mean were considered significant when $p < 0.05$.

Declaration: NO Animal or Human subjects were used in this study

Results

The physical characteristics such as odor, color, and taste of the different concentrations of Bridelia ferruginea stem bark extract mouthwash solutions are presented in Table 2.These characteristics are important for patients usage compliance. The intensity of

Table 2: Physical characteristics of the Bridelia ferruginea stem bark extract mouthwash solutions

Parameters	**Mouthwash Solutions**					
	A	B	C	D	E	F
Colour	Colorless	Brown	Brown	Brown	Brown	Brown
Odour	Odorless	Odorless	Odorless	Odorless	Odorless	Odorless
Taste	Sweet	Sweet and astringent	Sweet and astringent	Sweet and astringent	Sweet and astringent	Sweet and astringent

these organoleptic properties was found to be of increasing order across the concentration range. It is even possible that a natural pigment may be present in the stem bark contributing to the coloration. The mean pH values of the Bridelia ferruginea stem bark extract mouthwash solutions are presented in Table 3. The pH of the formulation must be compatible with body pH. Any adverse pH could lead to deleterious reactions. The revelation of the pH at pre—formulation stage could help in formulation re-engineering through the use of buffer solutions towards achieving an acceptable product. Results of the various viscosity values (Pa.S) obtained for the Bridelia ferruginea stem bark extract mouthwash solutions at spindle number 2 and rotations are represented in Figure 1. Rheological information often helps in being able to predict the performance of dosage formulations during storage whether they are able to retain their forms or otherwise. The flow pattern also determines the pourability of liquid systems. The diameter of the zone of inhibition in millimeters of the Bridelia ferruginea stem bark extract mouthwash solutions at different concentrations against Candida albicans is presented in Table 4.The choice of fungus was informed by the fact that it is often implicated in oral thrush for which the plant has been used ethnomedicinally. From Table 4, the observation was that lethal effect of the formulation increased as the concentration of the extract increased. This shows activity related to the concentration of secondary metabolites present in the plant.

Discussion

The plant Bridelia ferruginea Benth (Euphorbiaceae) is used in many parts of

Table 3: The pH of the mouthwash formulations.

Mouthwash Solutions (%w/v)	pH
0	5.48 ± 0.04
0.5	5.41 ± 0.07
1.0	5.46 ± 0.12
1.5	5.49 ± 0.03
2.0	5.63 ± 0.06
2.5	5.63 ± 0.03

Africa including Nigeria for the management of oral thrush (Dada-Adegbola et al. 2010.). Thus, the selection of the plant, the part used for this study and its extraction method are based on ethnomedicinal usage background parameters. This present study involved the preparation of mouthwash formulation for antifungal action against Candida albicans commonly implicated in oral thrush. The formulation consisted of aqueous extract of Bridelia ferruginea Benth stem bark in graded concentrations. The Bridelia ferruginea stem bark extract mouthwash solutions were brown in color and the color was more intense with increased extract concentration. This explains the exclusion of colorant in the formulation. The odor of the mouthwash formulation was agreeable, this tends towards the commonly used flavoring's odor in commercial mouthwash solutions. An acceptable odor is an essential feature of a mouthwash solutions because of the need to refresh the breath of the patient. In some other instances, the odor from the mouthwash is expected to mask bad breath or odor of some patients. Deliberately, use of flavoring was omitted in the present formulation because the natural odor satisfies the standard requirement for patient compliance. The taste was sweet and astringent. The sweetness was further strengthened by the inclusion of saccharin, a sweetener in the formulation. This is because generally mouthwash solutions are supposed to possess this characteristic since patients usually will have to hold the solution in the mouth for a few minutes, any unpleasantness in the taste may lead to loss of compliance. The pH of the mouthwash formulations ranged between 5.41 and 5.63, values which tend towards acidic. This could pose a potential problem in the use of the B. ferruginea stem bark extract mouthwash solutions, the pH level in the mouth affects the health of teeth and gums, and tooth decay can occur when the pH level in the mouth drops below 5.5. Also, when the mouth undergoes dramatic or long-lasting periods of low pH, it can cause cavity-causing bacteria to grow. Hence, there is a need for pH balance in the mouth. Modifications can be made to the pH of this formulation through the use of a buffer solution in order to obtain an ideal pH value which poses little or no risk to the mouth and the health of teeth and gums.

The viscosity values of the mouthwash formulations were obtained at various speeds of rotations with the different spindle no 2. Mouthwashes are Newtonian fluids and as such are expected to maintain a constant viscosity independent of the shear. The viscosity values were uniform for all the mouthwash concentrations at 60rpm and this is in accordance with the flow behavior of mouthwash solutions.

Table 4: Mean inhibition zones of Bridelia ferruginea stem bark extract mouthwash solutions (mm)

Mouthwash solutions (%w/v)	Candida albicans
0.0	0.00
0.5	10.50 ± 0.71
1.0	12.50 ± 0.71
1.5	15.25 ± 1.77
2.0	20.50 ± 2.12
2.5	25.50 ± 0.71
Positive control	28.00 ± 1.41

Positive control: Nystatin oral suspension

However, there were variations at other lower rotation speeds. This is because of the fact that the lower the speed of rotation, the higher the drag force against the driving force to bring about the real flow in a system. The antimicrobial properties of the B. ferruginea stem bark extract have been established by earlier workers (Dada-Adegbola et al. 2010; Jose and Kayode 2009). From the results of the antifungal activity of the mouthwash formulations against Candida albicans (Table 4), all the mouthwash formulations of the B.ferruginea stem bark extract had considerable activity against the organism and thus, its use in ethnomedicine for the treatment of oral thrush is justified. The mouthwash formulations inhibited the growth of Candida albicans in order of increasing extract concentrations $p=0.0005(p<0.05)$ contained in them, with the highest concentration (2.5%w/v) exhibiting the largest zone of inhibition. However, the difference in mean zones of inhibition of the B.ferruginea stem bark extract mouthwash solution with the highest concentration of the extract (2.5%w/v) and the reference compound (Nystatin oral suspension) is not statistically different with p-value being 0.15, which is greater than 0.05. Thus, this suggests that the 2.5%w/v B.ferruginea stem bark extract mouthwash solution is comparable in its inhibitory activity against Candida albicans with the reference standard (Nystatin oral suspension). This is similar to the earlier study by Dada-Adegbola et al (2010), where they used a decoction of the B.ferruginea stem bark extract in the treatment of oral thrush. In earlier studies by Mbah et al (2012), Araromi et al (2014), Ndam et al (2014), they were able to establish the presence of phytoconstituents such as saponins, tannin, alkaloids, anthraquinones, flavonoids, and terpenoids in the stem bark extract. The presence of these phytoconstituents may be responsible for the antimicrobial properties of the bark extract (Rains and Jain 2011). These phytochemicals are biologically active compounds (Kulkarni et al, 2014; Ajinkya and Manjusha, 2016, Azubuike et al, 2015) and have a broad range of biological activities. Phytochemicals such as tannins exhibit antimicrobial properties.

Conclusion

All formulated mouthwash solutions showed potential for use in the treatment of oropharyngeal candidiasis. The results of the study indicate that Bridelia ferruginea stem bark extract can be formulated into mouthwashes for oral use in treating oral thrush caused by Candida albicans.

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Conflict of interest.

All authors (O.I.Aremu and A.B.Adewoyin) declare that they did not have a conflict of interest.

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