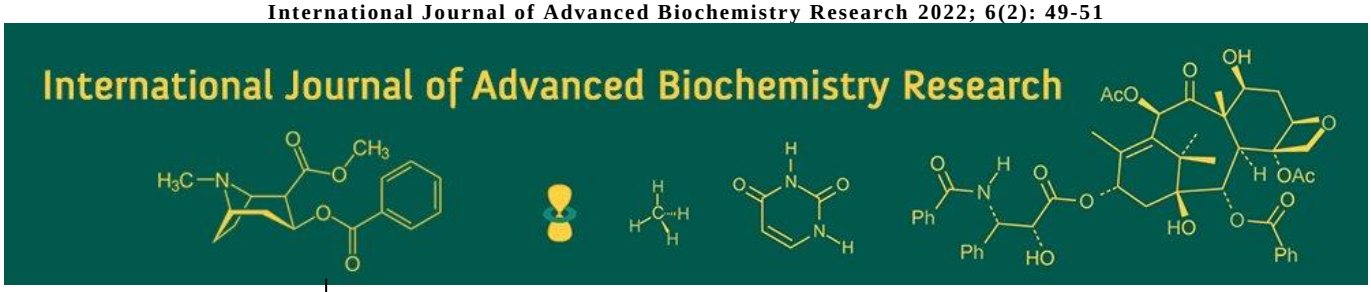

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Production of herbal toothpaste using *Moringa* root essential oil extract

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Abstract
In this study, antimicrobial toothpaste (dentifrice), which can compete with existing toothpaste, was produced using *Moringa oleifera* root essential oil extract. The oil extract 2 ml per 100 g obtained, gave peppermint flavor and was volatile. The phytochemical screening revealed a high concentration of flavonoid, a bioactive agent with antimicrobial activity in nature. 0.5 ml of the concentrate essential oil extract was used to produce 120 gm of a novel dentifrice. The antimicrobial activities of the novel herbal toothpaste were assessed against *Streptococcus mutans*, *Lactobacillus*, *Staphylococcus aureus*, and *Candida albicans* which are microorganisms involved in tooth plaque, using Oral-B (pro-health), commercial toothpaste as a positive control. The antimicrobial potential of the novel toothpaste was evaluated by the muller Hinton agar well diffusion method against the selected bacteria. The novel toothpaste showed higher inhibitory activity against *Streptococcus mutans* (31 mm), *Lactobacillus* (33 mm), *Staphylococcus aureus* (16 mm), and *Candida albicans* (16 mm) while, the Oral-B positive control toothpaste showed inhibition against the bacteria; *Streptococcus mutans* (22 mm), *Lactobacillus* (28 mm), *Staphylococcus aureus* (12 mm) and *Candida albicans* (15 mm) respectively. From the result, it was shown that the Moringa root herbal toothpaste has high antimicrobial potential than Oral-B herbal toothpaste against tooth plaque. It can be recommended that further studies on sensory analysis of food and dentifrice products containing Moringa root essential oil be carried out to evaluate its acceptability and shelf life.

Keywords: Antimicrobial, dentifrice, *Moringa oleifera*, essential oil, tooth plaque, toothache, phytochemicals

Introduction
Oral and dental health is an essential part of general health and is one of the public health hazards (Bhattacharya *et al*, 2003) [7]. Tooth decay, gingivitis, and periodontitis are the most common oral and dental health diseases which can be seen frequently in different age groups and different parts of the world, (Akltar and Bhakuni, 2004) [1].

In the development of dental health diseases, various microorganisms, such as *Streptococcus mutans* (SM), and *Lactobacillus* have significant effects. These microorganisms play a key role in the fermentation of sucrose resulting in lactic acid production which leads to demineralization of the enamel and dental plaque (Khare, 2007) [10]. Other microbes such as *Staphylococcus aureus*, *Streptococcus* mitis, and *Candida albicans* have been implicated in dental diseases (Hamada and Slade, 2010) [9].

Many dentifrices have been produced over the years, some focusing on marketing strategies to sell products such as offering whitening capabilities, (Maripandi *et al.*, 2011) [11]. The most essential dentifrices recommended by dentists are toothpaste used in conjunction with a toothbrush to help remove food debris and dental plaque. These bacteria elaborate various compounds (H_2S, NH_3, amines, toxins, enzymes, and antigens) which elicit an inflammatory response that is though protective but also responsible for the loss of periodontal tissues, pocket formations, and loosening and loss of teeth. This continues until late, when abscesses may occur. Bleeding gums and bad breath may occur (Hamada and Slade, 2010) [9]. The advent of modern technology brought in many brands of synthetic kinds of toothpaste with chemically based antimicrobial agents such as Chlorhexidine, triclosan, and fluoride compounds (Maripandi *et al*., 2011) [11]. Fluoridated toothpaste apart from not being recommended for children below 6 years of age, causes pigment of teeth and weakening of enamel, while Chlorhexidine, (Chlorophenylbiguanide), causes pigmentation of dental,

mouth, and tongue surroundings with an altered sense of taste, irritation and oral dryness, scaling of gingival and negative systemic effects in ingestion (Ghelichli, 2014) [8].

Herbal medicine has been considered an alternative formulation to chemically based orthodox formulations in every aspect of pharmaceutical products and dentifrices, with no exceptions. Although some literature has reported the antibacterial activities of Moringa oleifera, there are not many research studies on the use of Moringa plant extract in the formulation of dentifrices. Moringa oleifera (M. Oleifera), belongs to the family, Moringaceae and order, brassicales. It is popularly known as drumstick or horse radish in English. The seeds, fruits, leaves, and immature pods of this tree are used as highly nutritive vegetables in Nigeria (Answer *et al*, 2005) [3]. The root part is highly medicinal, and the extract has antimicrobial activities enough to subdue harmful microorganisms. It contains bioactive substances that affect living organisms, tissues, and cells (Barreto, *et al*, 2007) [6]. The root part contains essential oil and active substances enough to keep the mouth fresh if compounded in dentifrices.

The essential oil is made of phytochemicals most of which are volatile substances that gave a peppermint smell. (National Center for Complementary and Integrative Health, 2016) which was indigenous to Europe and the Middle East before it became common in other regions such as North America and Asia. Peppermint extract is commonly used in cooking as a dietary supplement, in herbal or alternative medicine, as a pest repellent, and as a flavor in cleaning products, cosmetics, mouthwash, chewing gum, and candies. It is used as an antiseptic, anti-viral, and stimulant (Tanu *et al*., 2016) [17], hence essential oil is effective as an antimicrobial agent. Moderate levels can be safely mixed into food items or applied topically or inhaled using aromatherapy (Santus, 2020) [16] and as such used as a remedy for a toothache (Worwood, 1991) [18].

In this regard, this research is aimed to provide information on how to formulate herbal toothpaste using Moringa root essential oil extract against the microorganism implicated in dental plaque.

Materials and Method

Collection/Preparation of Materials

Fresh Moringa roots were harvested from the plant growing in the botanical garden of Science Laboratory Technology, located at the permanent site of the Federal Polytechnic Oko. The harvested roots were washed and Sun-dried. One hundred grams of the dried root was ground to powdered mesh using a manually operated grinder and kept in a bottle container tightly closed.

Extraction

The oil was extracted by the Soxhlet extraction method, (Azhari, *et al*., 2018) [5].

50 g of powdered sample of Moringa root was extracted using 300 ml of 95% ethanol by a soxhlet extractor. The extract was concentrated to 2 ml dark brown viscous oil, using a rotary evaporator at 65 °C.

Phytochemical Analysis

0.5 ml of the extract was used for qualitative phytochemical screening by the method described by AOAC (2005) [4]. Bioactive agents screened for were flavonoids, alkaloids, phenolics, and tannins.

Formulation of Novel Toothpaste

Formulation of Moringa root extract-based toothpaste. All ingredients were weighed and turned into a stainless mixer as follows:

0.5 ml Moringa root extract
50 g calcium carbonate (Abrasive)
20 g sodium lauryl sulphate (Detergent)
0.1 g methyl cellulose (Binder/hardener)
1 g glycerine (Sweetener)
50 g water (Dispersion medium)

These materials were added slowly into the dispersion medium and mixed until a paste consistency was formed.

Comparative Sensitivity Tests

The pH of the novel toothpaste and that of Oral-B toothpaste were measured using a pH meter.

Antimicrobial Sensitivity Test

Microorganism selection

Four (4) bacteria strains identified in tooth plaque were obtained from the microbiology laboratory of science laboratory technology federal poly Oko namely *Streptococcus mutans* (SM), *Lactobacillus*, *Streptococcus* aureus, and *Candida albicans*.

The antimicrobial sensitivity test for novel toothpaste was carried out by the agar diffusion assay as described by Jorgenson *et al*., (2007). Briefly, bacteria strains were grown in Mueller-Hinton agar and broth. The strains were incubated at 36 ºC for 18h and were diluted to a final concentration. 106 cfu/ml. Each bacteria suspension was spread over the surface of Mueller Hinton agar, containing 3 wells of 7 mm diameter and filled with 0.25, 0.50, 0.75, and 1.00 ml of extract made by Serial dilute of the concentrate dissolved in the medium and incubated at 36 °C for 20 h. An Oral –B toothpaste was used as a positive control.

Results

The total yield of Moringa Novel Toothpaste was 120 gm.

The result of the confirmatory phytochemical analysis of the extract was presented in table 1.

Table 1: Phytochemical composition of Moringa root extract

Phytochemicals	Values
Flavonoids	+++
Alkaloids	++
Phenolics	+++
Tannis	++

Key: +++ = Highly Present
++ = Moderately Present
+ = Slightly Present
= Absent

Table 2: Physicochemical properties of Novel toothpaste and Oral-B toothpaste

Parameter	Novel toothpaste	Oral-B toothpaste (Control)
Colour	White	Blue
Clogging	Absent	Absent
Homogeneity	Homeogenous	Homogenous
Texture	Smooth	Smooth
% Moisture	50%	55%
Ph	8.00	7.70

Table 3: the results of the antimicrobial analysis of the sample and control against selected organisms are shown in table 3. Zones of inhibitions (mm) on the tested Microorganisms

Bacteria strain	Moringa toothpaste	(Oral-B)
Streptococcus mutans	31	22
Lactobacillus	33	28
Staphylococcus aureus	16	12
Candida albican	16	15

From the result, Novel Dentifrice showed higher inhibitory activity on microorganisms causing teeth plaque, than Oral-B. Zones of inhibitions on streptococcus, and *Lactobacillus* by the Novel toothpaste were higher than that of Oral-B commercial toothpaste.

Discussion

In the result, 50 g of Moringa root extract yielded 2 ml of essential oil, which is in line with earlier work by Azhari *et al*., (2018) [5], who reported a total Soxhlet extraction result of 1.5+0.12 ml. The phytochemical screening result showed the presence of flavonoids, alkaloids, phenolics, and tannins which can be effective on the microorganisms implicated in the tooth plaque (National Center for Complementary and Integrated Health (NCCIH, 2016) [12]. Dentifrices including toothpowder and paste are agents along with a toothbrush to clean and keep natural teeth healthy. (Zero, 2011) [19].

The antimicrobial sensitivity test showed that Moringa formulated toothpaste has higher efficacy than the control toothpaste considering its inhibition of *Streptococcus mutans* (31 mm) and *Lactobacillus* 33 mm compared to Oral-B with (22 mm) and (28 mm) respectively. This may be a result of high concentrations of flavonoid and phenolic groups in the plant extract. (Amaechi, and Loveren, 2013) [2]. The result showed that the essential oil of Moringa oleifera root back had antibacterial, activity against all the bacteria Strains tested and implicated in the teeth plaque. (Hamada and Slade 2010) [9], who reported similar activity on microorganisms of tooth plaque by herbal toothpaste. This result is in fair correlation with the studies in which essential oil has antibacterial activity against gram-positive and gram-negative bacteria as reported by Hamada and Slade (2010) [9]. The plant extract has advantages over chemical agents since it is organic and harmless to the body.

Conclusion

The result revealed that *Moringa oleifera* root back contained essential oil extract capable of inhibiting microorganisms implicated in dental plaque and as such can be a good source of active agent in the formulation of medicinal toothpaste.

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